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(54)【発明の名称】インターロイキン１５（ＩＬ－１５）に特異的なヒト抗体

(57)【要約】
IL-15（例えばヒトIL-15）に特異的に結合する単離されたヒトモノクローナル抗体及び関連する抗体ベースの組成物及び分子を開示する。本ヒト抗体は、V-D-J組換え及びアイソタイプ・スイッチングを起こすことにより複数のアイソタイプのヒトモノクローナル抗体を産生できる、トランスフェクトーマ又はトランスジェニック・マウスなどの非ヒトトランスジェニック動物で産生させることができる。さらに、本ヒト抗体を含む医薬組成物、本ヒト抗体を産生する非ヒトトランスジェニック動物及びハイブリドーマ、並びに本ヒト抗体を用いた治療法及び診断法も開示されている。

【特許請求の範囲】
【請求項１】
ヒトIL-15に特異的に結合してIL-15誘導性炎症誘発作用を阻害する単離されたヒトモノクローナル抗体。
【請求項２】
IL-15誘導性TNFα産生又はT細胞増殖を阻害する、請求項１に記載の抗体。
【請求項３】
IL-15誘導性T細胞増殖を、増殖阻害検定で判定したときにほぼ100nM未満のIC_{50}値で阻害する、請求項２に記載の抗体。
【請求項４】
IL-15誘導性T細胞増殖を、増殖阻害検定で判定したときにほぼ10nM未満のIC_{50}値で阻害する、請求項２に記載の抗体。
【請求項５】
組換えヒトIL-15を分析物とし、抗体をリガンドとして用いた表面プラスモン共鳴（SPR）技術で判定したときに、10^{-7}M未満の解離平衡定数（K_D）でヒトIL-15に結合する、請求項１に記載の抗体。
【請求項６】
前記抗体が、ヒトIL-15のβ鎖又はγ鎖相互作用ドメイン上に位置するエピトープに特異的に結合する、請求項１に記載の抗体。
【請求項７】
前記抗体が、ヒトIL-15のγ鎖相互作用ドメイン上に位置するエピトープに特異的に結合する、請求項６に記載の抗体。
【請求項８】
前記抗体が、ヒトIL-15のAsp^{8}がヒトIL-15受容体のβユニットへ結合すること、又は、ヒトIL-15のGln^{108}がヒトIL-15受容体のγユニットへ結合すること、に干渉する、請求項１に記載の抗体。
【請求項９】
前記抗体が、受容体に結合したヒトIL-15に特異的に結合する、請求項１に記載の抗体。
【請求項１０】
それぞれ図２（配列番号:1）及び図３（配列番号:3)並びにそれらの保存的配列改変に記載された通りのヌクレオチド配列を可変領域に含むヒトIgG重鎖及びヒトカッパ軽鎖核酸にコードされた、ヒトIL-15に特異的に結合する単離されたヒトモノクローナル抗体。
【請求項１１】
それぞれ図２（配列番号:2）及び図３（配列番号:4)並びにそれらの保存的配列改変に示すアミノ酸配列を含むヒトIgG重鎖及びカッパ軽鎖可変領域を有する、ヒトIL-15に特異的に結合する単離されたヒトモノクローナル抗体。
【請求項１２】
（ａ）それぞれ図２（配列番号:2）及び図３（配列番号:4)並びにそれらの保存的配列改変に示すアミノ酸配列CDR1を含むCDR1ドメイン、
（ｂ）それぞれ図２（配列番号:2）及び図３（配列番号:4)並びにそれらの保存的配列改変に示すアミノ酸配列CDR2を含むCDR2ドメイン、及び
（ｃ）それぞれ図２（配列番号:2）及び図３（配列番号:4)並びにそれらの保存的配列改変に示すアミノ酸配列CDR3を含むCDR3ドメイン、
から成る群より選択されるCDRドメインを含む、ヒトIL-15に特異的に結合する単離されたヒトモノクローナル抗体であって、
前記CDRドメインが抗体フレームワーク内に挿入されているか、又は、合成リンカにより接合されている、単離されたヒトモノクローナル抗体。
【請求項１３】
前記抗体が、IgG1、IgG2、IgG3、IgG4、IgM、IgA1、IgA2、IgAsec、IgD、及びIgE 抗体から成る群より選択される、請求項１に記載の抗体。

【請求項１４】
IgG1重鎖を含む、請求項１に記載の抗体。
【請求項１５】
一抗体フラグメント又は一本鎖抗体である、請求項１に記載の抗体。
【請求項１６】
完全抗体である、請求項１に記載の抗体。
【請求項１７】
ヒト重鎖導入遺伝子及びヒト軽鎖導入遺伝子を含むゲノムを有する非ヒトトランスジェニック動物由来のB細胞を不死化細胞に融合させて含有するハイブリドーマにより産生される、請求項１に記載の抗体。
【請求項１８】
ヒトIL-15に特異的に結合して、IL-15の、その受容体結合時の炎症誘発作用誘導能を阻害する、単離されたヒトモノクローナル抗体。
【請求項１９】
ヒトIL-15に特異的に結合する単離されたヒトモノクローナル抗体にIL-15を接触させるステップを含む、T細胞又は単球において、IL-2誘導性TNFα産生は阻害せずに、IL-15誘導性TNFα産生を阻害する方法。
【請求項２０】
T細胞の存在下で、ヒトIL-15に特異的に結合する単離されたヒトモノクローナル抗体にIL-15を接触させるステップを含む、IL-2誘導性T細胞増殖は阻害せずに、IL-15誘導性T細胞増殖を阻害する方法。
【請求項２１】
前記T細胞が、末梢血単核細胞（PBMC）又はCTLL-2細胞である、請求項２０に記載の方法。
【請求項２２】
ヒト重鎖導入遺伝子及び軽鎖導入遺伝子を含むゲノムを有する非ヒトトランスジェニック動物由来のB細胞を不死化細胞に融合させて含有するハイブリドーマであって、ヒトIL-15に特異的に結合するヒトモノクローナル抗体を産生する、ハイブリドーマ。
【請求項２３】
ヒトIgG重鎖及びヒトカッパ軽鎖核酸にコードされたヒトモノクローナル抗体を産生する、請求項２２に記載のハイブリドーマ。
【請求項２４】
それぞれ図２（配列番号:1）及び図３（配列番号:3)並びにそれらの保存的配列改変に記載されたヌクレオチド配列を可変領域に含むヒトIgG重鎖及びヒトカッパ軽鎖核酸にコードされたヒトモノクローナル抗体を産生するハイブリドーマ。
【請求項２５】
それぞれ図２（配列番号:2）及び図３（配列番号:4)並びにそれらの保存的配列改変に示すアミノ酸配列を含むIgG重鎖及びカッパ軽鎖可変領域を有するヒトモノクローナルを産生するハイブリドーマ。
【請求項２６】
ヒト重鎖及びヒト軽鎖をコードする核酸を含むトランスフェクトーマにより産生される、請求項１に記載の単離されたヒト抗体。
【請求項２７】
前記トランスフェクトーマが、検出可能な量の請求項１に記載のモノクローナル抗体を産生する、ヒト重鎖及びヒト軽鎖をコードする核酸を含むトランスフェクトーマ。
【請求項２８】
それぞれ配列番号:1 及び配列番号:3又はこれらの保存的配列改変に記載されたヌクレオチド配列を可変領域に含む、ヒト重鎖及びヒト軽鎖をコードする核酸を含む、請求項２７に記載のトランスフェクトーマ。
【請求項２９】

ヒトIL-15に特異的に結合するヒトモノクローナル抗体を発現する非ヒトトランスジェニック動物であって、ヒト重鎖導入遺伝子及びヒト軽鎖導入遺伝子を含むゲノムを有する、非ヒトトランスジェニック動物。

【請求項３０】

ヒト重鎖導入遺伝子及びヒト軽鎖導入遺伝子を含むゲノムを有する非ヒトトランスジェニック動物を、ヒトIL-15又はヒトIL-15発現細胞で、前記動物のB細胞により抗体が産生されるように、免疫するステップと；

前記動物のB細胞を単離するステップと；

前記B細胞を骨髄腫細胞に融合させて、IL-15に特異的なヒトモノクローナル抗体を分泌する不死のハイブリドーマ細胞を形成するステップと；

前記ハイブリドーマの培養上清から、IL-15に特異的なヒトモノクローナル抗体を単離するステップと

を含む、ヒトIL-15に特異的に結合するヒトモノクローナル抗体を作製する方法。

【請求項３１】

請求項１に記載の抗体及び治療薬を含む免疫結合体。

【請求項３２】

前記治療薬が免疫抑制剤である、請求項３１に記載の免疫結合体。

【請求項３３】

前記治療薬が、ステロイド系抗炎症薬、非ステロイド系抗炎症薬及びDMARDから成る群より選択される抗炎症薬である、請求項３１に記載の免疫結合体。

【請求項３４】

前記治療薬が細胞毒である、請求項３１に記載の免疫結合体。

【請求項３５】

請求項１に記載の抗体及び薬学的に許容可能な担体を含む、医薬組成物。

【請求項３６】

治療薬をさらに含む、請求項３５に記載の組成物。

【請求項３７】

前記治療薬が免疫抑制剤である、請求項３６に記載の組成物。

【請求項３８】

前記免疫抑制剤がシクロスポリンである、請求項３７に記載の組成物。

【請求項３９】

前記治療薬が、ステロイド系抗炎症薬及び非ステロイド系抗炎症薬から成る群より選択される抗炎症薬である、請求項３６に記載の組成物。

【請求項４０】

前記治療薬が、メトトレキセート、エタネルセプト、及びインフリキシマブから成る群より選択されるDMARDである、請求項３６に記載の組成物。

【請求項４１】

前記治療薬が、ドキソルビシン、シスプラチン、ブレオマイシン、カルムスチン、シクロホスファミド、及びクロラムブシル、から成る群より選択される化学療法薬である、請求項３６に記載の組成物。

【請求項４２】

前記治療薬が乾癬の治療薬である、請求項３６に記載の組成物。

【請求項４３】

前記治療薬が抗体である、請求項３６に記載の組成物。

【請求項４４】

前記抗体が、CD4特異抗体及びIL-2特異抗体から成る群より選択される、請求項４３に記載の組成物。

【請求項４５】

請求項１に記載の抗体を、ヒトIL-15により媒介される障害を治療又は予防するのに有効量、対象に投与するステップを含む、前記障害を治療又は予防する方法。

【請求項４６】
前記障害が、乾癬、関節炎、炎症性腸疾患、癌、移植片拒絶及び感染性疾患から成る群より選択される、請求項４５に記載の方法。
【請求項４７】
前記関節炎がリウマチ性関節炎である、請求項４６に記載の方法。
【請求項４８】
治療薬の同時投与をさらに含む、請求項４５に記載の方法。
【請求項４９】
前記治療薬が免疫抑制剤。シクロスポリンである、請求項４８に記載の方法。
【請求項５０】
前記免疫抑制剤がシクロスポリンである、請求項４９に記載の方法。
【請求項５１】
前記治療薬が、ステロイド系抗炎症薬及び非ステロイド系抗炎症薬から成る群より選択される抗炎症薬である、請求項４８に記載の方法。
【請求項５２】
前記治療薬が、メトトレキセート、エタネルセプト、及びインフリキシマブから成る群より選択されるDMARDである、請求項４８に記載の方法。
【請求項５３】
前記治療薬が、ドキソルビシン、シスプラチン、ブレオマイシン、カルムスチン、シクロホスファミド、及びクロラムブシル、から成る群より選択される化学療法薬である、請求項４８に記載の方法。
【請求項５４】
前記治療薬が乾癬の治療薬である、請求項４８に記載の方法。
【請求項５５】
前記治療薬が抗体である、請求項４８に記載の方法。
【請求項５６】
前記抗体が、CD4特異抗体及びIL-2特異抗体から成る群より選択される、請求項５５に記載の方法。
【請求項５７】
ヒトIL-15に特異的に結合して、その受容体への結合時にIL-15の炎症誘発作用誘導能を阻害する単離されたヒトモノクローナル抗体を対象に投与するステップを含む、乾癬を治療又は予防する方法。
【請求項５８】
前記抗体が、IL-15の錯角化症誘導能を阻害する、請求項５７に記載の方法。
【請求項５９】
前記抗体が、IL-15の表皮肥厚誘導能を阻害する、請求項５７に記載の方法。
【請求項６０】
前記抗体が、IL-15のケラチノサイト増殖誘導能を阻害する、請求項５７に記載の方法。
【請求項６１】
ヒトIL-15に特異的に結合して、その受容体への結合時にIL-15の炎症誘発作用誘導能を阻害する単離されたヒトモノクローナル抗体を対象に投与するステップを含む、リウマチ性関節炎を治療又は予防する方法。
【請求項６２】
前記抗体が、IL-15の活性化白血球遊走誘導能を阻害する、請求項６１に記載の方法。
【請求項６３】
試料中のIL-15抗原又はIL-15発現細胞の存在を検出することにより、IL-15媒介性疾患を診断する方法であって、
前記試料及びコントロール試料を、請求項１に記載のヒト抗体に、前記抗体もしくはその一部分とIL-15との間での複合体形成が可能な条件下で接触させるステップと、
複合体形成を検出するステップと、

を含み、前記コントロール試料に比較したときの前記試料の複合体形成の差が、前記試料中のIL-15の存在の指標である、方法。

【請求項６４】

IL-15の炎症誘発作用を阻害するヒトモノクローナル抗体の可変領域をコードするヌクレオチド配列を含む核酸。

【請求項６５】

ヒトIL-15に特異的に結合するヒトモノクローナル抗体の可変領域をコードするヌクレオチド配列を含む核酸であって、前記ヌクレオチド配列が、配列番号:1 及び配列番号:3並びにこれらの保存的配列改変から成る群より選択される、核酸。

【請求項６６】

ヒトIL-15に結合するヒト抗体の軽鎖、重鎖、又は軽鎖及び重鎖の両方、の可変領域をコードするヌクレオチド配列を含む発現ベクタ。

【請求項６７】

IL-15に結合するヒト抗体の軽鎖、重鎖、又は軽鎖及び重鎖の両方、のCDR領域をコードするヌクレオチド配列をさらに含む、請求項６６に記載の発現ベクタ。

【請求項６８】

それぞれ配列番号:2 及び 配列番号:4並びにこれらの保存的配列改変に示すアミノ酸配列を含む重鎖及び軽鎖可変領域をコードするヌクレオチド配列を含む発現ベクタ。

【請求項６９】

それぞれ配列番号:2 及び 配列番号:4並びにこれらの保存的改変に示すアミノ酸配列を含む重鎖及び軽鎖可変領域をコードするヌクレオチド配列を含む発現ベクタ。

【請求項７０】

請求項６６に記載の発現ベクタを含むトランスフェクトーマ。

【発明の詳細な説明】

【発明の背景】

【０００１】

インターロイキン-15 (IL-15) は炎症誘発性サイトカインであり、14乃至15kDの糖タンパク質である。単球及びマクロファージ、線維芽細胞、ケラチノサイト及び樹状細胞を含む多種の細胞及び組織で、構成的発現が報告されている (Waldmann and Tagaya, 1999; Fehniger and Caligiuri, 2001)。その発現は、IFN-γ及びLPSで刺激を受けたときや、又はウィルス、細菌もしくは原虫感染によって刺激を受けたときの単球で報告されているように、炎症状態で上方調節される(Kirman et al., 1998; Waldmann et al., 1998; Waldmann and Tagaya, 1999; Fehniger and Caligiuri, 2001)。さらに、リウマチ性関節炎などの慢性炎症性疾患では、局所的に産生されるIL-15 が、滑膜T細胞の動員及び活性化によって炎症を増幅しているようである。IL-15により誘導されるこの作用が、疾患の病因で中心的な役割を果たしていることが示唆されている (Kirman et al., 1998; McInnes et al., 1996; McInnes et al., 1997; McInnes and Liew, 1998; Fehniger and Caligiuri, 2001)。

【０００２】

In vitro研究では、IL-15はIL-2と共通の生物活性をいくつか有し、その理由はこれらに共通の受容体成分があるためであることが示されている。T細胞上に存在するIL-15受容体は、固有のα鎖であるIL-15Rαから成るが、そのβ鎖及びγ鎖はIL-2Rと共通である。その結果、両者の受容体は同じJak/STATシグナリング配列を用いる。しかしながら、IL-2及びIL-15並びにそれらの受容体の複雑な調節及び示差的発現に基づき、in vivoでの機能には重要な違いがあることが報告されている (Kirman et al., 1998; Waldmann and Tagaya, 1999; Waldmann et al., 2001)。さらに、ナチュラルキラー(NK)細胞、NK-T細胞及び上皮内リンパ球の発生、生存、増殖及び機能においては、IL-15に特有の役割があることに注目することも重要である(Kennedy et al., 2000; Liu et al.,2000)。

【０００３】

McInnes及び共同研究者ら (McInnes et al., 1997; McInnes and Liew, 1998) は、リウ

マチ性関節炎患者由来のT細胞において、IL-15を刺激した後にTNF-α産生が誘導されたことを報告した。さらに、IL-15で活性化した末梢血T細胞が、マクロファージによる著しいTNF-α産生を、細胞接触依存的な機序を通じて誘導することが示された。リウマチ性関節炎におけるTNF-αの破壊的役割のために、このサイトカインの阻害があると疾患の活性が低下する (Bathon et al., 2000; Klippel, 2000; Lovell et al., 2000; Maini and Taylor, 2000)。

【発明の開示】

【０００４】

発明の概要

本発明は、ヒトIL-15に特異的に結合すると共に、IL-15の誘導する炎症誘発作用を阻害する完全ヒトモノクローナル抗体の初めての作製及び単離や、このような新規な抗体の特徴付け、並びに、多種のIL-15媒介性疾患を治療する上でのそれらの治療上の価値の実証、に基づくものである。例えばここで解説するように、本ヒト抗体は、その両者が炎症性の障害に一体に関与しているTNFα産生及びT細胞増殖の両方を阻害することが示されている。従って、本発明のヒト抗体は、このような疾患（及び他の何らかのIL-15媒介性障害）を治療及び予防する優れた手段となる。これらは、その固有の特異性（例えばエピトープ及び種特異性）、親和性、構造、機能的活性に起因すると共に、これらが完全ヒトであるために、ヒト患者に投与した場合に予め作製された他のIL-15抗体（例えばマウス及びヒト化抗体）よりも、著しく免疫原性が低く、またより治療効果が高く有用であるという事実に起因するものである。さらに本発明は、リウマチ性関節炎、乾癬、移植片拒絶及び癌などの炎症性疾患の治療を含め、ここで解説するヒト抗体などのIL-15阻害性抗体の新しい治療上用途の発見にも基づく。

【０００５】

本発明の単離されたヒト抗体には、IgG1、IgG2、IgG3、IgG4、IgM、 IgA1、 IgA2、 IgAsec、IgD、及び IgEなどの多種の抗体アイソタイプが含まれる。典型的には、これらにはIgG1 (例えば IgG1k)、IgG3 及び IgM アイソタイプが含まれる。本抗体は完全長（例えば IgG1 又はIgG3 抗体）でもよく、又は、抗原結合部分（例えばFab、$F(ab')_2$、Fv、一本鎖Fv フラグメント、単離された相補性決定領域 (CDR)又は２種以上の単離されたCDRの組合せ、など）のみを含むものでもよい。

【０００６】

ある実施態様では、本ヒト抗体は組換え抗体である。ある具体的な実施態様では、本ヒト抗体は、それぞれ配列番号:1 及び 配列番号:3並びにそれらの保存的配列改変に記載された通りのヌクレオチド配列をそれらの可変領域に含むヒトIgG重鎖及びヒトカッパ軽鎖核酸にコードされている。別の実施態様では、本ヒト抗体は、それぞれ配列番号:2 及び 配列番号:4並びにそれらの保存的配列改変に示すアミノ酸配列を含むIgG重鎖及びカッパ軽鎖可変領域を有する。

【０００７】

本発明のヒト抗体は、当該抗体の重鎖及び軽鎖をコードする核酸を含有するトランスフェクトーマ（例えば不死化CHO細胞又はリンパ球から成るトランスフェクトーマ）など、ホスト細胞内で組換えにより産生させることも、あるいは、当該抗体を発現するハイブリドーマ（例えば当該抗体をコードするヒト重鎖導入遺伝子及びヒト軽鎖導入遺伝子を含むゲノムを有するトランスジェニック・マウスなどの非ヒトトランスジェニック動物から得たＢ細胞を不死化細胞に融合させて含有するもの）から直接得ることもできる。具体的な実施態様では、本抗体を、ここで146B7と言及するハイブリドーマか、又は、それぞれ配列番号: 1 及び 3並びにそれらの保存的改変に記載された通りのヌクレオチド配列をそれらの可変領域に含むヒト重鎖及びヒト軽鎖核酸を含有するホスト細胞（例えばCHO細胞）トランスフェクトーマに、産生させる。具体的な実施態様では、本抗体を、ここで146B7、146H5, 404E4、及び404A8と言及されるハイブリドーマに産生させる。ある好適な実施態様では、本抗体は、IL-15のβ-及び／又はγ-鎖相互作用ドメイン上に位置するエピトープに特異的に結合するものである。

【０００８】
別の実施態様では、本発明のヒト抗体は、ヒトIL-15に特異的に結合して、IL-15の炎症誘発作用誘導能を阻害するものであり、例えばIL-15がIL-15受容体に結合した時に、TNFαの産生を阻害したり、及び／又は、PBMC又はCTLL-2 T細胞などのT細胞の増殖を阻害する。典型的には、本ヒト抗体は、組換えヒトIL-15を分析物及び本抗体をリガンドとして用いて、BIACORE 3000装置で表面プラスモン共鳴（CPR）技術で判定した場合に、例えばほぼ10^{-8}M、10^{-9}M又は10^{-10}M又はそれ未満など、ほぼ10^{-7}M未満の解離平衡定数（K_D）でIL-15に結合する。ある具体的な実施態様では、本抗体はヒトIL-15に、ほぼ6.5×10^{-8}Mの解離平衡定数（K_D）で結合する。

【０００９】
別の局面では、本発明は、本発明の抗体又は抗原結合部分をコードする核酸分子を提供するものである。従って、本発明の抗体をコードする核酸を含有する組換え発現ベクタや、このようなベクタをトランスフェクトしたホスト細胞も、これらのホスト細胞を培養することで本発明の抗体を作製する方法と同様、本発明の包含するところである。

【００１０】
さらに本発明は、それぞれ配列番号:2 及び配列番号:4並びにこれらの保存的改変に示すアミノ酸配列を含む重鎖及び軽鎖可変領域をコードするヌクレオチド配列を含む発現ベクタにも関する。このような発現ベクタは当業で公知である。その例には、例えば網状赤血球ライセートを用いたin vitro 転写／翻訳ベクタがある。

【００１１】
さらに別の局面では、本発明は、IL-15に特異的に結合する多種のアイソタイプ（例えばIgG、IgA及び／又はIgM）のヒトモノクローナル抗体を発現できる、トランスジェニック・マウスなどの非ヒトトランスジェニック動物由来の単離されたB細胞を提供するものである。好ましくは、前記単離されたB細胞が、IL-15抗原の精製もしくは濃縮製剤、及び／又は、IL-15発現細胞、で免疫してあるトランスジェニック・マウスなどの非ヒトトランスジェニック動物から得られるとよい。好ましくは、トランスジェニック・マウスなどの非ヒトトランスジェニック動物が、ヒト重鎖導入遺伝子及びヒト軽鎖導入遺伝子を含むゲノムを有するとよい。次に、この単離されたB細胞を不死化して、IL-15に対する比とモノクローナル抗体の供給源（例えばハイブリドーマ）とする。

【００１２】
従って、本発明は、さらに、IL-15に特異的に結合するヒトモノクローナル抗体を産生できるハイブリドーマを提供する。ある実施態様では、当該ハイブリドーマは、ヒト重鎖導入遺伝子及びヒト軽鎖導入遺伝子を含むゲノムを有する、トランスジェニック・マウスなどの非ヒトトランスジェニック動物から得たB細胞を不死化細胞に融合させて含有するものである。この非ヒトトランスジェニック動物は、抗体産生ハイブリドーマを作製するために、IL-15抗原の精製もしくは濃縮製剤、及び／又は、IL-15発現細胞で免疫することができる。本発明が提供する具体的なハイブリドーマには、146B7、146H5、404E4、及び404A8がある。

【００１３】
さらに別の局面では、本発明は、IL-15に特異的に結合するヒトモノクローナル抗体を発現する、トランスジェニック・マウスなどの非ヒトトランスジェニック動物を提供するものである。具体的な実施態様では、当該の非ヒトトランスジェニック動物は、ヒト重鎖導入遺伝子及びヒト軽鎖導入遺伝子を含むゲノムを有するトランスジェニック・マウスである。当該非ヒトトランスジェニック動物は、IL-15抗原の精製もしくは濃縮製剤、及び／又は、IL-15発現細胞で免疫することができる。好ましくは、トランスジェニック・マウスなどの非ヒトトランスジェニック動物が、V-D-J組換え及びアイソタイプ・スイッチングを起こすことにより、IL-15に対して複数のアイソタイプ（例えば IgG、IgA及び／又は IgM)のヒトモノクローナル抗体を産生できるとよい。アイソタイプ・スイッチングは、例えば古典的アイソタイプ・スイッチングで起きるものでも、又は非古典的アイソタイプ・スイッチングで起きるものでもよい。

【００１４】
別の局面では、本発明は、IL-15と特異的に反応するヒトモノクローナル抗体を作成する方法を提供するものである。ある実施態様では、本方法は、ヒト重鎖導入遺伝子及びヒト軽鎖導入遺伝子を含むゲノムを有する、トランスジェニック・マウスなどの非ヒトトランスジェニック動物を、L-15抗原の精製もしくは濃縮製剤、及び／又は、IL-15発現細胞で免疫するステップを含む。次にこの動物のB細胞（例えば脾B細胞）を得、骨髄腫細胞に融合させて、IL-15に対するヒトモノクローナル抗体を分泌する不死のハイブリドーマ細胞を形成する。
【００１５】
別の局面では、本発明は、例えば細胞傷害性薬物、酵素活性毒素、又はそのフラグメントなどの治療成分、放射性同位体又は低分子抗癌剤などの治療的成分に結合されたヒト抗IL-15抗体を特徴とする。
【００１６】
別の局面では、本発明は、薬学的に許容可能な担体と、IL-15に特異的に結合する、少なくとも一種の本発明のヒトモノクローナル抗体とを含んで成る、例えば医薬組成物及び診断用組成物などの組成物を提供するものである。当該組成物にはさらに、他の免疫抑制剤、又は化学療法薬などの他の治療薬を含めることができる。
【００１７】
さらに別の局面では、本発明は、例えばIL-15誘導性TNFα産生及び／又はT細胞増殖を阻害するなど、好ましくは構造上関連するタンパク質／サイトカイン（例えばIL-2）の活性（例えばTNFα産生及び／又はT細胞増殖）を阻害することなく、本発明の一種以上のヒト抗体を用いてIL-15の炎症誘発作用を阻害する方法を提供するものである。
【００１８】
本発明のヒト抗体は、多種のIL-15媒介性疾患を、このような疾患に罹患した患者に本抗体を投与することにより治療及び／又は予防するために用いることができる。
【００１９】
本発明の方法及び組成物を用いて治療（例えば寛解）又は予防できる疾患の例には、限定はしないが、炎症性障害、例えば関節炎（例えば乾癬性関節炎並びに活動性リウマチ性関節炎及び若年性リウマチ性関節炎を含むリウマチ性関節炎）、炎症性腸疾患、がある。例えば、本抗体が、錯角化症を減少させ、表皮の肥厚を減少させ、乾癬でケラチノサイトの増殖を低下させることが示されている。また本抗体は、リウマチ性関節炎に伴う炎症を低下させる、及び／又は、活性化白血球の細胞遊走を防ぐことが、示されている。さらに本抗体は、HIV感染などの感染性疾患の治療にも用いることができる。さらに本抗体は移植片拒絶を治療するためにも使用できる。またさらに本抗体は、腫瘍成長及び癌、例えばT細胞白血病など、IL-15媒介性血管新生の関与する多種の疾患の治療にも、用いることができる。
【００２０】
また本発明のヒト抗体を、例えば抗炎症剤、DMARD（疾患改良抗リウマチ薬）、免疫抑制剤、化学療法薬、及び乾癬薬などの一種以上の付加的な治療薬と組み合わせてもよい。
【００２１】
ある実施態様では、例えばステロイド系薬物又はNSAID（非ステロイド系抗炎症薬）などの抗炎症性薬剤など、本抗体による炎症誘発作用阻害を高める一種以上の薬剤で、対象を付加的に治療することができる。好適な薬剤には、例えばアスピリン及び他のサリチル酸塩、Cox-2阻害剤、例えばロフェコキシブ（ヴィオックス）及びセレコキシブ（セレブレックス）、NSAID、例えばイブプロフェン（モトリン、アドビル）、フェノプロフェン（ナルフォン）、ナプロキセン(ナプロシン)、スリンダック(クリノリル)、ジクロフェナック（ヴォルタレン）、ピロキシカム（フェルデン）、ケトプロフェン（オルディス）、ジフルニザール（ドロビド）、ナブメトン（レラフェン）、エトドラック（ロジン）、オキサプロジン（デイプロ）、及びインドメタシン（インドシン）、がある。
【００２２】

別の実施態様では、本発明のヒト抗体を、例えばメトトレキセート（リューマトレックス）、ヒドロキシクロロキン（プラケニル）スルファサラジン（アスルフィジン）、ピリミジン合成阻害剤、例えばレフルノミド（アラバ）、IL-1受容体遮断剤、例えばアナキンラ（キネレット）、及びTNF-α遮断剤、例えばエタネルセプト（エンブレル）、インフリキシマブ（レミケイド）及びアダリムマブなどの一種以上のDMARDと組み合わせて投与することができる。

【００２３】

別の実施態様では、本発明のヒト抗体を、例えばシクロスポリン（サンジムン、ネオラール）及びアザチオプリン（イムラール）などの一種以上の免疫抑制剤と組み合わせて投与することができる。

【００２４】

別の実施態様では、本発明のヒト抗体を、例えばドキソルビシン（アドリアマイシン）、シスプラチン（プラチノール）、ブレオマイシン（ブレノキサン）、カルムスチン（グリアデル）、シクロホスファミド（シトキサン、プロサイトックス、ネオサール）、及びクロラムブシル（ロイケラン）などの一種以上の化学療法薬と組み合わせて投与することができる。本発明によるヒト抗体を、放射線治療と併用投与することもできる。

【００２５】

別の実施態様では、本発明のヒト抗体を、例えばコールタール、ビタミンＡ、コルチゾン又は他のコルチコステロイドを含有する局所用医薬や、例えばコルチコステロイド、メトトレキセート、レチノイド、例えばアシクレチン（ネオギタゾン）又はシクロスポリン（サンジムン、ネオラール）などの経口用又は注射用医薬など、一種以上の乾癬治療薬と組み合わせて投与することができる。他の治療法には、日光への暴露又は光線療法が含まれるであろう。

【００２６】

別の実施態様では、本発明のヒト抗体を、例えばCD4特異抗体及びIL-2特異抗体などの他の抗体と組み合わせて投与することができる。本ヒト抗体と、CD4特異抗体又はIL-2特異抗体との組合せは、自己免疫疾患及び移植片拒絶の治療にとって特に有用であると考えられる。

【００２７】

さらに別の局面では、本発明は、例えばIL-15媒介性疾患を診断するためなど、試料中のIL-15抗原の存在をin vitro又はin vivoで検出する方法を提供するものである。ある実施態様では、これは、検査対象の試料を、コントロール試料と並行して、本発明のヒトモノクローナル抗体又はその抗原結合部分に、前記抗体及びIL-15間の複合体形成が可能な条件下で接触させることにより、行われる。次に複合体形成を両方の試料で（例えばELISAを用いて）検出し、これら試料間で複合体形成に何らかの統計上有意な違いがあれば、検査試料中にIL-15抗原が存在することの指標である。

【００２８】

本発明の他の特徴及び長所は、以下の詳細な説明及び請求の範囲から明白となるであろう。

【００２９】

発明の詳細な説明

本発明は、IL-15が媒介する多種の障害（即ち、IL-15の炎症誘発作用が引き起こす障害）を治療及び診断するための新規な抗体ベースの治療薬を提供するものである。ここで用いる用語「IL-15の炎症誘発作用」には、例えばTNFα及び他の炎症媒介物質の産生や、T細胞の動員／増殖など、IL-15により誘導されるあらゆる体液性又は細胞媒介性の免疫応答が含まれる。本発明の治療法では、IL-15上に存在するエピトープに特異的に結合する単離されたヒトモノクローナル抗体を利用する。

【００３０】

ある実施態様では、本ヒト抗体は、V-D-J組換え及びアイソタイプ・スイッチングを起こすことにより、IL-15に対して複数のアイソタイプ（例えば IgG, IgA 及び／又は IgE)の

ヒトモノクローナル抗体を産生できる、トランスジェニック・マウスなどの非ヒトトランスジェニック動物で産生される。従って、本発明の多様な局面には、抗体及びその医薬組成物や、このようなモノクローナル抗体を作製するための非ヒトトランスジェニック動物、B細胞、ホスト細胞トランスフェクトーマ及びハイブリドーマが含まれる。本発明の抗体を用いて、IL-15が結合する細胞を検出する方法、及び／又は、IL-15媒介性機能をin vitro又はin vivoで阻害する方法も、本発明の包含するところである。IL-15が結合する細胞に薬剤を標的指向させる方法も包含されている。

【００３１】

本発明がより容易に理解されるよう、いくつかの用語をまず定義しておく。更なる定義は、詳細な説明欄全体に記載されている。

【００３２】

用語「IL-15」、「IL-15抗原」及び「インターロイキン 15」はここでは交換可能に用いられており、細胞が天然で発現するあらゆるバリアント又はアイソフォームを包含するものである。

【００３３】

ここで言及する用語「抗体」には、抗体全体や、そのあらゆる抗原結合フラグメント（即ち「抗原結合部分」）又は一本鎖が含まれる。「抗体」とは、少なくとも２本の重（Ｈ）鎖及び２本の軽（Ｌ）鎖をジスルフィド結合で相互に接続して含む糖タンパク質、又はその抗原結合部分、を言う。各重鎖は、重鎖可変領域（ここではV_Hと省略される）と重鎖定常領域とから成る。重鎖定常領域はCH1、CH2及びCH3という３つのドメインから成る。各軽鎖は軽鎖可変領域（ここではV_Lと省略される）及び軽鎖定常領域から成る。軽鎖定常領域は一つのドメインCLから成る。V_H及びV_L領域はさらに、より保存されたフレームワーク領域（FR）と呼ばれる領域間に介在する相補性決定領域（CDR）と呼ばれる超可変領域に小さく分割することができる。各V_H及びV_Lは、以下の順序：FR1、CDR1、FR2、CDR2、FR3、CDR3、FR4でアミノ末端からカルボキシ末端まで並んだ３つのCDR及び４つのFRから成る。重鎖及び軽鎖の可変領域は、抗原と相互作用する結合ドメインを含有する。抗体の定常領域は、免疫系の多種の細胞（例えばエフェクタ細胞）を含むホスト組織又は因子や、古典的な補体系の第一コンポーネント（C1q）に対する免疫グロブリンの結合を媒介していると考えられる。

【００３４】

抗体の「抗原結合部分」（又は簡単に「抗体部分」）という用語は、ここで用いる場合、抗原（例えばIL-15）への特異的結合能を維持した、抗体のうちの一つ以上のフラグメントを言う。抗体の抗原結合機能は、完全長抗体のうちの数フラグメントに行わせることができることが示されている。抗体の「抗原結合部分」という用語に包含される結合フラグメントの例には、（ｉ）V_L、V_H、C_L及びCH1ドメインから成る一価のフラグメントであるFabフラグメント；（ｉｉ）ヒンジ領域でジスルフィド架橋により連結された２つのFabフラグメントから成る二価のフラグメントである$F(ab')_2$フラグメント；（ｉｉｉ）V_H及びCH1ドメインから成るFdフラグメント；（ｉｖ）抗体の一本の腕のV_L及びV_Hドメインから成るFvフラグメント；（ｖ）V_Hドメインから成るdAbフラグメント(Ward et al., (1989) Nature 341:544-546)；及び（ｖｉ）単離された相補性決定領域(CDR)もしくは又は（ｖｉｉ）選択に応じて合成リンカにより接合してもよい単離されたCDR、がある。さらに、Fvフラグメントの２つのドメインV_L及びV_Hは別々の遺伝子にコードされているが、これらは、V_L及びV_H領域が対を成して一価の分子を形成するような一個のタンパク質鎖に作製できるようにする合成リンカーにより、組換え法を用いて接合することができる（一本鎖Fv（scFv）として知られる；例えば Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883を参照されたい）。このような一本鎖抗体はまた、抗体の「抗原結合部分」という用語に包含されるものと、意図されている。これらの抗体フラグメントは、当業者に公知の従来技術を用いて得られ、それらのフラグメントは、インタクト抗体と同じ態様で実用性についてスクリーニングされている。

【００３５】
ここで用いる用語「モノクローナル抗体」は、単一の結合特異性及び親和性を特定のエピトープに対して示す抗体を言う。従って、用語「ヒトモノクローナル抗体」とは、単一の結合特異性を示すと共に、ヒト生殖細胞系免疫グロブリン配列を由来とする可変領域及定常領域を有するような抗体を言う。ある実施態様では、ヒトモノクローナル抗体は、ヒト重鎖導入遺伝子及び軽鎖導入遺伝子を含むゲノムを有する、トランスジェニックマウスなどの非ヒトトランスジェニック動物から得られたＢ細胞を、不死化細胞に融合させて含むハイブリドーマにより産生される。

【００３６】
用語「組換えヒト抗体」は、ここで用いる場合、例えば（ａ）ヒト免疫グロブリン遺伝子についてトランスジェニック又はトランスクロモゾマルな動物（例えばマウス）から、又は、それから調製されたハイブリドーマから、単離された抗体（以下の項Ｉでさらに解説する）、（ｂ）当該抗体を発現するように形質転換させたホスト細胞から、例えばトランスフェクトーマから、単離された抗体、（ｃ）組換えのコンビナトリアルヒト抗体ライブラリから単離された抗体、及び（ｄ）ヒト免疫グロブリン遺伝子配列の他のDNA配列へのスプライシングに関与する何らかの他の手段により調製、発現、創出又は単離された抗体など、組換え手段により調製、発現、創出又は単離されたあらゆるヒト抗体を包含する。このような組換えヒト抗体は、ヒト生殖細胞系免疫グロブリン配列を由来とする可変及び定常領域を有する。しかしいくつかの実施態様では、このような組換えヒト抗体にin vitro変異誘発を行って（あるいはヒトIg配列についてトランスジェニックな動物を用いる場合にはin vivoで体細胞変異誘発を行って）、組換え抗体のV_H及びV_L領域のアミノ酸配列を、ヒト生殖細胞系V_H及びV_L配列を由来とし、かつ関連はしているが、in vivoのヒト抗体生殖細胞系レパートリに天然では存在しないと思われる配列にすることもできる。

【００３７】
ここで用いる「異種抗体」とは、このような抗体を産生する非ヒトトランスジェニック生物との関連から定義されている。この用語は、当該非ヒトトランスジェニック動物を構成しない生物に見られるものに相当すると共に、当該非ヒトトランスジェニック動物のそれ以外の種を概ね由来とするアミノ酸配列又はコーディング核酸配列を有する抗体を言う。

【００３８】
ここで用いる「単離された抗体」とは、異なる抗原特異性を有する他の抗体を実質的に含まない抗体を言うものと、意図されている（例えばIL-15に特異的に結合する単離された抗体は、IL-15以外の抗原に特異的に結合する抗体を実質的に含まない）。しかしIL-15のエピトープに特異的に結合する単離された抗体は、他の関連するサイトカイン又は異なる種を由来とする他のIL-15タンパク質に対して交差反応性を有していてもよい。しかしながら、本抗体は好ましくはヒトIL-15に常に結合するものであるとよい。加えて、単離された抗体は、典型的には、他の細胞物質及び／又は化学物質を実質的に含まない。本発明の一実施態様では、異なるIL-15特異性を有する複数の「単離された」モノクローナル抗体の組合せを、良く定義された組成で配合する。

【００３９】
ここで用いる「特異的結合」とは、所定の抗原に対して抗体が結合することを言う。典型的には、本抗体は、組換えヒトIL-15を分析物及び本抗体をリガンドとして用いて、BIACORE 3000装置で表面プラスモン共鳴（CPR）技術で判定した場合に、例えばほぼ10^{-8}M、10^{-9}M又は10^{-10}M未満又はさらに低い値など、ほぼ10^{-7}M未満の親和性（K_D）で結合し、所定の抗原に対しては、所定の抗原又は密接に関連する抗原以外の非特異的抗原（例えばBSA、カゼイン）に対するその結合親和性よりも、少なくとも２倍高い親和性で結合する。文言「ある抗原を認識する抗体」及び「ある抗原に特異的な抗体」は、ここでは、「ある抗原に特異的に結合する抗体」と交換可能に用いられている。

【００４０】
用語「K_D」は、ここで用いる場合、特定の抗体－抗原間相互作用の解離平衡定数を言うものと、意図されている。

【００４１】
ここで用いる「アイソタイプ」とは、重鎖定常領域遺伝子にコードされた抗体クラス（例えばIgM又はIgG1）を言う。
【００４２】
ここで用いる「アイソタイプ・スイッチング」とは、抗体のクラス又はアイソタイプが、ある１つのIgクラスから他のIgクラスの１つへ変化する現象を言う。
【００４３】
ここで用いる「スイッチングなしのアイソタイプ」とは、アイソタイプ・スイッチングが起きなかったときに産生されるアイソタイプ・クラスの重鎖を言う。スイッチングなしのアイソタイプをコードするCH遺伝子は、典型的には、機能的再編成の起きるVDJ遺伝子のすぐ下流にある１番目のCH遺伝子である。アイソタイプ・スイッチングは古典的又は非古典的アイソタイプ・スイッチングに分類されてきた。古典的アイソタイプ・スイッチングは、導入遺伝子中の少なくとも１つのスイッチ配列領域が関与する組換え事象により起きる。非古典的なアイソタイプ・スイッチングは、例えばヒトσμとヒトΣμとの間の相同組換え（δ関連欠失）などで起きることがある。また導入遺伝子間及び／又は染色体間での組換えなど、その他の非古典的なスイッチング機序が起きてアイソタイプ・スイッチングにつながることもある。
【００４４】
ここで用いる用語「スイッチ配列」とは、スイッチ組換えを担うDNA配列を言う。「スイッチ・ドナー」配列は典型的にはμスイッチ領域であり、スイッチ組換えの際に欠失するコンストラクト領域の5'側（即ち上流）にあるであろう。「スイッチ・アクセプタ」領域は、欠失することになるコンストラクト領域と、置換定常領域（例えばγ、ε、等）との間にあるであろう。組換えが常に起きるという特定の部位はないため、最終的な遺伝子配列は典型的にはコンストラクトからは予測不能であろう。
【００４５】
ここで用いる「糖付加パターン」とは、タンパク質、より具体的には免疫グロブリンタンパク質、に共有結合した糖単位のパターンであると定義しておく。ある異種抗体の糖付加パターンを、導入遺伝子のCH遺伝子の由来となった元の種の糖付加パターンよりも、非ヒトトランスジェニック動物の種における糖付加パターンにより似ていると当業者が認識するのであれば、その異種抗体の糖付加パターンは、非ヒトトランスジェニック動物の種の産生する抗体上に天然で存在する糖付加パターンに実質的に似ている、と特徴付けることができる。
【００４６】
ある物質についてここで用いる用語「天然で発生する」とは、ある物質が天然で見られるという事実を言う。例えば、（ウィルスを含む）生物中に存在し、天然の源から単離でき、研究室で人為的に改変されていないポリペプチド又はポリヌクレオチド配列は天然で発生したものである。
【００４７】
ここで用いる用語「再編成される」とは、Vセグメントが、D-J又はJセグメントのすぐ隣に位置して、それぞれ完全V_H又はV_Lドメインを実質的にコードするコンホメーションとなっているような重鎖又は軽鎖免疫グロブリン遺伝子座の配置を言う。再編成の起きた免疫グロブリン遺伝子座は、生殖細胞DNAに比較することで特定できる；再編成の起きた遺伝子座は少なくとも一つの組換えられた７量体／９量体相同配列を有するであろう。
【００４８】
Vセグメントに関してここで用いる用語「再編成のない」又は「生殖細胞の配置」とは、Vセグメントが組換えられておらず、D又はJセグメントのすぐ隣にあるような配置を言う。
【００４９】
ここで用いる用語「核酸分子」には、DNA分子及びRNA分子が包含されるものと、意図されている。核酸分子は一本鎖でも、又は二本鎖でもよいが、好ましくは二本鎖DNAである。
【００５０】

IL-15に結合する抗体又は抗体部分（例えばV_H、V_L、CDR3など）をコードする核酸に関してここで用いる用語「単離された核酸分子」とは、当該抗体又は抗体部分をコードするヌクレオチド配列が、IL-15以外の抗原に結合する抗体又は抗体部分をコードする、天然ではヒトゲノムDNA中で当該核酸をフランクしているであろう他のヌクレオチド配列を含まないことを言うものと、意図されている。配列番号: 1-4は、本発明のヒト抗IL-15抗体146B7の重鎖（V_H）及び軽鎖（V_L）可変領域を含むヌクレオチド及びアミノ酸配列に相当する。具体的には、配列番号:1 及び 2 は146B7抗体のV_Hに相当し、配列番号:3及び4 は146B7抗体のV_Lに相当する。

【００５１】

さらに本発明は、配列番号: 1-4に記載した通りの配列の「保存的配列改変」、即ち、前記ヌクレオチド配列にコードされた、又は、前記アミノ酸配列を含有する、当該抗体の結合特性に大きく影響又は変化させないようなヌクレオチド及びアミノ酸配列改変、も包含するものである。このような保存的配列改変にはヌクレオチド及びアミノ酸の置換、追加及び欠失が含まれる。例えば部位指定変異誘発及びPCR媒介変異誘発法など、当業で公知の標準的技術により、改変を配列番号:1-4に導入することができる。保存的アミノ酸置換には、アミノ酸残基が類似の側鎖を有するアミノ酸残基に置換されるものが含まれる。類似の側鎖を有するアミノ酸残基のファミリーが当業で定義されている。これらのファミリーには、塩基性の側鎖を持つアミノ酸（例えばリジン、アルギニン、ヒスチジン）、酸性の側鎖を持つアミノ酸（例えばアスパラギン酸、グルタミン酸）、無電荷の極性側鎖を持つアミノ酸（例えばグリシン、アスパラギン、グルタミン、セリン、スレオニン、チロシン、システイン、トリプトファン）、非極性の側鎖を持つアミノ酸（例えばアラニン、バリン、ロイシン、イソロイシン、プロリン、フェニルアラニン、メチオニン）、ベータ分枝側鎖を持つアミノ酸（例えばスレオニン、バリン、イソロイシン）及び芳香族側鎖を持つアミノ酸（例えばチロシン、フェニルアラニン、トリプトファン、ヒスチジン）、がある。このように、ヒト抗IL-15抗体の中で予測される重要でないアミノ酸残基を、同じ側鎖ファミリーの別のアミノ酸残基に置換することが好ましい。

【００５２】

代替的には、別の実施例では、例えば飽和変異誘発法などにより、抗IL-15抗体コーディング配列の全部又は一部にわたって変異を無作為に導入することができ、その結果改変された抗IL-15抗体を結合活性についてスクリーニングすることができる。

【００５３】

従って、ここで開示する（重鎖及び軽鎖可変領域）ヌクレオチド配列にコードされた抗体、及び／又は、ここに開示する（即ち配列番号: 1-4)（重鎖及び軽鎖可変領域）アミノ酸配列を含有する抗体には、保存的に改変され、類似の配列にコードされた、又は、類似の配列を含有する、実質的に類似の抗体が含まれる。このような実質的に類似の抗体を、ここに配列番号:1-4として開示された部分的（即ち重鎖及び軽鎖可変領域）配列に基づいてどのように作製できるかを、以下にさらに論じる。

【００５４】

核酸の場合、用語「実質的な相同性」は、最適にアライメントして比較した場合の２つの核酸又はそのうちの指示した配列が、適当なヌクレオチド挿入又は欠失がありながらも、ヌクレオチドの少なくとも約80%、通常はヌクレオチドの少なくとも約90%乃至95%、そしてより好ましくは少なくとも約98%乃至99.5%が、同一であることを指すものである。代替的には、数セグメントが選択的ハイブリダイゼーション条件下で当該鎖の相補配列にハイブリダイズするときに、実質的な相同性が存在することとする。

【００５５】

二つの配列間のパーセント同一性は、これら二つの配列を最適にアライメントするのに導入せねばならないギャップの数、及び各ギャップの長さを考慮に入れたときの、これら配列に共通の同一位置の数の関数である（即ち、％相同性＝同一位置の数／位置の総数×100）。二つの配列間の配列の比較及びパーセント同一性の決定は、以下の非限定的な例に解説するように、数学的アルゴリズムを用いて行うことができる。

【００５６】
二つのヌクレオチド間のパーセント同一性は、GCGソフトウェア・パッケージ（http://www.gcg.comで入手できる）のGAPプログラムを用い、NWSgapdna.CMP マトリックスを用いて、ギャップ・ウェイトを40、50、60、70、又は80 にし、そしてレングス・ウェイトを1、2、3、4、5、又は6にして決定することができる。二つのヌクレオチド又はアミノ酸配列間のパーセント同一性はまた、ALIGNプログラム（バージョン2.0)に組み込まれた E. マイヤース及びW. ミラーのアルゴリズム(Comput. Appl. Biosci., 4:11-17 (1988)) を用い、PAM120 ウェイト残基表を用いて、ギャップ・レングス・ペナルティを12、そしてギャップ・ペナルティを4にして、決定することもできる。さらに、二つのアミノ酸配列間のパーセント同一性は、GCGソフトウェア・パッケージ（http://www.gcg.comで入手できる)のGAPプログラムに組み込まれたニードルマン及びワンシュ (J. Mol. Biol. (48):444-453 (1970))のアルゴリズムを用い、Blossum 62 マトリックス又はPAM250マトリックスのいずれかを用いて、ギャップ・ウェイトを16、14、12、10、8、6、又は4にし、レングス・ウェイトを1、2、3、4、5、又は6にして、決定することができる。

【００５７】
さらに本発明の核酸及びタンパク質の配列を「クエリー配列」として利用して、公開データベースの検索を行って、例えば関連する配列を同定することなどができる。このような検索は、Altschul, et al. (1990) J. Mol. Biol. 215:403-10のNBLAST 及びXBLASTプログラム（バージョン2.0）を利用すれば行える。BLASTヌクレオチド検索を、NBLASTプログラムを用い、スコア= 100、ワード長= 12 にして行うと、本発明の核酸分子に相同なヌクレオチド配列を得ることができる。BLASTタンパク質検索を、 XBLASTプログラムを用い、スコア= 50、ワード長= 3にして行うと、本発明のタンパク質分子に相同なアミノ酸配列を得ることができる。比較を目的としてギャップのあるアライメントを行うには、Gapped BLAST をAltschul et al., (1997) Nucleic Acids Res. 25(17):3389-3402が解説するとおりに利用できる。BLAST及びギャップドBLASTプログラムを利用する場合、各プログラムの（例えばXBLAST 及びNBLAST)のデフォルト・パラメータを利用できる。http://www.ncbi.nlm.nih.gov. を参照されたい。

【００５８】
当該核酸は全細胞中にあっても、細胞ライセート中にあっても、又は部分的に精製されたもしくは実質的に純粋な形で存在してもよい。核酸は、アルカリ／SDS処理、CsClバンディング、カラム・クロマトグラフィ、アガロースゲル電気泳動法、及び当業で公知の他の技術を含む標準的な技術により、例えば他の細胞内核酸又はタンパク質など、他の細胞成分又は他の混入物質を取り除いて精製されている場合に、「単離されている」又は「実質的に純粋にされている」ことになる。 F. Ausubel, et al., ed. Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York (1987)を参照されたい。

【００５９】
cDNA、ゲノム又はこれらの混合物由来である本発明の核酸組成物は、しばしば天然配列（改変された制限部位等を除き）のままであり、遺伝子配列を提供する標準的技術に従って変異させてもよい。コーディング配列の場合、これらの変異は、必要に応じアミノ酸配列を左右するものでもよい。具体的には、ここで解説した天然V、D、J、定常、スイッチ及び他のこのような配列に実質的に相同又は由来とするDNA配列が考えられる（「由来する」が、ある配列が別の配列と同一か、もしくは別の配列から改変されていることを指す場合）。

【００６０】
核酸は、別の核酸配列と機能的な関係に置かれたときに「作動的に連結された」ことになる。例えば、あるプロモータ又はエンハンサが、あるコーディング配列の転写を左右するのであれば、その配列に作動的に連結されていることになる。転写制御配列に関する場合、作動的に連結されたとは、連結しようとするDNA配列が連続していることを意味し、また２つのタンパク質コーディング領域を接合するために必要な場合には、連続し、かつ読

み取り枠内にあることを意味する。スイッチ配列の場合には、作動的に連結された、とは、当該配列がスイッチ組換えを起こし得ることを指す。

【００６１】

ここで用いる用語「ベクタ」とは、連結された先の別の核酸を輸送できる核酸分子を言うものと、意図されている。ベクタの一種が、付加的なDNAセグメントを連結できる環状の二本鎖DNAループを言う「プラスミド」である。ベクタのもう一つの種類がウィルスベクタであり、この場合、付加的なDNAセグメントは、ウィルスゲノム内に連結させることができる。いくつかのベクタは導入された先のホスト細胞内で自律的複製が可能である（例えば細菌由来の複製開始点を有する細菌ベクタや、エピソームほ乳類ベクタなど）。他のベクタ（例えば非エピソームほ乳類ベクタなど）は、ホスト細胞に導入されるや、ホスト細胞のゲノムに組み込まれるため、ホストゲノムと一緒に複製される。さらに、いくつかのベクタは、作動的に連結された先の遺伝子の発現を命令することができる。このようなベクタをここでは「組換え発現ベクタ」（又は単に「発現ベクタ」）と呼ぶ。一般的に、組換えDNA技術で実用性のある発現ベクタは、しばしばプラスミドの形である。本明細書では、プラスミドが最も普通に用いられている形のベクタであるため、「プラスミド」及び「ベクタ」を交換可能に用いている場合がある。しかしながら、本発明には、例えばウィルスベクタ（例えば複製欠陥レトロウィルス、アデノウィルス及びアデノ随伴ウィルス）など、同等の機能を果たす他の形の発現ベクタも包含されることが、意図されている。

【００６２】

ここで用いる用語「組換えホスト細胞（又は単に「ホスト細胞」）とは、組換え発現ベクタが導入された細胞を言うものと、意図されている。このような用語は、特定の対象細胞だけでなく、このような細胞の後代も言うものと意図されていることは、理解されねばならない。突然変異又は環境による影響が原因で、特定の改変が継代に起きる場合があるため、このような後代は実際には親細胞と同一でないかも知れないが、それでも尚、ここで用いる用語「ホスト細胞」の範囲内に含まれる。

【００６３】

ここで用いる用語「対象」にはヒト又は非ヒト動物が含まれる。例えば本発明の方法及び組成物は、リウマチ性関節炎などの関節炎など、炎症性疾患患者を治療するために用いることができる。用語「非ヒト動物」には、例えばヒト以外の霊長類、ヒツジ、イヌ、ウシ、ニワトリ、両生類、は虫類など、ほ乳類及び非ほ乳類などのあらゆる脊椎動物が含まれる。

【００６４】

本発明の多様な局面を、以下の小項でさらに詳述する。

【００６５】

Ｉ．IL-15に対するヒト抗体の作製

本発明のヒトモノクローナル抗体は、Kohler and Milstein (1975) Nature 256: 495に解説された標準的な体細胞ハイブリダイゼーション技術など、多様な公知の技術により作製できる。体細胞ハイブリダイゼーション法が基本的には好適であるが、モノクローナル抗体を作製する他の技術、例えばＢリンパ球のウィルス又は腫瘍形成性形質転換、ヒト抗体遺伝子のライブラリを用いたファージ・ディスプレイ技術、も利用できる。

【００６６】

本発明のヒトモノクローナル抗体を産生するハイブリドーマを作製する好適な動物系はマウス系である。免疫プロトコルや、免疫後の脾細胞を単離及び融合させる技術を含め、マウスにおけるハイブリドーマ作製は公知である。

【００６７】

ある実施態様では、IL-15を狙ったヒトモノクローナル抗体を、マウス系ではなくヒト免疫系の一部を持つトランスジェニック又はトランスクロモゾマル・マウスを用いて作製する。ある実施態様では、本発明は、ここで「HuMAb」マウスと呼ばれる、再編成していないヒト重鎖（μ及びγ）及びκ軽鎖免疫グロブリン配列をコードするヒト免疫グロブリン遺伝子最小遺伝子座を、内因性のμ及びκ鎖遺伝子座を不活性化する標的設定された変異

と一緒に含有する(Lonberg, et al. (1994) Nature 368(6474): 856-859)トランスジェニックマウスを利用する。従って、このマウスの示すマウスIgM又はκの発現は低く、免疫処置に応答して、導入されたヒト重鎖及び軽鎖導入遺伝子がクラス・スイッチング及び体細胞変異を起こすことにより高親和ヒトIgGκモノクローナル抗体が生じる (Lonberg, N. (1994) Handbook of Experimental Pharmacologyで113:49-101レビューされた 上記のLonberg, N. et al. (1994) ; Lonberg, N. and Huszar, D. (1995) Intern. Rev. Immunol. Vol. 13: 65-93, 及びHarding, F. and Lonberg, N. (1995) Ann. N.Y. Acad. Sci 764:536-546)。HuMAbマウスの作製は、下の項ＩＩ及びTaylor, L. et al. (1992) Nucleic Acids Research 20:6287-6295; Chen, J. et al. (1993) International Immunology 5: 647-656; Tuaillon et al. (1993) Proc. Natl. Acad. Sci USA 90:3720-3724; Choi et al. (1993) Nature Genetics 4:117-123; Chen, J. et al. (1993) EMBO J. 12: 821-830; Tuaillon et al. (1994) J. Immunol. 152:2912-2920; Lonberg et al., (1994) Nature 368(6474): 856-859; Lonberg, N. (1994) Handbook of Experimental Pharmacology 113:49-101; Taylor, L. et al. (1994) International Immunology 6: 579-591; Lonberg, N. and Huszar, D. (1995) Intern. Rev. Immunol. Vol. 13: 65-93; Harding, F. and Lonberg, N. (1995) Ann. N.Y. Acad. Sci 764:536-546; Fishwild, D. et al. (1996) Nature Biotechnology 14: 845-851に詳細に解説されている。さらに、すべてLonberg及びKay、並びにジェンファーム・インターナショナル社に付与された米国特許第5,545,806号；第5,569,825号；第5,625,126号；第5,633,425号；第5,789,650号；第5,877,397号；第5,661,016号；第5,814,318号；第5,874,299号；及び第5,770,429号；Surani et al.のto 米国特許第5,545,807号；１９９８年６月１１日に公開された国際公報WO 98/24884；１９９４年１１月１０日に公開されたWO 94/25585；１９９３年６月２４日に公開された WO 93/1227；１９９２年１２月２３日に公開されたWO 92/22645；１９９２年３月１９日に公開されたWO 92/03918を参照されたい。特にHCO12トランスジェニックHuMabマウスの作製法を実施例２で解説する。

【００６８】

免疫処置

IL-15に対する完全ヒトモノクローナル抗体を作製するためには、ヒト免疫グロブリン遺伝子（例えばHCo12、HCo7又はKMマウス）を含有するトランスジェニック又はトランスクロモゾマル・マウスをLonberg, N. et al. (1994) Nature 368(6474): 856-859; Fishwild, D. et al. (1996) Nature Biotechnology 14: 845-851 及び WO 98/24884などに解説されたようにIL-15抗原の精製もしくは濃縮製剤及び／又はIL-15発現細胞で免疫することができる。代替的には、ヒトIl-15をコードするDNAでマウスを免疫することもできる。好ましくは、当該マウスは１回目の輸注時に６乃至１６週齢であるとよい。例えば、IL-15抗原の精製もしくは濃縮製剤（5乃至50μg）を用いて、HuMAbマウスを腹腔内により免疫することができる。IL-15抗原の精製もしくは濃縮製剤を用いた免疫処置でも抗体が生じない場合、細胞株など、IL-15発現細胞でマウスを免疫して、免疫応答を促進することもできる。

【００６９】

多様な抗原を用いて蓄積した経験では、HuMAbトランスジェニックマウスは、まず抗原を完全フロイント・アジュバントに入れて腹腔内（IP）又は皮下（SC）免疫し、その後不完全フロイントアジュバントに抗原を入れて１週置きに（最高で合計１０回）腹腔内／皮下免疫処置したときに最も良く応答することが示された。免疫応答は、眼窩後方の採血で得た血漿試料で、免疫プロトコルの経過にわたって観察することができる。血漿はELISA（以下に解説するように）でスクリーニングすることができ、充分な抗体価の抗IL-15ヒト免疫グロブリンを持つマウスを融合に用いることができる。マウスは、と殺及び脾臓の摘出から３日前に抗原を静注して追加免疫することができる。

【００７０】

IL-15に対するヒトモノクローナル抗体を産生するハイブリドーマの作製

IL-15に対するヒトモノクローナル抗体を産生するハイブリドーマを作製するには、免疫

後のマウスから脾細胞及びリンパ節細胞を単離し、マウス骨髄腫細胞株などの適した不死化細胞株に融合させることができる。こうして出来たハイブリドーマを次に、抗原特異的抗体の産生についてスクリーニングすることができる。例えば免疫後のマウス由来の脾臓リンパ球の単個細胞懸濁液を、50% PEG（w/v）で、SP2/0-Ag8.653非分泌性マウス骨髄腫細胞 (ATCC, CRL 1580)に融合させることができる。細胞を平底の微量定量プレートに約1×10^5になるようにプレートし、通常の試薬の他に10%胎児クローン血清、5-10%オリゲン・ハイブリドーマ・クローニング・ファクター（アイジェン社）、及び1×HAT（シグマ社）を含有する選択培地で２週間インキュベートすることができる。ほぼ２週間後、HATをHTに取り替えた培地で細胞を培養できる。次に個々のウェルをELISAによりヒト抗IL-15モノクローナルIgM及びIgG抗体についてスクリーニングすることができる。広汎なハイブリドーマ成長が起きたら培地を通常１０乃至１４日後に観察できる。抗体を分泌しているハイブリドーマを再度プレートし、再度スクリーニングし、ヒトIgGについてまだ尚陽性であれば、限界希釈により少なくとも２回、抗IL-15モノクローナル抗体をサブクローニングすることができる。次に安定なサブクローンをin vitroで培養して、抗体を組織培養培地中に生じさせ、特徴付けに向けることができる。

【００７１】

IL-15に対するヒトモノクローナル抗体を産生するトランスフェクトーマの作製

本発明のヒト抗体は、当業で公知のように、組換えDNA技術及び遺伝子トランスフェクション法の組合せなどを用いて、ホスト細胞トランスフェクトーマで作製することもできる（Morrison, S. (1985) Science 229:1202)。

【００７２】

例えばある実施態様では、ヒト抗体遺伝子などの目的の遺伝子を、例えばWO 87/04462、WO 89/01036 及び EP 338 841 に開示されたGS遺伝子発現系又は当業で公知の他の発現系で用いられるものなどの真核性発現プラスミドなどの発現ベクタ内に連結することができる。クローンされた抗体遺伝子を持つ精製済みプラスミドを、例えばCHO細胞又はNSO細胞などの真核性ホスト細胞や、又は代替的には、植物由来細胞、真菌もしくは酵母細胞などの他の真核細胞に導入することができる。これらの遺伝子を導入するために用いる方法は、例えば電気穿孔法、リポフェクチン、リポフェクタミン又は他のものなど、当業で解説された方法でよいであろう。これらの抗体遺伝子をホスト細胞内に導入後、当該抗体を発現している細胞を同定及び選抜することができる。これらの細胞が、その後それらの発現レベルを増幅して抗体の生産率を上げることのできるトランスフェクトーマである。これらの培養上清及び／又は細胞から、組換え抗体を単離及び精製することができる。

【００７３】

代替的には、これらのクローンされた抗体遺伝子を、E. coliなどの他の発現系や又は完全な生物中で発現させたり、あるいは合成により発現させることもできる。

【００７４】

インタクト抗体を発現させるための部分的抗体配列の利用

抗体は、６つの重鎖及び軽鎖相補性決定領域（CDR）に位置するアミノ酸残基を主に通じて標的抗原と相互作用する。そのため、CDR内のアミノ酸配列は、CDRの外にある配列よりも、個々の抗体間の違いが大きい。CDR配列は大半の抗体－抗原相互作用を担っているため、特定の天然発生型の抗体を由来とするCDR配列を、異なる性質を持つ異なる抗体由来のフレームワーク配列に移植した状態で含有するような発現ベクタを構築すると、特定の天然発生型抗体の性質を模倣する組換え抗体を発現させることができる（例えばRiechmann, L. et al., 1998, Nature 332:323-327; Jones, P. et al., 1986, Nature 321:522-525; and Queen, C. et al., 1989, Proc. Natl. Acad. See. U.S.A. 86:10029-10033を参照されたい）。このようなフレームワーク配列は生殖細胞抗体遺伝子配列を含む公開DNAデータベースから得ることができる。これらの生殖細胞配列は成熟型の抗体遺伝子配列とは異なるが、それはなぜなら、それらには、Ｂ細胞成熟の過程でV(D)J接合により形成される、完全にアセンブルされた可変遺伝子が含まれていないからである。生殖細胞遺伝子配列はまた、可変領域全体にわたって、高親和二次レパートリー抗体の配列とも、個々の

レベルで均一に異なる。例えば、体細胞変異は、フレームワーク領域のアミノ末端部分では比較的に頻度が低い。例えば、体細胞変異は、フレームワーク領域1のアミノ末端部分及びフレームワーク領域4のカルボキシ末端部分では比較的に頻度が低い。さらに、数多くの体細胞変異は、抗体の結合特性に大きく影響するものではない。そのために、もとの抗体のものと同様な結合特性を有するインタクト組換え抗体を作り直す際に、特定の抗体のDNA配列全体を得る必要はない（１９９９年３月１２日出願のPCT/US99/05535 を参照されたい）。典型的には、CDR領域にわたる部分的重鎖及び軽鎖配列があれば、この目的にとって充分である。この部分的配列を用いて、どの生殖細胞可変遺伝子及びジョイニング遺伝子セグメントが、組換え後の抗体可変遺伝子に寄与したかを決定する。次にこの生殖細胞配列を用いて、可変領域の欠けている部分を充填する。重鎖及び軽鎖リーダ配列はタンパク質成熟の過程で切断され、最終的な抗体の特性には寄与しない。欠けている配列を追加するためには、クローンされたcDNA配列を、ライゲーション又はPCR増幅法により、合成オリゴヌクレオチドに組み合わせることができる。代替的には、可変領域全体を一組の短い、重複のあるオリゴヌクレオチドとして合成し、PCR増幅法で組み合わせて、完全に人工的な可変領域クローンを作製することもできる。このプロセスは、特定の制限部位を削除又は含有させたり、あるいは特定のコドンを最適化するなどのいくつかの利点を有する。

【００７５】

ハイブリドーマからの重鎖及び軽鎖転写産物のヌクレオチド配列を用いて、重複組の合成オリゴヌクレオチドをデザインし、天然配列と同一のアミノ酸コーディング能を持つ合成V配列を作製する。この合成重鎖及びカッパ鎖配列は３つの方法で天然配列と異なってもよい：一続きの反復ヌクレオチド塩基に中断を加えてオリゴヌクレオチド合成及びPCR増幅がし易いようにする；最適な翻訳開始部位をコザックの規則に従い導入する(Kozak, 1991, J. Biol. Chem. 266L19867019870)；そしてHindIII部位をこの翻訳開始部位の上流に操作する。

【００７６】

重鎖及び軽鎖可変領域の両方について、最適化されたコーディング鎖配列及び対応する非コーディング鎖配列を、この対応する非コーディングオリゴヌクレオチドのほぼ中間点で３０乃至５０ヌクレオチドに分割する。従って各鎖毎に、オリゴヌクレオチドを、１５０乃至４００個のヌクレオチドのセグメントにわたる重複する二本鎖の組に組み立てることができる。次にこのプールをテンプレートとして用いて、１５０乃至４００個のヌクレオチドから成るPCR増幅産物を作製する。典型的には、一個の可変領域オリゴヌクレオチドの組を２つのプールに分割し、これらのプールを別々に増幅して２つの重複するPCR産物を作製することになるであろう。次にこれらの重複する産物をPCR増幅で組み合わせて完全な可変領域を形成する。また、重鎖又は軽鎖定常領域（カッパ軽鎖のBbsI部位、又はγ重鎖のAgeI部位を含む）の重複するフラグメントをPCR増幅に含めることが、発現ベクタコンストラクト内に容易にクローンできるフラグメントを作製するには望ましいであろう。

【００７７】

次に、再構築された重鎖及び軽鎖可変領域を、クローンされたプロモータ配列、リーダ配列、翻訳開始配列、リーダ配列、定常領域配列、3'側非翻訳配列、ポリアデニレーション配列、及び転写終了配列に組み合わせて、発現ベクタコンストラクトを形成する。重鎖及び軽鎖発現コンストラクトを単一のベクタ内で組み合わせることも、同時トランスフェクトすることも、順にトランスフェクトすることも、あるいはホスト細胞内に別々にトランスフェクトしてからこのホスト細胞を融合して、両方の鎖を発現するホスト細胞を形成することもできる。

【００７８】

ヒトIgGκのための発現ベクタの構築に用いるプラスミドを以下に解説する（実施例１）。当該プラスミドは、PCR増幅後のV重鎖及びVカッパ軽鎖cDNA配列を用いると完全重鎖及び軽鎖最小遺伝子を再構築できるように構築された。これらのプラスミドは、完全ヒトIg

$G_1\kappa$又は$IgG_4\kappa$抗体を発現させるために用いることができる。本発明の完全ヒト及びキメラ抗体には、さらにIgG2、IgG3、IgE、IgA、IgM、及びIgD抗体も含まれる。同様なプラスミドは、他の重鎖アイソタイプを発現させたり、又は、ラムダ軽鎖を含む抗体を発現させるためにも、構築することができる。

【００７９】

このように、本発明の別の局面では、本発明のヒト抗IL-15抗体、146B7、147H5、404A8及び404E4、の構造上の特徴を用いて、IL-15に結合するなどの本発明の抗体の少なくとも一つの機能的特性を維持した、構造上関連するヒト抗IL-15抗体を作製する。より具体的には、146B7、147H5、404A8及び404E4の一つ以上のCDR領域を、既知のヒトフレームワーク領域及びCDRに組換えにより組み合わせることで、更なる、組換え操作された、本発明のヒト抗IL-15抗体を作製することができる。

【００８０】

従って、別の実施態様では、本発明は：

（１）ヒト重鎖フレームワーク領域及びヒト重鎖CDRであって、前記ヒト重鎖CDRのうちの少なくとも一つが、図２に示された（又は配列番号：2の対応アミノ酸残基）CDRのアミノ酸配列から選択されるアミノ酸配列を含む、ヒト重鎖フレームワーク領域及びヒト重鎖CDRと；（２）ヒト軽鎖フレームワーク領域及びヒト軽鎖CDRであって、前記ヒト重鎖CDRのうちの少なくとも一つが、図３に示された（又は配列番号： 4のアミノ酸残基に相当する）CDRのアミノ酸配列から選択されるアミノ酸配列を含む、ヒト軽鎖フレームワーク領域及びヒト軽鎖CDRと、を含む抗体を調製するステップ

を含み、

但し前記抗体がIL-15への結合能を維持している、抗IL-15抗体を調製する方法を提供するものである。

【００８１】

当該抗体のIL-15への結合能は、実施例に記載したものなど、標準的な結合検定法（例えばELISA）を用いて判定することができる。

【００８２】

抗体の重鎖及び軽鎖CDR3ドメインは特に重要な役割を、抗原に対する抗体の結合特異性／親和性において果たすことが当業で公知であるため、上述のように調製された本発明の組換え抗体は、好ましくは、146B7、147H5、404A8及び404E4の重鎖及び軽鎖CDR3を含むとよい。本抗体にさらに146B7、147H5、404A8及び404E4のCDR2を含めることもできる。本抗体にはさらに146B7、147H5、404A8及び404E4のCDR1を含めることもできる。本抗体にはさらに、前記CDRのいかなる組合せをも含めることができる。

【００８３】

従って、別の局面では、さらに本発明は、（１）ヒト重鎖フレームワーク領域、ヒト重鎖CDR1領域、ヒト重鎖CDR2領域、及びヒト重鎖CDR3領域であって、但し前記ヒト重鎖CDR3領域が146B7、147H5、404A8及び404E4のCDR3、例えば図２に示す（又は配列番号：2の対応アミノ酸残基）146B7のヒト重鎖CDR領域、から成る群より選択される、ヒト重鎖フレームワーク領域、ヒト重鎖CDR1領域、ヒト重鎖CDR2領域、及びヒト重鎖CDR3領域と、（２）ヒト軽鎖フレームワーク領域、ヒト軽鎖CDR1領域、ヒト軽鎖CDR2領域、及びヒト軽鎖CDR3領域であって、前記ヒト軽鎖CDR3領域が146B7、147H5、404A8及び404E4のCDR3、例えば図３に示す（又は配列番号：4の対応アミノ酸残基）146B7のヒト軽鎖CDR領域、から成る群より選択される、ヒト軽鎖フレームワーク領域、ヒト軽鎖CDR1領域、ヒト軽鎖CDR2領域、及びヒト軽鎖CDR3領域と、を含む抗IL-15抗体、であって、前記抗体がIL-15に結合する、抗IL-15抗体、を提供するものである。さらに本抗体に、146B7、147H5、404A8及び404E4の重鎖CDR2及び／又は軽鎖CDR2を含めてもよい。さらに本抗体に、146B7、147H5、404A8及び404E4の重鎖CDR1及び／又は軽鎖CDR1を含めてもよい。

【００８４】

上述の操作された抗体のCDR1、2、及び／又は3領域に、ここに開示した146B7、147H5、404A8及び404E4のそれと全く同じアミノ酸配列を含めることができる。しかしながら、当業

者であれば、本抗体のIL-15への結合能が事実上保持されれば、146B7、147H5、404A8及び404E4通りのCDR配列から何らかの逸脱（例えば保存的配列改変）があってもよいことは理解されよう。従って、別の実施態様では、操作後の抗体は、146B7、147H5、404A8及び404E4の一つ以上のCDRに対し、例えば90%、95%、98% 又は99.5% 同一な一つ以上のCDRから成ってもよい。

【００８５】

IL-15へ単に結合することだけでなく、上述したものなどの操作された抗体を、本発明の抗体の他の機能上の特徴：例えば：

（１）ヒトIL-15への結合、及び、IL-15誘導性炎症誘発作用の阻害；

（２）IL-15誘導性TNFα産生又はT細胞増殖の阻害；

（３）組換えヒトIL-15を分析物とし、当該抗体をリガンドとして用いたBIACORE 3000装置による表面プラスモン共鳴（SPR）技術で判定した場合、ほぼ10^{-7}M未満の解離平衡定数（K_D）でのヒトIL-15への結合；

（４）ヒトIL-15のβ及び／又はγ鎖相互作用ドメイン上に位置するエピトープへの結合；

（５）ヒトIL-15のAsp^{8}の、ヒトIL-15受容体のβユニットへの結合、及び／又は、ヒトIL-15のGln^{108}の、ヒトIL-15受容体のγユニットへの結合、に対する干渉：

（６）受容体に結合したヒトIL-15への結合；

（７）ヒトIL-15への結合、及びヒトIL-15の錯角化症誘導能の阻害；

（８）ヒトIL-15への結合、及びヒトIL-15の表皮肥厚誘導能の阻害；

（９）ヒトIL-15への結合、及びヒトIL-15のケラチノサイト増殖誘導能の阻害；及び／又は

（１０）ヒトIL-15への結合、及びヒトIL-15の活性化白血球遊走誘導能の阻害；

などの保持について、選抜してもよい。

【００８６】

IL-15に対するヒトモノクローナル抗体の特徴付け

本発明のヒトモノクローナル抗体は、多種の公知の技術を用いてIL-15への結合について特徴付けることができる。一般的には、当該抗体をELISAでまず特徴付ける。簡単に説明すると、微量定量プレートを、PBSに入れた精製IL-15で被覆した後、PBSで希釈したウシ血清アルブミン（BSA)などの無関係のタンパク質で遮断することができる。IL-15免疫マウスから採った血漿の希釈液を各ウェルに加え、３７℃で１乃至２時間、インキュベートする。このプレートをPBS／Tween 20で洗浄した後、アルカリホスファターゼに結合させたヤギ抗ヒトIgG Fc特異ポリクローナル試薬と一緒に３７℃で１時間、インキュベートする。洗浄後、プレートをABTS基質で展開させ、405のODで分析する。好ましくは、最も高い抗体価を生じるマウスを融合に用いるとよい。

【００８７】

上述のELISA検定は、抗体、ひいてはIL-15免疫原との陽性反応性を示す抗体を産生するハイブリドーマを探すスクリーニングに用いることができる。こうして、IL-15に、好ましくは高い親和性で、結合するハイブリドーマをサブクローンし、さらに特徴付けることもできる。次に親細胞との反応性を維持した（ELISAにより）各ハイブリドーマから採った一個のクローンを選択し、細胞バンクを作製し、抗体精製に向けることができる。

【００８８】

ヒト抗IL-15抗体を精製するには、選抜されたハイブリドーマを、ローラ瓶、２リットル入りスピナー・フラスコ、又は他の培養系で成長させることができる。上清を濾過し、濃縮してからプロテインＡ－セファロース（ニュージャージー州ピスカタウェイ、ファルマシア社）によるアフィニティ・クロマトグラフィにかけて当該タンパク質を精製することができる。緩衝液をPBSに交換し、1.43の吸光係数を用いたOD_{280}により、又は好ましくはネフェロメータ分析により、濃度を判定できる。IgGはゲル電気泳動法及び抗原特異的方法で検査することができる。

【００８９】

選抜されたヒト抗IL-15モノクローナル抗体が固有のエピトープに結合するかを調べるためには、各抗体を市販の試薬（イリノイ州ロックフォード、ピアース社）を用いてビオチン化することができる。ビオチン化MAbの結合は、ストレプトアビジン標識したプローブで検出できる。精製された抗体のアイソタイプを調べるには、公知の技術を用いてアイソタイプELISAを行うことができる。例えば、微量定量プレートのウェルを10μg/ml の抗ヒトIg で一晩かけて４℃で被覆できる。5% BSAで遮断した後、プレートを10μg/mlのモノクローナル抗体又は精製済みのアイソタイプ・コントロールに、周囲温度で２時間、反応させる。次にこのウェルをヒトIgGl 又は他のヒトアイソタイプ特異結合プローブに反応させることができる。プレートを展開させ、上述したように分析する。

【００９０】

IL-15発現生存細胞へのモノクローナル抗体の結合を検査するためには、フローサイトメトリを利用できる。簡単に説明すると、膜結合IL-15を発現する細胞株及び／又はヒトPBMC（標準的な成長条件下で成長させたもの）を、0.1% BSA及び0.01% NaN3を含有する多様な濃度のモノクローナル抗体PBS溶液に４℃で１時間、混合する。洗浄後、細胞を、フルオレセインで標識された抗ヒトIgG抗体に、一次抗体染色と同じ条件下で反応させる。これら試料を、FACScan装置により、単個細胞を開口させる光及び側光散乱特性を用いて分析することができ、標識された抗体の結合を判定する。蛍光顕微鏡法を用いた代替的な検定法を（このフローサイトメトリ検定法に加えて又は代わりに）用いてもよい。細胞は上述した通りに染色し、蛍光顕微鏡法で調べることができる。この方法では、個々の細胞の観察が可能であるが、抗原の密度によっては感受性が劣るかも知れない。

【００９１】

さらに抗IL-15ヒトIgGは、IL-15抗原との反応性についてウェスタン・ブロット法でもテストすることができる。簡単に説明すると、IL-15発現細胞からの細胞抽出物を調製し、ドデシル硫酸ナトリウムポリアクリルアミドゲル電気泳動法にかけることができる。電気泳動後、分離した抗原をニトロセルロース・メンブレンに写し取り、20%マウス血清で遮断し、テスト対象のモノクローナル抗体でプローブする。ヒトIgGの結合は、抗ヒトIgGアルカリホスファターゼを用いて検出し、BCIP/NBT基質錠剤（ミズーリ州セントルイス、シグマ・ケミカルズ社）で展開させることができる。

【００９２】

ＩＩ．　ヒトモノクローナル抗IL-15抗体を産生する非ヒトトランスジェニック及びトランスクロモゾマル動物の作製

さらに別の局面では、本発明は、IL-15に特異的に結合するヒトモノクローナル抗体を発現できるトランスジェニックもしくはトランスクロモゾマルマウスなどの非ヒトトランスジェニックもしくはトランスクロモゾマル動物を提供するものである。ある具体的な実施態様では、本発明は、IL-15抗原及び／又はIL-15発現細胞で免疫したときにマウスがヒト抗IL-15抗体を産生するように、ヒト重鎖導入遺伝子を含むゲノムを有するトランスジェニックもしくはトランスクロモゾマル・マウスを提供する。前記ヒト重鎖導入遺伝子は、ここで詳細に解説し、例示する通りのHuMAbマウスなど、トランスジェニックの場合と同様に、マウスの染色体DNAに組み込ませることができる。反対に、前記ヒト重鎖導入遺伝子を、WO 02/43478（２００２年６月６日公開）に記載されたようなトランスクロモゾマル（例えばKM）マウスの場合と同様に、染色体外に維持することもできる。このようなトランスジェニック及びトランスクロモゾマル・マウスは、V-D-J組換え及びアイソタイプ・スイッチングを起こすことにより、IL-15に対して複数のアイソタイプ(例えばIgG、IgA 及び／又は IgE)のヒトモノクローナル抗体を産生できる。アイソタイプ・スイッチングは、例えば古典的又は非古典的なアイソタイプ・スイッチングなどで起きるものでもよい。

【００９３】

外来の抗原刺激に対して異種抗体レパートリーで応答する非ヒトトランスジェニックもしくはトランスクロモゾマル動物をデザインするには、トランスジェニック動物内に含まれた異種免疫グロブリン導入遺伝子がＢ細胞発生の経路全体にわたって正確に機能する必要

がある。これには、例えば、異種重鎖導入遺伝子のアイソタイプ・スイッチングが含まれる。従って、導入遺伝子は、抗体のアイソタイプ・スイッチングと、以下：（１）高レベルかつ細胞種特異的な発現、（２）機能遺伝子の再編成、（３）アレル排除の活性化及びアレル排除への応答、（４）充分な一次レパートリーの発現、（５）シグナル伝達、（６）体細胞の超変異、及び（７）免疫応答の際の導入遺伝子抗体遺伝子座の優性、のうちの一つ以上とが生じるように、構築される。

【００９４】

前述の基準の全てを満たす必要はない。例えば、トランスジェニック動物の内因性免疫グロブリン遺伝子座を機能的に破壊した実施態様では、この導入遺伝子はアレル排除を活性化する必要はない。さらに、導入遺伝子が機能的に再編成された重鎖及び／又は軽鎖免疫グロブリン遺伝子を含む実施態様では、機能遺伝子の再編成という二番目の基準は、少なくとも導入遺伝子が既に再編成されている限りにおいて、不要である。分子免疫学の背景については、Fundamental Immunology, 2nd edition (1989), Paul William E., ed. Raven Press, N.Y.を参照されたい。

【００９５】

いくつかの実施態様では、本発明のヒトモノクローナル抗体を作製するために用いる非ヒトトランスジェニックもしくはトランスクロモゾマル動物は、再編成された、再編成のない、又は再編成された及び再編成のないものの組合せの異種免疫グロブリン重鎖及び軽鎖導入遺伝子を、このトランスジェニック動物の生殖細胞に含有する。重鎖導入遺伝子のそれぞれは少なくとも一つのC_H遺伝子を含む。加えて、この重鎖導入遺伝子が、このトランスジェニック動物のＢ細胞中で、複数のC_H遺伝子をコードする異種導入遺伝子のアイソタイプ・スイッチングを支援できる機能的アイソタイプ・スイッチ配列を含有してもよい。このようなスイッチ配列は、導入遺伝子C_H遺伝子の源として役立てた種由来の生殖細胞免疫グロブリン遺伝子座に天然で存在するものであってもよく、あるいはこのようなスイッチ配列は、導入遺伝子コンストラクトを受け取る側の種（トランスジェニック動物）にあるものを由来としてもよい。例えば、トランスジェニックマウスを作製するために用いるヒト導入遺伝子コンストラクトは、マウス重鎖遺伝子座に天然で存在するものと類似のスイッチ配列が導入されている場合には、より高頻度でアイソタイプ・スイッチング事象を起こすと思われる。これはおそらく、マウススイッチ・リコンビナーゼ酵素系で機能するにはこのようなマウススイッチ配列は最適であるが、ヒトスイッチ配列はそうでないからであろう。スイッチ配列は従来のクローニング法で単離及びクローンしてもよく、又は、免疫グロブリンスイッチ領域配列に関して公開された配列情報に基づいてデザインされた重複合成オリゴヌクレオチドからde novo合成してもよい (Mills et al., Nucl. Acids Res. 15:7305-7316 (1991); Sideras et al., Intl. Immunol. 1:631-642 (1989))。前述のトランスジェニック動物のそれぞれの場合、機能的に再編成された異種重鎖及び軽鎖免疫グロブリン導入遺伝子が、このトランスジェニック動物のＢ細胞の大部分で見られる（少なくとも１０パーセント）。

【００９６】

本発明のトランスジェニック動物を作製するために用いる導入遺伝子は、少なくとも一つの可変遺伝子セグメント、一つの多様性遺伝子セグメント、一つのジョイニング遺伝子セグメント、及び少なくとも一つの定常領域遺伝子セグメント、をコードするDNAを含む重鎖導入遺伝子を含む。免疫グロブリン軽鎖導入遺伝子は、少なくとも一つの可変遺伝子セグメント、一つのジョイニング遺伝子セグメント、及び少なくとも一つの定常領域遺伝子セグメント、をコードするDNAを含む。前記軽鎖及び重鎖遺伝子セグメントをコードする遺伝子セグメントは、当該の非ヒトトランスジェニック動物を構成しない種を由来とする免疫グロブリン重鎖及び軽鎖遺伝子セグメントをコードするDNAを由来とするか、又は、このようなDNAに相当するという点で、この非ヒトトランスジェニック動物にとって異種である。本発明の一局面では、これら個々の遺伝子セグメントが再編成されないように、即ち、機能的免疫グロブリン軽鎖又は重鎖をコードするよう、再編成されないように、導入遺伝子を構築する。このような再編成のない導入遺伝子は、IL-15抗原に暴露したとき

に、V、D、及びJ遺伝子セグメントの組換え（機能的再編成）を支援し、好ましくは、本非ヒトトランスジェニック動物内のD領域遺伝子セグメントの全部又は一部が再編成後の免疫グロブリン重鎖へ取り込まれることを支援するとよい。

【００９７】

代替的な実施態様では、当該導入遺伝子は再編成のない「最小遺伝子座」を含むものである。このような導入遺伝子は典型的に、C、D、及びJセグメントの大部分や、V遺伝子セグメントのサブセットを含む。このような導入遺伝子コンストラクトにおいては、多様な調節配列、例えばプロモータ、エンハンサ、クラス・スイッチ領域、RNAプロセッシングの際のスプライス－ドナー及びスプライス－アクセプタ配列、組換えシグナル等、は、当該の異種DNA由来の対応する配列を含む。このような調節配列は、この導入遺伝子に、本発明で用いられる非ヒト動物と同じ種から、又は、関連する種から、導入してよい。例えば、ヒト免疫グロブリン遺伝子セグメントを導入遺伝子内でげっ歯類免疫グロブリンエンハンサ配列に組み合わせて、トランスジェニックマウスでの利用に向けてもよい。代替的には、哺乳動物のゲノムに天然で存在することが公知の機能的DNA配列にとって同種でないような合成調節配列を、導入遺伝子に組み込んでもよい。合成調節配列は、例えばスプライス－アクセプタ部位又はプロモータ／エンハンサ・モチーフの許容可能な配列を明示したものなど、コンセンサスの規則に従ってデザインされる。例えば、最小遺伝子座は、天然で発生する生殖細胞Ig遺伝子座に比較して、必須でないDNA部分（例えば介在配列；イントロン又はその一部分）に、少なくとも一つの中間（即ち当該部分の末端ではない）の欠失を有するゲノム免疫グロブリン遺伝子座部分を含む。

【００９８】

本発明のある好適な実施態様では、IL-15に対するヒト抗体を作製するために用いるトランスジェニックもしくはトランスクロモゾマル動物は、WO98/24884の実施例5、6、8、又は14に解説された軽鎖導入遺伝子を１コピー含有する動物と、WO98/24884の実施例10で解説されたJ_H欠失動物と交配したその仔で育種したWO98/24884の実施例１２で解説された導入遺伝子（例えばpHC1又はpHC2）のコピーを少なくとも１つ、典型的には２乃至１０、そして時には２５乃至５０又はより以上、含有する。動物は、これら３種の形質のそれぞれについてホモ接合型となるよう、交配する。このような動物は以下の遺伝子型：（WO98/24884の実施例１２に解説された）ヒト重鎖の再編成のない最小遺伝子座 の一個のコピー（染色体の１ハプロイド当たり）、（WO98/24884の実施例１４に解説された）再編成されたヒトK軽鎖コンストラクトの一個のコピー（染色体の１ハプロイド当たり）、及び（WO98/24884の実施例１０に解説された）機能的J_Hセグメントの全てを除去する各内因性マウス重鎖遺伝子座での欠失、を有する。このような動物を、J_Hセグメントの欠失についてホモ接合型になったマウス（WO98/24884の実施例１０）と交配して、J_H欠失についてホモ接合型、そしてヒト重鎖及び軽鎖コンストラクトについてヘミ接合型となった仔を作る。その動物に抗原を注射して、これらの抗原に対するヒトモノクローナル抗体の作製に用いる。

【００９９】

このような動物から単離されたＢ細胞は、ヒト重鎖及び軽鎖について単一特異的であるが、それはこれらが各遺伝子のコピーを１つしか含有しないからである。さらに、これらはヒト又はマウス重鎖についても単一特異的となるであろうが、それは、内因性マウス重鎖遺伝子コピーの両方が、WO98/24884の実施例9及び12に解説するように導入されたJ_H領域全般の欠失のために、機能を失っているからである。さらに、Ｂ細胞の大部分が、ヒト又はマウス軽鎖について単一特異的となるであろうが、それは、再編成されたヒトκ軽鎖遺伝子の前記単一コピーが発現することで、Ｂ細胞の大部分において、内因性マウスκ及びラムダ鎖遺伝子の再編成がアレル及びアイソタイプの上、排除されることになるからである。

【０１００】

本発明で用いるトランスジェニック及びトランスクロモゾマル・マウスは、大きなレパートリー、理想的には天然マウスのそれと実質的に同様なレパートリーで、免疫グロブリン

産生を示す。このように、例えば内因性Ig遺伝子が不活化されている実施態様では、総免疫グロブリンレベルは、血清の約0.1 から10 mg/ml、好ましくは0.5 から5 mg/mlの範囲、理想的には少なくとも約1.0 mg/mlであろう。IgMからIgGへのスイッチを行うことのできる導入遺伝子がトランスジェニックマウスに導入されている場合、成体マウスの血清IgG対IgMの比は好ましくは約１０：１である。IgG対IgMのこの比は幼若マウスではずっと低くなるであろう。おおざっぱに言って、当該脾臓及びリンパ節Ｂ細胞の約10%を越えるもの、好ましくは40乃至80%が、ヒトIgGタンパク質のみを発現する。

【０１０１】

前記レパートリーは、理想的には、天然マウスが示すものに、通常は少なくとも約10%、好ましくは25乃至50%又はそれ以上、近いとよいであろう。概して、少なくとも約１００００種の異なる免疫グロブリン（理想的にはIgG）、好ましくは10^4乃至10^6又はそれ以上の種類が、主にマウスゲノムに導入された様々なV、J及びD領域の数に応じて産生されるとよいであろう。これらの免疫グロブリンは、典型的には、例えばブドウ球菌プロテインAなど、抗原性の高いタンパク質の約半分以上を認識するであろう。典型的には、前記免疫グロブリンは、所定の抗原に対し、例えば10^{-8}M、10^{-9}M又は10^{-10}M未満又はそれ未満など、10^{-7}M未満の親和性（K_D）を示すであろう。

【０１０２】

いくつかの実施態様では、所定の抗原種に対する抗体応答で現れるV遺伝子の選択幅を制限するために、予め決められたレパートリーを持つマウスを作製することが好ましいであろう。予め決められたレパートリーを有する重鎖導入遺伝子には、例えばヒトにおいて所定の抗原種に対する抗体応答で優先的に用いられるヒトV_H遺伝子などを含めてもよい。代替的には、いくつかのV_H遺伝子を、多様な理由（例えば所定の抗原に対して親和性の高いV領域をコードする可能性が低い；体細胞変異及び親和性尖鋭化を起こす傾向が小さい；又は、特定のヒトに対して免疫原性である、など）のために、規定のレパートリーから除外してもよい。このように、多様な重鎖又は軽鎖遺伝子セグメントを含有する導入遺伝子が再編成される前に、このような遺伝子セグメントを、当該トランスジェニック動物以外の生物種由来であるとして、例えばハイブリダイゼーション又はDNA配列決定法などにより、容易に特定できよう。

【０１０３】

上述したトランスジェニック及びトランスクロモゾマル・マウスは、前述したように、例えばIL-15抗原の精製もしくは濃縮製剤、及び／又は、IL-15発現細胞で免疫することができる。選択的には、当該トランスジェニック・マウスを、ヒトIL-15をコードするDNAで免疫することもできる。このマウスが産生するＢ細胞は、導入遺伝子内スイッチ組換え（cisスイッチング）を通じてクラス・スイッチングを起こして、IL-15と反応性の免疫グロブリンを発現するであろう。この免疫グロブリンはヒト抗体（「ヒト配列抗体」とも呼ぶ）でもよく、そのときその重鎖及び軽鎖ポリペプチドは、ヒト導入遺伝子配列にコードされていてもよいが、前記ヒト導入遺伝子配列は、体細胞変異及びV領域組換えジョイント由来の配列や、生殖細胞にコードされた配列を含んでもよい。これらのヒト抗体は、ヒトV_L又はV_H遺伝子セグメント及びヒトJ_L又はD_H及びJ_Hセグメントにコードされたポリペプチド配列と実質的に同一であると言うことができ、ただし他の非生殖細胞配列も、体細胞変異及び示差的なV-J 及びV-D-J組換えジョイントの結果として存在してもよい。各抗体鎖の可変領域は、典型的に、ヒト生殖細胞V、J遺伝子セグメントに、そして重鎖の場合はD遺伝子セグメントに、少なくとも８０パーセント、コードされている。しばしば可変領域の少なくとも８５パーセントが、導入遺伝子上に存在するヒト生殖細胞配列にコードされている。可変領域配列のうちのしばしば９０又は９５パーセント又はそれ以上が、導入遺伝子上に存在するヒト生殖細胞配列にコードされている。しかしながら、体細胞変異並びにVJ及びVDJジョイニングでは非生殖細胞配列が導入されるために、当該ヒト配列抗体は、しばしば、このマウスの生殖細胞中のヒト導入遺伝子に見られるようなヒトV、D又はJ遺伝子セグメントにはコードされていない何らかの可変領域配列を（そして頻度は劣るが定常領域配列を）有するであろう。典型的には、このような非生殖細胞配列（又は個々のヌ

クレオチド位置）は、CDR中か、又はCDR近傍、あるいは体細胞変異が集中的に起きることが知られている領域に集まるであろう。

【０１０４】

所定の抗原に結合するヒト抗体は、例えばヒト配列γ鎖（例えばγ1、γ2a、γ2B、又はγ3）及びヒト配列軽鎖（例えばカッパ）を含むヒト抗体が生じるようなアイソタイプ・スイッチングにより生じさせることができる。このようなアイソタイプ・スイッチングの起きたヒト抗体は、親和性成熟及び抗原によるＢ細胞の選択の結果として、特に二次（又は後続）抗原刺激の結果として、一箇所以上の体細胞変異を、典型的には可変領域、そしてしばしばCDRの内部か、又はCDRから約１０残基以内に、しばしば含有する。これらの高親和性ヒト抗体は、例えば10^{-8}M、10^{-9}M又は10^{-10}Mもしくはそれ未満など、10^{-7}M未満の結合親和性(K_D）を有するであろう。

【０１０５】

本発明の別の局面は、ここで解説したトランスジェニック又はトランスクロモゾマル・マウス由来のＢ細胞を包含するものである。このＢ細胞を、高親和性（例えば10^{-7}M未満）でIL-15に結合するヒトモノクローナル抗体を発現するハイブリドーマを作製するために使用することができる。このように、別の実施態様では、本発明は、組換えヒトIL-15を分析物とし、当該抗体をヒトIL-15への結合のリガンドとして用いて、BIACORE 3000装置により表面プラスモン共鳴法（SPR）で判定したときの親和性(K_D）が、例えば10^{-8}M、10^{-9}M又は10^{-10}Mもしくはそれ未満など、10^{-7}M未満のヒト抗体を産生するハイブリドーマを提供し、このとき当該抗体は：

（１）ヒトV_L遺伝子セグメント及びヒトJ_Lセグメントにコードされたポリペプチド配列に実質的に同一なポリペプチド配列を有する軽鎖可変領域、及び（２）ヒトC_L遺伝子セグメントにコードされたポリペプチド配列に実質的に同一なポリペプチド配列を有する軽鎖定常領域、から成るヒト配列軽鎖と；

（１）ヒトV_H遺伝子セグメント、選択的にD領域、及びヒトJ_Hセグメントにコードされたポリペプチド配列に実質的に同一なポリペプチド配列を有する重鎖可変領域、及び（２）ヒトC_H遺伝子セグメントにコードされたポリペプチド配列に実質的に同一なポリペプチド配列を有する定常領域、から成るヒト配列重鎖と

を含む。

【０１０６】

IL-15に対する高親和ヒトモノクローナル抗体の開発は、組み込まれたヒト免疫グロブリン導入遺伝子を含むゲノムを有するトランスジェニックマウス中で、ヒト可変領域遺伝子セグメントのレパートリーを拡大する方法により容易になるが、当該方法は、前記組み込まれたヒト免疫グロブリン導入遺伝子には存在しないV領域遺伝子セグメントを含むV遺伝子導入遺伝子を前記ゲノムに導入するステップを含む。しばしば前記V領域導入遺伝子は、ヒトゲノムに天然で存在するような、又は、組換え法で一緒に別にスプライスされるような、ヒトV_H又はV_L（V_K）遺伝子セグメント・アレイの一部分を含む酵母人工染色体であり、この酵母人工染色体の含有するV遺伝子セグメントは順序が狂っていても、又は省略されていてもよい。しばしば少なくとも５つ以上の機能的V遺伝子セグメントが前記YAC上に含有されている。このバリエーションでは、前記Vレパートリー拡大法で生じるトランスジェニックマウスを作製することが可能であり、このとき当該マウスは、V領域導入遺伝子上に存在するV領域遺伝子セグメントにコードされた可変領域配列と、ヒトIg導入遺伝子にコードされたC領域とを含む免疫グロブリン鎖を発現する。Vレパートリー拡大法により、少なくとも５個の異なるV遺伝子を有するトランスジェニックマウスを作製でき、また少なくとも約２４個又はそれ以上のV遺伝子を含有するマウスも作製できる。いくつかのV遺伝子セグメントは非機能的であってもよい（例えば偽遺伝子等）。これらのセグメントを維持してもよいが、あるいは、必要に応じて当業者に可能な組換え法により選択的に欠失させてもよい。

【０１０７】

マウス生殖細胞を操作して、J及びC遺伝子セグメントを含有するヒトIg導入遺伝子には実

質的に存在しない拡大されたVセグメント・レパートリーを有する機能的YACを含有させたら、拡大されたVセグメント・レパートリーを有する機能的YACを、異なるヒトIg導入遺伝子を有するマウス生殖細胞に交雑するバックグラウンドを含め、他の遺伝的バックグラウンドにこの形質を伝播及び交雑することができる。拡大されたVセグメント・レパートリーを有する複数の機能的YACを、１つのヒトIg導入遺伝子（又は複数のヒトIg導入遺伝子）と一緒に働かせるために生殖細胞に交雑してよい。ここではYAC導入遺伝子と言及するが、ゲノムに組み込んだときのこのような導入遺伝子は、酵母で自律的複製を行うのに必要な配列など、酵母配列を実質的に欠いていてもよい。このような配列は、選択に応じて、酵母での複製がもはや必要でなくなってから（即ちマウスES細胞又はマウス前接合子への導入前に）遺伝子操作（例えば制限消化及びパルス界ゲル電気泳動法又は他の適した方法など）により取り除いてもよい。ヒト配列免疫グロブリン発現の形質を伝播させる方法には、ヒトIg導入遺伝子を有し、そして選択的には、拡大されたVセグメント・レパートリーを有する機能的YACもさらに有するようなトランスジェニックマウスを育種する方法がある。V_H及びV_L遺伝子セグメントの両方がYAC上に存在してもよい。当該トランスジェニックマウスは、ヒトIg導入遺伝子、及び／又は、他のヒトリンパ球たんぱくをコードする導入遺伝子を含め、他のヒト導入遺伝子を持つバックグラウンドを含む、開業医が希望するいかなるバックグラウンドに交雑してもよい。さらに本発明は、拡大されたV領域レパートリーYAC導入遺伝子を有するトランスジェニックマウスにより産生される高親和ヒト配列免疫グロブリンを提供するものである。前の記載では本発明のトランスジェニック動物の好適な実施態様を解説したが、以下、４つのカテゴリーに分類された他の実施態様も考察されている：

Ｉ．再編成のない重鎖及び再編成される軽鎖免疫グロブリン導入遺伝子を含有するトランスジェニック動物；

ＩＩ．再編成のない重鎖及び再編成のない軽鎖免疫グロブリン導入遺伝子を含有するトランスジェニック動物；

ＩＩＩ．再編成される重鎖及び再編成のない軽鎖免疫グロブリン導入遺伝子を含有するトランスジェニック動物；及び

ＩＶ．　再編成される重鎖及び再編成される軽鎖免疫グロブリン導入遺伝子を含有するトランスジェニック動物。

【０１０８】

これらのカテゴリーのトランスジェニック動物のうち、好適な優先度は以下、ＩＩ＞Ｉ＞ＩＩＩ＞ＩＶ（この場合の内因性の軽鎖遺伝子（又は少なくともK遺伝子）は、相同組換え（又は他の方法）によりノックアウトされている）、及びＩ＞ＩＩ＞ＩＩＩ＞ＩＶ（この場合の内因性軽鎖遺伝子はノックアウトされておらず、アレル排除により劣性とならなければならない）の通りである。

【０１０９】

ＩＩＩ．　抗体結合体／イムノトキシン

別の局面では、本発明は、細胞毒、薬物（例えば免疫抑制剤）又は放射性同位元素などの治療部分に結合させたヒト抗IL-15モノクローナル抗体を特徴とする。細胞毒に結合させた場合、これらの抗体結合体は「イムノトキシン」と呼ばれる。細胞毒又は細胞傷害性薬剤には、細胞にとって有害（例えば致死させる）なあらゆる物質が含まれる。 例にはタキソール、シトカラシンＢ、グラミシジンＤ、臭化エチジウム、エメチン、ミトマイシン、エトポシド、テノポシド、ビンクリスチン、ビンブラスチン、コルヒチン、ドキソルビシン、ダウノルビシン、ジヒドロキシアントラシンジオン、ミトキサントロン、ミトラマイシン、アクチノマイシンＤ、1-デヒドロテストステロン、糖質コルチコイド、プロカイン、テトラカイン、リドカイン、プロプラノロール、及びプロマイシン並びにこれらの類似体又は同族体、がある。治療的薬剤には、限定はしないが、抗代謝産物（例えばメトトレキセート、6-メルカプトプリン、6-チオグアニン、シタラビン、5-フルオロウラシルデカルバジン)、アルキル化剤（例えばメクロレタミン、チオテパクロラムブシル、メルファラン、カルムスチン（BSNU）及びロムスチン（CCNU)、シクロトスファミド、ブスルフ

ァン、ジブロモマンニトール、ストレプトゾトシン、ミトマイシンＣ、及びcis-ジクロロジアミンプラチナム(II) (DDP) シスプラチン)、アントラサイクリン（例えばダウノルビシン（以前のダウノマイシン）、及びドキソルビシン）、抗生物質（例えばダクチノマイシン（以前のアクチノマイシン）、ブレオマイシン、ミトラマイシン、及びアントラマイシン（AMC))、及び抗有糸分裂剤（例えばビンクリスチン及びビンブラスチン）、がある。本発明の抗体を、放射性ヨウ素などの放射性同位元素に結合させて、癌などのIL-15関連異常を治療するための細胞傷害性放射性医薬品を作製することもできる。

【０１１０】

本発明の抗体結合体を用いて所定の生物学的応答を修飾することができる。前記治療的部分は、古典的な化学療法薬に限定されるものと、捉えられてはならない。例えば当該の薬物成分は、所望の生物活性を有するタンパク質又はポリペプチドであってもよい。このようなタンパク質には、例えばアブリン、リシンＡ、シュードモナス・エキソトキシン、又はジフテリア毒素などの酵素活性のある毒素又はその活性フラグメント；腫瘍壊死因子又はインターフェロン-γなどのタンパク質；又は、例えばリンホカイン、インターロイキン-1（「IL-1」）、インターロイキン-2（「IL-2」）、インターロイキン-6（「IL-6」）、顆粒球マクロファージコロニー刺激因子（「GM-CSF」）、顆粒球コロニー刺激因子（「G-CSF」）、又は他のサイトカイン又は成長因子などの生物学的応答修飾物質、が含まれよう。

【０１１１】

このような治療成分を抗体に結合させる技術は公知であり、例えばArnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), 及びThorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982)を参照されたい。

【０１１２】

ＩＶ．　医薬組成物

別の局面では、本発明は、薬学的に許容可能な担体と一緒に調合された、本発明のヒトモノクローナル抗体又はその抗原結合部分を１つ又は組み合わせで含有する、医薬組成物などの組成物を提供するものである。ある好適な実施態様では、本組成物は、複数（例えば二種以上）の単離された本発明のヒト抗体の組合せを含有する。好ましくは、本組成物の抗体の各々が、IL-15の異なる、予め選択されたエピトープに結合するとよい。

【０１１３】

さらに本発明の医薬組成物を併用療法で投与することもでき、即ち他の薬剤と組み合わせることができる。例えばこの併用療法には、本発明の医薬組成物と、例えば抗炎症薬、DMARDs（疾患改変抗リウマチ薬）、免疫抑制剤、化学療法薬及び乾癬薬、などの少なくとも一種以上の付加的治療薬とを含めることができる。本発明の医薬組成物は、さらに、放射線療法と併用投与することもできる。CD4特異抗体及びIL-2特異抗体などの他の抗体との同時投与も本発明の包含するところである。CD4特異抗体又はIL-2特異抗体とのこのような組合せは、特に自己免疫疾患及び移植片拒絶などの治療に特に有用であると考えられる。

【０１１４】

ここで用いる「薬学的に許容可能な担体」には、生理学的に適合性あるあらゆる溶媒、分散媒、コーティング、抗菌剤及び抗カビ剤、等張剤及び吸収遅延剤等が含まれる。好まし

くは、当該の担体が静脈内、筋肉内、皮下、腸管外、脊髄もしくは表皮投与（例えば注射又は輸注により）に適しているとよい。投与経路によっては、活性化合物、即ち抗体、二重特異的及び多重特異的分子を、当該化合物を不活化しかねない酸及び他の天然条件の作用から当該化合物を保護する物質で被覆してもよい。

【０１１５】

「薬学的に許容可能な塩」とは、親化合物の所望の生物活性を保持しつつも、望ましくない毒性作用を与えないような塩を言う（例えばBerge, S.M., et al. (1977) J. Pharm. Sci. 66:1-19を参照されたい）。このような塩の例には、酸添加塩及び塩基添加塩がある。酸添加塩には、非毒性の無機酸、例えば塩酸、硝酸、リン酸、硫酸、臭化水素酸、ヨウ化水素酸、リン等から誘導されたものや、非毒性の有機酸、例えば脂肪族モノカルボン酸及びジカルボン酸、フェニル置換アルカン酸、ヒドロキシアルカン酸、芳香族の酸、脂肪族及び芳香族のスルホン酸等から誘導されたものがある。塩基添加塩には、ナトリウム、カリウム、マグネシウム、カルシウム等のアルカリ土類金属から誘導されたものや、N,N'-ジベンジルエチレンジアミン、N-メチルグルカミン、クロロプロカイン、コリン、ジエタノールアミン、エチレンジアミン、プロカイン等の非毒性の有機アミンから誘導されたものがある。

【０１１６】

本発明の組成物は、当業で公知の多種の方法で投与することができる。当業者であれば理解されるように、投与の経路及び／又は形態は、所望の結果に応じて様々であろう。当該活性化合物は、インプラント、経皮パッチ、及びマイクロ封入送達系を含め、制御放出製剤などの急速な放出から当該化合物を保護する担体と一緒に調製することができる。エチレン酢酸ビニル、ポリ無水物、ポリグリコール酸、コラーゲン、ポリオルトエステル、及びポリ乳酸など、生分解性で生体適合性あるポリマを用いることができる。このような製剤の調製法が数多く、特許付与されており、当業者に広く公知である。 例えばSustained and Controlled Release Drug Delivery Systems, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978を参照されたい。

【０１１７】

特定の投与経路で本発明の化合物を投与するには、当該化合物の失活を防ぐ物質でそれを被覆するか、又は当該化合物と同時投与することが必要な場合がある。例えば本化合物を、適したリポソームなどの担体又は希釈剤に入れて対象に投与してもよい。薬学的に許容可能な希釈剤には生理食塩水及び水性の緩衝液がある。リポソームには水中油中水CGFエマルジョンや、従来のリポソームがある(Strejan et al. (1984) J. Neuroimmunol. 7:27)。

【０１１８】

薬学的に許容可能な担体には無菌の水溶液又は分散液並びに、無菌の注射液又は分散液の即時調製用の無菌粉末がある。このような媒質及び薬剤の、薬学的に活性な物質のための使用は当業で公知である。従来の媒質又は薬剤が当該活性化合物にとって不適合でない限り、本発明の医薬組成物中のその使用は考察されたところである。補助的な活性化合物も、本組成物中に組み込むことができる。

【０１１９】

治療用の組成物は典型的に無菌でなければならず、また製造及び保管条件下で安定でなければならない。本組成物は、高い薬物濃度に適した溶液、マイクロ乳液、リポソーム、又は他の秩序ある構造として調合することができる。当該の担体は、例えば水、エタノール、ポリオール（例えばグリセロール、プロピレングリコール、及び液体ポリエチレングリコール等）、及びこれらの適した混合物などを含有する溶媒又は分散媒であってよい。適した流動性は、例えばレシチンなどのコーティングを用いたり、分散液の場合には必要な粒子の大きさを維持したり、そして界面活性剤を使用するなどにより、維持できる。多くの場合、例えば糖類、マンニトール、ソルビトールなどの多価アルコール、又は塩化ナトリウムなどの等張剤を組成物中に含めることが好ましいであろう。注射用組成物の吸収を長引かせるには、モノステアリン酸塩及びゼラチンなど、吸収を遅らせる薬剤を組成物中

に含めることにより、可能である。

【０１２０】

無菌の注射用溶液は、必要量の活性化合物を適した溶媒に、必要に応じて上に列挙した成分の１つ又は組み合わせと一緒に 加えた後、滅菌マイクロ濾過を行うことにより、調製できる。分散液は一般的には、塩基性の分散媒と、上に列挙したものの中で必要な他の成分とを含有する無菌の賦形剤に当該活性化合物を加えることで、調製されている。無菌の注射用溶液の調製用の無菌粉末の場合、好適な調製法は真空乾燥及び凍結乾燥（凍結乾燥）であり、その結果、活性成分及び付加的な所望の成分の粉末が、予め殺菌濾過されたその溶液から生じる。

【０１２１】

投薬計画は、最適な所望の応答（例えば治療的応答）が得られるように調節される。例えば単一の巨丸剤を投与してもよく、複数に分割された用量を一定期間にわたって投与しても、又は、治療状況の緊急度を指標として用量を比率的に増減させてもよい。例えば本発明のヒト抗体を、皮下注射により１週間当たり１回又は２回、あるいは皮下注射により１ヶ月間当たり１回又は２回、投与してもよい。投与の容易さ及び投薬量の均一性のためには、非経口用組成物を単位剤形で調合することが特に有利である。ここで用いる単位剤形とは、治療しようとする対象にとって単位型の投薬量として調整された物理的に別個の単位を言う。各単位は、必要な薬品用担体との関連から所望の治療効果を生ずるよう計算された所定量の活性化合物を含有する。本発明の単位剤形の詳細は、（ａ）活性化合物の固有の特徴、及び、達成しようとする特定の治療効果、及び（ｂ）このような活性化合物を、個体の感受性の治療に向けて配合する技術に内在する限界、によって決定され、またこれらに直接依存する。

【０１２２】

薬学的に許容可能な抗酸化剤の例には：（１）水溶性の抗酸化剤、例えばアスコルビン酸、塩酸システイン、重硫酸ナトリウム、メタ重硫酸ナトリウム、亜硫酸ナトリウム等；（２）油溶性抗酸化剤、例えばアスコルビン酸パルミテート、ブチル化ヒドロキシアニソール（BHA)、ブチル化ヒドロキシトルエン(BHT)、レシチン、没食子酸プロピル、アルファ-トコフェロール、等；及び（３）金属キレート剤、例えばクエン酸、エチレンジアミン四酢酸(EDTA)、ソルビトール、酒石酸、リン酸等がある。

【０１２３】

治療用組成物の場合、本発明の調合物には、経口、鼻孔、局所（口腔内及び舌下を含む）、直腸、膣及び／又は非経口投与に適したものが含まれる。当該調合物は適宜、単位剤形で提供してもよく、製薬業で公知のいずれの方法で調製してもよい。一個分の剤形を作製するために担体物質と組み合わせることのできる活性成分の量は、治療しようとする対象、及び特定の投与形態に応じて様々であろう。一個分の剤形を作製するために担体物質と組み合わせることのできる活性成分の量は、一般に、治療効果を生む組成物量となるであろう。概して、100パーセントのうちで、この量は約0.001パーセント乃至約99パーセントの活性成分、好ましくは約0.005パーセント乃至約70パーセント、最も好ましくは約0.01パーセント乃至約30パーセントの範囲であろう。

【０１２４】

経膣投与に適した本発明の調合物には、さらに、当業で適していることが公知の担体を含有するペッサリ、タンポン、クリーム、ゲル、ペースト、フォーム又はスプレー調合物がある。本発明の組成物の局所もしくは経皮投与用の剤形には、粉末、スプレー、軟膏、ペースト、クリーム、ローション、ゲル、溶液、パッチ及び吸入剤、がある。当該の活性化合物は、薬学的に許容可能な担体や、必要に応じて何らかの保存剤、緩衝剤、又は推進剤と、無菌条件下で混合してよい。

【０１２５】

ここで用いる文言「非経口投与」及び「非経口的に投与する」とは、通常は注射による、腸管内及び局所投与以外の投与形態を意味し、その中には、限定はしないが、静脈内、筋肉内、動脈内、鞘内、嚢内、眼窩内、心臓内、皮内、腹腔内、経気管、皮下、表皮下、関

節内、被膜下、くも膜下、髄腔内、硬膜外及び胸骨内注射及び輸注、がある。

【０１２６】

本発明の医薬組成物中に用いてもよい適した水性及び非水性の担体の例には、水、エタノール、ポリオール（例えばグリセロール、プロピレングリコール、ポリエチレングリコール等）、及びこれらの適した混合物、オリーブ油などの植物油、及びオレイン酸エチルなどの注射可能な有機エステル、がある。適正な流動性は、例えばレシチンなどのコーティング材料を用いたり、分散液の場合には必要な粒子の大きさを維持したり、そして界面活性剤を使用するなどにより、維持できる。

【０１２７】

これらの組成物には、保存剤、湿潤剤、乳濁剤及び分散剤などのアジュバントを含有させてもよい。微生物の存在を防ぐには、上述の滅菌法と、パラベン、クロロブタノール、フェノールソルビン酸等の多種の抗菌剤及び抗カビ剤の含有の両方を行うと、確実になろう。例えば糖類、塩化ナトリウム等の等張剤を組成物に含めることも好ましいであろう。加えて、注射用の薬形の吸収を長引かせるには、モノステアリン酸アルミニウム及びゼラチンなど、吸収を遅らせる薬剤を含めることにより、可能である。

【０１２８】

本発明の化合物を製薬としてヒト及び動物に投与する場合、これらを単独で与えることもできるが、又は、例えば0.001%乃至90%（より好ましくは0.005%乃至70%、例えば0.01乃至30%）の活性成分を薬学的に許容可能な担体と組み合わせて含有する医薬組成物としても、与えることができる。

【０１２９】

選択した投与経路に関係なく、適した水和型で用いてもよい本発明の化合物、及び／又は、本発明の医薬組成物は、当業者に公知の常法により、薬学的に許容可能な剤形に調合される。

【０１３０】

本発明の医薬組成物中の活性成分の実際の投薬量レベルは、特定の患者、組成物、及び投与形態にとって、患者に毒性となることなく所望の治療応答を得るために有効量の活性成分が得られるよう、変更してもよい。選択される投薬量レベルは、用いる本発明の特定の組成物又は、そのエステル、塩又はアミドの活性、投与経路、投与機関、用いる特定の化合物の排出速度、治療期間、用いる特定の組成物と併用する他の薬物、化合物及び／又は物質、治療する患者の年齢、性別、体重、状態、全身の健康及び以前の医療歴等、医業で公知の因子を含め、多種の薬物動態学的因子に依拠することとなろう。当業において通常の技術を有する医師又は獣医であれば、本医薬組成物の必要な有効量を容易に決定及び処方することができる。例えば、この医師又は獣医は、当該医薬組成物中に用いる本発明の化合物の用量を、所望の治療効果を得るのに必要なそれより少ないレベルで開始し、この投薬量を所望の効果が得られるまで次第に増加させていってもよい。一般的には、本発明の組成物の適した一日当たりの用量は、治療効果を生むために有効な最も少ない用量である化合物量であろう。このような有効量は一般に、上で解説した因子に依拠するであろう。投与は、静脈内、筋肉内、腹腔内、又は皮下によることが好ましく、好ましくは標的部位の近位に投与するとよい。必要に応じ、治療用組成物の有効な一日分の用量を、２回、３回、４回、５回、６回又はそれ以上の小分けした用量に分けて別々に、全日にわたって適当な間隔を置きながら、選択的には単位剤形で、投与してもよい。本発明の化合物を単独で投与することも可能であるが、本化合物を医薬調合物（組成物）として投与することが好ましい。

【０１３１】

治療用組成物は当業で公知の医療器具を用いて投与できる。例えばある好適な実施態様では、本発明の治療用組成物を、例えば米国特許第5,399,163号；第5,383,851号；第5,312,335号；第5,064,413号；第4,941,880号；第4,790,824号；又は第4,596,556号に開示された器具などの無針皮下注射器具で投与することができる。本発明で有用な公知のインプラント及びモジュールの例には、制御された速度で薬品を分配するインプラント可能なマイ

クロ輸注ポンプを開示する米国特許第4,487,603号；薬品を皮膚を透過させて投与する治療器具を開示する米国特許第4,486,194号；精確な輸注速度で医薬を送達する医療用輸注ポンプを開示する米国特許第4,447,233号；継続的な薬物送達のための可変流量式のインプラント可能な輸注装置を開示する米国特許第4,447,224号；多チャンバ・コンパートメントを有する浸透圧薬物送達系を開示する米国特許第4,439,196号；及び浸透圧薬物送達系を開示する米国特許第4,475,196号、がある。数多くの他のこのようなインプラント、送達系、及びモジュールが当業者に公知である。

【０１３２】

いくつかの実施態様では、本発明のヒトモノクローナル抗体を、in vivoで確実に適正に分布するように調合することができる。例えば血液脳関門（ＢＢＢ）は数多くの親水性化合物を排除する。本発明の治療用化合物がＢＢＢを確実に透過するようにする（望ましい場合）には、これらを例えばリポソーム中に調合することができる。リポソームの製造方法については、例えば米国特許第4,522,811号; 第5,374,548号；及び第5,399,331号を参照されたい。前記リポソームには、特定の細胞又は臓器に選択的に輸送されて指向性ある薬物送達を高めるような１つ以上の成分を含めてもよい (例えばV.V. Ranade (1989) J. Clin. Pharmacol. 29:685)。標的指向成分の例には、葉酸又はビオチン（例えばLow et al.の米国特許第5,416,016号を参照されたい)；マンノシド（Umezawa et al., (1988) Biochem. Biophys. Res. Commun. 153:1038)；抗体 (P.G. Bloeman et al. (1995) FEBS Lett. 357:140; M. Owais et al. (1995) Antimicrob. Agents Chemother. 39:180)；その様々な種が本発明の調合物や、本発明の分子の構成成分を成していてもよいサーファクタントプロテインＡ受容体 (Briscoe et al. (1995) Am. J. Physiol. 1233:134)；p120 (Schreier et al. (1994) J. Biol. Chem. 269:9090);があり、さらにK. Keinanen; M.L. Laukkanen (1994) FEBS Lett. 346:123; J.J. Killion; I.J. Fidler (1994) Immunomethods 4:273を参照されたい。本発明の一実施態様では、本発明の治療用化合物をリポソーム中に調合する。より好適な実施態様では、当該リポソームが標的決定成分を含む。最も好適な実施態様では、当該リポソーム中の治療用化合物を、腫瘍又は感染に近位の部位への大量注射により送達する。当該組成物は、注射筒での注入が容易な程度に流動性でなくてはならない。それは、製造及び保管条件下で安定でなくてはならず、細菌及び真菌などの微生物の汚染作用から守られていなくてはならない。

【０１３３】

リウマチ性関節炎のための「治療上有効量」とは、好ましくは、患者においてACR20のプリリミナリ・ディフィニション・オブ・インプルーブメント（原語：Preliminary Definition of Improvement）に至る量、より好ましくは、ACR50のプリリミナリ・ディフィニション・オブ・インプルーブメントに至る量、そしてさらにより好ましくはARCD70のプリリミナリ・ディフィニション・オブ・インプルーブメントに至る量であろう。

【０１３４】

ACR20のプリリミナリ・ディフィニション・オブ・インプルーブメントは：
20%以上の改善がテンダー・ジョイント・カウント（原語：Tender Joint Count）（TCJ）及びスウォレン・ジョイント・カウント（原語：Swollen Joint Count）（SWJ）にあり、そして、20%以上の改善が以下、５つの評価：ペイシェント・ペイン・アセスメント（原語：Patient Pain Assessment）（VAS）、ペイシェント・グローバル・アセスメント（原語：Patient Global Asessment）（VAS）、フィジシャン・グローバル・アセスメント（原語：Physician Global Assessment）（VAS）、パテント・セルフ－アセッスド・ディスアビリティ（原語：Patient Self-Assessed Disability）（HAQ）、アキュート・フェイズ・リアクタント（原語：Acute Phase Reactant）（CRP又はESR）のうちの３つにあること、と定義されている。

【０１３５】

ACR50 及び ACR70 も同様にそれぞれ50%以上及び70%以上の改善、と定義されている。更なる詳細についてはFelson et al. in American College of Rheumatology Preliminary Definition of Improvement in Rheumatoid Arthritis; Arthritis Rheumatism (1995) 3

8: 727-735を参照されたい。

【０１３６】

ある化合物の癌阻害能は、ヒト腫瘍での効験を予測する動物モデル系で評価できる。代替的には、ある組成物のこのような性質は、当業者に公知の検定でin vitroでの阻害を調べることで当該化合物の阻害能を検査することでも評価できる。ある治療用化合物の治療上有効量は、腫瘍の大きさを減少させるものでも、又は、対象の症状を寛解させるものでもよい。当業者であれば、このような量を、対象の体格、対象の症状の重篤度、及び特定の組成物又は選択された投与経路などの因子に基づいて決定できよう。

【０１３７】

さらに、当該抗体の、乾癬を治療又は予防する能力も、当業で公知の方法に従って評価できる。

【０１３８】

本組成物は、無菌、かつ、本組成物を注射筒で送達可能な程度に流動性でなくてはならない。当該の担体は、水に加え、等張の緩衝生理食塩水、エタノール、ポリオール（例えばグリセロール、プロピレングリコール、及び液体ポリエチレングリコール等）、及びこれらの適した混合物であってよい。適正な流動性は、例えばレシチンなどのコーティングを用いたり、分散液の場合には必要な粒子サイズを維持したり、そして界面活性剤を利用するなどにより、維持できる。多くの場合、糖類、マンニトール又はソルビトールなどの多価アルコール、及び塩化ナトリウムなどの等張剤を組成物中に含めることが好ましい。注射用組成物の長期吸収は、モノステアリン酸アルミニウム又はゼラチンなど、吸収を遅らせる物質を組成物中に含めると、可能である。

【０１３９】

活性化合物を上述のように適切に保護すれば、当該化合物を、例えば不活性の希釈剤又は同化可能な食用担体と一緒に経口投与してもよい。

【０１４０】

Ｖ．　発明の用途及び方法

本発明による、IL-15に対するヒト抗IL-15抗体（この抗体の誘導体及び結合体も含む）及び前記抗体を含有する組成物は、多種のin vitro及びin vivoでの診断用途及び治療用途で用いることができる。

【０１４１】

ある実施態様では、本発明のヒト抗体を用いて、T細胞及び／又は単球／マクロファージによるIL-15誘導性TNFα産生を、好ましくはIL-2などの他のサイトカインが誘導するTNFα産生を阻害することなく、阻害する。本抗体をIL-15に接触させる（例えば対象に本抗体を投与する）ことにより、IL-15がIL-15受容体を通じてシグナル伝達する能力が阻害され、こうしてT細胞及び／又は単球／マクロファージによるTNFα産生も阻害される。好適な抗体は、IL-15に特異的なエピトープ（例えばガンマサブユニットなどの特定のサブユニット）に結合することで、IL-15誘導性TNFα産生は阻害するが、IL-2などの構造上関連するサイトカインによるTNFα産生には干渉しないものである。

【０１４２】

別の実施態様では、本発明のヒト抗体を用いて、IL-15誘導性T細胞動員及び／又は増殖を、好ましくは、IL-2などの構造上関連する他のサイトカインが誘導するT細胞増殖を阻害することなく、阻害する。TNFα産生の場合と同様に、本抗体をIL-15に接触させる（例えば対象に本抗体を投与する）ことにより、IL-15がIL-15受容体を通じてシグナル伝達する能力が阻害され、こうしてIL-15によるT細胞刺激が阻害される。

【０１４３】

従って、さらに別の実施態様では、本発明は、IL-15により媒介される障害（例えば自己免疫疾患、例えば乾癬、リウマチ性関節炎、又は炎症性腸疾患、又は感染性疾患、例えばHIV）を、前記障害を治療又は予防するのに有効量の本発明のヒト抗体を対象に投与することにより、治療又は予防する方法を提供するものである。本抗体は単独で投与することも、又は、当該IL-15媒介性疾患を治療又は予防するために本抗体と協働する又は相乗的

に作用する、例えばステロイド系又は非ステロイド系炎症薬などの抗炎症薬や、又はサイトカインなど、別の治療薬と一緒に投与することもできる。

【０１４４】

ある具体的な実施態様では、本発明のヒト抗体を、リウマチ性関節炎(RA)を治療又は予防するために用いる。本抗体は、RAなどの疾患に伴う炎症の進行においてIL-15が果たす役割を制限する。T細胞、特にCD4＋Tヘルパー細胞は、RAにおいて炎症プロセスの開始及び維持に関与している。別のサイトカインであるTNFαもこの炎症経路に関与しており、最終的にはRA患者の関節の破壊及び廃疾を引き起こす。局所でのIL-15合成は、T細胞の活性化及び動員や、TNFα及び他の炎症性サイトカインの誘導の両方で鍵となる役割を果たす。RAの進行におけるIL-15の役割には、マクロファージにより合成されるIL-15がT細胞動員を誘導するプロセスが関与している。活性化したT細胞は次に：（１）マクロファージ活性化を維持し；そして（２）TNFα産生を誘導する。刺激を受けたマクロファージはより多くのIL-15合成及びT細胞活性化を促進し、こうしてこのサイクルが続く。IL-15は、そのTNFα及びマクロファージに対する作用に加え、好中球を活性化して、局所的なB細胞による免疫グロブリン分泌、特にリウマチ因子合成、に影響を与える。

【０１４５】

従って、本発明の抗IL-15抗体は、RAを起こすIL-15の前述の作用を防止又は遮断するために用いることができ、こうしてこの疾患の予防又は治療に用いることができる。例えば本発明の抗IL-15抗体を用いて、RAに関与する炎症を阻害する、及び／又は、活性化白血球の遊走を防ぐことができる。

【０１４６】

本発明のヒト抗体を、メトトレキセートへの応答が乏しかったリウマチ性関節炎患者において構造損傷の進行を阻害したり、DMARDでの治療に失敗しなかった患者も含め、中程度から重度の活動性リウマチ関節炎患者において兆候及び症状を軽減し、構造損傷を遅らせるために、用いてよい。

【０１４７】

さらに本発明のヒト抗体は、IL-15の他の作用を遮断又は阻害するためにも、用いることができる。IL-15は単球及びマクロファージ、線維芽細胞、樹状細胞及びケラチノサイトを含め、多種の細胞及び組織で発現する。ケラチノサイトは、表皮や、粘膜組織の表皮内層の主要な構成要素である。ケラチノサイトの成長の制御は、そのいくつかがケラチノサイト自身によって産生されるサイトカイン及び成長因子の複雑なネットワークによって媒介されている。乾癬性プラークでは、ケラチノサイト由来IL-15がT細胞の蓄積、増殖及び生存に寄与している。ケラチノサイト数が増加し、その結果、関連する疾患症状のうちの少なくともいくつかの原因となる表皮過形成が引き起こされるような数多くの疾患が公知である。これらの疾患の中には、乾癬及びアトピー性皮膚炎などの慢性疾患や、慢性手湿疹、接触性皮膚炎、ウィルス性疣贅（HPV随伴）、皮膚性T細胞リンパ腫などの状態、糖尿病を原因とする創傷治癒不全などの治癒不全、がある。従って、本発明は、このような障害を治療又は予防するのに有効量の本発明のヒト抗IL-15抗体を患者に投与することにより、当該障害を治療又は予防する方法を提供するものである。例えば本発明の抗IL-15抗体を、乾癬における錯角化症を遮断又は阻害したり、乾癬における表皮肥厚を減少させたり、そして乾癬におけるケラチノサイトの増殖を減少させるために、用いることができる。

【０１４８】

IL-15はまた、腸管表皮細胞の機能も変調する（Reinecker, et al. (1996) Gastroenterology 111:1706-13)。具体的には、IL-15は、粘膜表皮細胞上の修飾や、腸管表皮細胞内層の修飾を引き起こすことができ、従って、セリアック病などの炎症性腸疾患の病理発生に関与している。このような疾患でのIL-15の役割は、未処置のセリアック病患者の小腸でのIL-15＋細胞の選択的過剰表現により示されている (WO 00/02582)。このように、IL-15がセリアック病の発症及び維持に直接関与していることが示されている。従って別の実施態様では、本発明の抗IL-15ヒト抗体（即ち、IL-15の炎症誘発作用を阻害するもの）を、

セリアック病を治療又は予防するのに有効量の本抗体を患者に投与することにより、この障害を治療及び／又は予防するために用いることができる。

【０１４９】

加えて、IL-15はさらに、新しい血管の形成という、新血管形成又は脈管形成と呼ばれるプロセスを促進することが、本発明の発明者により見出された。従って、本発明の抗体のさらに別の用途には、血管新生の関与する疾患の予防又は治療がある。これらの疾患には、炎症性疾患に加え、血管新生に依拠する、又は、血管新生を特徴とする多種の癌が含まれる。

【０１５０】

さらに本発明のヒト抗体を、HIVなどの感染性疾患に関係したIL-15の作用を遮断又は阻害するために用いることもできる。従って、本発明の抗体の他の用途には、HIV-1などの感染性疾患の予防又は治療が含まれる。

【０１５１】

例えば、IL-15が媒介する多種の疾患を診断するために、本抗体をin vitro又はin vivoで用いることができる。具体的には、本抗体を用いて、IL-15のレベルや、又は、細胞膜表面上にIL-15を含有する、もしくは、その上の受容体にIL-15を結合させた（受容体結合型ヒトIL-15）細胞のレベルを検出することができる。その後、IL-15のこのようなレベルの検出を特定の疾患の症状と相関付けることができる。代替的には、本抗体を用いて、IL-15機能を阻害又は遮断でき、ひいてはIL-15機能により引き起こされる疾患症状を防止又は寛解させることができる。

【０１５２】

前述したように、本発明のヒト抗IL-15抗体を、全体的な抗炎症作用を高めるために、例えば免疫抑制剤又は抗炎症薬などの一種以上の他の治療薬と同時投与することができる。本抗体を作用物質に（免疫複合体として）連結することも、又は、前記作用物質とは別に投与することもできる。後者（別の投与）の場合、本抗体は、前記作用物質の前でも、後でも、又は同時にも投与することができる。適した治療薬には、とりわけ、抗炎症薬、DMARD(疾患改変抗リウマチ薬)、免疫抑制剤、化学療法薬、及び乾癬薬、がある。さらに本発明に基づくヒト抗体を放射線療法と併用投与することもできる。

【０１５３】

別の実施態様では、本発明のヒト抗体を、例えばCD4特異抗体及びIL-2特異抗体などの他の抗体と組み合わせて投与することができる。本発明の抗体をCD4特異抗体又はIL-2特異抗体と組み合わせると、自己免疫疾患及び移植片拒絶を治療するために特に有用であると考えられる。

【０１５４】

さらに、本発明のヒト抗IL-15抗体と、選択的に使用上の指示とを含むキットも、本発明の範囲内にある。本キットには、さらに、例えば免疫抑制剤などの一種以上の付加的な試薬、又は、一種以上の付加的な本発明のヒト抗体（例えば、IL-15抗原上の、第一のヒト抗体とは異なるエピトープに結合する補完的な活性を有するヒト抗体など）、を含めることができる。

【０１５５】

従って、本発明の抗体で処置した患者に、本ヒト抗体の治療効果を高める又は増大させる、例えば抗炎症薬などの別の治療薬を、（本発明のヒト抗体の投与前、投与と同時、又は投与後に）付加的に投与することができる。

【０１５６】

さらに別の実施態様では、化合物（例えば治療薬、標識、細胞毒、免疫抑制剤等）を本抗体に連結させることで、表面に（膜に結合した、又は、IL-15受容体に結合した）IL-15が結合した状態で有する細胞にこのような化合物を標的指向させるために、本発明のヒト抗体を用いることができる。このように、本発明は、IL-15発現細胞及びIL-15受容体発現細胞をex vivo、in vivo又はin vitroで（例えば放射性同位体、蛍光化合物、酵素、又は酵素コファクタなどの検出可能な標識を用いて）定位する方法も提供する。

【０１５７】
本発明の他の実施態様を以下の実施例の項で解説する。
【０１５８】
本発明を以下の実施例でさらに説明することとするが、以下の実施例をさらに限定的なものと捉えられてはならない。本出願全体を通じて引用された配列表、図面及び全参考文献、特許及び公開済み特許出願の内容を、引用をもってここに援用することを明示しておく。
【０１５９】
実施例
実施例１　Cmu標的化マウスの作製
CMDターゲティング・ベクタの構築
プラスミドpICEmuは、mu遺伝子に延びる、Balb/Cゲノム・ラムダ・ファージディスプレイから得られたマウスIg重鎖遺伝子座のEcoRI/XhoI断片を含有する(Marcu et al. Cell 22: 187, 1980)。このゲノム断片をプラスミドpICEMI9HのXhoI/EcoRI部位にサブクローンした (Marsh et al; Gene 32, 481-485, 1984)。pICEmuに含まれたこれら重鎖配列は、muイントロン・エンハンサのちょうど3'側に位置するEcoRI部位の下流から、mu遺伝子の最後の膜貫通エキソンのほぼ1kb下流に位置するXhoI部位まで延びる。しかし、このmuスイッチ反復領域の大半は、E. coliを通過させて欠失させてある。
【０１６０】
ターゲティング・ベクタは以下の通りに構築された。1.3 kb のHindIII/SmaI 断片をpICEmuから切り出し、HindIII/SmaIで消化したpBluescript（カリフォルニア州ラホーヤ、ストラタジーン社）内にサブクローンした。このpICEmu断片は、Cmu1のほぼ1 kb 5'側に位置するHindIII部位からCmu1内にあるSmaI部位まで延びる。その結果得られたプラスミドをSmaI/SpeIで消化し、pICEmu由来の、Cmu1の3'側のSmaI部位から最後のCmuエキソンのちょうど下流に位置するXbaI部位まで延びる約4 kb のSmaI/XbaI 断片を挿入した。その結果得られたプラスミドpTAR1をSmaI部位で直線化し、neo発現カセットを挿入した。このカセットは、マウスホスホグリセレートキナーゼ（pgk）プロモータ（XbaI/Taq1断片Adra et al. (1987) Gene 60: 65-74）の転写制御下にあると共に、pgkポリアデニレーション部位(PvuII/HindIII 断片；Boer et al. (1990) Biochemical Genetics 28: 299-308)を含有するneo遺伝子から成る。このカセットは、プラスミドpKJ1 (Tybulewicz et al. (1991) Cell 65: 1153-1163に解説がある)から得たが、このプラスミドから前記neoカセットをEcoRI/HindIII断片として切り出し、EcoRI/HindIIIで消化したpGEM-7Zf（＋）内にサブクローンして、pGEM-7 (KJ1)を作製した。このneoカセットを、pGEM-7 (KJ1)からEcoRI/SalI消化により切り出し、平滑末端にしてから、プラスミドpTAR1のSmaI部位にゲノムCmu配列とは反対の方向でサブクローンした。その結果得られたプラスミドをNot Iで直線化し、単純疱疹ウィルスチミジンキナーゼ(tk)カセットを挿入して、Mansour et al. (1988) Nature 336: 348-352が解説した通りに、相同組換え体を持つESクローンを濃縮できるようにした。このカセットは、Tybulewicz et al. (1991) Cell 65: 1153-1163が解説したように、マウスpgkプロモータ及びポリアデニレーション部位を両端に持つtk遺伝子のコーディング配列から成る。その結果得られたCMDターゲティング・ベクタは、前記重鎖遺伝子座に合計でほぼ5.3 kb の相同性を含有し、neo発現カセットが一番目のCmuエキソンの非反復SmaI部位に挿入された変異mu遺伝子を生ずるよう、デザインされている。このターゲティング・ベクタを、ES細胞内に電気穿孔注入する前に、プラスミド配列内で切断するPvuIで直線化した。
【０１６１】
標的設定されたES細胞の作製及び分析
AB-1 ES 細胞 (McMahon, A. P. and Bradley, A., (1990) Cell 62: 1073-1085) を有糸分裂不活性期のSNL76/7 細胞支持細胞層（同書）上で、基本的には解説 (Teratocarcinomas and Embryonic Stem Cells: a Practical Approach (E. J. Robertson, ed.) Oxford: IRL Press, p. 71-112のRobertson, E. J. (1987))された通りに成長させた。直線化さ

せたCMDターゲティング・ベクタを、電気穿孔法でAB-1細胞に、Hasty et al. (Hasty, P. R. et al. (1991) Nature 350: 243-246)で解説された方法により注入した。注入後の細胞を100 mm 皿に、1-2×10^6細胞／皿の密度になるようにプレートした。２４時間後、G418 (200マイクログラム／mlの活性成分）及び FIAU (5×10^{-7}M) を培地に加え、薬物耐性クローンを８乃至９日間、展開させた。クローンを摘みだし、トリプシン処理し、２つの部分に分割し、さらに展開させた。その後各クローン由来の細胞の半分を凍結させ、残りの半分を、ベクタと標的配列との間の相同組換えについて分析した。

【０１６２】

DNA解析をサザン・ブロット・ハイブリダイゼーションで行った。DNAはLaird et al. (Laird, P. W. et al., (1991) Nucleic Acids Res. 19 : 4293)が解説した通りに前記クローンから単離した。単離されたゲノムDNAをSpeIで消化し、muイントロン・エンハンサとmuスイッチ領域との間の配列にハイブリダイズするプローブA（図１）である915 bp のSacI 断片でプローブした。プローブＡは、野生型遺伝子座の9.9 kbのSpeI断片と、CMDターゲティング・ベクタと相同組換えを起こしたmu遺伝子座の判断材料となる7.6kbのバンドとを検出する（neo発現カセットはSpeI部位を含有する)。サザン・ブロット解析でスクリーニングした1132 個のG418及びFIAU 耐性クローンのうち、3個が、mu遺伝子座での相同組換えを示す7.6 kb のSpeI バンドを示した。これら3個のクローンを酵素BglI、BstXI、及びEcoRI でさらに消化して、当該ベクタがmu遺伝子に相同的に組み込まれたことを確認した。プローブＡとハイブリダイズした場合、BglI、BstXI、又はEcoRI で消化した野生型DNAのサザン・ブロットでは、それぞれ15.7、7.3、及び12.5 kbの断片が生ずるが、標的にしたmuアレルの存在は、それぞれ7.7、6.6、及び14.3 kbの断片で示される。SpeI消化により検出された３個の陽性クローンはすべて、neoカセットがCmu1エキソンへ挿入されたことの判断材料となる、予想通りのBglI、BstXI、及びEcoRI制限断片を示した。

【０１６３】

変異mu遺伝子を持つマウスの作製

264番、272番及び408番と指定した３つの標的設定されたESクローンを解凍し、C57BL/6J胚盤胞にBradley (Teratocarcinomas and Embryonic Stem Cells: a Practical Approach. (E. J. Robertson, ed.) Oxford: IRL Press, p. 113-151のBradley, A. (1987))の解説通りに注入した。注入された胚盤胞を偽妊娠メスの子宮に移して、注入されたES細胞及びホストの胚盤胞を由来とする細胞の混合物であるキメラマウスを作製した。このキメラへのES細胞の寄与度は、黒色のC57BL/6Jバックグラウンド上に見える、ES細胞系由来の野鼠色の皮の量により視覚的に判断できる。クローン272及び408では、ごく低いパーセンテージでキメラが生じた（即ち、野鼠色の着色率が低い）が、クローン264では、高率でオスのキメラが生じた。これらのキメラをC57BL/6Jメスと交配し、ES細胞ゲノムの生殖細胞伝播を示す野鼠色の仔を作った。尾の生検で得たDNAをBgII消化し、サザン・ブロット分析して、標的設定されたmu遺伝子のスクリーニングを行った（ES細胞DNAの分析について上述した通り）。野鼠色の仔のほぼ５０％が、野生型のバンド15.7kbに加え、ハイブリダイズした7.7kbのBgIIバンドを示し、標的設定されたmu遺伝子の生殖細胞伝播が実証された。

【０１６４】

mu遺伝子の機能不活性化に関するトランスジェニックマウスの分析

neoカセットをCmu1に挿入したことでIg重鎖遺伝子が不活性化したかどうかを調べるために、クローン264キメラを、JH遺伝子セグメントを欠失させて重鎖発現を不活性化させるJHD変異がホモ接合型となったマウスと交配した(Chen et al, (1993) Immunol. 5: 647-656)。４匹の野鼠色の仔を得た。血清を１月齢のこれら動物から得、ELISAで検定してマウスIgMの存在を調べた。４匹の仔のうち２匹がIgMを完全に欠いていた（表１を参照されたい）。４匹の動物を、尾の生検で得たDNAをBgII消化し、プローブＡ（図１を参照されたい）にハイブリダイズさせ、さらにStuI消化し、475bpのEcoRI/StuI断片（同書）にハイブリダイズさせる、といったサザン・ブロット分析で遺伝子型決定したところ、血清IgMを発現できない当該動物は、重鎖遺伝子座の一方のアレルがJHD変異を持ち、他方のアレ

ルがCmu1変異を持つものであることが実証された。JHD変異がヘテロ接合型となったマウスは野生型のレベルの血清Igを示す。これらのデータは、Cmu1変異はmu遺伝子の発現を不活性化することを実証するものである。

【０１６５】

【表１】

マウス	血清IgM (マイクログラム/ml)	Ig H鎖遺伝子型
42	<0 002	CMD/JHD
43	196	+/JHD
44	<0 002	CMD/JHD
45	174	+/JHD
129 x BL6 F1	153	+/+
JHD	<0 002	JHD/JHD

【０１６６】

表１は、CMD及びJHD 変異の両方を持つマウス (CMD/JHD)、JHD変異についてヘテロ接合型のマウス (+/JHD)、野生型 (129Sv ×C57BL/6J)F1 マウス(+/+)、及び、JHD変異がホモ接合型のB細胞欠損マウス(JHD/JHD)について、ELISAで検出された血清IgMレベルを示す。

【０１６７】

実施例２　HCO12トランスジェニックマウスの作製

HCO12ヒト重鎖導入遺伝子

80 kb のpHC2 のインサート(Taylor et al., 1994, Int. Immunol., 6: 579-591)及び25 kbのpVx6のインサートを同時注入することで、HCO12導入遺伝子を作製した。プラスミドpVx6 を以下に解説するように構築した。

【０１６８】

生殖細胞ヒトV_H1-18 (DP-14)遺伝子を、ほぼ2.5 kb の5' 側フランキング及び5 kb の3' 側フランキングゲノム配列と一緒に含む、8.5 kb のHindIII/SalI DNA 断片をプラスミド・ベクタpSP72 (ウィスコンシン州マジソン、プロメガ社）内にサブクローンして、プラスミドp343.7.16を作製した。生殖細胞ヒトVH5-51 (DP-73)遺伝子を、ほぼ5 kbの5' 側フランキング及び1 kb の3' 側フランキングゲノム配列と一緒に含む、7 kbのBamHI/HindIII DNA断片を、pBR322ベースのプラスミド・クローニング・ベクタpGP1f (Taylor et al. 1992, Nucleic Acids Res. 20: 6287-6295)内にクローンして、プラスミドp251fを作製した。pGP1f、pGP1k (配列番号:13)を由来とする新しいクローニング・ベクタを、EcoRV/BamHIで消化し、生殖細胞ヒトVH3-23 (DP47)遺伝子をほぼ4 kbの5' 側フランキング及び5 kb の3' 側フランキングゲノム配列と一緒に含む、10 kbのEcoRV/BamHI DNA 断片に連結した。その結果得られたプラスミドp112.2RR.7をBamHI/SalI で消化し、p251fの7 kbの精製済みBamHI/SalI インサートに連結した。その結果得られたプラスミドpVx4をXhoIで消化し、p343.7.16の8.5 kbの XhoI/SalI インサートに連結した。

【０１６９】

V_H1-18遺伝子を他の２つのV遺伝子と同じ方向で持つクローンを得た。このクローンをpVx6と命名した後、NotIで消化し、精製されたその26 kbのインサートを、pHC2の精製済み80 kbのNotIインサートと一緒に、1:1のモル比で、Hogan et al. (B. Hogan et al., Manipulating the Mouse Embryo, A Laboratory Manual, 2nd edition, 1994, Cold Spring Harbor Laboratory Press, Plainview NY)が解説したように半日齢(C57BL/6J x DBA/2J)F2 胚の前核に同時注入した。これらの注入を受けた胚から発生したマウスから、V×6及びHC2の両方を由来とする配列を含むトランスジェニックマウスの３つの個別の株を確立した。これらの株を(HCO12)14881、(HCO12)15083、及び(HCO12)15087と命名する。次にこれらの３つの株のそれぞれを、実施例１で解説したCMD変異、JKD 変異 (Chen et al. 1993, EMBO J. 12: 811-820)、及び(KCo5) 9272 導入遺伝子(Fishwild et al. 1996, Nature Biotechnology 14: 845-851)を含むマウスに交配した。その結果得られるマウスは、ヒト重鎖及びカッパ軽鎖導入遺伝子を、内因性マウス重鎖及びカッパ軽鎖遺伝子座の破壊につい

てホモ接合型であることをバックグラウンドとして発現する。

【０１７０】

実施例３　IL-15に対するヒトモノクローナル抗体の作製

上述の通りに作製され、米国カリフォルニア州サンホセのメダレックス社より提供されたHCo12 及び HCo7 トランスジェニックマウスを、完全フロイント・アジュバント（CFA、米国ミシガン州デトロイト、ディフコ・ラボラトリーズ社、ロット番号121024LA）又は不完全フロイント・アジュバント（ICFA、ディフコ社、ロット番号121195LA）を添加したヒト組換えIL-15 (hIL-15、米国、シアトル、イムネックス社）で皮下的（SC）腹腔内（IP)又は静脈内（IV)により免疫した。いくつかの場合では、KLHに結合させたhIL-15を免疫処置に用いた。完全もしくは不完全フロイント・アジュバントを添加したhIL-15で数回、追加し激した後、マウスの血清を、IL-15に対するヒト抗体の存在について検査した。

【０１７１】

最終的なクローン146B7、146H5、404E4及び404A8を生じたトランスジェニック・マウスの免疫処置スキーム

マウス番号 146 (HCo12)、ID 995-146、メス

170699 SC 12μg hIL-15 のCFA (ディフコ社、ロット番号121024LA)溶液

010799 SC 12μg hIL-15 のICFA (ディフコ社、ロット番号121195LA)溶液

150799 SC 12μg hIL-15 のICFA溶液

020899 SC 12μg hIL-15-KLH のICFA溶液

070999 SC 12μg hIL-15-KLH のICFA溶液

280999 SC 12μg hIL-15-KLH のCFA溶液

111099 IV 30μg hIL-15 のPBS溶液

121099 IV 30μg hIL-15 のPBS溶液

151099 このマウスのリンパ節及び脾細胞のSP2/0との融合

【０１７２】

マウス番号404 (HCo7)、ID 997-404、メス

201099 IP 25μg hIL-15-KLHのCFA (ディフコ社、ロット番号121024LA)

031199 IP 12.5μg hIL-15、12.5μg hIL-15-KLH、25μgのICFA溶液 (ディフコ 社、ロット番号121195LA)

101199 IV 12.5μg hIL-15、12.5μg hIL-15-KLH

121199 IV 12.5μg hIL-15、 12.5μg hIL-15-KLH

191199 このマウスのリンパ節及び脾細胞のSP2/0との融合

【０１７３】

培地

融合相手の培地（FPM）：

イスコーブの改変ダルベッコ培地に、100 IU/ml ペニシリン、100μg/ml ストレプトマイシン、1 mM ピルビン酸ナトリウム、0.5 mM β-メルカプトエタノール（スコットランド、ペーズリー、ライフ・テクノロジーズ社）及び10% 熱不活化ウシ胎児血清（米国ユタ州、ハイクローン社）を添加した。

【０１７４】

融合選択培地（FSM）：

30 ml オリゲン・ハイブリドーマ・クローニング・ファクター（米国メリーランド州、ガイザーズバーグ、アイジェン社）、HAT （1バイアル、メーカの推奨濃度、米国ミズーリ州セントルイス、シグマ・ケミカル社）及び0.5 mg/ml カナマイシン（スコットランド、ペーズリー、ライフ・テクノロジーズ社）を添加したFPM。

【０１７５】

融合クローニング培地（FCM）：

20 ml オリゲン・ハイブリドーマ・クローニング・ファクター（米国メリーランド州、ガイザーズバーグ、アイジェン社）、HAT （1バイアル、メーカの推奨濃度、米国ミズーリ州セントルイス、シグマ・ケミカル社）及び0.5 mg/ml カナマイシン（スコットランド、

ペーズリー、ライフ・テクノロジーズ社）を添加した培地。

【０１７６】

ハイブリドーマの調製：脾細胞及びリンパ節細胞のSP2/0 骨髄腫細胞との融合

ハイブリドーマを得るために、脾臓、鼠径及び大動脈周囲リンパ節をマウスから摘出した。脾臓及びリンパ節細胞の単個細胞懸濁液をSP2/0骨髄腫細胞に細胞比１：２で混合した。細胞を遠心分離で沈降させ、そのペレットを３７℃の１ml ポリエチレングリコール（PBSによる50% w/v溶液、英国アーヴィン、シグマ－アルドリッチ社）中に静かに再懸濁させた。細胞を６０秒間、渦流させた後、25 ml FPM-2 を加え、細胞を３７℃で３０乃至６０分間、インキュベートした。インキュベート後、細胞を９６ウェル・プレートでFSMに入れて1ウェル当たり（100μl中）0,75×10^5細胞　の細胞密度で培養した。３日後、100μl FSM を各ウェルに加えた。

【０１７７】

hIL-15で免疫したHCo7及びHCo12マウスの脾臓及びリンパ節を融合させたところ、IL-15を指向する抗体を産生するハイブリドーマがいくつか、生じた。完全ヒト抗IL-15抗体を産生する以下の４つの安定なクローンを単離した：（１）146LyD7F7B7改名後：146B7;（２）146DE2E12A3H5 改名後：146H5;(3) 404CG11B7E4 改名後：404E4；及び（４）404FB12E7A8 改名後：404A8。これらのクローンは、ヒトIgG1/kサブクラスのすべてだった。

【０１７８】

ハイブリドーマのスクリーニング

融合後７日目乃至１１日目の間にウェルをヒト抗体の存在について以下のELISAを用いてスクリーニングした：

【０１７９】

培養上清中のヒトIgGの存在をスクリーニングするためのELISA

ヒトIgG抗体の存在を検出するELISAを行うために、100μl/ウェルの0.9μg/ml ウサギ-α-k-軽鎖抗体（デンマーク、グロストラップ、ダコ社）をリン酸緩衝生理食塩水（PBS）に入れてNunc Maxisorp ELISA-プレートに加えた（インキュベーションは室温で一晩）。ニワトリ血清（2%；スコットランド、ペーズリー、ライフ・テクノロジーズ社）及びTween-20 (0.05 %；PBSTC)を添加したPBSでこのプレートを遮断後、培養上清を加えた。１．５時間のインキュベート後、プレートを洗浄し、PBSTCで希釈した0.5μg/mlの西洋わさびペルオキシダーゼ（デンマーク、グロストラップ、ダコ社）に結合させたウサギ-α-ヒトIgG (Fab2-フラグメント）を加えた。１時間のインキュベート後、ウェルを洗浄し、基質であるABTS (2,2'-アジノビス-3-エチルベンズチアゾリン-スルホン酸、ドイツ、マンハイム、ロシュ・ダイアグノスティックス社）をメーカのプロトコル通りに加え、抗体結合を405nmで、EL808 ELISAリーダ（米国ヴァーモント州、ウィヌースキ、バイオ－テック・インスツルメンツ社）で評価した。

【０１８０】

IL-15特異抗体の存在をスクリーニングするためのELISA

ヒトIgG/k抗体を含有するウェルを、さらにヒト抗IL-15抗体の存在について、IL-15特異的ELISAで検査した。このELISAを行うために、100μl/ウェルの1μg/ml IL-15 をリン酸緩衝生理食塩水(PBS)に入れてNunc Maxisorp ELISAプレート（室温で一晩、インキュベート）に加えた。ニワトリ血清（2%；スコットランド、ペーズリー、ライフ・テクノロジーズ社）及びTween-20 (0.05 %；PBSTC)を添加したPBSでこのプレートを遮断後、培養上清を加えた。１．５時間のインキュベート後、プレートを洗浄し、PBSTCで1/5000に希釈した西洋わさびペルオキシダーゼ（米国ペンシルヴァニア州、ウェスト・グローブ、ジャクソン・イムノ・リサーチ社）に結合させたα-ヒトIgG Fc を加えた。１時間のインキュベート後、ウェルを洗浄し、基質であるABTS (2,2'-アジノビス-3-エチルベンズチアゾリン－スルホン酸、ドイツ、マンハイム、ロシュ・ダイアグノスティックス社）をメーカのプロトコル通りに加え、抗体結合を405nmで、EL808 ELISAリーダ（米国ヴァーモント州、ウィヌースキ、バイオ－テック・インスツルメンツ社）で評価した。

【０１８１】

ハイブリドーマのサブクローニング

安定な抗IL-15細胞株を得るために、前記ハイブリドーマを細胞の（0.5細胞／ウェルまでの）限界希釈により、９６ウェル・プレートでサブクローンした。

【０１８２】

これらのサブクローンを、ほぼ１０日後に上述のIL-15 ELISAで検査した。いくつかのサブクローニング法ではFSMをFCMからFPMに相変化させた。サブクローンのアイソタイプは、以下に解説するELISAで判定された。

【０１８３】

ELISAによる抗IL-15抗体のアイソタイプ決定

アイソタイプELISAを行うために、100μl/ウェルの1μg/ml 抗ヒトFc（ジャクソン・イムノ・リサーチ社）をリン酸緩衝生理食塩水(PBS)に入れてNunc Maxisorp ELISAプレート（室温で一晩、インキュベート）に加えた。ニワトリ血清（2%；スコットランド、ペーズリー、ライフ・テクノロジーズ社）及びTween-20 (0.05 %；PBSTC)を添加したPBSでこのプレートを遮断後、培養上清を加えた。１．５時間のインキュベート後、プレートを洗浄し、アルカリホスファターゼ（ランド、プラーツ、ザイメッド社）に結合させたマウス-α-HuIgG1又は、西洋わさびペルオキシダーゼ（ザイメッド社）に結合させたマウス-α-HuIgG3を加えた。１時間のインキュベート後、ウェルを洗浄し、基質であるABTS (2,2'-アジノビス-3-エチルベンズチアゾリン－スルホン酸、ドイツ、マンハイム、ロシュ・ダイアグノスティックス社）をメーカのプロトコル通りに加えた。抗体結合を405nmで、EL808 ELISAリーダ（米国ヴァーモント州、ウィヌースキ、バイオ－テック・インスツルメンツ社）で評価した。

【０１８４】

実施例４　完全ヒト抗IL-15抗体のエピトープ特異性

治療的に機能し、そしてIL-15誘導性炎症誘発作用を阻害するためには、IL-15特異抗体は、IL-15受容体のIL-2Rβ鎖及び／又はγ鎖との相互作用に関与するIL-15エピトープを認識する必要がある。

【０１８５】

（Pettit et al.が解説した）変異タンパク質を用いて、 完全ヒト抗IL-15抗体、146B7、146H5、404A8及び404E4のエピトープ特異性を評価した。用いたIL-15変異体には、IL-15変異体Q108S（残基108位のGlnがSerに置換されたもの；γ鎖相互作用部位での変異）及び変異体D8SQ108S（残基108位のGlnをSerに、そして８位のAspをSerに置換したもの；IL-15のβ鎖及びγ鎖相互作用部位の両方での変異）が含まれていた。

【０１８６】

hIL-15及び変異型IL-15タンパク質に対するhIL-15特異抗体146B7、147H5、404A8及び404E4の結合を判定するためのELISA

このELISAを行うために、100μlの1μg/ml IL-15 又はhIL-15 変異型タンパク質のリン酸緩衝生理食塩水(PBS)をNunc Maxisorp ELISAプレートに加えて被覆した。ニワトリ血清（2%；スコットランド、ペーズリー、ライフ・テクノロジーズ社）及びTween-20 (0.05 %；PBSTC)を添加したPBSでこのプレートを遮断後、hIL-15特異抗体の連続希釈液をインキュベートした。洗浄後、PBSTCで1/5000に希釈したペルオキシダーゼ（米国ペンシルヴァニア州、ウェスト・グローブ、ジャクソン・イムノ・リサーチ社）に結合させたα-ヒトIgG Fcを加えた。洗浄後、基質であるABTS (2,2'-アジノビス-3-エチルベンズチアゾリン－スルホン酸、ドイツ、マンハイム、ロシュ・ダイアグノスティックス社）をメーカのプロトコルに従って加え、抗体結合を405 nmでEL808 ELISAリーダ（米国ヴァーモント州、ウィヌースキ、バイオ－テック・インスツルメンツ社）で評価した。

【０１８７】

完全ヒトIL-15特異抗体 146B7、146H5、404A8及び404E4 のhIL-15 並びにIL-15変異型タンパク質Q108S 及びD8SQ108Sへの結合を図１に示す。146B7又は146H5のいずれも、これらの変異型IL-15タンパク質に結合できなかった。両方の変異型ともQ108S変異を持つため、146B7及び146H5により認識されるエピトープは、IL-15のうちで、IL-15受容体のγ鎖と相

互作用する重要なドメイン内にある。404A8及び404E4は両者とも、これら変異型タンパク質に結合できたため、これらの抗体は、IL-15のβ鎖及びγ鎖相互作用ドメイン外にあるエピトープを認識するものである。146B7及び146H5は両者とも、IL-15に、IL-15受容体のγ鎖と相互作用する領域で結合する。このことは、本発明の完全ヒト抗IL-15抗体を用いた増殖検定で得たデータと一致する。以下に詳述するように、404A8 又は404E4 のいずれも、CTLL-2細胞及びヒトPBMCのIL-15誘導性増殖を阻害できなかった。146B7 及び146H5は両者とも、 IL-15誘導性増殖を阻害できた。さらに、増殖の阻害は、IL-15と、IL-15受容体のγサブユニットとの相互作用を遮断することにより、達成される。

【０１８８】

実施例５　146B7のV_H及びV_L領域の配列

146B7の再編成のあるV_H及びV_Lドメインのヌクレオチド配列及び推定アミノ酸配列を以下の手法を用いて決定した。これらの配列は、用いるV_H及びV_L生殖細胞系ファミリーに関する情報を提供する； これらの生殖細胞配列中の点変異は、動物の免疫処置の際のB細胞の親和性成熟が原因である。

【０１８９】

RNAの調製

総RNAを5×10^6個の146B7ハイブリドーマ細胞から、RNAzol（英国プール、バイオジェネシス社）を用いてメーカのプロトコルに従って調製した。

【０１９０】

cDNAの調製

146B7由来のRNAのcDNAを、3μgの総RNAから、緩衝剤（ドイツ、マンハイム、ロシュ・ダイアグノスティックス社）、オリゴ$d(T)_{15}$（米国ウィスコンシン州、マジソン、プロメガ社）、dNTP（米国、ベーリンガー・マンハイム社）及びRNAsin（プロメガ社）を加えたAMVリバース・トランスクリプターゼを用いてメーカのプロトコルに従って調製した。

【０１９１】

クローニング用のV_H及びV_L領域を増幅するために用いたPCRプライマ

用いたプライマ対：

V_H：

FR1 5' プライマ

(1) AB62 CAg gTK CAg CTg gTg CAg TC

(2) AB63 SAg gTg CAg CTg KTg gAg TC

(3) AB65 gAg gTg CAg CTg gTg CAg TC

V_Hリーダ5'プライマ

(4) AB85 ATg gAC Tgg ACC Tgg AgC ATC

(5) AB86 ATg gAA TTg ggg CTg AgC Tg

(6) AB87 ATg gAg TTT ggR CTg AgC Tg

(7) AB88 ATg AAA CAC CTg Tgg TTC TTC

(8) AB89 ATg ggg TCA ACC gCC ATC CT

V_H3'プライマ

(9) AB90 TgC CAg ggg gAA gAC CgA Tgg

V_K：

FR1 5'プライマ

(1) AB8 RAC ATC CAg ATg AYC CAg TC

(2) AB9 gYC ATC YRg ATg ACC CAg TC

(3) AB10 gAT ATT gTg ATg ACC CAg AC

(4) AB11 gAA ATT gTg TTg ACR CAg TC

(5) AB12 gAA ATW gTR ATg ACA CAg TC

(6) AB13 gAT gTT gTg ATg ACA CAG TC

(7) AB14 gAA ATT gTg CTg ACT CAg TC

V_Kリーダ5'プライマ：

(8) AB123 CCC gCT Cag CTC CTg ggg CTC CTg
(9) AB124 CCC TgC TCA gCT CCT ggg gCT gC
(10) AB125 CCC AgC gCA gCT TCT CTT CCT CCT gC
(11) AB126 ATg gAA CCA Tgg AAg CCC CAg CAC AgC
V_K3'プライマ
(12) AB16 Cgg gAA gAT gAA gAC AgA Tg

【０１９２】

クローニング用のV_H及びV_L領域を増幅するために用いたPCR条件

PCR反応を、AmpliTaq ポリメラーゼ（パーキン・エルマー社）をGeneAmp PCRシステム9700 (米国カリフォルニア州、フォスター・シティ、パーキン・エルマー・アプライド・バイオシステムズ社)で用いて行った。

【０１９３】

PCRサイクル・プロトコル：
94° 2'
11サイクル94° 30"
１サイクル毎 65° 30"にマイナス 1°
72° 30"
30 サイクル94° 30"
55° 30"
72° 30"
72° 10"
4°まで冷却

【０１９４】

pGEMT-ベクタ系IでのV_H及びV_Lのクローニング

アガロース・ゲルでPCR産物を解析後、この産物を S-400 もしくは S300マイクロスピン・カラム (米国ニュージャージー州ピスカタウェイ、アマーシャム・ファルマシア・バイオテック社)か、又はQIAEX II ゲル・エクストラクション・キット（ドイツ、ヒルデン、キアゲン社）で精製した。各実験につき、各V_H及びV_L領域のうちで、FR1又はリーダ・プライマを用いて２つの個別に増幅されたPCR産物を、メーカのプロトコルに従ってpGEMT-ベクタ系（プロメガ社）でクローンした。

【０１９５】

E. coli DH5αへの形質転換後、個々のコロニを、T7 及びSP6プライマを用いたコロニPCRにより、55℃で30サイクル通してスクリーニングした。各個別のコロニから採ったプラスミドDNAをQiaprepスピン・ミニプレップ・キット（キアゲン社）を用いて精製した。さらに分析するために、Nco1/Not1 (英国NEバイオ・ラブズ社及びロシュ・ダイアグノスティックス社）消化を行い、アガロース・ゲルで分析した。

【０１９６】

配列決定

V領域は、pGEMTベクタ系Iでクローニングした後に配列決定された。T7及びSp6プライマ（ベルギー、リュイック、ユーロジェンテック社）を、配列決定キット：ABIプリズムBigDyeターミネータ・サイクル・シーケンシング・レディ・リアクション・キット（英国ウォリントン、アプライド・バイオシステムズ社）とプロトコルに従って組み合わせて用いた。反応をABI PRISM 377 シーケンサ(PEアプライド・バイオシステムズ社）で行わせ、配列をプログラムDNAStar, SeqmanIIで解析した。次にこの配列を生殖細胞V遺伝子配列にVBASE (www.mrc-cpe.cam.ac.uk/imt-doc/public/intro.htm)でアライメントした。

【０１９７】

146B7のV_H及びV_L領域のクローニング及び配列決定

ハイブリドーマ146B7由来のV_H及びV_L領域をPCRで増幅し、pGEMTベクタ系Iでクローンして、そのcDNA配列を決定した。当該ヌクレオチド配列及び対応するアミノ酸配列を、それぞれ図２ (配列番号: 1 及び2) 及び図３(配列番号: 3 及び4)に示す。フレームワーク(FR)

及び相補性決定領域(CDR)も示す。Vbaseでのアライメントに基づく146B7のV_H領域の生殖細胞ファミリー：V_H5-51 (V_H5-サブグループ)、D2-15/D2 (D_H-セグメント）、JH4b (J_H-セグメント）。Vbaseでのアライメントに基づく146B7のV_L領域の生殖細胞ファミリー： A27 (V_KIII-サブグループ）及びJ_K2 (J_K-セグメント)。V_H及びV_Lドメインに関する更なる情報は、Kabatデータベース http://immuno.bme.nwu.edu/ 又はhttp://www.Vbase.comに見られる。

【０１９８】

実施例６　146B7の親和結合特性

146B7の親和性を表面プラスモン共鳴(SPR)技術により、BIACORE 3000 装置を用いて解析して、以下の手法に従って生体分子タンパク質間相互作用を調べた。生体分子結合により起きる表面層上でのSPRシグナルの変化を検出し、表面層での質量密度の変化とする。親和性は以下の定義を用いて表現されている：k_a=結合速度定数 ($M^{-1}sec^{-1}$）；k_d=解離速度定数（sec^{-1}）；K_A=結合平衡定数=k_a/k_d（M^{-1}）；及びK_D=解離平衡定数=k_d/k_a (M)。

【０１９９】

ヒトIL-15（hIL-15）に対する146B7の親和性を得るために異なる手法を行った。２つの異なる提供業者（米国シアトル、イムネックス社及び米国ニュージャージー州、ロッキーヒル、ペプロテック社）から得たヒト組換えIL-15をCM5センサ・チップに結合させた。センサ・チップに結合させたこの化合物をリガンドと定義する。他の実験では146B7をリガンドとして用いた。

【０２００】

各動態解析において、センサチップに結合させたリガンドに適合させた解析物146B7又はhIL-15の結合を、基準コントロールCM5センサ・チップへの結合に比較した。解析物の連続希釈液を検査した(0、3.125、6.25、12.5、25、50μg/ml)。結合曲線及び解離曲線を、ラングミュアのモデル1:1で単量体での相互作用に合わせて、k_a及びk_dを決定し、K_A及びK_Dを計算した。データはすべて、BIAエバリュエーション、バージョン3.1を用いて解析した。二価の相互作用に関しては、モデル「二価解析物」を用いた。解析はすべて、基線の変動分について補正した。

【０２０１】

146B7の抗体親和性を判定するために、抗体146B7の親和性を、イムネックス社及びペプロテック社という２つの異なる提供業者から得たヒト組換えIL-15について、BIACORE 3000で測定した。146B7をリガンドとして、そしてhIL-15を分析物として用いて、一価相互作用を判定した（曲線はラングミュア1:1で調整）。

【０２０２】

IL-15（イムネックス社製）に対する146B7の親和性を以下の通りに測定した：

結合速度定数k_a： $1.07 (\pm 0.17) \times 10^5 M^{-1} sec^{-1}$

解離速度定数k_d： $6.56 (\pm 0.09) \times 10^{-3} sec^{-1}$

結合平衡定数K_A： $1.55 (\pm 0.21) \times 10^7 M^{-1}$

解離平衡定数K_D： $6.59 (\pm 0.88) \times 10^{-8} M$

【０２０３】

146B7の結合力を調べるために、IL-15（イムネックス社製）をリガンドとして用い、そして146B7 を分析物として用いた。得られたデータを、抗体の二価相互作用を調整するラングミュア（1:1）曲線を用いて表現して分析する場合、抗体の結合力を判定した。

【０２０４】

146B7のIL-15（イムネックス社製）に対する結合力を以下の通りに測定した：

結合速度定数k_a： $7.30 (\pm 0.81) \times 10^5 M^{-1} sec^{-1}$

解離速度定数k_d： $1.45 (\pm 2.05) \times 10^{-3} sec^{-1}$

結合平衡定数K_A： $5.03 (\pm 3.40) \times 10^8 M^{-1}$

解離平衡定数K_D： $1.55 (\pm 1.24) \times 10^{-9} M$

【０２０５】

ペプロテック社製IL-15に対する146B7の親和性及び結合力も判定した。異なる供給源のIL

-15間で、親和性にも、又は結合力にも大きな違いは何ら見られなかった。

【０２０６】

CTLL-2細胞及びPBMCのヒトインターロイキン-15（hIL-15）誘導性増殖の、完全ヒト抗IL-15抗体による阻害に関する下記の実施例で解説するように、［^{3}H］-チミジン取り込みで測定したところ、146B7 は用量依存的にIL-15誘導性増殖を阻害した。50%の阻害があったときの濃度であるIC50という、親和性を調べるためのより機能的な方法である数値を、これらの増殖阻害実験で計算したところ、3.1±0.91 nMだった。このIC50は、BIACORE 3000（K_D 1.5 nM)で、146B7をリガンドとして用い、そして組換えヒトIL-15を分析物として用いて測定された結合力と一致し、ここで得られた親和性及び結合力の測定値の裏付けとなった。

【０２０７】

実施例７　hIL-15誘導性TNF-α産生の、完全ヒト抗IL-15抗体による阻害

完全ヒト抗IL-15抗体146B7、146H5、404E4及び404A8がIL-15誘導性TNF-α産生に及ぼす作用を、以下の手法を用い、健康なボランティアから採った末梢血由来単核細胞（PBMC）を用いて研究した。IL-15に対する特異性を評価するために、これらの抗体がIL-2媒介性TNF-α産生に及ぼす作用も調べた。

【０２０８】

細胞培養

培養株は、2 mM L-グルタミン、100 IU/ml ペニシリン、100μg/ml ストレプトマイシン（すべてスコットランド、ペーズリー、ライフ・テクノロジーズ社から得た）及び10% 熱不活化ウシ胎児血清（米国ユタ州ハイクローン社）を加えたRPMI-1640中に維持された。

【０２０９】

末梢血単核細胞（PBMC）の精製

新鮮なヒト血液を健康なボランティアからインフォームド・コンセント後に採血し、ヘパリンを加えて凝固を防いだ。PBMCの精製をフィッコール（スウェーデン、ウプサラ、ファルマシア社）を用いた密度勾配遠心分離により行った。

【０２１０】

テスト化合物

HIL-15、ロット番号：6870-011、米国ワシントン州、シアトル、イムネックス社。 hIL-2、オランダ、アムステルダム、チロン・ベネルクス社。

用いた完全ヒト抗体：146B7（バッチ：070101）及び146B7RDJW07、404A8（バッチ：030101）及び404E4（バッチ：080101）、そしてアイソタイプ・コントロールとして抗体T1（97-2B11-2B12、バッチ：190900)。

【０２１１】

PBMCによるヒトIL-15（hIL-15)もしくはhIL-2誘導性TNF-α産生の抗IL-15抗体による阻害

PBMCを三重又は四重にして９６ウェルの平底プレートで、１ウェル当たり1.5×10^{5}個の細胞にして、hIL-2又はhIL-15の存在下もしくは非存在下、そして抗IL-15抗体を加えて、又は加えずに、培養した。 アイソタイプ・コントロール抗体(T1)を陰性コントロールとして含めた。コンカナバリンＡ（2.5μg/ml、カルビオケム社）を増殖に関する陽性コントロールとして加えた。細胞を７２時間、３７℃で5% のCO_2下でインキュベートした。上清を採集して、ヒトTNF-αの量をELISA（オランダ、ユトレヒト、U-サイテック社）で定量した。

【０２１２】

146B7及びアイソタイプ・コントロール抗体が、PBMCによるIL-15媒介性TNF-α産生に及ぼす作用を検査した。146B7はhIL-15媒介性TNF-α産生を用量依存的に阻害したが、他方、当該アイソタイプ・コントロール抗体はhIL-15誘導性TNF-α産生を阻害しなかった（図６）。二人の健康なボランティアのデータを示す。404E4 及び404A8はhIL-15誘導性TNF-α産生を阻害できなかった。

【０２１３】

抗IL-15抗体の特異性を確認するために、それらがhIL-2媒介性TNF-α産生に及ぼす作用を

評価した。IL-2媒介性TNF-α産生の阻害は、146B7によっては誘導されなかった（図７）。hIL-2媒介性TNF-α産生において、404E4 又は404A8のいずれでも、用量依存的な阻害は見られなかった。

【０２１４】

hIL-15媒介性TNF-α産生の用量依存的な阻害は、146B7によってのみ、見られ、404E4及び404A8では見られなかった。この阻害作用はhIL-15に特異的だった。IL-2媒介性TNF-α産生は阻害されなかった。

【０２１５】

実施例８　CTLL細胞及びPBMCのヒトインターロイキン-15 (hIL-15)誘導性増殖の、完全ヒト抗IL-15抗体による阻害

抗体146B7、146H5、404E4及び404A8を、それらのT細胞増殖阻害能について、CTLL-2細胞(Gillis et al., 1978)及び末梢血単核細胞(PBMC) を用い、以下の手法を用いて検査した。

【０２１６】

細胞培養

培養株は、2 mM L-グルタミン、100 IU/ml ペニシリン、100μg/ml ストレプトマイシン（スコットランド、ペーズリー、ライフ・テクノロジーズ社から得たもの）及び10% 熱不活化ウシ胎児血清（米国ユタ州、ハイクローン社）を加えたRPMI-1640中に維持された。CTLL-2細胞(Gillis et al., 1978) は、36 単位 hIL-2/ml (オランダ、アムステルダム、チロン・ベネルクス社）を添加した上述の培地中に維持し、３乃至４日間、hIL-2を枯渇させてから、実験を開始した。CTLL-2細胞は使用前に三回、洗浄した。

【０２１７】

末梢血単核細胞（PBMC）の精製

新鮮なヒト血液を健康なボランティアからインフォームド・コンセント後に採血し、ヘパリンを加えて凝固を防いだ。PBMCの精製をフィッコール（スウェーデン、ウプサラ、ファルマシア社）を用いた密度勾配遠心分離により行った。

【０２１８】

テスト化合物

HIL-15、ロット番号：6870-011、米国ワシントン州、シアトル、イムネックス社。 hIL-2、オランダ、アムステルダム、チロン・ベネルクス社。

図８に示すこの報告でCTLL-2検定に用いた抗IL-15抗体：146B7、146H5、404A8、404E4。PBMC検定に用いた抗IL-15抗体：146B7 (バッチ：070101)、404A8 (バッチ：030101) 及び 404E4 (バッチ：080101)。

【０２１９】

ヒトIL-15 (hIL-15) 又はhIL-2誘導性CTLL-2増殖の抗IL-15抗体による阻害

各実験において、細胞を三重にして９６ウェルプレートに、hIL-2 又はhIL-15のいずれかの存在下又は非存在下で１ウェル当たり5×10^3個の細胞になるように接種した。増殖に対する作用を評価するために４種類の抗IL-15抗体のそれぞれを加えた。細胞を１６時間、３７℃で5% CO_2下でインキュベートした。[^{3}H] チミジン (1μCi/ウェル、英国バッキンガムシャイア、リトル・チャルフォント、アマーシャム・ライフ・サイエンセズ社）を加えてから４時間後に回収した(米国コネチカット州オレンジ、トムテック社、ハーベスター 96 Mach II M）。

【０２２０】

図８に示すように、CTLL-2細胞のIL-15誘導性増殖は、[^{3}H] -チミジン取り込みの減少に反映されるように、146B7及び146H5により用量依存的に低下した。404E4及び404A8の両者とも、CTLL-2細胞のIL-15誘導性増殖を阻害できなかった。

【０２２１】

hIL-15 (hIL-15) 又はhIL-2誘導性のPBMCの増殖の抗IL-15抗体による阻害

PBMCを三重にして96ウェルのU型底プレート（Nunc、デンマーク、ナルジ・ナンク・インターナショナル社）で、１ウェル当たり5×10^4個の細胞にして、hIL-2もしくはhIL-15及

び抗IL-15抗体の存在下又は非存在下で培養した。コンカナバリンＡ（2.5μg/ml、カルビオケム社）を増殖の陽性コントロールのために加えた。細胞を７２時間、３７℃で5% CO_2下でインキュベートした。［^{3}H］チミジン（1μCi/ウェル、英国バッキンガムシャイア、リトル・チャルフォント、アマーシャム・ライフ・サイエンセズ社）を加えてから１６時間後に回収した(米国コネチカット州オレンジ、トムテック社、ハーベスター 96）。

【０２２２】

146B7 はIL-15誘導性［^{3}H]-チミジン取り込みを用量依存的に阻害でき、従って増殖を阻害した(IC50=3.1±0.91 nM)。404E4及び404A8は両者とも、hIL-15誘導性PBMC増殖を阻害できなかった。146H5は、前に行った実験で得たデータに従って検査されなかった。146B7、404E4及び404A8のIL-15に対する特異性を確認するために、これらの抗体を、IL-2媒介性増殖に対するそれらの作用についても評価した。検査した抗IL-15抗体のいずれも、IL-2誘導性増殖に対する作用を示さなかった（図９）。

【０２２３】

実施例９　ヒト抗IL-15抗体146B7はヒトPBMC上に存在するヒトIL-15に結合する

テスト化合物

ヒトPBMCを、インフォームド・コンセント後に健康なボランティアから得た。

抗体 146B7 (バッチ番号 MDX015)、米国カリフォルニア州ミルピタス、メダレックス社。

【０２２４】

146B7及びヒトIgGのビオチン化

N-ヒドロキシスクシンイミド-ビオチン（シグマ社）をまずDMSO (最終希釈液：100 mg/ml）で希釈し、次に0.1 M $NaHCO_3$（最終希釈液：1 mg/ml、シグマ社）で希釈した。 抗体１mg当たり（1mlに希釈したもの）、600μlのビオチン溶液を加えた（暗、２時間、RT）。抗体－ビオチン溶液をスライド-a-ライザー^{TM}透析カセット（オランダ、ペルビオ・サイエンス、ピアース社、10,000 MWCO）（４℃で一晩）で透析して未標識のビオチンを取り除いた。翌日、ビオチン化抗体の濃度を分光光度法（Ultrospec 2100pro）で280nmのODで判定した。

【０２２５】

末梢血の刺激

IL-15を誘導するために、健康なボランティアから静脈穿刺で血液を得た。PBMCを、ペニシリン(5 U/ml)、ストレプトマイシン（50μg/ml)、L-グルタミン (2mM) (バイオホイッテカー・ヨーロッパ社）及び10% ウシ胎児血清(オプティマム C241、マルチセル、ウィセント社）を添加したRPMI 1640 (バイオホイッテカー・ヨーロッパ社）で、最長２日間（３７℃）で培養し、500 U/ml IFNγ（ベーリンガー・インゲルハイム社）で刺激した。

【０２２６】

フローサイトメトリ

細胞を、ペニシリン（5 U/ml)、ストレプトマイシン（50μg/ml)、L-グルタミン（2mM）(バイオホイッテカー・ヨーロッパ社）及び10% ウシ胎児血清（オプチマム C241、マルチセル、ウィセント社）を添加したRPMI 1640 (バイオホイッテカー・ヨーロッパ社）中で、10%ヒトAB血清（オランダ、アムステルダム、CLB社）と一緒にプレインキュベートした。透過化（カリフォルニア州サンディエゴ、ベクトン・ディッキンソン社、Cytofix/Cytoperm^{TM}キット中で２０分間、４℃）及びPerm/Wash^{TM}（Cytofix/Cytoperm^{TM}キット）緩衝液で洗浄した後、フローサイトメトリにより、PBMCにIL-15の染色を施した。この染色処置全般に渡って Perm/Wash^{TM}緩衝液 (Cytofix/Cytoperm^{TM}キット）を用いることにより、継続的な透過性を保つことに成功した。これら細胞をビオチン化146B7又はビオチン化hIgG1 (20μg/ml、３０分間、４℃）と一緒にインキュベートし、Perm/Wash^{TM}緩衝液で洗浄した後、細胞をストレプトアビジン-フィコエリトリン (ダコ社）と一緒に３０分間（４℃）でインキュベートした。分析後に１試料当たり少なくとも5000個の細胞の蛍光強度をフローサイトメトリ (FACS Calibur、ベクトン・ディッキンソン社）で調べ、CellQuest Pro ソフトウェアを用いて単球の通門を調べた。データは以下：S.I.＝（平均蛍光陽性染色）／（平均蛍光バックグラウンド染色）の通りに計算された刺激指数（S.I.）を示す。

【０２２７】
免疫細胞化学法
ヒト単球に存在するIL-15を検出するために、サイトスピン・プレパラートを全血試料から作製した。 5×10^4個の細胞（200μｌ）を遠心してSuperfrost(R)-プラス顕微鏡用スライド（メンゼル社）上に沈降させた後、スライドを空気乾燥（＜６０分間）し、2%パラホルムアルデヒド／PBS(８分間、４℃)で固定し、PBSで洗浄し、再び空気乾燥した。染色前にサイトスピン・プレパラートをPBS （＋0.1% サポニン；PBSS)で透過化し、これをその後染色処置全般に用いた。内因性ペルオキシダーゼ活性を阻害するために、サイトスピン・プレパラートを、クエン酸／リン酸緩衝液（pH5.8、２０分間、RT）で希釈した0.05% (v/v) 過酸化水素（H_2O_2）と一緒にインキュベートした。PBSSで洗浄後、内因性ビオチン活性をメーカの指示（ビオチン・ブロッキング・キット、ダコ社、ベクタ・ラブズ）に従って阻害した。PBSSで洗浄後、このサイトスピン・プレパラートを、PBSSに溶かした10% (v/v)ヒトプールAB血清（オランダ、アムステルダム、CLB社）と一緒にインキュベートする（３０分間）ことで非特異的結合部位を遮断した。その後、サイトスピン・プレパラートをビオチン化一次抗体（６０分間、RT）と一緒にインキュベートし、PBSSで洗浄した後に、ビオチン化西洋わさびペルオキシダーゼ(streptABComplex/HRP、ダコ社；2% ヒトAB血清を含有するPBSSによる1:100溶液；３０分間、RT）と複合体形成させたストレプトアビジンと一緒にインキュベートした。PBSSで洗浄した後、サイトスピン・プレパラートを、3-アミノ-9-エチルカルバゾール (0.5 mg/ml)及びH_2O_2（0.01%)の酢酸ナトリウム緩衝液（50 mM、pH 4.9）溶液と一緒に１０分間（RT）、インキュベートして、HRP活性の検出に向けた。サイトスピンを水道水で５分間、洗浄し、ヘマトキシリン（ダコ社）で１分間、対比染色し、水道水でさらに５分間、洗浄し、ファラマウント又はグリセルゲル（ダコ社）中に包埋した。
【０２２８】
フローサイトメトリ
IFNγで刺激したヒト単球への146B7の結合を図１２に示す。ビオチン化146B7は未刺激の単球に結合することから、未刺激の細胞にIL-15が存在することが分かる。単球をIFNγで刺激すると、146B7のこれら細胞への結合が増し、培養一日目で最大に達する。コントロール抗体であるhIgG1は未刺激の単球への結合をほとんど示さない。IFNγで刺激すると、単球上の Fcγ受容体の発現が増加することで、hIgG1の結合が増す。
【０２２９】
免疫細胞化学法
図１３は、146B7又はコントロール抗体hIgG1によるヒト単球の染色を示す。細胞質の明確な赤い染色が、細胞を146B7と一緒にインキュベートした後では観察されるが、コントロール抗体とインキュベートした後では観察されない。従って、146B7は単球中のhIL-15に結合し、この結合はIFNγでの刺激後に上方調節される。図１３ではさらに、IL-15の染色が主に細胞内で起きていることも示す。
【０２３０】
実施例１０　ヒト抗IL-15抗体146B7は組織中のIL-15に免疫組織化学的に結合する
テスト化合物
ヒト乾癬皮膚　－　組織試料をインフォームド・コンセント後に得た。デンマーク、コペンハーゲン、ゲントフテ大学病院皮膚科、ルイース・ヴィラーゼン。
抗体146B7 (バッチ番号. MDX015)、米国カリフォルニア州、ミルピタス、メダレックス社。
【０２３１】
146B7及びヒトIgGのビオチン化
N-ヒドロキシスクシンイミド-ビオチン（シグマ社）をまずDMSO (最終希釈液：100 mg/ml)で希釈し、次に0.1 M $NaHCO_3$（最終希釈液：1 mg/ml、シグマ社）で希釈した。 抗体１mg当たり（1mlに希釈したもの）、600μｌのビオチン溶液を加えた（暗、２時間、RT）。抗体－ビオチン溶液をスライド-a-ライザー^{TM}透析カセット（オランダ、ペルビオ・サイ

エンス、ピアース社、10,000 MWCO）（４℃で一晩）で透析して未標識のビオチンを取り除いた。翌日、ビオチン化抗体の濃度を分光光度法（Ultrospec 2100pro）で280nmのODで判定した。

【０２３２】

免疫組織化学法

検定まで組織を－８０℃で保存した。解凍後、組織切片をアセトン中に固定し（１０分間、RT）、空気乾燥した。内因性ペルオキシダーゼ活性を阻害するために、クエン酸／リン酸緩衝液（pH5.8、２０分間、RT）で希釈した0.05%(v/v)過酸化水素（H_2O_2）と一緒に切片をインキュベートした。PBS-Tween 20 (PBST、0.05% v/v)で洗浄後、内因性ビオチン活性をメーカの指示（ビオチン・ブロッキング・キット、ダコ社、ベクター・ラブ）に従って阻害した。PBSTで洗浄後、PBSTに溶かした10%(v/v)ヒトプールAB血清（オランダ、アムステルダム、CLB社）と一緒に組織切片をインキュベート（３０分間）することで、非特異的結合部位を遮断した。血清を吸い取り、次に切片を、2%ヒトAB血清を含有するPBSで希釈したビオチン化一次抗体（146B7又はhIgG1）と一緒に６０分間（RT）、インキュベートした。切片をPBSTで洗浄した。PBSTで洗浄した後、すべての組織切片を、streptABComplex/HRP (ダコ社；2%ヒトAB血清を含有するPBSで 1:100に希釈したもの；３０分間、RT)と一緒にインキュベートした。PBSTで洗浄した後、酢酸ナトリウム緩衝液（50mM、pH4.9）に溶かした3-アミノ-9-エチルカルバゾール (0.5 mg/ml) 及びH_2O_2（0.01%)と一緒に切片を１０分間（RT）、インキュベートして、HRP活性の検出に向けた。切片を水道水で５分間、洗浄し、ヘマトキシリン（ダコ社）で１分間、対比染色し、水道水でさらに５分間、洗浄し、最後にファラマウント又はグリセルゲル（ダコ社）中に包埋した。

【０２３３】

結果

明確な細胞質の染色が、乾癬皮膚のケラチノサイトに、146B7による組織切片染色後に観察されたが、コントロール抗体による染色では観察されなかった（図１４；146B7は、乾癬プラークから得られたIL-15陽性ケラチノサイトを染色する）。

【０２３４】

実施例１１　ヒト抗IL-15抗体146B7 は、SCIDマウス－ヒト組織キメラ中のIL-15を遮断する：関節炎組織及び乾癬組織の両方における炎症の著しい阻害

テスト化合物

滑膜組織　－　若年性リウマチ性関節炎患者から、インフォームド・コンセント後に得た；米国オハイオ州、シンシナティ、チルドレンズ・ホスピタル・メディカル・センター、小児リウマチ科アレクセイ・グロム。

角膜切開刀生検　－　組織試料をインフォームド・コンセント後に得た。デンマーク、コペンハーゲン、ゲントフテ大学病院皮膚科、ルイース・ヴィラーゼン。

抗体146B7 (バッチ番号。MDX015)、乾癬実験用に米国カリフォルニア州ミルピタス、メダレックス社から。

抗体146B7 (バッチ番号。15-00RDJW07)、リウマチ性関節炎実験用に米国カリフォルニア州ミルピタス、メダレックス社から。

【０２３５】

SCIDマウス中のIL-15の阻害　－　ヒト滑膜組織キメラ

新鮮な滑膜組織試料を、若年性リウマチ性関節炎患者から関節置換手術後に得た。試料を無菌状態で採集した。全滑膜組織試料から採った刻んだ組織断片を完全に混合して各プレパラートが確実に均質になるようにした。刻んだ試料（動物１匹当たり2乃至4移植片；一箇所当たり100mg）をSCID/NODマウス（ジャクソン・ラボラトリーズ）の背面に皮下的に植え付けた。移植片の移植日、及び移植後７日目、１４日目、及び２１日目に各動物に146B7 (500μg、i.p.) 又はPBS を投与した。移植後２８日目に動物をと殺した。滑膜移植片を切除し、Ｈ＆Ｅ染色に向けてホルマリン上に配置した。

【０２３６】

SCIDマウス　－　ヒト滑膜組織キメラ由来の組織のＨ＆Ｅ染色の定量(Modified from Leh

r et al., J. Histochem. Cytochem. 1997, 45, 1559)

SCIDマウス－ヒト滑膜組織キメラから得た切片のデジタル画像（2600×2060、jpg）を１０倍対物レンズ（ツァイス顕微鏡；アキシオビジョン・ソフトウェア）を用いて得た後、Photoshop バージョン6.0（カリフォルニア州マウンテンビュー、Adobe システムズ社）を用いてデータをコンピュータ解析し、1300×1300ピクセルに縮小した。各切片内で６つの１０倍野を選び出し、スライド全体の上の組織の染色全体が最良に反映されるようにした。着色した核を全部選抜した後（許容差１０で暗色の核を魔法の杖で）、所定の面積の光学密度表を作製し、平均染色強度を記録した（同様な／画像ヒストグラムコマンドの選択の後）。次にバックグラウンドを選択し、染色を定量した（許容差１０でバックグランド上に魔法の杖）。核染色及びバックグラウンド染色間の差として、染色強度を計算した。これを任意の単位を持つ細胞化学的指数として指定した。データは平均及びs.e.m.として示されている。データをスチューデントのt検定で解析した。

【０２３７】

SCIDマウス－ヒト乾癬組織キメラにおけるIL-15の阻害

角膜切開刀生検を二人の患者の乾癬プラークから得、分割し、C.B-17 SCID（ジャクソン・ラボラトリーズ）マウスに移植した。移植後３週間目にマウスにPBS（プラセボ）、CsA（シクロスポリンＡ）（サンドス社）を１５日間の間１日置きに10mg/kgの用量、又は、146B7を１日目に20mg/kg、そして８日目及び１５日目に10 mg/kgの用量、投与した。最後の注射から１週間後にマウスをと殺し、4mmの穿孔生検を各異種移植片から採取した。パラフィン包埋用に生検をホルマリンに固定し、Ｈ＆ＥでKi-67 核内抗原について染色した。

【０２３８】

SCIDマウス－ヒト乾癬組織キメラ由来の組織の免疫組織化学染色の定量

Ｈ＆Ｅ染色切片を表皮の厚さ（μｍ）、 錯角化症の等級（０から３までの等級）、及び上側真皮の炎症性単核細胞の数、について評価した。Ki-67について染色した切片を、1mm^{2}切片当たりのサイクリング（原語：cycling）ケラチノサイト数について評価した。各処理群中の４匹のマウスの平均値を計算し、各患者から採ったデータを平均及びs.e.m.として要約した。

【０２３９】

SCID/RA モデル

切片の顕微鏡観察から、最も濃く染色された核は浸潤細胞のものであることが分かった。従って、（相対的表面積で測定したときの）核の数を、浸潤の測定値とみなしている。146B7を注射すると、炎症性滑膜組織への浸潤細胞の数が、賦形剤処理に比較して減少する（図１５ａ、$p<0.05$)。図１５ｂは、異種移植された滑膜組織への細胞浸潤に対する146B7の作用を示し、賦形剤処理に比較したときの、暗色の核を持つ細胞数の減少を示す。

【０２４０】

SCID/乾癬モデル

図１６は、146B7又はコントロール処理で処理したSCID／乾癬マウスを示す。賦形剤PBSに比較して、146B7を注射すると、角質層からリート・ペグ（原語：rete pegs）の始まりにかけて測定された表皮厚さで評価したときの乾癬の重篤度が低下した（図１６Ａ））：PBS (177.8$^{\pm}$ 42.2μｍ)、CsA (91.0$^{\pm}$ 15.2μｍ)、146B7 (62.5$^{\pm}$ 9.1μｍ)。厚さの減少はさらに、角質層からリート・ペグの最深部までを測定した場合にも観察された（図１６Ｂ）：PBS (433.8$^{\pm}$ 32.1μｍ)、CsA (303.8$^{\pm}$ 62.9μｍ) 及び146B7 (208.0$^{\pm}$ 33.8μｍ)。さらに、錯角化症の等級が146B7処理により低下した（図１６Ｃ）：PBS (1.6$^{\pm}$ 0.4)、CsA (1.3$^{\pm}$ 0.3)、146B7 (0.5$^{\pm}$ 0.3)。その上、146B7は上側真皮中の炎症性単核細胞の数を減少させる（図１６Ｄ）：PBS (33.3$^{\pm}$ 1.9 単核細胞)、CsA (19.4$^{\pm}$ 8.5)、146B7 (16.4$^{\pm}$ 0.1)。ヒトKi-67タンパク質の発現は細胞増殖と深い関係がある。分裂間期においてこの抗原を核内でのみ、検出することができるが、他方、有糸分裂時にはこのタンパク質の大半が染色体表面上に移動する。Ki-67タンパク質は細胞周期のすべての活動期 (G(1)、S、G(2)、及び有糸分裂)で存在するが、休止期 (G(0))では存在しないという事実から、これを、あ

る任意の細胞集団のうちのいわゆる成長群を判定する優れたマーカとすることができる。146B7はKi-67^{+}サイクリング（原語：cycling）ケラチノサイトの数を減少させる（図１６Ｅ）：PBS (247.9±77.0)、CsA (116.0±24.1)、146B7 (73.8±9.9)。

【０２４１】

リウマチ性関節炎のヒトSCIDモデルでは、146B7で処理すると、炎症性細胞の炎症性組織への浸潤が阻害された。さらに、ヒト乾癬プラークを移植されたSCIDマウスでは、146B7処理により、CsA処理に比べ、乾癬の重篤度が軽減した。実際、146B7による処理の結果、炎症、表皮の厚さ、分裂ケラチノサイト数、及び錯角化症の重篤度が、ヒト／SCIDマウスで大きく低下した。

【０２４２】

実施例１２　ヒト抗IL-15抗体146B7は、受容体に結合したIL-15を認識する

テスト化合物

hIgG1 - ヒトコントロール抗体（シグマ社）。

抗体146B7　メダレックス社、MDX015。

IL-15Rαを構成的に発現するRaji細胞(英国サザンプトン、サザンプトン・ゼネラル病院、テノバス・リサーチ・ラボラトリー、マーティン・グレニー）。

【０２４３】

146B7及びヒトIgGのビオチン化

N-ヒドロキシスクシンイミド-ビオチン（シグマ社）をまずDMSO（最終希釈液：100 mg/ml）で希釈し、次に0.1 M $NaHCO_3$（最終希釈液：1 mg/ml、シグマ社）で希釈した。 抗体１mg当たり（1mlに希釈したもの）、600μlのビオチン溶液を加えた（暗、２時間、RT）。抗体－ビオチン溶液をスライド-a-ライザー^{TM}透析カセット（オランダ、ペルビオ・サイエンス、ピアース社、10,000 MWCO）（４℃で一晩）で透析して未標識のビオチンを取り除いた。翌日、ビオチン化抗体の濃度を分光光度法（Ultrospec 2100pro）で280nmのODで判定した。

【０２４４】

ELISAによる146B7のIL-15－IL-15Rα複合体への結合

平底マイクロタイタ・プレート（グライナー社）をIL-15Rα（米国ミネソタ州、ミネアポリス、Ｒ＆Ｄシステムズ社）で被覆（室温で一晩）した後、プレートをPBS及びニワトリ血清（2%、RT、６０分間）と一緒にインキュベートした。PBS （＋0.05% Tween 20：PBST)で洗浄後、プレートを、未標識のIL-15 (50μl、RT、米国シアトル、イムネックス社)のいくつかの希釈液と一緒にインキュベートした。１０分後、ビオチン化抗体を様々な濃度になるようにウェル（50μl）に加えた（室温で９０分間）。PBSTで洗浄した後、プレートを、PBST-C (PBST 及び2% ニワトリ血清)に1:10,000になるように希釈したストレプトアビジン-ポリ-西洋わさびペルオキシダーゼ (オランダ、アムステルダム、CLB社）と一緒にインキュベート（室温で６０分間）した。最後に、プレートを洗浄し、次に、メーカのプロトコルに従って、ABTS緩衝液にしたABTS (アジノビス-3-エチルベンズチアゾリン－スルホン酸、ドイツ、マンハイム、ロシュ・ダイアグノスティックス社)と一緒にインキュベートした。呈色反応を2% シュウ酸（50μl）で停止させた。結合を 405 nmでEL808 ELISA-リーダ (米国ヴァーモント州、ウィヌースキ、バイオ-テック・インスツルメンツ社）で評価した。

【０２４５】

Raji細胞上のIL-15－IL-15R 複合体への146B7 の結合

Raji細胞を、10% ヒトプールAB血清（オランダ、アムステルダム、CLB社）のFACS 緩衝液（PBS、0.05%BSA、0.02% $NaNO_3$）溶液と一緒にプレインキュベートした（４℃で２０分間）。Raji 細胞（1-2*10^5細胞/ml）をウェル内に入れ、50μlの未標識のIL-15 を数種類の濃度で加えた (10% ヒトAB血清を加えたFACS緩衝液に希釈して）。この細胞を３０分間（４℃）インキュベートし、FACS緩衝液で２回、洗浄した後、50μlのビオチン化抗体 (146B7 又は hIgG1)をウェルに加えた（４℃で３０分間）。FACS緩衝液で２回、洗浄した後、50μlのストレプトアビジン-フィコエリトリンを各ウェルに加えた（４℃で３０分間）。

FACS 緩衝液で２回、洗浄した後、細胞を200μlのFACS緩衝液中に取り出し、１試料当たり少なくとも5000個の細胞の蛍光強度を、CellQuestソフトウェアを用いたフローサイトメトリ（FACS Calibur、ベクトン・ディッキンソン社）による分析後に判定した。データは、以下の通り：
S.I. =（平均蛍光陽性染色）／（平均蛍光バックグラウンド染色）
に計算された刺激指数（S.I.）を示す。

【０２４６】

ELISA

ELISAにおける146B7のIL-15/IL-15R 複合体への結合を図１９に示す。146B7の結合は、その受容体に結合しているIL-15の濃度が増すにつれ、高まる。コントロール抗体のIL-15又はIL-15Rへの結合の作用は何ら、観察されなかった。

【０２４７】

IL-15R発現Raji細胞への結合

Raji細胞上のIL-15/IL-15R複合体への146B7結合を図２０に示す。146B7はIL-15/IL-15R複合体に用量依存的に結合する。Raji細胞上のIL-15/IL-15R複合体へのhIgG1の結合は何ら、観察されなかった（図２０）。

【０２４８】

146B7はIL-15に、このサイトカインがその受容体に結合した後で結合することができる。146B7は、IL-15上のうちで、受容体への結合に関与していないエピトープに結合する。

【０２４９】

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【０２５０】

均等物

当業者であれば、日常的な実験によって、ここに解説した本発明の具体的な実施態様の均等物を数多く、認識し、又は確認できることであろう。このような均等物は以下の請求の範囲の包含するところと、意図されている。従属請求項に開示した実施例のいかなる組合せも、本発明の範囲内にあると考えられる。

【０２５１】

引用による援用

ここで引用する全公開文献、特許及び係属中の特許出願の全文を、引用をもってここに援用することとする。

【図面の簡単な説明】

【０２５２】

【図１】図１は、ヒトIL-15特異抗体146B7、147H5、404A8 及び404E4の、ヒトIL-15 (hIL-15)並びに変異型IL-15タンパク質Q108S及びD8SQ108Sへの結合を示すグラフを含む。抗体の連続希釈液を、それらのhIL-15又は変異型IL-15タンパク質D8SQ108S 及びQ108S への結合に関してELISAで調べた。

【図２】図２及び３は、抗体146B7由来のそれぞれV_H及びV_L領域のアミノ酸（配列番号:2及び4) 並びにヌクレオチド (配列番号:1 及び3) 配列を示す。フレームワーク領域(FR)及び相補性決定領域 (CDR)を示す。

【図３】図２及び３は、抗体146B7由来のそれぞれV_H及びV_L領域のアミノ酸（配列番号:2及び4) 並びにヌクレオチド (配列番号:1 及び3) 配列を示す。フレームワーク領域(FR)及び相補性決定領域 (CDR)を示す。

【図４】図４－Ｄは、IL-15媒介性TNF-α放出の、抗体146B7による阻害を示すグラフを含む。ヒトPBMCを、146B7抗体又はアイソタイプ・コントロール抗体(0.1、1、10μg/ml)と組み合わせたhIL-15 (0、50、100ng/ml)と一緒に７２時間、インキュベートした。産生されたTNF-αの量をELISAで測定した。２人の健康なボランティアのデータを示す。

【図５】図５は、IL-2もしくはIL-15媒介性TNF-α産生に対する抗体146B7の作用を示すグ

ラフである。ヒトPBMCを、146B7抗体(0.1、1、10μg/ml)と組み合わせたhIL-15 (0、50、100ng/ml)又はhIL-2（100ng/ml）と一緒に７２時間、インキュベートした。産生されたTNF-αの量をELISAで測定した。

【図６】図６は、hIL-15誘導性CTLL-2増殖に対する抗体146B7、146H5、404E4 及び404A8の阻害活性を示すグラフである。hIL-2を枯渇させたCTLL-2細胞を、146B7、146H5、404E4 及び404A8の連続希釈液と組み合わせた hIL-15 (60 pg/ml) と一緒に４８時間、インキュベートした。[^{3}H] -チミジン取り込みを測定して増殖を表現した（cpm）。結果を平均値として示す。

【図７】図７乃至９は、 IL-15誘導性PBMC増殖に対する抗体146B7（図７）、404E4 （図８）及び404A8（図９）の阻害活性を示すグラフを含む。ヒトPBMCを、0.1、1、10μg/mlの146B7 （図７)、404E4 （図８）又は 404A8 （図９）と組み合わせたhIL-15 (0、25、100 ng/ml；それぞれ図７Ａ、８Ａ、及び９Ａ）又はhIL-2 (0、10、100 ng/ml；それぞれ図７Ｂ、８Ｂ、及び９Ｂ）と一緒に７２時間、インキュベートした。[^{3}H] -チミジン取り込みを測定して増殖を表現した（cpm）。

【図８】図７乃至９は、 IL-15誘導性PBMC増殖に対する抗体146B7（図７）、404E4 （図８）及び404A8（図９）の阻害活性を示すグラフを含む。ヒトPBMCを、0.1、1、10μg/mlの146B7 （図７)、404E4 （図８）又は 404A8 （図９）と組み合わせたhIL-15 (0、25、100 ng/ml；それぞれ図７Ａ、８Ａ、及び９Ａ）又はhIL-2 (0、10、100 ng/ml；それぞれ図７Ｂ、８Ｂ、及び９Ｂ）と一緒に７２時間、インキュベートした。[^{3}H] -チミジン取り込みを測定して増殖を表現した（cpm）。

【図９】図７乃至９は、 IL-15誘導性PBMC増殖に対する抗体146B7（図７）、404E4 （図８）及び404A8（図９）の阻害活性を示すグラフを含む。ヒトPBMCを、0.1、1、10μg/mlの146B7 （図７)、404E4 （図８）又は 404A8 （図９）と組み合わせたhIL-15 (0、25、100 ng/ml；それぞれ図７Ａ、８Ａ、及び９Ａ）又はhIL-2 (0、10、100 ng/ml；それぞれ図７Ｂ、８Ｂ、及び９Ｂ）と一緒に７２時間、インキュベートした。[^{3}H] -チミジン取り込みを測定して増殖を表現した（cpm）。

【図１０】図１０は、IFNγで刺激した単球に対する抗体146B7の結合を示すグラフである。ヒトPBMCを IFNγ（500 U/ml）の存在下で最長２日間（３７℃）、培養した。一試料当たり少なくとも5000個の細胞の蛍光強度を、フローサイトメトリ及び単球の通門による分析後に調べた。データは刺激指数（S.I.＝（平均蛍光陽性染色）／（平均蛍光バックグラウンド染色）を示す。

【図１１】図１１は、抗体146B7（パネルＢ）又はアイソタイプ・コントロール抗体（パネルＡ）とのヒト単球の結合を示す。ヒトPBMCを単離し、この細胞をIFNγ（500U/ml）と一緒に培養後にサイトスピンを作製した。細胞をヘマトキシリンで対比染色した。

【図１２】図１２は、ヒト乾癬皮膚の、146B7（パネルＢ）又はアイソタイプ・コントロール抗体（hIgG1）（パネルＡ）との結合を示す。ヒト乾癬プラークを患者からインフォームド・コンセント後に得、検定まで－８０℃で保存した。組織をビオチン化抗体で染色し、西洋わさびペルオキシダーゼの活性化後に観察した。

【図１３】図１３Ａは、SCIDマウスを146B7又は賦形剤で処理した後のリウマチ性関節組織中の有核細胞のパーセンテージを示すグラフである。組織をヘマトキシリン及びエオシン（Ｈ＆Ｅ）で染色し、Photo Shop バージョン 6.0で解析した。データは、146B7処理（n=4）又は賦形剤処理（n=2）後のマウスの核（全面積のパーセンテージ）の平均及びs.e.m.として示されている。図１３Ｂは、146B7（パネルＢ）又はPBS（パネルＡ）で処理した後の、SCIDマウス中に異種移植されたRA組織の代表的Ｈ＆Ｅ染色を示す。

【図１４】図１４は、SCID／乾癬マウスにおける抗体146B7処理の作用を示すグラフを含む。パラフィン包埋に向けて生検をホルマリン固定し、Ｈ＆ＥでKi-67核内抗原ついて染色した。図１４Ａは、角質層からリート・ペッグズ（原語：rete pegs）の始まりまで測定された表皮の厚さにより評価された、乾癬の重篤度を示す。図１４Ｂは、角質層からリート・ペッグズの最深部まで測定された表皮の厚さを示す。図１４Ｃは、錯角化症の等級を示す。図１４Ｄは、上側真皮中の炎症性単核細胞数を示す。図１４Ｅは、Ki-67+サイク

リング・ケラチノサイトの数を示す。

【図１５】図１５は、抗体146B7（パネルＣ）、CsA（パネルB)、又は賦形剤（パネルＡ）で処理した後のSCIDマウスに移植されたヒト乾癬皮膚のＨ＆Ｅ染色を示す。移植から３週間後にマウスにPBS（プラセボ)、CsA（シクロスポリンＡ）（サンドス社）を、１５日間にわたって１日置きに10mg/kgの用量、又は146B7を１日目に20 mg/kg、そして８日目及び１５日目に10 mg/kgの用量、投与した。最後の注射から１週間後にマウスをと殺し、4 mm の穿孔生検を各異種移植片から採取した。パラフィン包埋に向けて生検をホルマリン固定し、Ｈ＆Ｅで染色した。

【図１６】図１６は、146B7（パネルＣ)、CsA（パネルＢ)、又は賦形剤(パネルＡ)で処理後のSCIDマウスに移植されたヒト乾癬皮膚のKi-67染色を示す。移植から３週間後にマウスにPBS（プラセボ)、CsA（シクロスポリンＡ）（サンドス社）を、１５日間にわたって１日置きに10mg/kgの用量、又は146B7を１日目に20 mg/kg、そして８日目及び１５日目に10 mg/kgの用量、投与した。最後の注射から１週間後にマウスをと殺し、4 mm の穿孔生検を各異種移植片から採取した。パラフィン包埋に向けて生検をホルマリン固定し、Ki-67核内抗原について染色した。

【図１７】図１７は、抗体146B7の、受容体に結合したIL-17への結合を示すグラフである。プレートをIL-15Rαで被覆し、IL-15と一緒にインキュベートした。１０分後、ビオチン化146B7をウェルに加えた。146B7の、受容体に結合したIL-15への結合を405 nm でELISA-リーダで評価した。

【図１８】図１８は、IL-15が、Raji細胞上に発現したその受容体への結合後の、抗体146B7のIL-15への結合を示すグラフである。IL-15R発現Raji細胞をIL-15と一緒にインキュベート後、ビオチン化146B7 を１０分後にこれら細胞に加えた。146B7 の、受容体に結合したIL-15への結合をFACS分析で評価した。

【図１】

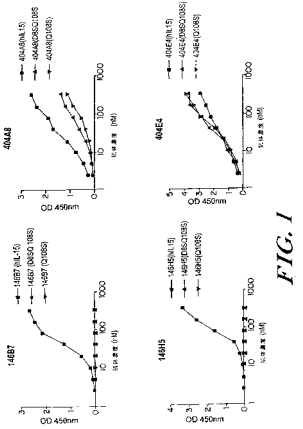

FIG. 1

【図２】

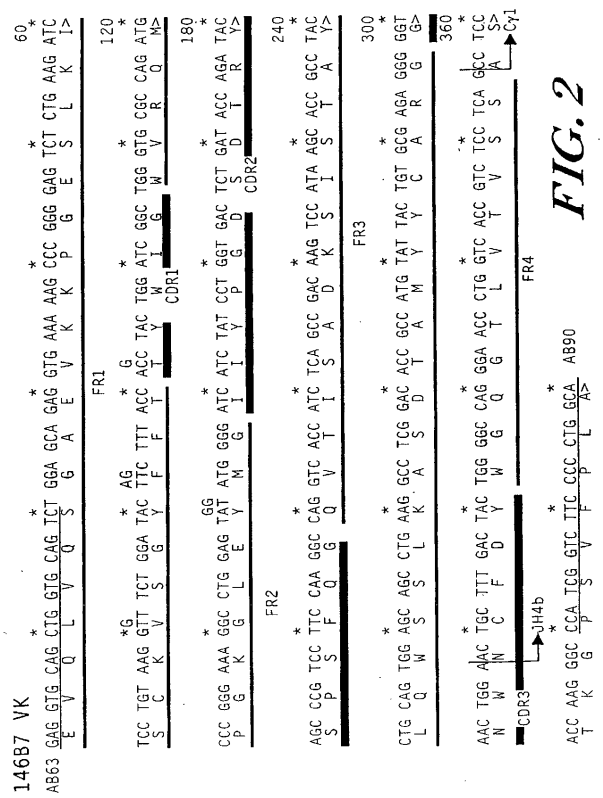

FIG. 2

【図３】

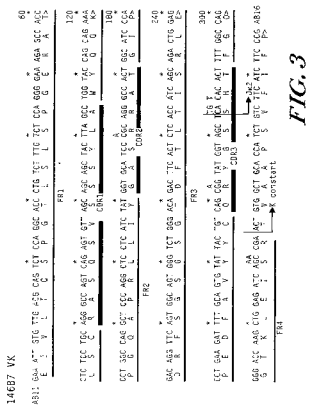

FIG. 3

【図４Ａ】

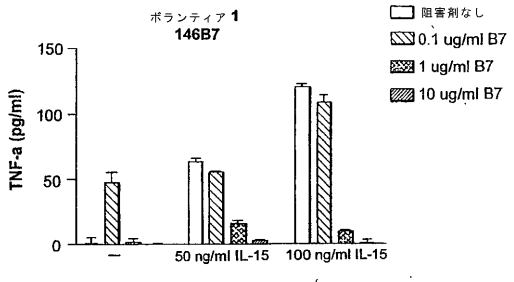

FIG. 4A

【図４Ｂ】

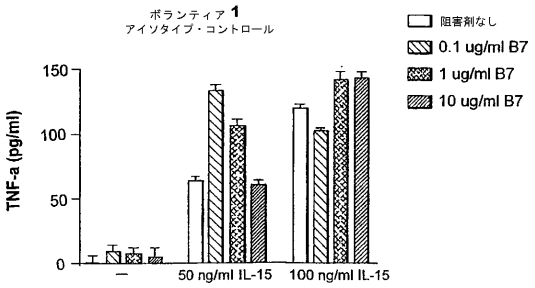

FIG. 4B

【図４Ｃ】

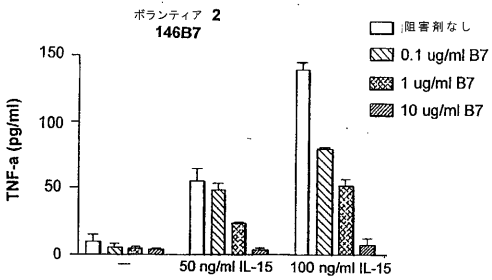

FIG. 4C

【図４Ｄ】

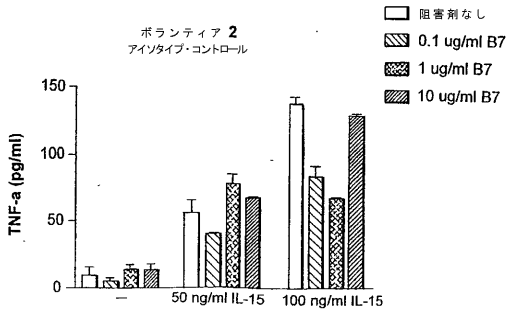

FIG. 4D

【図６】

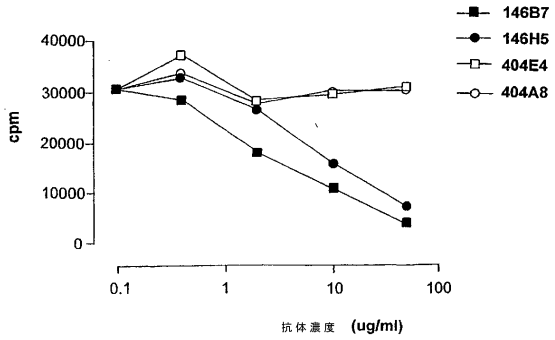

FIG. 6

【図５】

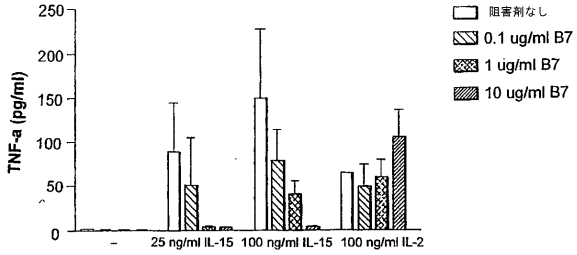

FIG. 5

【図７Ａ】

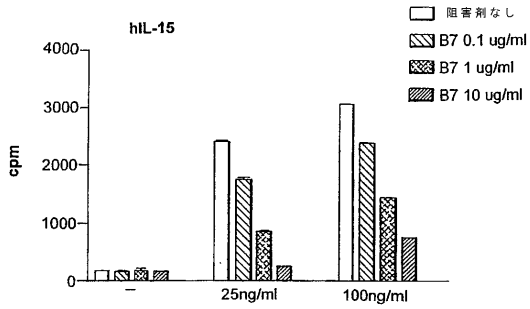

FIG. 7A

【図７Ｂ】

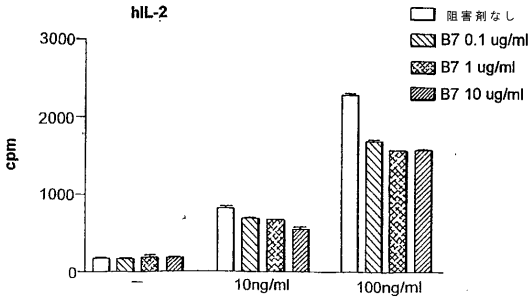

FIG. 7B

【図９Ａ】

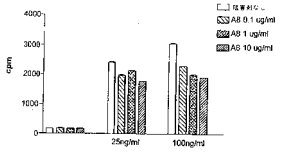

FIG. 9A

【図９Ｂ】

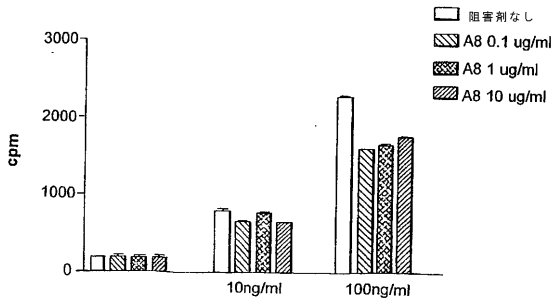

FIG. 9B

【図８Ａ】

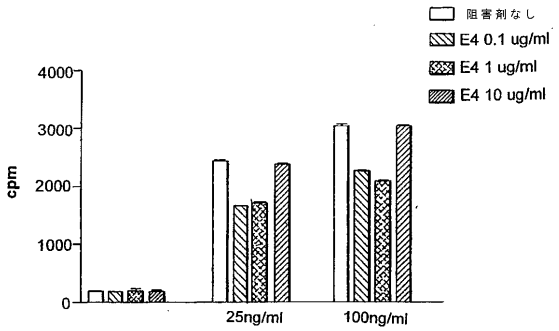

FIG. 8A

【図８Ｂ】

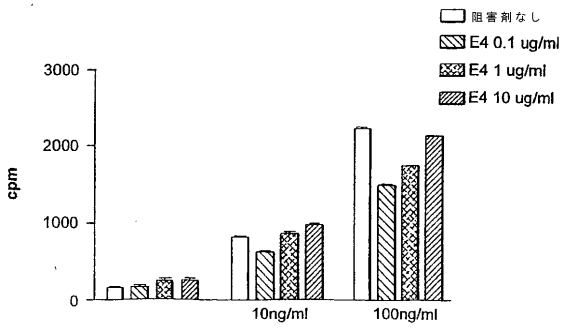

FIG. 8B

【図１０】

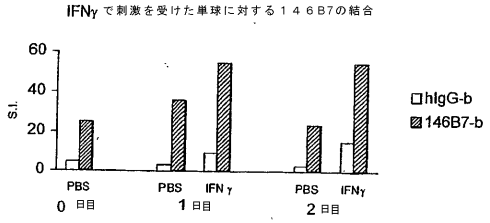

FIG. 10

【図１１】

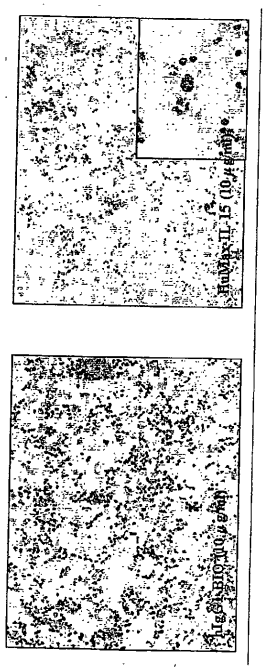

FIG. 11B

FIG. 11A

【図１２】

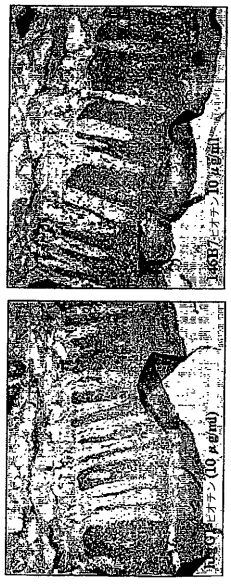

FIG. 12B

FIG. 12A

【図１３Ａ】

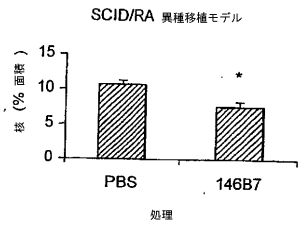

FIG. 13A

【図１３ＢＣ】

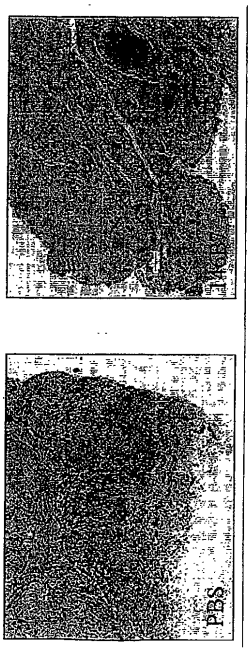

FIG. 13C

FIG. 13B

【図１４Ａ】

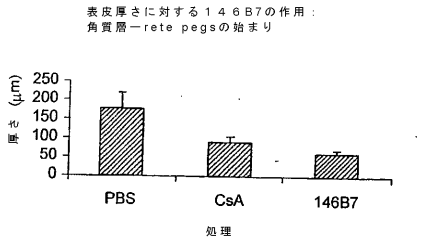

FIG. 14A

【図１４Ｂ】

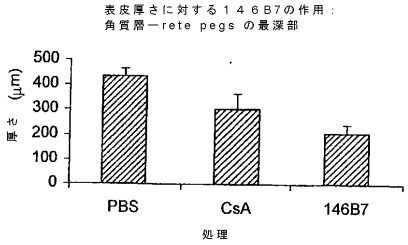

FIG. 14B

【図１４Ｃ】

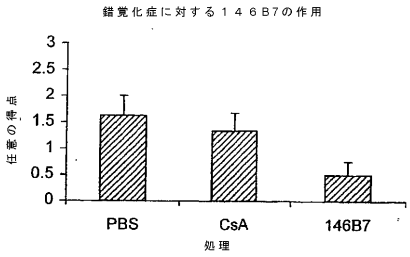

FIG. 14C

【図１４Ｄ】

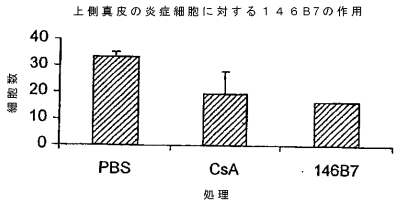

FIG. 14D

【図１４Ｅ】

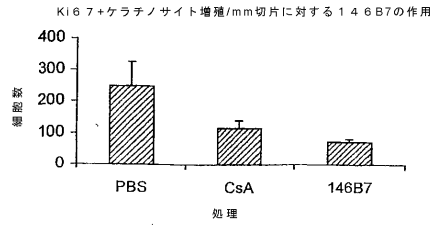

FIG. 14E

【図１５】

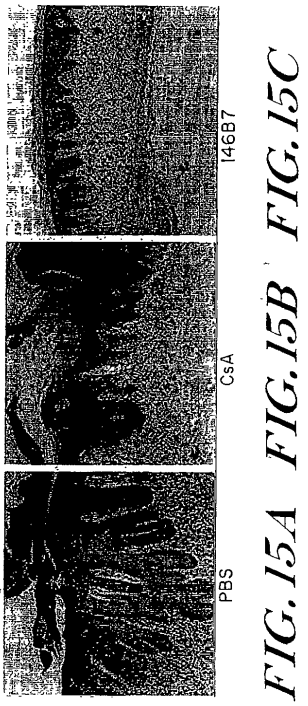

FIG. 15A FIG. 15B FIG. 15C

【図１６】

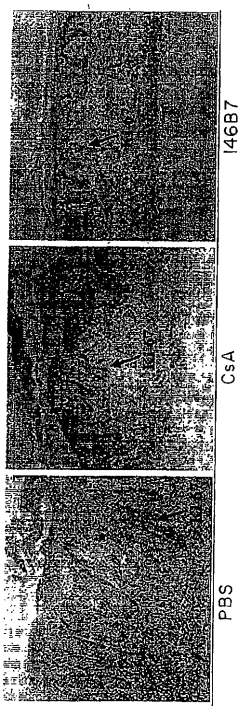

FIG. 16A FIG. 16B FIG. 16C

【図１７】

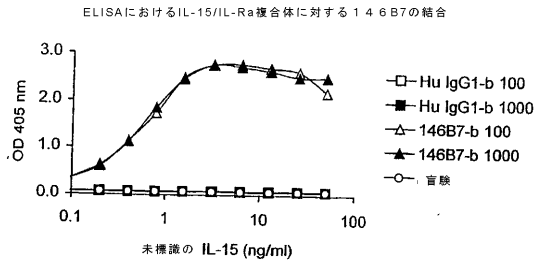

FIG. 17

【図１８】

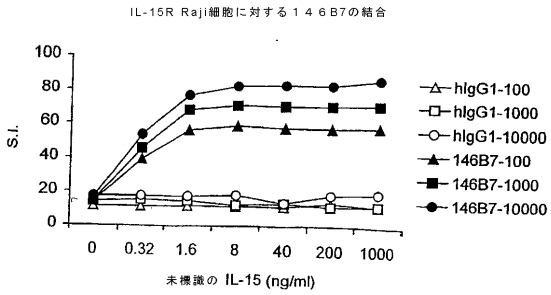

FIG. 18

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(54) Title: HUMAN ANTIBODIES SPECIFIC FOR INTERLEUKIN 15 (IL-15)

(57) Abstract: Isolated human monoclonal antibodies which specifically bind to IL-15 (e.g., human IL-15), and related antibody-based compositions and molecules, are disclosed. The human antibodies can be produced in a transfectoma or in a non-human transgenic animal, e.g., a transgenic mouse, capable of producing multiple isotypes of human monoclonal antibodies by undergoing V-D-J recombination and isotype switching. Also disclosed are pharmaceutical compositions comprising the human antibodies, non-human transgenic animals, and hybridomas which produce the human antibodies, and therapeutic and diagnostic methods for using the human antibodies.

HUMAN ANTIBODIES SPECIFIC FOR INTERLEUKIN 15 (IL-15)

Background of the Invention

Interleukin-15 (IL-15) is a pro-inflammatory cytokine, a glycoprotein of 14-15 kD. Constitutive expression has been reported in various cells and tissues including monocytes and macrophages, fibroblasts, keratinocytes and dendritic cells (Waldmann and Tagaya, 1999; Fehniger and Caligiuri, 2001). The expression is upregulated under inflammatory conditions, as reported for monocytes stimulated with IFN-γ and LPS or by infection with viruses, bacteria or protozoans (Kirman *et al.*, 1998; Waldmann *et al.*, 1998; Waldmann and Tagaya, 1999; Fehniger and Caligiuri, 2001). Furthermore, in chronic inflammatory diseases such as rheumatoid arthritis, locally produced IL-15 is likely to amplify inflammation by the recruitment and activation of synovial T-cells. This IL-15-induced effect has been suggested to play a pivotal role in disease pathogenesis (Kirman *et al.*, 1998; McInnes *et al.*, 1996; McInnes *et al.*, 1997; McInnes and Liew, 1998; Fehniger and Caligiuri, 2001).

In vitro studies have shown that IL-15 shares several biological activities with IL-2, due to shared receptor components. The IL-15 receptor present on T-cells consists of an unique α-chain, IL-15Rα, but shares the β-chain and the γ-chain with IL-2R. As a consequence, both receptors use the same Jak/STAT-signaling elements. However, based on complex regulation and differential expression of IL-2 and IL-15 and their receptors, critical differences in the *in vivo* functions have been reported (Kirman *et al.*, 1998; Waldmann and Tagaya, 1999; Waldmann *et al.*, 2001). It is also important to note the non-redundant role for IL-15 in natural killer (NK) cell, NK-T cell and intraepithelial lymphocyte development, survival, expansion and function (Kennedy *et al.*, 2000; Liu *et al.*,2000).

McInnes and coworkers (McInnes *et al.*, 1997; McInnes and Liew, 1998) reported the induction of TNF-α production after IL-15 stimulation in T-cells derived from rheumatoid arthritis patients. Furthermore, peripheral blood T cells activated by IL-15 were shown to induce significant TNF-α production by macrophages via a cell-contact-dependent mechanism. Because of the destructive role of TNF-α in rheumatoid arthritis, inhibition of this cytokine decreases disease activity (Bathon *et al.*, 2000; Klippel, 2000; Lovell *et al.*, 2000; Maini and Taylor, 2000).

Summary of the Invention

The present invention is based on the generation and isolation, for the first time, of fully human monoclonal antibodies which specifically bind to human IL-15 and which inhibit the proinflammtory effects induced by IL-15, as well as the characterization of such novel antibodies and the demonstration of their therapeutic value in treating a variety of IL-15 mediated diseases. For example, as described herein, the human antibodies have been shown to inhibit both TNFα production and T cell proliferation, both of which are integrally involved in inflammatory disorders. Accordingly, the human antibodies of the present invention provide an improved means for treating and preventing such disorders (and any other IL-15 mediated disorder), attributable in part to their unique specificity (*e.g.*, epitope and species specificity), affinity, structure, functional activity and the fact that they are fully human, making them significantly less immunogenic and more therapeutically effective and useful when administered to human patients than other IL-15 antibodies previously generated (*e.g.*, murine and humanized antibodies). The present invention is also based on the discovery of new therapeutic applications, including treatment of inflammatory diseases, such as rheumatoid arthritis, psoriasis, transplant rejections and cancers, for IL-15 inhibiting antibodies such as the human antibodies described herein.

Isolated human antibodies of the invention include a variety of antibody isotypes, such as IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgAsec, IgD, and IgE. Typically, they include IgG1 (*e.g.*, IgG1k), IgG3 and IgM isotypes. The antibodies can be full-length (*e.g.*, an IgG1 or IgG3 antibody) or can include only an antigen-binding portion (*e.g.*, a Fab, $F(ab')_2$, Fv, a single chain Fv fragment, an isolated complementarity determining region (CDR) or a combination of two or more isolated CDRs).

In one embodiment, the human antibodies are recombinant antibodies. In a particular embodiment, the human antibody is encoded by human IgG heavy chain and human kappa light chain nucleic acids comprising nucleotide sequences in their variable regions as set forth in SEQ ID NO:1 and SEQ ID NO:3, respectively, and conservative sequence modifications thereof. In another embodiment, the human antibody includes IgG heavy chain and kappa light chain variable regions which comprise the amino acid sequences shown in SEQ ID NO:2 and SEQ ID NO:4, respectively, and conservative sequence modifications thereof.

Human antibodies of the invention can be produced recombinantly in a host cell, such as a transfectoma (*e.g.*, a transfectoma consisting of immortalized CHO cells or lymphocytic cells) containing nucleic acids encoding the heavy and light chains of the antibody, or be obtained directly from a hybridoma which expresses the antibody (*e.g.*, which includes a B cell obtained from a transgenic non-human animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a human light chain transgene that encode the antibody, fused to an immortalized cell). In a particular embodiment, the antibodies are produced by a hybridoma referred to herein as 146B7 or by a host cell (*e.g.*, a CHO cell) transfectoma containing human heavy chain and human light chain nucleic acids which comprise nucleotide sequences in their variable regions as set forth in SEQ ID NOs: 1 and 3, respectively, and conservative modifications thereof. In particular embodiments, the antibodies are produced by hybridomas referred to herein as 146B7, 146H5, 404E4, and 404A8. In a preferred embodiment, the antibody specifically binds to an epitope located on the β- and/or γ-chain interacting domain of IL-15.

In another embodiment, the human antibodies of the present invention specifically bind to human IL-15 and inhibit the ability of IL-15 to induce proinflammatory effects, *e.g.*, inhibit the production of TNFα and/or inhibit the proliferation of T cells, such as PBMC or CTLL-2 T cells, upon IL-15 binding to the IL-15 receptor. Typically, the human antibodies bind to IL-15 with a dissociation equilibrium constant (K_D) of less than approximately 10^{-7} M, such as less than approximately 10^{-8} M, 10^{-9} M or 10^{-10} M or even lower when determined by surface plasmon resonance (SPR) technology in a BIACORE 3000 instrument using recombinant human IL-15 as the analyte and the antibody as the ligand. In a particular embodiment, the antibody binds to human IL-15 with a dissociation equilibrium constant (K_D) of approximately 6.5×10^{-8} M.

In another aspect, the invention provides nucleic acid molecules encoding the antibodies, or antigen-binding portions, of the invention. Accordingly, recombinant expression vectors which include the antibody-encoding nucleic acids of the invention, and host cells transfected with such vectors, are also encompassed by the invention, as are methods of making the antibodies of the invention by culturing these host cells.

The invention also relates to an expression vector comprising a nucleotide sequence encoding heavy and light variable regions which comprise the amino acid sequences shown in SEQ ID NO:2 and SEQ ID NO:4, respectively, and conservative modifications thereof. Such expression vectors are well known in the art. Examples hereof include *in vitro* transcription/translation vectors using, for example, reticulocyte lysates.

In yet another aspect, the invention provides isolated B-cells from a transgenic non-human animal, *e.g.*, a transgenic mouse, which are capable of expressing various isotypes (*e.g.*, IgG, IgA and/or IgM) of human monoclonal antibodies that specifically bind to IL-15. Preferably, the isolated B cells are obtained from a transgenic non-human animal, *e.g.*, a transgenic mouse, which has been immunized with a purified or enriched preparation of IL-15 antigen and/or cells expressing IL-15. Preferably, the transgenic non-human animal, *e.g.*, a transgenic mouse, has a genome comprising a human heavy chain transgene and a human light chain transgene. The isolated B-cells are then immortalized to provide a source (*e.g.*, a hybridoma) of human monoclonal antibodies to IL-15.

Accordingly, the present invention also provides a hybridoma capable of producing human monoclonal antibodies that specifically bind to IL-15. In one embodiment, the hybridoma includes a B cell obtained from a transgenic non-human animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a human light chain transgene fused to an immortalized cell. The transgenic non-human animal can be immunized with a purified or enriched preparation of IL-15 antigen and/or cells expressing IL-15 to generate antibody-producing hybridomas. Particular hybridomas provided by the invention include 146B7, 146H5, 404E4, and 404A8.

In yet another aspect, the invention provides a transgenic non-human animal, such as a transgenic mouse, which expresses human monoclonal antibodies that specifically bind to IL-15. In a particular embodiment, the transgenic non-human animal is a transgenic mouse having a genome comprising a human heavy chain transgene and a human light chain transgene. The transgenic non-human animal can be immunized with a purified or enriched preparation of IL-15 antigen and/or cells expressing IL-15. Preferably, the transgenic non-human animal, *e.g.*, the transgenic mouse, is capable of producing multiple isotypes of human monoclonal antibodies to IL-

15 (*e.g.*, IgG, IgA and/or IgM) by undergoing V-D-J recombination and isotype switching. Isotype switching may occur by, *e.g.*, classical or non-classical isotype switching.

In another aspect, the present invention provides methods for producing human monoclonal antibodies which specifically react with IL-15. In one embodiment, the method includes immunizing a transgenic non-human animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a human light chain transgene, with a purified or enriched preparation of IL-15 antigen and/or cells expressing IL-15. B cells (*e.g.*, splenic B cells) of the animal are then obtained and fused with myeloma cells to form immortal, hybridoma cells that secrete human monoclonal antibodies against IL-15.

In another aspect, the present invention features a human anti-IL-15 antibody conjugated to a therapeutic moiety, *e.g.*, a cytotoxic drug, an enzymatically active toxin, or a fragment thereof, a radioisotope, or a small molecule anti-cancer drug.

In another aspect, the present invention provides compositions, *e.g.*, pharmaceutical and diagnostic compositions, comprising a pharmaceutically acceptable carrier and at least one human monoclonal antibody of the invention which specifically binds to IL-15. The composition can further include other therapeutic agents, such as other immunosuppressive agents, or chemotherapeutic agents.

In yet another aspect, the invention provides methods for inhibiting the proinflammatory effects of IL-15, such as inhibiting IL-15 induced TNFα production and/or T cell proliferation, preferably without inhibiting the activity (*e.g.*, TNFα production and/or T cell proliferation) of structurally related proteins/cytokines (*e.g.*, IL-2) using one or more human antibodies of the invention.

Human antibodies of the present invention can be used to treat and/or prevent a variety of IL-15 mediated diseases by administering the antibodies to patients suffering from such diseases.

Exemplary diseases that can be treated (*e.g.*, ameliorated) or prevented using the methods and compositions of the invention include, but are not limited to, inflammatory disorders, such as arthritis (*e.g.*, psoriatic arthritis and rheumatoid arthritis including active rheumatoid arthritis and juvenile rheumatoid arthritis), inflammatory bowel disease. For example, the antibodies have been shown to reduce parakeratosis, reduce epidermal thickness and reduce proliferation of keratinocytes in psoriasis. The

antibodies also have been shown to reduce inflammation and/or prevent chemotaxis of activated leukocytes involved in rheumatoid arthritis. The antibodies also can be used to treat infectious diseases, such as HIV infection. Furthermore, the antibodies can be used to treat transplant rejection. Still further, the antibodies can be used to treat a variety of diseases involving IL-15 mediated neovascularization, such as tumor growth and cancers, *e.g.* T-cell leukaemia.

The human antibodies of the present invention may also be combined with one or more additional therapeutic agents, such as anti-inflammatory agents, DMARDs (disease-modifying anti-rheumatic drugs), immunosuppressive agents, chemotherapeutics, and psoriasis agents.

In one embodiment, the subject can be additionally treated with one or more agents that enhance the inhibition of the proinflammatory effect of the antibodies, *e.g.*, an anti-inflammatory agent, such as a steroidal drug or a NSAID (nonsteroidal anti-inflammatory drug). Preferred agents include, for example, aspirin and other salicylates, Cox-2 inhibitors, such as rofecoxib (Vioxx) and celecoxib (Celebrex), NSAIDs such as ibuprofen (Motrin, Advil), fenoprofen (Nalfon), naproxen (Naprosyn), sulindac (Clinoril), diclofenac (Voltaren), piroxicam (Feldene), ketoprofen (Orudis), diflunisal (Dolobid), nabumetone (Relafen), etodolac (Lodine), oxaprozin (Daypro), and indomethacin (Indocin).

In another embodiment, the human antibodies of the invention can be administered in combination with one or more DMARDs, such as methotrexate (Rheumatrex), hydroxychloroquine (Plaquenil), sulfasalazine (Asulfidine), pyrimidine synthesis inhibitors, *e.g.* leflunomide (Arava), IL-1 receptor blocking agents, *e.g.* anakinra (Kineret), and TNF-α blocking agents, *e.g.* etanercept (Enbrel), infliximab (Remicade) and adalimumab.

In another embodiment, the human antibodies of the invention can be administered in combination with one or more immunosuppressive agents, such as cyclosporine (Sandimmune, Neoral) and azathioprine (Imural).

In another embodiment, the human antibodies of the invention can be administered in combination with one or more chemotherapeutics, such as doxorubicin (Adriamycin), cisplatin (Platinol), bleomycin (Blenoxane), carmustine (Gliadel), cyclophosphamide (Cytoxan, Procytox, Neosar), and chlorambucil (Leukeran). The

human antibodies according to the invention can also be administered in conjuction with radiation therapy.

In another embodiment, the human antibodies of the invention can be administered in combination with one or more agents for treating psoriasis, such as topical medications containing coal tar, A vitamin, cortisone or other corticosteroids, oral or injected medications, such as corticosteroids, methotrexate, retinoids, e.g. acicretin (Neogitason) or cyclosporine (Sandimmune, Neoral). Other treatments may include exposure to sunlight or phototherapy.

In another embodiment, the human antibodies of the invention can be administered in combination with other antibodies, such as CD4 specific antibodies and IL-2 specific antibodies. A combination of the present human antibodies with CD4 specific antibodies or IL-2 specific antibodies are considered particularly useful for treating autoimmune diseases and transplant rejections.

In yet another aspect, the present invention provides a method for detecting *in vitro* or *in vivo* the presence of the IL-15 antigen in a sample, *e.g.*, to diagnose IL-15-mediated diseases. In one embodiment, this is achieved by contacting a sample to be tested, along with a control sample, with a human monoclonal antibody of the invention, or an antigen-binding portion thereof under conditions that allow for formation of a complex between the antibody and IL-15. Complex formation is then detected (*e.g.*, using an ELISA) in both samples, and any statistically significant difference in the formation of complexes between the samples is indicative of the presence of the IL-15 antigen in the test sample.

Other features and advantages of the instant invention will be apparent from the following detailed description and claims.

Brief Description of the Drawings

Figure 1 includes graphs showing the binding of the human IL-15 specific antibodies, 146B7, 147H5, 404A8 and 404E4, to human IL-15 (hIL-15) and to the mutant IL-15 proteins, Q108S and D8SQ108S. Serial dilutions of the antibodies were examined for their binding to hIL-15 or the mutant IL-15 proteins D8SQ108S and Q108S in an ELISA.

Figures 2 and 3 show amino acid (SEQ ID NOs:2 and 4) and nucleotide (SEQ ID NOs:1 and 3) sequences of the V_H and V_L-regions, respectively, from antibody 146B7. The framework (FR) and complementarity determining regions (CDR) are indicated.

Figures 4A-D include graphs showing the inhibition of IL-15-mediated TNF-α release by antibody 146B7. Human PBMC were incubated with hIL-15 (0, 50, 100 ng/ml) in combination with 146B7 antibody or with an isotype control antibody (0.1, 1, 10 μg/ml) for 72 hours. The amount of TNF-α produced was measured by ELISA. Data from two healthy volunteers are shown.

Figure 5 is a graph showing the effect of antibody 146B7 on IL-2 or IL-15-mediated TNF-α production. Human PBMC were incubated with hIL-15 (0, 50, 100 ng/ml) or with hIL-2 (100 ng/ml) in combination with 146B7 (0.1, 1, 10 μg/ml) for 72 hours. The amount of TNF-α produced was measured by ELISA.

Figure 6 is a graph showing the inhibitory activity of antibodies 146B7, 146H5, 404E4 and 404A8 on hIL-15 induced CTLL-2 proliferation.
CTLL-2 cells starved for hIL-2 were incubated with hIL-15 (60 pg/ml) combined with serial dilutions of 146B7, 146H5, 404E4 and 404A8 for 48 hours. [^{3}H]-Thymidine incorporation was measured to express proliferation (cpm). The results are presented as mean values.

Figures 7-9 include graphs showing the inhibitory activity of antibodies 146B7 (Figure 7), 404E4 (Figure 8) and 404A8 (Figure 9) on IL-15 induced PBMC proliferation. Human PBMC were incubated with hIL-15 (0, 25, 100 ng/ml; Figures 7A, 8A, and 9A, respectively) or hIL-2 (0, 10, 100 ng/ml; Figures 7B, 8B, and 9B, respectively) in combination with 146B7 (Figure 7), 404E4 (Figure 8) or 404A8 (Figure 9) at 0.1, 1, 10 μg/ml for 72 hours. [3H]-Thymidine incorporation was measured to express proliferation (cpm).

Figure 10 is a graph showing the binding of antibody 146B7 to IFNγ-stimulated monocytes. Human PBMCs were cultured in the presence of IFNγ (500 U/ml) for up to 2 days (37°C). Fluorescence intensity of at least 5000 cells per sample was determined after analysis by flow cytometry and gating on the monocytes. Data show the stimulation index (S.I. = (mean fluorescence positive staining)/(mean fluorescence background staining)).

Figure 11 shows binding of human monocytes with antibody 146B7 (panel B) or with the isotype control antibody (panel A). Human PBMCs were isolated and cytospins were made after culturing the cells with IFNγ (500U/ml). Cells were counterstained with haematoxylin.

Figure 12 shows binding of human psoriatic skin with 146B7 (panel B) or with the isotype control antibody (hIgG1) (panel A). Human psoriatic plaques were obtained from patients after informed consent, and stored at –80°C until assay. Tissues were stained with biotinylated antibodies and visualized after activation of horse radish peroxidase.

Figure 13A is a graph showing the percentage of nucleated cells in rheumatoid arthritic tissue after treatment of SCID mice with 146B7 or with vehicle. Tissues were stained with haematoxilin and eosin (H&E) and analysed with Photo Shop version 6.0. Data are shown as mean and s.e.m. of nuclei (as percentage of total area) of mice after 146B7 treatment (n=4) or vehicle treatment (n=2). *Figure 13B* shows a representative H&E staining of xenografted RA tissue in SCID mice, after treatment with 146B7 (panel B) or with PBS (panel A).

Figure 14 includes graphs showing the effects of antibody 146B7 treatment in SCID/psoriasis mice. Biopsies were fixed in formalin for paraffin embedding and stained in H&E and for Ki-67 nuclear antigen. *Figure 14A* shows the severity of psoriasis evaluated by epidermal thickness which was measured from the stratum corneum to the beginning of the rete pegs. *Figure 14B* shows the epidermal thickness which was measured from the stratum corneum to the deepest part of the rete

pegs. *Figure 14C* shows the grade of parakeratosis. *Figure 14D* shows the number of inflammatory mononuclear cells in upper dermis. *Figure 14E* shows the number of Ki-67+ cycling keratinocytes.

Figure 15 shows H&E staining of human psoriatic skin engrafted in SCID mice, after treatment with antibody 146B7 (panel C), with CsA (panel B), or with vehicle (panel A). Three weeks after transplantation mice received PBS (placebo), CsA (cyclosporine A) (Sandoz) at a dose of 10 mg/kg every second day for 15 days, or 146B7 at a dose of 20 mg/kg on day 1 and 10 mg/kg on days 8 and 15. One week after the last injection, mice were sacrificed, and a 4 mm punch biopsy was taken from each xenograft. Biopsies were fixed in formalin for paraffin embedding and stained in H&E.

Figure 16 shows Ki-67 staining of human psoriatic skin engrafted in SCID mice, after treatment with 146B7 (panel C), with CsA (panel B), or with vehicle (panel A). Three weeks after transplantation mice received PBS (placebo), CsA (cyclosporine A) (Sandoz) at a dose of 10 mg/kg every second day for 15 days, or 146B7 at a dose of 20 mg/kg on day 1 and 10 mg/kg on days 8 and 15. One week after the last injection, mice were sacrificed, and a 4 mm punch biopsy was taken from each xenograft. Biopsies were fixed in formalin for paraffin embedding and stained for Ki-67 nuclear antigen.

Figure 17 is a graph showing the binding of antibody 146B7 to receptor-bound IL-15. Plates were coated with IL-15Rα and incubated with IL-15. After 10 minutes, biotinylated 146B7 was added to the wells. Binding of 146B7 to receptor-bound IL-15 was evaluated at 405 nm in an ELISA-reader.

Figure 18 is a graph showings the binding of antibody 146B7 to IL-15, after binding of IL-15 to its receptor expressed on Raji cells. After incubation of IL-15R-expressing Raji cells with IL-15, biotinylated 146B7 was added to the cells after 10 minutes. Binding of 146B7 to receptor-bound IL-15 was evaluated by FACS analysis.

Detailed Description of the Invention

The present invention provides novel antibody-based therapeutics for treating and diagnosing a variety of disorders mediated by IL-15 (*i.e.*, disorders caused by the proinflammatory effects of IL-15). As used herein, the term "proinflammatory effects of IL-15" includes any humoral or cell-mediated immune response induced by IL-15, such as production of TNFα and other inflammatory mediators, and recruitment/proliferation of T-cells. Therapies of the invention employ isolated human monoclonal antibodies which specifically bind to an epitope present on IL-15.

In one embodiment, the human antibodies are produced in a non-human transgenic animal, *e.g.*, a transgenic mouse, capable of producing multiple isotypes of human monoclonal antibodies to IL-15 (*e.g.*, IgG, IgA and/or IgE) by undergoing V-D-J recombination and isotype switching. Accordingly, various aspects of the invention include antibodies and pharmaceutical compositions thereof, as well as non-human transgenic animals, B-cells, host cell transfectomas, and hybridomas for making such monoclonal antibodies. Methods of using the antibodies of the invention to detect cells to which IL-15 is bound, and/or to inhibit IL-15 mediated functions either *in vitro* or *in vivo*, are also encompassed by the invention. Methods for targeting agents to cells to which IL-15 is bound are also included.

In order that the present invention may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

The terms "IL-15," "IL-15 antigen" and "Interleukin 15" are used interchangeably herein, and include any variants or isoforms which are naturally expressed by cells.

The term "antibody" as referred to herein includes whole antibodies and any antigen binding fragment (*i.e.*, "antigen-binding portion") or single chain thereof. An "antibody" refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The V_H and V_L

regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system.

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, IL-15). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and CH1 domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a V_H domain; and (vi) an isolated complementarity determining region (CDR) or (vii) a combination of two or more isolated CDRs which may optionally be joined by a synthetic linker. Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

The term "monoclonal antibody" as used herein, refers to an antibody which displays a single binding specificity and affinity for a particular epitope. Accordingly, the term "human monoclonal antibody" refers to an antibody which displays a single binding specificity and which has variable and constant regions derived from human germline immunoglobulin sequences. In one embodiment, human monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic non-human animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

The term "recombinant human antibody", as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom (described further in Section I, below), (b) antibodies isolated from a host cell transformed to express the antibody, *e.g.*, from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial human antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

As used herein, a "heterologous antibody" is defined in relation to the transgenic non-human organism producing such an antibody. This term refers to an antibody having an amino acid sequence or an encoding nucleic acid sequence corresponding to that found in an organism not consisting of the transgenic non-human animal, and generally from a species other than that of the transgenic non-human animal.

An "isolated antibody", as used herein, is intended to refer to an antibody which is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds to IL-15 is substantially free of antibodies that specifically bind antigens other than IL-15). An isolated antibody that specifically binds to an epitope of IL-15 may, however, have cross-reactivity to other related cytokines or to other IL-15 proteins from different species. However, the antibody preferably always binds to human IL-15. In addition, an isolated antibody is typically substantially free of other cellular material and/or chemicals. In one embodiment of the invention, a combination of "isolated" monoclonal antibodies having different IL-15 specificities are combined in a well defined composition.

As used herein, "specific binding" refers to antibody binding to a predetermined antigen. Typically, the antibody binds with an affinity (K_D) of approximately less than 10^{-7} M, such as approximately less than 10^{-8} M, 10^{-9} M or 10^{-10} M or even lower when determined by surface plasmon resonance (SPR) technology in a BIACORE 3000 instrument using recombinant human IL-15 as the analyte and the antibody as the ligand, and binds to the predetermined antigen with an affinity that is at least two-fold greater than its affinity for binding to a non-specific antigen (*e.g.*, BSA, casein) other than the predetermined antigen or a closely-related antigen. The phrases "an antibody recognizing an antigen" and " an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen".

The term "K_D", as used herein, is intended to refer to the dissociation equilibrium constant of a particular antibody-antigen interaction.

As used herein, "isotype" refers to the antibody class (*e.g.*, IgM or IgGl) that is encoded by heavy chain constant region genes.

As used herein, "isotype switching" refers to the phenomenon by which the class, or isotype, of an antibody changes from one Ig class to one of the other Ig classes.

As used herein, "nonswitched isotype" refers to the isotypic class of heavy chain that is produced when no isotype switching has taken place; the CH gene encoding the nonswitched isotype is typically the first CH gene immediately downstream from the functionally rearranged VDJ gene. Isotype switching has been classified as classical or non-classical isotype switching. Classical isotype switching

occurs by recombination events which involve at least one switch sequence region in the transgene. Non-classical isotype switching may occur by, for example, homologous recombination between human σ_μ and human $\sum_\mu$ (δ-associated deletion). Alternative non-classical switching mechanisms, such as intertransgene and/or interchromosomal recombination, among others, may occur and effectuate isotype switching.

As used herein, the term "switch sequence" refers to those DNA sequences responsible for switch recombination. A "switch donor" sequence, typically a μ switch region, will be 5' (*i.e.*, upstream) of the construct region to be deleted during the switch recombination. The "switch acceptor" region will be between the construct region to be deleted and the replacement constant region (*e.g.*, γ, ε, etc.). As there is no specific site where recombination always occurs, the final gene sequence will typically not be predictable from the construct.

As used herein, "glycosylation pattern" is defined as the pattern of carbohydrate units that are covalently attached to a protein, more specifically to an immunoglobulin protein. A glycosylation pattern of a heterologous antibody can be characterized as being substantially similar to glycosylation patterns which occur naturally on antibodies produced by the species of the nonhuman transgenic animal, when one of ordinary skill in the art would recognize the glycosylation pattern of the heterologous antibody as being more similar to said pattern of glycosylation in the species of the nonhuman transgenic animal than to the species from which the CH genes of the transgene were derived.

The term "naturally-occurring" as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally-occurring.

The term "rearranged" as used herein refers to a configuration of a heavy chain or light chain immunoglobulin locus wherein a V segment is positioned immediately adjacent to a D-J or J segment in a conformation encoding essentially a complete V_H or V_L domain, respectively. A rearranged immunoglobulin gene locus can be identified by comparison to germline DNA; a rearranged locus will have at least one recombined heptamer/nonamer homology element.

The term "unrearranged" or "germline configuration" as used herein in reference to a V segment refers to the configuration wherein the V segment is not recombined so as to be immediately adjacent to a D or J segment.

The term "nucleic acid molecule", as used herein, is intended to include DNA molecules and RNA molecules. A nucleic acid molecule may be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term "isolated nucleic acid molecule", as used herein in reference to nucleic acids encoding antibodies or antibody portions (*e.g.*, V_H, V_L, CDR3) that bind to IL-15, is intended to refer to a nucleic acid molecule in which the nucleotide sequences encoding the antibody or antibody portion are free of other nucleotide sequences encoding antibodies or antibody portions that bind antigens other than IL-15, which other sequences may naturally flank the nucleic acid in human genomic DNA. SEQ ID NOS: 1-4 correspond to the nucleotide and amino acid sequences comprising the heavy chain (V_H) and light chain (V_L) variable regions of the human anti-IL-15 antibody 146B7 of the invention. In particular, SEQ ID NO:1 and 2 correspond to the V_H of the 146B7 antibody, SEQ ID NO:3 and 4 correspond to the V_L of the 146B7 antibody.

The present invention also encompasses "conservative sequence modifications" of the sequences set forth in SEQ ID NOs: 1-4, *i.e.*, nucleotide and amino acid sequence modifications which do not significantly affect or alter the binding characteristics of the antibody encoded by the nucleotide sequence or containing the amino acid sequence. Such conservative sequence modifications include nucleotide and amino acid substitutions, additions and deletions. Modifications can be introduced into SEQ ID NOs:1-4 by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions include ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a human

anti-IL-15 antibody is preferably replaced with another amino acid residue from the same side chain family.

Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a anti-IL-15 antibody coding sequence, such as by saturation mutagenesis, and the resulting modified anti-IL-15 antibodies can be screened for binding activity.

Accordingly, antibodies encoded by the (heavy and light chain variable region) nucleotide sequences disclosed herein and/or containing the (heavy and light chain variable region) amino acid sequences disclosed herein (*i.e.*, SEQ ID NOs: 1-4) include substantially similar antibodies encoded by or containing similar sequences which have been conservatively modified. Further discussion as to how such substantially similar antibodies can be generated based on the partial (*i.e.*, heavy and light chain variable regions) sequences disclosed herein as SEQ ID Nos:1-4 is provided below.

For nucleic acids, the term "substantial homology" indicates that two nucleic acids, or designated sequences thereof, when optimally aligned and compared, are identical, with appropriate nucleotide insertions or deletions, in at least about 80% of the nucleotides, usually at least about 90% to 95%, and more preferably at least about 98% to 99.5% of the nucleotides. Alternatively, substantial homology exists when the segments will hybridize under selective hybridization conditions, to the complement of the strand.

The percent identity between two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % homology = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

The percent identity between two nucleotide sequences can be determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. The percent identity between two nucleotide or amino acid sequences can also be determined using the algorithm of E.

Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to the nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to the protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov.

The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. A nucleic acid is "isolated" or "rendered substantially pure" when purified away from other cellular components or other contaminants, *e.g.*, other cellular nucleic acids or proteins, by standard techniques, including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis and others well known in the art. *See*, F. Ausubel, *et al.*, ed. Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York (1987).

The nucleic acid compositions of the present invention, while often in a native sequence (except for modified restriction sites and the like), from either cDNA, genomic or mixtures thereof may be mutated, in accordance with standard techniques to provide gene sequences. For coding sequences, these mutations, may affect amino acid

sequence as desired. In particular, DNA sequences substantially homologous to or derived from native V, D, J, constant, switches and other such sequences described herein are contemplated (where "derived" indicates that a sequence is identical or modified from another sequence).

A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence. With respect to transcription regulatory sequences, operably linked means that the DNA sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame. For switch sequences, operably linked indicates that the sequences are capable of effecting switch recombination.

The term "vector", as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The term "recombinant host cell" (or simply "host cell"), as used herein, is intended to refer to a cell into which a recombinant expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications

may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

As used herein, the term "subject" includes any human or non-human animal. For example, the methods and compositions of the present invention can be used to treat a subject with an inflammatory disease, such as arthritis, *e.g.*, rheumatoid arthritis. The term "non-human animal" includes all vertebrates, *e.g.*, mammals and non-mammals, such as non-human primates, sheep, dog, cow, chickens, amphibians, reptiles, etc.

Various aspects of the invention are described in further detail in the following subsections.

I. Production of Human Antibodies to IL-15

Human monoclonal antibodies of the invention can be produced using a variety of known techniques, such as the standard somatic cell hybridization technique described by Kohler and Milstein, Nature 256: 495 (1975). Although somatic cell hybridization procedures are preferred, in principle, other techniques for producing monoclonal antibodies also can be employed, *e.g.*, viral or oncogenic transformation of B lymphocytes, phage display technique using libraries of human antibody genes.

The preferred animal system for generating hybridomas which produce human monoclonal antibodies of the invention is the murine system. Hybridoma production in the mouse is well known in the art, including immunization protocols and techniques for isolating and fusing immunized splenocytes.

In one embodiment, human monoclonal antibodies directed against IL-15 are generated using transgenic or transchromosomal mice carrying parts of the human immune system rather than the mouse system. In one embodiment, the invention employs transgenic mice, referred to herein as "HuMAb mice" which contain a human immunoglobulin gene miniloci that encodes unrearranged human heavy (μ and γ) and κ light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and κ chain loci (Lonberg, N. *et al.* (1994) *Nature* 368(6474): 856-859). Accordingly, the mice exhibit reduced expression of mouse IgM or κ, and in response to immunization, the introduced human heavy and light chain transgenes

undergo class switching and somatic mutation to generate high affinity human IgGκ monoclonal antibodies (Lonberg, N. *et al.* (1994), *supra*; reviewed in Lonberg, N. (1994) *Handbook of Experimental Pharmacology* 113:49-101; Lonberg, N. and Huszar, D. (1995) *Intern. Rev. Immunol.* Vol. 13: 65-93, and Harding, F. and Lonberg, N. (1995) *Ann. N.Y. Acad. Sci* 764:536-546). The preparation of HuMAb mice is described in detail in Section II below and in Taylor, L. *et al.* (1992) *Nucleic Acids Research* 20:6287-6295; Chen, J. *et al.* (1993) *International Immunology* 5: 647-656; Tuaillon *et al.* (1993) *Proc. Natl. Acad. Sci USA* 90:3720-3724; Choi *et al.* (1993) *Nature Genetics* 4:117-123; Chen, J. *et al.* (1993) *EMBO J.* 12: 821-830; Tuaillon *et al.* (1994) *J. Immunol.* 152:2912-2920; Lonberg *et al.*, (1994) *Nature* 368(6474): 856-859; Lonberg, N. (1994) *Handbook of Experimental Pharmacology* 113:49-101; Taylor, L. *et al.* (1994) *International Immunology* 6: 579-591; Lonberg, N. and Huszar, D. (1995) *Intern. Rev. Immunol.* Vol. 13: 65-93; Harding, F. and Lonberg, N. (1995) *Ann. N.Y. Acad. Sci* 764:536-546; Fishwild, D. *et al.* (1996) *Nature Biotechnology* 14: 845-851. See further, U.S. Patent Nos. 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,877,397; 5,661,016; 5,814,318; 5,874,299; and 5,770,429; all to Lonberg and Kay, and GenPharm International; U.S. Patent No. 5,545,807 to Surani *et al.*; International Publication Nos. WO 98/24884, published on June 11, 1998; WO 94/25585, published November 10, 1994; WO 93/1227, published June 24, 1993; WO 92/22645, published December 23, 1992; WO 92/03918, published March 19, 1992. The preparation of HCO12 transgenic HuMAb mice, in particular, is described in Example 2.

Immunizations

To generate fully human monoclonal antibodies to IL-15, transgenic or transchromosomal mice containing human immunoglobulin genes (*e.g.*, HCo12, HCo7 or KM mice) can be immunized with a purified or enriched preparation of the IL-15 antigen and/or cells expressing IL-15, as described, for example, by Lonberg *et al.* (1994) *Nature* 368(6474): 856-859; Fishwild *et al.* (1996) *Nature Biotechnology* 14: 845-851 and WO 98/24884. Alternatively, mice can be immunized with DNA encoding human IL-15. Preferably, the mice will be 6-16 weeks of age upon the first infusion. For example, a purified or enriched preparation (5-50 μg) of the IL-15 antigen can be used to immunize the HuMAb mice intraperitoneally. In the event that immunizations using a purified or enriched preparation of the IL-15 antigen do not result in antibodies,

mice can also be immunized with cells expressing IL-15, *e.g.*, a cell line, to promote immune responses.

Cumulative experience with various antigens has shown that the HuMAb transgenic mice respond best when initially immunized intraperitoneally (IP) or subcutaneously (SC) with antigen in complete Freund's adjuvant, followed by every other week IP/SC immunizations (up to a total of 10) with antigen in incomplete Freund's adjuvant. The immune response can be monitored over the course of the immunization protocol with plasma samples being obtained by retroorbital bleeds. The plasma can be screened by ELISA (as described below), and mice with sufficient titers of anti-IL-15 human immunoglobulin can be used for fusions. Mice can be boosted intravenously with antigen 3 days before sacrifice and removal of the spleen.

Generation of Hybridomas Producing Human Monoclonal Antibodies to IL-15

To generate hybridomas producing human monoclonal antibodies to IL-15, splenocytes and lymph node cells from immunized mice can be isolated and fused to an appropriate immortalized cell line, such as a mouse myeloma cell line. The resulting hybridomas can then be screened for the production of antigen-specific antibodies. For example, single cell suspensions of splenic lymphocytes from immunized mice can be fused to SP2/0-Ag8.653 nonsecreting mouse myeloma cells (ATCC, CRL 1580) with 50% PEG (w/v). Cells can be plated at approximately 1 x 10^5 in flat bottom microtiter plate, followed by a two week incubation in selective medium containing besides usual reagents 10% fetal Clone Serum, 5-10% origen hybridoma cloning factor (IGEN) and 1X HAT (Sigma). After approximately two weeks, cells can be cultured in medium in which the HAT is replaced with HT. Individual wells can then be screened by ELISA for human anti-IL-15 monoclonal IgM and IgG antibodies. Once extensive hybridoma growth occurs, medium can be observed usually after 10-14 days. The antibody secreting hybridomas can be replated, screened again, and if still positive for human IgG, anti-IL-15 monoclonal antibodies can be subcloned at least twice by limiting dilution. The stable subclones can then be cultured *in vitro* to generate antibody in tissue culture medium for characterization.

Generation of Transfectomas Producing Human Monoclonal Antibodies to IL-15

Human antibodies of the invention also can be produced in a host cell transfectoma using, for example, a combination of recombinant DNA techniques and gene transfection methods as is well known in the art (Morrison, S. (1985) Science 229:1202).

For example, in one embodiment, the gene(s) of interest, *e.g.*, human antibody genes, can be ligated into an expression vector such as a eukaryotic expression plasmid such as used by GS gene expression system disclosed in WO 87/04462, WO 89/01036 and EP 338 841 or other expression systems well known in the art. The purified plasmid with the cloned antibody genes can be introduced in eukaryotic host cells such as CHO-cells or NSO-cells or alternatively other eukaryotic cells like a plant derived cells, fungi or yeast cells. The method used to introduce these genes could be methods described in the art such as electroporation, lipofectine, lipofectamine or other. After introducing these antibody genes in the host cells, cells expressing the antibody can be identified and selected. These cells represent the transfectomas which can then be amplified for their expression level and upscaled to produce antibodies. Recombinant antibodies can be isolated and purified from these culture supernatants and/or cells.

Alternatively these cloned antibody genes can be expressed in other expression systems such as *E. coli* or in complete organisms or can be synthetically expressed.

Use of Partial Antibody Sequences to Express Intact Antibodies

Antibodies interact with target antigens predominantly through amino acid residues that are located in the six heavy and light chain complementarity determining regions (CDRs). For this reason, the amino acid sequences within CDRs are more diverse between individual antibodies than sequences outside of CDRs. Because CDR sequences are responsible for most antibody-antigen interactions, it is possible to express recombinant antibodies that mimic the properties of specific naturally occurring antibodies by constructing expression vectors that include CDR sequences from the specific naturally occurring antibody grafted onto framework sequences from a different antibody with different properties (see, *e.g.*, Riechmann, L. *et al.*, 1998, *Nature* 332:323-327; Jones, P. *et al.*, 1986, *Nature* 321:522-525; and Queen, C. *et al.*, 1989, *Proc. Natl. Acad. See.* U.S.A. 86:10029-10033). Such framework

sequences can be obtained from public DNA databases that include germline antibody gene sequences. These germline sequences will differ from mature antibody gene sequences because they will not include completely assembled variable genes, which are formed by V(D)J joining during B cell maturation. Germline gene sequences will also differ from the sequences of a high affinity secondary repertoire antibody at individual evenly across the variable region. For example, somatic mutations are relatively infrequent in the amino-terminal portion of framework region. For example, somatic mutations are relatively infrequent in the amino terminal portion of framework region 1 and in the carboxy-terminal portion of framework region 4. Furthermore, many somatic mutations do not significantly alter the binding properties of the antibody. For this reason, it is not necessary to obtain the entire DNA sequence of a particular antibody in order to recreate an intact recombinant antibody having binding properties similar to those of the original antibody (see PCT/US99/05535 filed on March 12, 1999). Partial heavy and light chain sequence spanning the CDR regions is typically sufficient for this purpose. The partial sequence is used to determine which germline variable and joining gene segments contributed to the recombined antibody variable genes. The germline sequence is then used to fill in missing portions of the variable regions. Heavy and light chain leader sequences are cleaved during protein maturation and do not contribute to the properties of the final antibody. To add missing sequences, cloned cDNA sequences can be combined with synthetic oligonucleotides by ligation or PCR amplification. Alternatively, the entire variable region can be synthesized as a set of short, overlapping, oligonucleotides and combined by PCR amplification to create an entirely synthetic variable region clone. This process has certain advantages such as elimination or inclusion or particular restriction sites, or optimization of particular codons.

The nucleotide sequences of heavy and light chain transcripts from a hybridoma are used to design an overlapping set of synthetic oligonucleotides to create synthetic V sequences with identical amino acid coding capacities as the natural sequences. The synthetic heavy and kappa chain sequences can differ from the natural sequences in three ways: strings of repeated nucleotide bases are interrupted to facilitate oligonucleotide synthesis and PCR amplification; optimal translation initiation sites are incorporated according to Kozak's rules (Kozak, 1991, J. Biol. Chem. 266L19867019870); and, HindIII sites are engineered upstream of the translation initiation sites.

For both the heavy and light chain variable regions, the optimized coding, and corresponding non-coding, strand sequences are broken down into 30 – 50 nucleotide approximately the midpoint of the corresponding non-coding oligonucleotide. Thus, for each chain, the oligonucleotides can be assembled into overlapping double stranded sets that span segments of 150 – 400 nucleotides. The pools are then used as templates to produce PCR amplification products of 150 – 400 nucleotides. Typically, a single variable region oligonucleotide set will be broken down into two pools which are separately amplified to generate two overlapping PCR products. These overlapping products are then combined by PCR amplification to form the complete variable region. It may also be desirable to include an overlapping fragment of the heavy or light chain constant region (including the BbsI site of the kappa light chain, or the AgeI site if the gamma heavy chain) in the PCR amplification to generate fragments that can easily be cloned into the expression vector constructs.

The reconstructed heavy and light chain variable regions are then combined with cloned promoter, leader sequence, translation initiation, leader sequence, constant region, 3' untranslated, polyadenylation, and transcription termination, sequences to form expression vector constructs. The heavy and light chain expression constructs can be combined into a single vector, co-transfected, serially transfected, or separately transfected into host cells which are then fused to form a host cell expressing both chains.

Plasmids for use in construction of expression vectors for human IgGκ are described below (Example 1). The plasmids were constructed so that PCR amplified V heavy and V kappa light chain cDNA sequences could be used to reconstruct complete heavy and light chain minigenes. These plasmids can be used to express completely human $IgG_1\kappa$ or $IgG_4\kappa$ antibodies. Fully human and chimeric antibodies of the present invention also include IgG2, IgG3, IgE, IgA, IgM, and IgD antibodies. Similar plasmids can be constructed for expression of other heavy chain isotypes, or for expression of antibodies comprising lambda light chains.

Thus, in another aspect of the invention, the structural features of an human anti-IL-15 antibodies of the invention, 146B7, 147H5, 404A8 and 404E4, are used to create structurally related human anti-IL-15 antibodies that retain at least one functional property of the antibodies of the invention, such as binding to IL-15. More specifically, one or more CDR regions of 146B7, 147H5, 404A8 and 404E4 can be

combined recombinantly with known human framework regions and CDRs to create additional, recombinantly-engineered, human anti-IL-15 antibodies of the invention.

Accordingly, in another embodiment, the invention provides a method for preparing an anti-IL-15 antibody comprising:

preparing an antibody comprising (1) human heavy chain framework regions and human heavy chain CDRs, wherein at least one of the human heavy chain CDRs comprises an amino acid sequence selected from the amino acid sequences of CDRs shown in Figure 2 (or corresponding amino acid residues in SEQ ID NO: 2); and (2) human light chain framework regions and human light chain CDRs, wherein at least one of the human heavy chain CDRs comprises an amino acid sequence selected from the amino acid sequences of CDRs shown in Figure 3 (or corresponding amino acid residues in SEQ ID NO: 4);

wherein the antibody retains the ability to bind to IL-15.

The ability of the antibody to bind IL-15 can be determined using standard binding assays, such as those set forth in the Examples (*e.g.*, an ELISA).

Since it is well known in the art that antibody heavy and light chain CDR3 domains play a particularly important role in the binding specificity/affinity of an antibody for an antigen, the recombinant antibodies of the invention prepared as set forth above preferably comprise the heavy and light chain CDR3s of 146B7, 147H5, 404A8 and 404E4. The antibodies further can comprise the CDR2s of 146B7, 147H5, 404A8 and 404E4. The antibodies further can comprise the CDR1s 146B7, 147H5, 404A8 and 404E4. The antibodies can further comprise any combinations of the CDRs.

Accordingly, in another embodiment, the invention further provides anti-IL-15 antibodies comprising: (1) human heavy chain framework regions, a human heavy chain CDR1 region, a human heavy chain CDR2 region, and a human heavy chain CDR3 region, wherein the human heavy chain CDR3 region is selected from the CDR3s of 146B7, 147H5, 404A8 and 404E4, for example, a human heavy chain CDR region of 146B7 as shown in Figure 2 (or corresponding amino acid residues in SEQ ID NO: 2); and (2) human light chain framework regions, a human light chain CDR1 region, a human light chain CDR2 region, and a human light chain CDR3 region, wherein the human light chain CDR3 region is selected from the CDR3s of 146B7, 147H5, 404A8 and 404E4, for example, a human light chain CDR region of 146B7 as shown in Figure 3 (or corresponding amino acid residues in SEQ ID NO: 4), wherein the antibody binds

IL-15. The antibody may further comprise the heavy chain CDR2 and/or the light chain CDR2 of 146B7, 147H5, 404A8 and 404E4. The antibody may further comprise the heavy chain CDR1 and/or the light chain CDR1 of 146B7, 147H5, 404A8 and 404E4.

The CDR1, 2, and/or 3 regions of the engineered antibodies described above can comprise the exact amino acid sequence(s) as those of 146B7, 147H5, 404A8 and 404E4 disclosed herein. However, the ordinarily skilled artisan will appreciate that some deviation from the exact CDR sequences of 146B7, 147H5, 404A8 and 404E4 may be possible while still retaining the ability of the antibody to bind IL-15 effectively (*e.g.*, conservative sequence modifications). Accordingly, in another embodiment, the engineered antibody may be composed of one or more CDRs that are, for example, 90%, 95%, 98% or 99.5% identical to one or more CDRs of 146B7, 147H5, 404A8 and 404E4.

In addition to simply binding IL-15, engineered antibodies such as those described above may be selected for their retention of other functional properties of antibodies of the invention, such as:

(1) binding to human IL-15 and inhibiting IL-15 induced proinflammatory effects;

(2) inhibiting IL-15 induced TNFα production or T cell proliferation;

(3) binding to human IL-15 with a dissociation equilibrium constant (K_D) of less than approximately 10^{-7} M when determined by surface plasmon resonance (SPR) technology in a BIACORE 3000 instrument using recombinant human IL-15 as the analyte and the antibody as the ligand;

(4) binding to an epitope located on the β- and/or γ-chain interacting domain of human IL-15;

(5) interfering with the binding of Asp^8 of human IL-15 to the β-unit of the human IL-15 receptor and/or of Gln^{108} of human IL-15 to the γ-unit of human IL-15 receptor;

(6) binding to receptor-bound human IL-15;

(7) binding to human IL-15 and inhibiting the ability of human IL-15 to induce parakeratosis;

(8) binding to human IL-15 and inhibiting the ability of human IL-15 to induce epidermal thickening;

(9) binding to human IL-15 and inhibiting the ability of human IL-15 to induce proliferation of keratinocytes; and/or

(10) binding to human IL-15 and inhibiting the ability of human IL-15 to induce chemotaxis of activated leukocytes.

Characterization of Human Monoclonal Antibodies to IL-15

Human monoclonal antibodies of the invention can be characterized for binding to IL-15 using a variety of known techniques. Generally, the antibodies are initially characterized by ELISA. Briefly, microtiter plates can be coated with purified IL-15 in PBS, and then blocked with irrelevant proteins such as bovine serum albumin (BSA) diluted in PBS. Dilutions of plasma from IL-15-immunized mice are added to each well and incubated for 1-2 hours at 37°C. The plates are washed with PBS/Tween 20 and then incubated with a goat-anti-human IgG Fc-specific polyclonal reagent conjugated to alkaline phosphatase for 1 hour at 37°C. After washing, the plates are developed with ABTS substrate, and analyzed at OD of 405. Preferably, mice which develop the highest titers will be used for fusions.

An ELISA assay as described above can be used to screen for antibodies and, thus, hybridomas that produce antibodies that show positive reactivity with the IL-15 immunogen. Hybridomas that bind, preferably with high affinity, to IL-15 can than be subcloned and further characterized. One clone from each hybridoma, which retains the reactivity of the parent cells (by ELISA), can then be chosen for making a cell bank, and for antibody purification.

To purify human anti-IL-15 antibodies, selected hybridomas can be grown in roller bottles, two-liter spinner-flasks or other culture systems. Supernatants can be filtered and concentrated before affinity chromatography with protein A-sepharose (Pharmacia, Piscataway, NJ) to purify the protein. After buffer exchange to PBS, the concentration can be determined by OD_{280} using 1.43 extinction coefficient or preferably by nephelometric analysis. IgG can be checked by gel electrophoresis and by antigen specific method..

To determine if the selected human anti-IL-15 monoclonal antibodies bind to unique epitopes, each antibody can be biotinylated using commercially available reagents (Pierce, Rockford, IL).. Biotinylated MAb binding can be detected with a streptavidin labeled probe. To determine the isotype of purified antibodies, isotype

ELISAs can be performed using art recognized techniques. For example, wells of microtiter plates can be coated with 10 μg/ml of anti-human Ig overnight at 4°C. After blocking with 5% BSA, the plates are reacted with 10 μg/ml of monoclonal antibodies or purified isotype controls, at ambient temperature for two hours. The wells can then be reacted with either human IgGl or other human isotype specific conjugated probes. Plates are developed and analyzed as described above.

To test the binding of monoclonal antibodies to live cells expressing IL-15, flow cytometry can be used. Briefly, cell lines and/or human PBMCs expressing membrane-bound IL-15 (grown under standard growth conditions) are mixed with various concentrations of monoclonal antibodies in PBS containing 0.1% BSA and 0.01% NaN3 at 4°C for 1 hour. After washing, the cells are reacted with Fluorescein-labeled anti-human IgG antibody under the same conditions as the primary antibody staining. The samples can be analyzed by FACScan instrument using light and side scatter properties to gate on single cells and binding of the labeled antibodies is determined. An alternative assay using fluorescence microscopy may be used (in addition to or instead of) the flow cytometry assay. Cells can be stained exactly as described above and examined by fluorescence microscopy. This method allows visualization of individual cells, but may have diminished sensitivity depending on the density of the antigen.

Anti-IL-15 human IgGs can be further tested for reactivity with the IL-15 antigen by Western blotting. Briefly, cell extracts from cells expressing IL-15 can be prepared and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis. After electrophoresis, the separated antigens will be transferred to nitrocellulose membranes, blocked with 20% mouse serum, and probed with the monoclonal antibodies to be tested. Human IgG binding can be detected using anti-human IgG alkaline phosphatase and developed with BCIP/NBT substrate tablets (Sigma Chem. Co., St. Louis, MO).

II. Production of Transgenic and Transchromosomal Nonhuman Animals Which Generate Human Monoclonal Anti-IL-15 Antibodies

In yet another aspect, the invention provides transgenic and transchromosomal non-human animals, such as transgenic or transchromosomal mice, which are capable of expressing human monoclonal antibodies that specifically bind to

IL-15. In a particular embodiment, the invention provides a transgenic or transchromosomal mouse having a genome comprising a human heavy chain transgene, such that the mouse produces human anti-IL-15 antibodies when immunized with IL-15 antigen and/or cells expressing IL-15. The human heavy chain transgene can be integrated into the chromosomal DNA of the mouse, as is the case for transgenic, *e.g.*, HuMAb mice as described in detail herein and exemplified. Alternatively, the human heavy chain transgene can be maintained extrachromosomally, as is the case for transchromosomal (*e.g.*, KM) mice as described in WO 02/43478 (published June 6, 2002). Such transgenic and transchromosomal mice are capable of producing multiple isotypes of human monoclonal antibodies to IL-15 (*e.g.*, IgG, IgA and/or IgE) by undergoing V-D-J recombination and isotype switching. Isotype switching may occur by, *e.g.*, classical or non-classical isotype switching.

The design of a transgenic or transchromsomal non-human animal that responds to foreign antigen stimulation with a heterologous antibody repertoire, requires that the heterologous immunoglobulin transgenes contained within the transgenic animal function correctly throughout the pathway of B-cell development. This includes, for example, isotype switching of the heterologous heavy chain transgene. Accordingly, transgenes are constructed so as to produce isotype switching and one or more of the following of antibodies: (1) high level and cell-type specific expression, (2) functional gene rearrangement, (3) activation of and response to allelic exclusion, (4) expression of a sufficient primary repertoire, (5) signal transduction, (6) somatic hypermutation, and (7) domination of the transgene antibody locus during the immune response.

Not all of the foregoing criteria need be met. For example, in those embodiments wherein the endogenous immunoglobulin loci of the transgenic animal are functionally disrupted, the transgene need not activate allelic exclusion. Further, in those embodiments wherein the transgene comprises a functionally rearranged heavy and/or light chain immunoglobulin gene, the second criteria of functional gene rearrangement is unnecessary, at least for that transgene which is already rearranged. For background on molecular immunology, see, Fundamental Immunology, 2nd edition (1989), Paul William E., ed. Raven Press, N.Y.

In certain embodiments, the transgenic or transchromosomal non-human animals used to generate the human monoclonal antibodies of the invention contain rearranged, unrearranged or a combination of rearranged and unrearranged heterologous

immunoglobulin heavy and light chain transgenes in the germline of the transgenic animal. Each of the heavy chain transgenes comprises at least one C_H gene. In addition, the heavy chain transgene may contain functional isotype switch sequences, which are capable of supporting isotype switching of a heterologous transgene encoding multiple C_H genes in the B-cells of the transgenic animal. Such switch sequences may be those which occur naturally in the germline immunoglobulin locus from the species that serves as the source of the transgene C_H genes, or such switch sequences may be derived from those which occur in the species that is to receive the transgene construct (the transgenic animal). For example, a human transgene construct that is used to produce a transgenic mouse may produce a higher frequency of isotype switching events if it incorporates switch sequences similar to those that occur naturally in the mouse heavy chain locus, as presumably the mouse switch sequences are optimized to function with the mouse switch recombinase enzyme system, whereas the human switch sequences are not. Switch sequences may be isolated and cloned by conventional cloning methods, or may be synthesized *de novo* from overlapping synthetic oligonucleotides designed on the basis of published sequence information relating to immunoglobulin switch region sequences (Mills *et al.*, *Nucl. Acids Res.* 15:7305-7316 (1991); Sideras *et al.*, *Intl. Immunol.* 1:631-642 (1989)). For each of the foregoing transgenic animals, functionally rearranged heterologous heavy and light chain immunoglobulin transgenes are found in a significant fraction of the B-cells of the transgenic animal (at least 10 percent).

The transgenes used to generate the transgenic animals of the invention include a heavy chain transgene comprising DNA encoding at least one variable gene segment, one diversity gene segment, one joining gene segment and at least one constant region gene segment. The immunoglobulin light chain transgene comprises DNA encoding at least one variable gene segment, one joining gene segment and at least one constant region gene segment. The gene segments encoding the light and heavy chain gene segments are heterologous to the transgenic non-human animal in that they are derived from, or correspond to, DNA encoding immunoglobulin heavy and light chain gene segments from a species not consisting of the transgenic non-human animal. In one aspect of the invention, the transgene is constructed such that the individual gene segments are unrearranged, *i.e.*, not rearranged so as to encode a functional immunoglobulin light or heavy chain. Such unrearranged transgenes support recombination of the V, D, and J gene segments (functional rearrangement) and

preferably support incorporation of all or a portion of a D region gene segment in the resultant rearranged immunoglobulin heavy chain within the transgenic non-human animal when exposed to the IL-15 antigen.

In an alternate embodiment, the transgenes comprise an unrearranged "mini-locus". Such transgenes typically comprise a substantial portion of the C, D, and J segments as well as a subset of the V gene segments. In such transgene constructs, the various regulatory sequences, *e.g.* promoters, enhancers, class switch regions, splice-donor and splice-acceptor sequences for RNA processing, recombination signals and the like, comprise corresponding sequences derived from the heterologous DNA. Such regulatory sequences may be incorporated into the transgene from the same or a related species of the non-human animal used in the invention. For example, human immunoglobulin gene segments may be combined in a transgene with a rodent immunoglobulin enhancer sequence for use in a transgenic mouse. Alternatively, synthetic regulatory sequences may be incorporated into the transgene, wherein such synthetic regulatory sequences are not homologous to a functional DNA sequence that is known to occur naturally in the genomes of mammals. Synthetic regulatory sequences are designed according to consensus rules, such as, for example, those specifying the permissible sequences of a splice-acceptor site or a promoter/enhancer motif. For example, a minilocus comprises a portion of the genomic immunoglobulin locus having at least one internal (*i.e.*, not at a terminus of the portion) deletion of a non-essential DNA portion (*e.g.*, intervening sequence; intron or portion thereof) as compared to the naturally-occurring germline Ig locus.

In a preferred embodiment of the invention, the transgenic or transchromosomal animal used to generate human antibodies to IL-15 contains at least one, typically 2-10, and sometimes 25-50 or more copies of the transgene described in Example 12 of WO 98/24884 (*e.g.*, pHC1 or pHC2) bred with an animal containing a single copy of a light chain transgene described in Examples 5, 6, 8, or 14 of WO 98/24884, and the offspring bred with the J_H deleted animal described in Example 10 of WO 98/24884. Animals are bred to homozygosity for each of these three traits. Such animals have the following genotype: a single copy (per haploid set of chromosomes) of a human heavy chain unrearranged mini-locus (described in Example 12 of WO 98/24884), a single copy (per haploid set of chromosomes) of a rearranged human K light chain construct (described in Example 14 of WO 98/24884), and a deletion at each

endogenous mouse heavy chain locus that removes all of the functional J_H segments (described in Example 10 of WO 98/24884). Such animals are bred with mice that are homozygous for the deletion of the J_H segments (Examples 10 of WO 98/24884) to produce offspring that are homozygous for the J_H deletion and hemizygous for the human heavy and light chain constructs. The resultant animals are injected with antigens and used for production of human monoclonal antibodies against these antigens.

B cells isolated from such an animal are monospecific with regard to the human heavy and light chains because they contain only a single copy of each gene. Furthermore, they will be monospecific with regards to human or mouse heavy chains because both endogenous mouse heavy chain gene copies are nonfunctional by virtue of the deletion spanning the J_H region introduced as described in Example 9 and 12 of WO 98/24884. Furthermore, a substantial fraction of the B cells will be monospecific with regards to the human or mouse light chains because expression of the single copy of the rearranged human κ light chain gene will allelically and isotypically exclude the rearrangement of the endogenous mouse κ and lambda chain genes in a significant fraction of B-cells.

Transgenic and transchromsomal mice employed in the present invention exhibit immunoglobulin production with a significant repertoire, ideally substantially similar to that of a native mouse. Thus, for example, in embodiments where the endogenous Ig genes have been inactivated, the total immunoglobulin levels will range from about 0.1 to 10 mg/ml of serum, preferably 0.5 to 5 mg/ml, ideally at least about 1.0 mg/ml. When a transgene capable of effecting a switch to IgG from IgM has been introduced into the transgenic mouse, the adult mouse ratio of serum IgG to IgM is preferably about 10:1. The IgG to IgM ratio will be much lower in the immature mouse. In general, greater than about 10%, preferably 40 to 80% of the spleen and lymph node B cells express exclusively human IgG protein.

The repertoire will ideally approximate that shown in a native mouse, usually at least about 10% as high, preferably 25 to 50% or more. Generally, at least about a thousand different immunoglobulins (ideally IgG), preferably 10^4 to 10^6 or more, will be produced, depending primarily on the number of different V, J and D regions introduced into the mouse genome. These immunoglobulins will typically recognize about one-half or more of highly antigenic proteins, e.g., staphylococcus

protein A. Typically, the immunoglobulins will exhibit an affinity (K_D) for preselected antigens of below 10^{-7} M, such as of below 10^{-8} M, 10^{-9} M or 10^{-10} M or even lower.

In some embodiments, it may be preferable to generate mice with predetermined repertoires to limit the selection of V genes represented in the antibody response to a predetermined antigen type. A heavy chain transgene having a predetermined repertoire may comprise, for example, human V_H genes which are preferentially used in antibody responses to the predetermined antigen type in humans. Alternatively, some V_H genes may be excluded from a defined repertoire for various reasons (*e.g.*, have a low likelihood of encoding high affinity V regions for the predetermined antigen; have a low propensity to undergo somatic mutation and affinity sharpening; or are immunogenic to certain humans). Thus, prior to rearrangement of a transgene containing various heavy or light chain gene segments, such gene segments may be readily identified, *e.g.* by hybridization or DNA sequencing, as being from a species of organism other than the transgenic animal.

Transgenic and transchromosomal mice as described above can be immunized with, for example, a purified or enriched preparation of IL-15 antigen and/or cells expressing IL-15. Alternatively, the transgenic mice can be immunized with DNA encoding human IL-15. The mice will then produce B cells which undergo class-switching via intratransgene switch recombination (cis-switching) and express immunoglobulins reactive with IL-15. The immunoglobulins can be human antibodies (also referred to as "human sequence antibodies"), wherein the heavy and light chain polypeptides are encoded by human transgene sequences, which may include sequences derived by somatic mutation and V region recombinatorial joints, as well as germline-encoded sequences; these human antibodies can be referred to as being substantially identical to a polypeptide sequence encoded by a human V_L or V_H gene segment and a human J_L or D_H and J_H segment, even though other non-germline sequences may be present as a result of somatic mutation and differential V-J and V-D-J recombination joints. The variable regions of each antibody chain are typically at least 80 percent encoded by human germline V, J, and, in the case of heavy chains, D, gene segments; frequently at least 85 percent of the variable regions are encoded by human germline sequences present on the transgene; often 90 or 95 percent or more of the variable region sequences are encoded by human germline sequences present on the transgene. However, since non-germline sequences are introduced by somatic mutation and VJ and

VDJ joining, the human sequence antibodies will frequently have some variable region sequences (and less frequently constant region sequences) which are not encoded by human V, D, or J gene segments as found in the human transgene(s) in the germline of the mice. Typically, such non-germline sequences (or individual nucleotide positions) will cluster in or near CDRs, or in regions where somatic mutations are known to cluster.

Human antibodies which bind to the predetermined antigen can result from isotype switching, such that human antibodies comprising a human sequence γ chain (such as γl, γ2a, γ2B, or γ3) and a human sequence light chain (such as kappa) are produced. Such isotype-switched human antibodies often contain one or more somatic mutation(s), typically in the variable region and often in or within about 10 residues of a CDR) as a result of affinity maturation and selection of B cells by antigen, particularly subsequent to secondary (or subsequent) antigen challenge. These high affinity human antibodies may have binding affinities (K_D) of below 10^{-7} M, such as of below 10^{-8} M, 10^{-9} M or 10^{-10} M or even lower.

Another aspect of the invention includes B cells derived from transgenic or transchromosomal mice as described herein. The B cells can be used to generate hybridomas expressing human monoclonal antibodies which bind with high affinity (*e.g.*, lower than 10^{-7} M) to human IL-15. Thus, in another embodiment, the invention provides a hybridoma which produces a human antibody having an affinity (K_D) of below 10^{-7} M, such as of below 10^{-8} M, 10^{-9} M or 10^{-10} M or even lower when determined by surface plasmon resonance (SPR) technology in a BIACORE 3000 instrument using recombinant human IL-15 as the analyte and the antibody as the ligand for binding human IL-15, wherein the antibody comprises:

a human sequence light chain composed of (1) a light chain variable region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human V_L gene segment and a human J_L segment, and (2) a light chain constant region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human C_L gene segment; and

a human sequence heavy chain composed of a (1) a heavy chain variable region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human V_H gene segment, optionally a D region, and a human J_H

segment, and (2) a constant region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human C_H gene segment.

The development of high affinity human monoclonal antibodies against IL-15 can be facilitated by a method for expanding the repertoire of human variable region gene segments in a transgenic mouse having a genome comprising an integrated human immunoglobulin transgene, said method comprising introducing into the genome a V gene transgene comprising V region gene segments which are not present in said integrated human immunoglobulin transgene. Often, the V region transgene is a yeast artificial chromosome comprising a portion of a human V_H or V_L (V_K) gene segment array, as may naturally occur in a human genome or as may be spliced together separately by recombinant methods, which may include out-of-order or omitted V gene segments. Often at least five or more functional V gene segments are contained on the YAC. In this variation, it is possible to make a transgenic mouse produced by the V repertoire expansion method, wherein the mouse expresses an immunoglobulin chain comprising a variable region sequence encoded by a V region gene segment present on the V region transgene and a C region encoded on the human Ig transgene. By means of the V repertoire expansion method, transgenic mice having at least 5 distinct V genes can be generated; as can mice containing at least about 24 V genes or more. Some V gene segments may be non-functional (*e.g.*, pseudogenes and the like); these segments may be retained or may be selectively deleted by recombinant methods available to the skilled artisan, if desired.

Once the mouse germline has been engineered to contain a functional YAC having an expanded V segment repertoire, substantially not present in the human Ig transgene containing the J and C gene segments, the trait can be propagated and bred into other genetic backgrounds, including backgrounds where the functional YAC having an expanded V segment repertoire is bred into a mouse germline having a different human Ig transgene. Multiple functional YACs having an expanded V segment repertoire may be bred into a germline to work with a human Ig transgene (or multiple human Ig transgenes). Although referred to herein as YAC transgenes, such transgenes when integrated into the genome may substantially lack yeast sequences, such as sequences required for autonomous replication in yeast; such sequences may optionally be removed by genetic engineering (*e.g.*, restriction digestion and pulsed-field gel electrophoresis or other suitable method) after replication in yeast is no longer

necessary (*i.e.*, prior to introduction into a mouse ES cell or mouse prozygote). Methods of propagating the trait of human sequence immunoglobulin expression, include breeding a transgenic mouse having the human Ig transgene(s), and optionally also having a functional YAC having an expanded V segment repertoire. Both V_H and V_L gene segments may be present on the YAC. The transgenic mouse may be bred into any background desired by the practitioner, including backgrounds harboring other human transgenes, including human Ig transgenes and/or transgenes encoding other human lymphocyte proteins. The invention also provides a high affinity human sequence immunoglobulin produced by a transgenic mouse having an expanded V region repertoire YAC transgene. Although the foregoing describes a preferred embodiment of the transgenic animal of the invention, other embodiments are contemplated which have been classified in four categories:

I. Transgenic animals containing an unrearranged heavy and rearranged light immunoglobulin transgene;

II. Transgenic animals containing an unrearranged heavy and unrearranged light immunoglobulin transgene;

III. Transgenic animal containing rearranged heavy and an unrearranged light immunoglobulin transgene; and

IV. Transgenic animals containing rearranged heavy and rearranged light immunoglobulin transgenes.

Of these categories of transgenic animal, the preferred order of preference is as follows II > I > III > IV where the endogenous light chain genes (or at least the K gene) have been knocked out by homologous recombination (or other method) and I > II > III >IV where the endogenous light chain genes have not been knocked out and must be dominated by allelic exclusion.

III. Antibody Conjugates/Immunotoxins

In another aspect, the present invention features a human anti-IL-15 monoclonal antibody conjugated to a therapeutic moiety, such as a cytotoxin, a drug (*e.g.*, an immunosuppressant) or a radioisotope. When conjugated to a cytotoxin, these antibody conjugates are referred to as "immunotoxins." A cytotoxin or cytotoxic agent includes any agent that is detrimental to (*e.g.*, kills) cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide,

tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine). An antibody of the present invention can be conjugated to a radioisotope, *e.g.*, radioactive iodine, to generate cytotoxic radiopharmaceuticals for treating a IL-15-related disorder, such as a cancer.

The antibody conjugates of the invention can be used to modify a given biological response. The therapeutic moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, an enzymatically active toxin, or active fragment thereof, such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor or interferon-γ; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other cytokines or growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in

Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982).

IV. Pharmaceutical Compositions

In another aspect, the present invention provides a composition, *e.g.*, a pharmaceutical composition, containing one or a combination of human monoclonal antibodies, or antigen-binding portion(s) thereof, of the present invention, formulated together with a pharmaceutically acceptable carrier. In a preferred embodiment, the compositions include a combination of multiple (*e.g.*, two or more) isolated human antibodies of the invention. Preferably, each of the antibodies of the composition binds to a distinct, pre-selected epitope of IL-15.

Pharmaceutical compositions of the invention also can be administered in combination therapy, *i.e.*, combined with other agents. For example, the combination therapy can include a composition of the present invention with at least one or more additional therapeutic agents, such as anti-inflammatory agents, DMARDs (disease-modifying anti-rheumatic drugs), immunosuppressive agents, chemotherapeutics, and psoriasis agents. The pharmaceutical compositions of the invention can also be administered in conjunction with radiation therapy. Co-administration with other antibodies, such as CD4 specific antibodies and IL-2 specific antibodies, are also encompassed by the invention. Such combinations with CD4 specific antibodies or IL-2 specific antibodies are considered particularly useful for treating autoimmune diseases and transplant rejections.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (*e.g.*, by injection or infusion). Depending on the route of administration, the active compound, *i.e.*, antibody, bispecific and multispecific molecule, may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

A "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired

toxicological effects (see *e.g.*, Berge, S.M., *et al.* (1977) J. Pharm. Sci. 66:1-19). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

A composition of the present invention can be administered by a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. *See, e.g., Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

To administer a compound of the invention by certain routes of administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation. For example, the compound may be administered to a subject in an appropriate carrier, for example, liposomes, or a diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes (Strejan *et al.* (1984) *J. Neuroimmunol.* 7:27).

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is

incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Dosage regimens are adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. For example, the human antibodies of the invention may be administered once or twice weekly by subcutaneous injection or once or twice monthly by subcutaneous injection.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein

refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

For the therapeutic compositions, formulations of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated, and the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 0.001 per cent to about ninety percent of active ingredient, preferably from about 0.005 per cent to about 70 per cent, most preferably from about 0.01 per cent to about 30 per cent.

Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate. Dosage forms for the topical or transdermal administration of compositions of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a

pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, supra, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

When the compounds of the present invention are administered as pharmaceuticals, to humans and animals, they can be given alone or as a pharmaceutical composition containing, for example, 0.001 to 90% (more preferably, 0.005 to 70%, such as 0.01 to 30%) of active ingredient in combination with a pharmaceutically acceptable carrier.

Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into

pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts. A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, a suitable daily dose of a compositions of the invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. It is preferred that administration be intravenous, intramuscular, intraperitoneal, or subcutaneous, preferably administered proximal to the site of the target. If desired, the effective daily dose of a therapeutic compositions may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. While it is possible for a compound of the present invention to be administered alone, it is preferable to administer the compound as a pharmaceutical formulation (composition).

Therapeutic compositions can be administered with medical devices known in the art. For example, in a preferred embodiment, a therapeutic composition of the invention can be administered with a needleless hypodermic injection device, such as the devices disclosed in U.S. Patent Nos. 5,399,163, 5,383,851, 5,312,335, 5,064,413,

4,941,880, 4,790,824, or 4,596,556. Examples of well-known implants and modules useful in the present invention include: U.S. Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4.,486,194, which discloses a therapeutic device for administering medicants through the skin; U.S. Patent No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Patent No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and U.S. Patent No. 4,475,196, which discloses an osmotic drug delivery system. Many other such implants, delivery systems, and modules are known to those skilled in the art.

In certain embodiments, the human monoclonal antibodies of the invention can be formulated to ensure proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of the invention cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, *e.g.*, U.S. Patents 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (*see, e.g.*, V.V. Ranade (1989) *J. Clin. Pharmacol.* 29:685). Exemplary targeting moieties include folate or biotin (see, *e.g.*, U.S. Patent 5,416,016 to Low *et al.*); mannosides (Umezawa *et al.*, (1988) *Biochem. Biophys. Res. Commun.* 153:1038); antibodies (P.G. Bloeman *et al.* (1995) *FEBS Lett.* 357:140; M. Owais *et al.* (1995) *Antimicrob. Agents Chemother.* 39:180); surfactant protein A receptor (Briscoe *et al.* (1995) *Am. J. Physiol.* 1233:134), different species of which may comprise the formulations of the inventions, as well as components of the invented molecules; p120 (Schreier *et al.* (1994) *J. Biol. Chem.* 269:9090); see also K. Keinanen; M.L. Laukkanen (1994) *FEBS Lett.* 346:123; J.J. Killion; I.J. Fidler (1994) *Immunomethods* 4:273. In one embodiment of the invention, the therapeutic compounds of the invention are formulated in liposomes; in a more preferred embodiment, the liposomes include a targeting moiety. In a most preferred embodiment, the therapeutic compounds in the liposomes are delivered by bolus injection to a site proximal to the tumor or infection. The composition must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be

preserved against the contaminating action of microorganisms such as bacteria and fungi.

A "therapeutically effective dosage" for rheumatoid arthritis preferably will result in an ACR20 Preliminary Definition of Improvement in the patients, more preferred in an ACR50 Preliminary Definition of Improvement and even more preferred in an ARCD70 Preliminary Definition of Improvement.

ACR20 Preliminary Definition of Improvement is defined as: ≥ 20% improvement in: Tender Joint Count (TCJ) and Swollen Joint Count (SWJ) and ≥ 20% improvement in 3 of following 5 assessments: Patient Pain Assessment (VAS), Patient Global assessment (VAS), Physician Global Assessment (VAS), Patent Self-Assessed Disability (HAQ), Acute Phase Reactant (CRP or ESR).

ACR50 and ACR70 are defined in the same way with ≥ 50% and ≥ 70% improvements, respectively. For further details see Felson *et al.* in American College of Rheumatology Preliminary Definition of Improvement in Rheumatoid Arthritis; Arthritis Rheumatism (1995) 38: 727-735.

The ability of a compound to inhibit cancer can be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit, such inhibition *in vitro* by assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound can decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

The ability of the antibodies to treat or prevent psoriasis can also be evaluated according to methods well known in the art.

The composition must be sterile and fluid to the extent that the composition is deliverable by syringe. In addition to water, the carrier can be an isotonic buffered saline solution, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. Proper fluidity can be maintained, for example, by use of coating such as lecithin, by maintenance of required particle size in the case of dispersion and by use of surfactants. In many cases, it is preferable to include isotonic agents, for example, sugars,

polyalcohols such as mannitol or sorbitol, and sodium chloride in the composition. Long-term absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

When the active compound is suitably protected, as described above, the compound may be orally administered, for example, with an inert diluent or an assimilable edible carrier.

V. Uses and Methods of the Invention

Human anti-IL-15 antibodies to IL-15 of the present invention (including derivatives and conjugates of the antibodies) and compositions containing the antibodies can be used in a variety of *in vitro* and *in vivo* diagnostic and therapeutic applications.

In one embodiment, human antibodies of the invention are used to inhibit IL-15 induced TNFα production by T cells and/or monocytes/macrophages, preferably without inhibiting TNFα production induced by other cytokines, such as IL-2. By contacting the antibody with IL-15 (*e.g.*, by administering the antibody to a subject), the ability of IL-15 to signal through the IL-15 receptor is inhibited and, thus, the production of TNFα by T-cells and/or monocytes/macrophages also is inhibited. Preferred antibodies bind to epitopes (*e.g.*, particular subunits, such as the gamma subunit) which are specific to IL-15 and, thus, advantageously inhibit IL-15-induced TNFα production, but do not interfere with TNFα production by structurally related cytokines, such as IL-2.

In another embodiment, human antibodies of the invention are used to inhibit IL-15 induced T cell recruitment and/or proliferation, preferably without inhibiting T cell proliferation induced by other structurally related cytokines, such as IL-2. As with TNFα production, by contacting the antibody with IL-15 (*e.g.*, by administering the antibody to a subject), the ability of IL-15 to signal through the IL-15 receptor is inhibited and, thus, T cell stimulation by IL-15 is inhibited.

Accordingly, in yet another embodiment, the present invention provides a method for treating or preventing a disorder mediated by IL-15 (*e.g.*, an autoimmune disease, such as psoriasis, rheumatoid arthritis, or inflammatory bowel disease, or an infectious disease, such as HIV), by administering to a subject a human antibody of the

invention in an amount effective to treat or prevent the disorder. The antibody can be administered alone or along with another therapeutic agent, such as an anti-inflammatory agent, *e.g.*, a steroidal or nonsteroidal inflammatory agent, or a cytotoxin which acts in conjunction with or synergistically with the antibody to treat or prevent the IL-15 mediated disease.

In a particular embodiment, human antibodies of the present invention are used to treat or to prevent rheumatoid arthritis (RA). The antibodies limit the role that IL-15 plays in the progression of inflammation associated with diseases such as RA. T cells, particularly CD4+ T-helper cells, are involved in the initiation and maintenance of inflammatory processes in RA. TNF-α, another cytokine, is also involved in the inflammatory pathways which ultimately lead to joint destruction and incapacitation of the patient with RA. Local synthesis of IL-15 plays a key role both in the activation and recruitment of T cells and in the induction of TNF-α and other inflammatory cytokines. The role of IL-15 in the progression of RA involves a process whereby IL-15, which is synthesized by macrophages, induces T cell recruitment. The activated T cells then: (1) maintain macrophage activation; and (2) induce TNF-α production. Stimulated macrophages promote the synthesis of more IL-15 and T cell activation, thus, continuing the cycle. In addition to its effects on TNF-α and macrophages, IL-15 also activates neutrophils and affects local B cell immunoglobulin secretion, particularly rheumatoid factor synthesis.

Accordingly, anti-IL-15 antibodies of the invention can be used to prevent or block the foregoing effects of IL-15 which cause RA and, thus, can be used to prevent or treat this disease. For example, anti-IL-15 antibodies of the invention can be used to inhibit inflammation and/or prevent chemotaxis of activated leukocytes involved in RA.

The human antibodies of the present invention may be used for inhibition of progression of structural damage in patients with rheumatoid arthritis who have had an inadequate response to methotrexate or for reducing sign and symptoms and delaying structural damage in patients with moderately to severely active rheumatoid arthritis, including those who have not previously failed treatment with a DMARD.

Human antibodies of the present invention also can be used to block or inhibit other effects of IL-15. IL-15 is expressed in various cells and tissues including

monocytes and macrophages, fibroblasts, dendritic cells, and keratinocytes. Keratinocytes are major constituents of the epidermis and the epithelial lining of mucosal tissue. Control of keratinocyte growth is mediated by a complex network of cytokines and growth factors, some of which are produced by keratinocytes themselves. Keratinocyte-derived IL-15 contributes to T cell accumulation, proliferation, and survival in psoriatic plaques. Many diseases are known wherein the number of keratinocytes is increased which leads to epidermal hyperplasia which is responsible for at least some of the related disease symptoms. These diseases include chronic diseases such as psoriasis and atopic dermatitis, as well as conditions like chronic hand eczema, contact dermatitis, viral warts (HPV associated), cutaneous T cell lymphoma, impaired wound healing, such as impaired wound healing due to diabetes. Accordingly, the invention provides methods for treating or preventing such disorders by administering to patients a human anti-IL-15 antibody of the invention in an amount effective to treat or prevent the disorder. For example, anti-IL-15 antibodies of the invention can be used to block or inhibit parakeratosis in psoriasis, reduce epidermal thickness in psoriasis, and reduce proliferation of keratinocytes in psoriasis.

IL-15 also modulates the function of intestinal epithelial cells (Reinecker, *et al.* (1996) Gastroenterology 111:1706-13). Specifically, IL-15 can cause modifications on mucosal epithelial cells and on intestinal epithelial cell lines and, therefore, is involved in the pathogenesis of inflammatory bowel disease, *e.g.*, celiac disease. The role of IL-15 in such diseases is shown by the selective over-representation of IL-15+ cells in the small intestine of untreated patients with celiac disease (WO 00/02582). Thus, it has been shown that IL-15 is directly involved in the initiation and maintenance of celiac disease. Accordingly, in another embodiment, anti-IL-15 human antibodies of the present invention (*i.e.*, which inhibit the proinflammatory effects of IL-15) can be used to treat and/or to prevent celiac disease by administering the antibody to a patient in an amount effective to treat or prevent the disorder.

In addition, it has been found by the inventors of the present invention that IL-15 also promotes the formation of new blood vessels, a process called neovascularization or angiogenesis. Accordingly, yet another use for the antibodies of the invention includes the prevention or treatment of diseases involving neovascularization. These diseases include a variety of cancers which rely on or are characterized by neovascularization, in addition to inflammatory diseases.

Human antibodies of the present invention also can be used to block or inhibit the effects of IL-15 associated with infectious diseases, such as HIV. Accordingly, another use for the antibodies of the invention includes the prevention or treatment of infectious diseases, *e.g.*, HIV-1.

For example, the antibodies can be used *in vitro* or *in vivo* to diagnose a variety of diseases mediated by IL-15. Specifically, the antibodies can be used to detect levels of IL-15, or levels of cells which contain IL-15 on their membrane surface or linked to their receptors (receptor-bound human IL-15). The detection of such levels of IL-15 can then be correlated to certain disease symptoms. Alternatively, the antibodies can be used to inhibit or block IL-15 function which, in turn, can prevent or ameliorate disease symptoms caused by IL-15 function.

As previously described, human anti-IL-15 antibodies of the invention can be co-administered with one or other more therapeutic agents, *e.g.*, an immunosuppressive agent or an anti-inflammatory agent to increase the overall anti-inflammatory effect. The antibody can be linked to the agent (as an immunocomplex) or can be administered separate from the agent. In the latter case (separate administration), the antibody can be administered before, after or concurrently with the agent. Suitable therapeutic agents include, among others, anti-inflammatory agents, DMARDs (disease-modifying anti-rheumatic drugs), immunosuppressive agents, chemotherapeutics, and psoriasis agents. The human antibodies according to the invention can also be administered in conjunction with radiation therapy.

In another embodiment, the human antibodies of the invention can be administered in combination with other antibodies, such as CD4 specific antibodies and IL-2 specific antibodies. A combination of the present human antibodies with CD4 specific antibodies or IL-2 specific antibodies are considered particularly useful for treating autoimmune diseases and transplant rejections.

Also within the scope of the present invention are kits comprising human anti-IL-15 antibodies of the invention and, optionally, instructions for use. The kit can further contain one ore more additional reagents, such as an immunosuppressive reagent, or one or more additional human antibodies of the invention (*e.g.*, a human antibody having a complementary activity which binds to an epitope in the IL-15 antigen distinct from the first human antibody).

Accordingly, patients treated with antibodies of the invention can be additionally administered (prior to, simultaneously with, or following administration of a human antibody of the invention) with another therapeutic agent, such as an anti-inflammatory agent, which enhances or augments the therapeutic effect of the human antibodies.

In yet another embodiment, human antibodies of the invention can be used to target compounds (*e.g.*, therapeutic agents, labels, cytotoxins, immunosuppressants etc.) to cells which have IL-15 bound to their surface (*e.g.*, membrane bound or bound to IL-15 receptor by linking such compounds to the antibody. Thus, the invention also provides methods for localizing *ex vivo*, *in vivo* or *in vitro* cells expressing IL-15 and IL-15 receptor (*e.g.*, with a detectable label, such as a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor).

Other embodiments of the present invention are described in the following Examples.

The present invention is further illustrated by the following examples which should not be construed as further limiting. The contents of Sequence Listing, figures and all references, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

EXAMPLES

Example 1 Generation of Cmu targeted mice

Construction of a CMD targeting vector

The plasmid pICEmu contains an EcoRI/XhoI fragment of the murine Ig heavy chain locus, spanning the mu gene, that was obtained from a Balb/C genomic lambda phage library (Marcu *et al.* Cell 22: 187, 1980). This genomic fragment was subcloned into the XhoI/EcoRI sites of the plasmid pICEMI9H (Marsh et al; Gene 32, 481-485, 1984). The heavy chain sequences included in pICEmu extend downstream of the EcoRI site located just 3' of the mu intronic enhancer, to the XhoI site located approximately 1 kb downstream of the last transmembrane exon of the mu gene; however, much of the mu switch repeat region has been deleted by passage in *E. coli*.

The targeting vector was constructed as follows. A 1.3 kb HindIII/SmaI fragment was excised from pICEmu and subcloned into HindIII/SmaI digested pBluescript (Stratagene, La Jolla, CA). This pICEmu fragment extends from the HindIII site located approximately 1 kb 5' of Cmu1 to the SmaI site located within Cmu1. The resulting plasmid was digested with SmaI/SpeI and the approximately 4 kb SmaI/XbaI fragment from pICEmu, extending from the Sma I site in Cmu1 3' to the XbaI site located just downstream of the last Cmu exon, was inserted. The resulting plasmid, pTAR1, was linearized at the SmaI site, and a neo expression cassette inserted. This cassette consists of the neo gene under the transcriptional control of the mouse phosphoglycerate kinase (pgk) promoter (XbaI/TaqI fragment; Adra *et al.* (1987) Gene 60: 65-74) and containing the pgk polyadenylation site (PvuII/HindIII fragment; Boer *et al.* (1990) Biochemical Genetics 28: 299-308). This cassette was obtained from the plasmid pKJ1 (described by Tybulewicz *et al.* (1991) Cell 65: 1153-1163) from which the neo cassette was excised as an EcoRI/HindIII fragment and subcloned into EcoRI/HindIII digested pGEM-7Zf(+) to generate pGEM-7 (KJ1). The neo cassette was excised from pGEM-7 (KJ1) by EcoRI/SalI digestion, blunt ended and subcloned into the SmaI site of the plasmid pTAR1, in the opposite orientation of the genomic Cmu sequences. The resulting plasmid was linearized with Not I, and a herpes simplex virus thymidine kinase (tk) cassette was inserted to allow for enrichment of ES clones bearing homologous recombinants, as described by Mansour *et al.* (1988) Nature 336: 348-352. This cassette consists of the coding sequences of the tk gene bracketed by the mouse pgk promoter and polyadenylation site, as described by Tybulewicz *et al.* (1991) Cell 65: 1153-1163. The resulting CMD targeting vector contains a total of approximately 5.3 kb of homology to the heavy chain locus and is designed to generate a mutant mu gene into which has been inserted a neo expression cassette in the unique SmaI site of the first Cmu exon. The targeting vector was linearized with PvuI, which cuts within plasmid sequences, prior to electroporation into ES cells.

Generation and analysis of targeted ES cells

AB-1 ES cells (McMahon, A. P. and Bradley, A., (1990) Cell 62: 1073-1085) were grown on mitotically inactive SNL76/7 cell feeder layers (ibid.) essentially as described (Robertson, E. J. (1987) in Teratocarcinomas and Embryonic Stem Cells: a Practical Approach (E. J. Robertson, ed.) Oxford: IRL Press, p. 71-112). The linearized

CMD targeting vector was electroporated into AB-1 cells by the methods described Hasty *et al.* (Hasty, P. R. *et al.* (1991) Nature 350: 243-246). Electroporated cells were plated into 100 mm dishes at a density of 1-2 x 10^6 cells/dish. After 24 hours, G418 (200 micrograms/ml of active component) and FIAU (5 x 10^{-7} M) were added to the medium, and drug-resistant clones were allowed to develop over 8-9 days. Clones were picked, trypsinized, divided into two portions, and further expanded. Half of the cells derived from each clone were then frozen and the other half analyzed for homologous recombination between vector and target sequences.

DNA analysis was carried out by Southern blot hybridization. DNA was isolated from the clones as described by Laird *et al.* (Laird, P. W. *et al.*, (1991) Nucleic Acids Res. 19 : 4293). Isolated genomic DNA was digested with SpeI and probed with a 915 bp SacI fragment, probe A (see Figure 1), which hybridizes to a sequence between the mu intronic enhancer and the mu switch region. Probe A detects a 9.9 kb SpeI fragment from the wild type locus, and a diagnostic 7.6 kb band from a mu locus which has homologously recombined with the CMD targeting vector (the neo expression cassette contains a SpeI site). Of 1132 G418 and FIAU resistant clones screened by Southern blot analysis, 3 displayed the 7.6 kb Spe I band indicative of homologous recombination at the mu locus. These 3 clones were further digested with the enzymes BglI, BstXI, and EcoRI to verify that the vector integrated homologously into the mu gene. When hybridized with probe A, Southern blots of wild type DNA digested with BglI, BstXI, or EcoRI produce fragments of 15.7, 7.3, and 12.5 kb, respectively, whereas the presence of a targeted mu allele is indicated by fragments of 7.7, 6.6, and 14.3 kb, respectively. All 3 positive clones detected by the SpeI digest showed the expected BglI, BstXI, and EcoRI restriction fragments diagnostic of insertion of the neo cassette into the Cmu1 exon.

Generation of mice bearing the mutated mu gene

The three targeted ES clones, designated number 264, 272, and 408, were thawed and injected into C57BL/6J blastocysts as described by Bradley (Bradley, A. (1987) in Teratocarcinomas and Embryonic Stem Cells: a Practical Approach. (E. J. Robertson, ed.) Oxford: IRL Press, p. 113-151). Injected blastocysts were transferred into the uteri of pseudopregnant females to generate chimeric mice representing a mixture of cells derived from the input ES cells and the host blastocyst. The extent of

ES cell contribution to the chimera can be visually estimated by the amount of agouti coat coloration, derived from the ES cell line, on the black C57BL/6J background. Clones 272 and 408 produced only low percentage chimeras (*i.e.* low percentage of agouti pigmentation) but clone 264 produced high percentage male chimeras. These chimeras were bred with C57BL/6J females and agouti offspring were generated, indicative of germline transmission of the ES cell genome. Screening for the targeted mu gene was carried out by Southern blot analysis of BglI digested DNA from tail biopsies (as described above for analysis of ES cell DNA). Approximately 50% of the agouti offspring showed a hybridizing BglI band of 7.7 kb in addition to the wild type band of 15.7 kb, demonstrating a germline transmission of the targeted mu gene.

Analysis of transgenic mice for functional inactivation of mu gene

To determine whether the insertion of the neo cassette into Cmu1 has inactivated the Ig heavy chain gene, a clone 264 chimera was bred with a mouse homozygous for the JHD mutation, which inactivates heavy chain expression as a result of deletion of the JH gene segments (Chen et al, (1993) Immunol. 5: 647-656). Four agouti offspring were generated. Serum was obtained from these animals at the age of 1 month and assayed by ELISA for the presence of murine IgM. Two of the four offspring were completely lacking IgM (see Table 1). Genotyping of the four animals by Southern blot analysis of DNA from tail biopsies by BglI digestion and hybridization with probe A (see Figure 1), and by StuI digestion and hybridization with a 475 bp EcoRI/StuI fragment (ibid.) demonstrated that the animals which fail to express serum IgM are those in which one allele of the heavy chain locus carries the JHD mutation, the other allele the Cmu1 mutation. Mice heterozygous for the JHD mutation display wild type levels of serum Ig. These data demonstrate that the Cmu1 mutation inactivates expression of the mu gene.

TABLE 1

Mouse	Serum IgM (micrograms/ml)	Ig H chain genotype
42	<0.002	CMD/JHD
43	196	+/JHD
44	<0.002	CMD/JHD
45	174	+/JHD
129 x BL6 F1	153	+/+
JHD	<0.002	JHD/JHD

Table 1 shows the levels of serum IgM, detected by ELISA, for mice carrying both the CMD and JHD mutations (CMD/JHD), for mice heterozygous for the JHD mutation (+/JHD), for wild type (129Sv x C57BL/6J)F1 mice (+/+), and for B cell deficient mice homozygous for the JHD mutation (JHD/JHD).

Example 2 Generation of HCO12 transgenic mice

The HCO12 human heavy chain transgene

The HCO12 transgene was generated by coinjection of the 80 kb insert of pHC2 (Taylor *et al.*, 1994, Int. Immunol., 6: 579-591) and the 25 kb insert of pVx6. The plasmid pVx6 was constructed as described below.

An 8.5 kb HindIII/SalI DNA fragment, comprising the germline human V_H 1-18 (DP-14) gene together with approximately 2.5 kb of 5' flanking, and 5 kb of 3' flanking genomic sequence was subcloned into the plasmid vector pSP72 (Promega, Madison, WI) to generate the plasmid p343.7.16. A 7 kb BamHI/HindIII DNA fragment, comprising the germline human V_H 5-51 (DP-73) gene together with approximately 5 kb of 5' flanking and 1 kb of 3' flanking genomic sequence, was cloned into the pBR322 based plasmid cloning vector pGP1f (Taylor *et al.* 1992, Nucleic Acids Res. 20: 6287-6295), to generate the plasmid p251f. A new cloning vector derived from pGP1f, pGP1k (SEQ ID NO:13), was digested with EcoRV/BamHI, and ligated to a 10 kb EcoRV/BamHI DNA fragment, comprising the germline human V_H 3-23 (DP47) gene together with approximately 4 kb of 5' flanking and 5 kb of 3' flanking genomic

sequence. The resulting plasmid, p112.2RR.7, was digested with BamHI/SalI and ligated with the 7 kb purified BamHI/SalI insert of p251f. The resulting plasmid, pVx4, was digested with XhoI and ligated with the 8.5 kb XhoI/SalI insert of p343.7.16.

A clone was obtained with the V_H 1-18 gene in the same orientation as the other two V genes. This clone, designated pVx6, was then digested with NotI and the purified 26 kb insert coinjected--together with the purified 80 kb NotI insert of pHC2 at a 1:1 molar ratio--into the pronuclei of one-half day (C57BL/6J x DBA/2J)F2 embryos as described by Hogan *et al.* (B. Hogan *et al.*, Manipulating the Mouse Embryo, A Laboratory Manual, 2nd edition, 1994, Cold Spring Harbor Laboratory Press, Plainview NY). Three independent lines of transgenic mice comprising sequences from both Vx6 and HC2 were established from mice that developed from the injected embryos. These lines are designated (HCO12)14881, (HCO12)15083, and (HCO12)15087. Each of the three lines were then bred with mice comprising the CMD mutation described in Example 1, the JKD mutation (Chen *et al.* 1993, EMBO J. 12: 811-820), and the (KCo5)9272 transgene (Fishwild *et al.* 1996, Nature Biotechnology 14: 845-851). The resulting mice express human heavy and kappa light chain transgenes in a background homozygous for disruption of the endogenous mouse heavy and kappa light chain loci.

Example 3 Production of Human Monoclonal Antibodies Against IL-15

HCo12 and HCo7 transgenic mice, generated as described above and supplied from Medarex, San José, CA, USA, were immunised with human recombinant IL-15 (hIL-15, Immunex corp., Seattle, USA) supplemented with either Complete Freunds Adjuvant (CFA, lot no. 121024LA, Difco Laboratories, Detroit, Michigan, USA) or with Incomplete Freunds Adjuvant (ICFA, lot no. 121195LA, Difco, subcutaneously (SC) intraperitoneally (IP) or intravenously (IV). In several instances hIL-15 coupled to KLH was used for immunisation. After several boosts with hIL-15 supplemented with either Complete or Incomplete Freunds Adjuvant, the serum of the mice was tested for the presence of human antibodies directed against IL-15.

Immunisation schemes of the transgenic mice which resulted in final clones 146B7, 146H5, 404E4 and 404A8

Mouse no. 146 (HCo12), ID 995-146, Female

170699	SC	12 μg hIL-15 in CFA (Difco, Lot no. 121024LA)
010799	SC	12 μg hIL-15 in ICFA (Difco, Lot no. 121195LA)
150799	SC	12 μg hIL-15 in ICFA
020899	SC	12 μg hIL-15-KLH in ICFA
070999	SC	12 μg hIL-15-KLH in ICFA
280999	SC	12 μg hIL-15-KLH in CFA
111099	IV	30 μg hIL-15 in PBS
121099	IV	30 μg hIL-15 in PBS
151099		fusion of lymph node and spleen cells of this mouse with SP2/0

Mouse no. 404 (HCo7), ID 997-404, Female

201099	IP	25 μg hIL-15-KLH in CFA (Difco, lot no. 121024LA)
031199	IP	12.5 μg hIL-15, 12.5 μg hIL-15-KLH, 25 μg in ICFA (Difco, lot no. 121195LA)
101199	IV	12.5 μg hIL-15, 12.5 μg hIL-15-KLH
121199	IV	12.5 μg hIL-15, 12.5 μg hIL-15-KLH
191199		fusion of lymph node and spleen cells of this mouse with SP2/0

Culture media

Fusion Partner Medium (FPM):

Iscoves Modified Dulbecco's Medium was supplemented with 100 IU/ml penicillin, 100 μg/ml streptomycin, 1 mM Na-Pyruvate, 0.5 mM β-mercaptoethanol (Life Technologies, Paisley, Scotland) and 10% heat-inactivated fetal calf serum (HyClone, Utah, USA).

Fusion Selection Medium (FSM):

FPM supplemented with 30 ml Origen Hybridoma Cloning Factor (IGEN, Gaithersburg, MD, USA), HAT (1 vial, manufacturer's recommended concentration, Sigma Chemical Co., St. Louis, MO, USA) and 0.5 mg/ml kanamycin (Life Technologies, Paisley, Scotland).

Fusion Cloning Medium (FCM):

FPM supplemented with 20 ml Origen Hybridoma Cloning Factor (IGEN, Gaithersburg, MD, USA), HT (1 vial, manufacturer's recommended concentration, Sigma Chemical Co., St. Louis, MO, USA) and 0.5 mg/ml kanamycin (Life Technologies, Paisley, Scotland).

Hybridoma preparation: fusion of spleen and lymph node cells with SP2/0 myeloma cells

To obtain hybridomas, spleen, inguinal and para-aortic lymph nodes were removed from the mice. Single cells suspensions of spleen and lymph node cells were mixed with SP2/0 myeloma cells in a cell ratio 1:2. Cells were spun down and the pellet was resuspended gently in 1 ml polyethyleneglycol (50% w/v in PBS, Sigma-Aldrich, Irvine, UK) at 37°C. After swirling the cells for 60 seconds, 25 ml FPM-2 was added and cells were incubated at 37°C for 30-60 minutes. After incubation, cells were cultured at a cell concentration of $0,75 \times 10^5$ cells per well (in 100 µl) in 96-wells plates in FSM. After 3 days, 100 µl FSM was added to each well.

Fusion of spleen and lymph nodes of HCo7 and HCo12 mice immunised with hIL-15 resulted in the generation of several hybridomas producing antibodies directed against IL-15. The following four stable clones producing fully human anti-IL-15 antibodies were isolated: (1) 146LyD7F7B7 renamed: 146B7; (2) 146DE2E12A3H5 renamed: 146H5; (3) 404CG11B7E4 renamed: 404E4; and (4) 404FB12E7A8 renamed: 404A8. These clones were all of the human IgG1/k subclass.

Screening of the hybridomas

Between day 7 and 11 after the fusion, the wells were screened for the presence of human antibodies using the following ELISAs:

ELISA to screen for the presence of human IgG in the culture supernatants

To perform the ELISA to detect the presence of human IgG antibodies, 100 µl/well of 0.9 µg/ml rabbit-α-k-light chains antibodies (DAKO, Glostrup, Denmark) was added in phosphate buffered saline (PBS) to Nunc Maxisorp ELISA-plate (incubation overnight at room temperature). After blocking the plate with PBS supplemented with chicken serum (2 %; Life Technologies, Paisley, Scotland) and Tween-20 (0.05 %; PBSTC), culture supernatants were added. After incubation for 1.5 hour the plates were washed and rabbit-α-Human IgG (Fab2-fragments) conjugated with horse radish peroxidase (DAKO, Glostrup, Denmark) 0.5 µg/ml diluted in PBSTC was added. After incubation for 1 hour, the wells were washed and substrate, ABTS (2,2'-Azinobis-3-ethylbenzthiazoline-sulphonic-acid, Roche Diagnostics, Mannheim, Germany) was added according to the manufacturer's protocol and antibody binding was evaluated at 405 nm in an EL808 ELISA-reader (Bio-tek Instruments, Winooski, VT, USA).

ELISA to screen for the presence of IL-15 specific antibodies

Wells containing human IgG/k antibodies were further tested for the presence of human anti-IL-15 antibodies in an IL-15-specific ELISA. To perform the ELISA, 100 µl/well of 1 µg/ml IL-15 was added in phosphate buffered saline (PBS) to Nunc Maxisorp ELISA-plate (incubation overnight at room temperature). After blocking the plate with PBS supplemented with chicken serum (2 %; Life Technologies, Paisley, Scotland) and Tween-20 (0.05 %; PBSTC), culture supernatants were added. After incubation for 1.5 hours the plates were washed and α-Human IgG Fc conjugated with horse radish peroxidase (Jackson Immuno research, West Grove, Pennsylvania, USA) 1/5000 diluted in PBSTC was added. After incubation for 1 hour, the wells were washed and substrate, ABTS (2,2'-Azinobis-3-ethylbenzthiazoline-sulphonic-acid, Roche Diagnostics, Mannheim, Germany) was added according to the manufacturer's protocol and antibody binding was evaluated at 405 nm in an EL808 ELISA-reader (Bio-tek Instruments, Winooski, VT, USA).

Subcloning of the hybridomas

To obtain stable anti-IL-15 cell lines, the hybridomas were subcloned by a limiting dilution of the cells (to 0.5 cell/well) in 96-wells plates.

The subclones were tested after approximately 10 days with the above mentioned IL-15 ELISA. During the several subcloning procedures, FSM was changed in phases via FCM to FPM. The isotype of the subclones was determined with the ELISA described below.

Isotype determination of the anti-IL-15 antibodies by ELISA

To perform the isotype ELISA, 100 µl/well of 1 µg/ml anti-human Fc (Jackson Immuno research) was added in phosphate buffered saline (PBS) to Nunc Maxisorp ELISA-plate (incubation overnight at room temperature). After blocking the plate with PBS supplemented with chicken serum (2 %; Life Technologies, Paisley, Scotland) and Tween-20 (0.05 %; PBSTC), culture supernatants were added. After incubation for 1.5 hours the plates were washed and mouse-α-HuIgG1 conjugated with alkaline phosphatase (Zymed, plaats, land), or mouse-α-HuIgG3 conjugated with horse radish peroxidase (Zymed) was added. After incubation for 1 hour the wells were washed and substrate, ABTS (2,2'-Azinobis-3-ethylbenzthiazoline-sulphonic-acid, Roche Diagnostics, Mannheim, Germany) was added according to the manufacturer's protocol. Antibody binding was evaluated at 405 nm in an EL808 ELISA-reader (Bio-tek Instruments, Winooski, VT, USA.

Example 4 Epitope Specificity of Fully Human Anti-IL-15 Antibodies

To function therapeutically and to inhibit IL-15-induced proinflammatory effects, IL-15 specific antibodies need to recognize the IL-15 epitopes involved in interaction with the IL-2Rβ-chain and/or the γ-chain of IL-15 receptor.

Mutant proteins (described by Pettit *et al.*)were used to evaluate the epitope specificity of the fully human anti-IL-15 antibodies, 146B7, 146H5, 404A8 and 404E4. The IL-15 mutants used include IL-15 mutant Q108S (Gln at residue 108 was replaced by Ser; a mutation in the γ-chain interaction site) and mutant D8SQ108S (Gln

at residue 108 was replaced by Ser and Asp at position 8 was substituted for Ser; mutations in both the β and γ-chain interaction sites of IL-15).

ELISA to determine binding of the hIL-15 specific antibodies, 146B7, 147H5, 404A8 and 404E4, to hIL-15 and to mutant IL-15 proteins

To perform the ELISA, 100 µl of 1 µg/ml IL-15 or hIL-15 mutant protein, in phosphate buffered saline (PBS), was added to Nunc Maxisorp ELISA-plate for coating. After blocking the plate with PBS supplemented with chicken serum (2 %; Life Technologies, Paisley, Scotland) and Tween-20 (0.05 %; PBSTC), serial dilutions of the hIL-15 specific antibodies were incubated. After washing, α-Human IgG Fc conjugated with peroxidase (Jackson Immuno research, West Grove, Pennsylvania, USA) 1/5000 diluted in PBSTC was added. After washing substrate, ABTS (2,2'-Azinobis-3-ethylbenzthiazoline-sulphonic-acid, Roche Diagnostics, Mannheim, Germany) was added according to the manufacturer's protocol and antibody binding was evaluated at 405 nm in an EL808 ELISA-reader (Bio-tek Instruments, Winooski, VT, USA).

The binding of the fully human IL-15 specific antibodies 146B7, 146H5, 404A8 and 404E4 to hIL-15 and to the IL-15 mutant proteins Q108S and D8SQ108S is shown in Figure 1. Neither 146B7 nor 146H5 were able to bind to these mutant IL-15 proteins. Since both mutants carry the Q108S mutation, the epitope recognized by 146B7 and 146H5 is within the critical domains of IL-15 which interact with the γ-chain of the IL-15 receptor. 404A8 and 404E4 were both able to bind the mutant proteins, therefore, these antibodies recognize an epitope outside the β- and γ-chain interacting domains of IL-15. Both 146B7 and 146H5 bind to IL-15 at the region that interacts with the γ-chain of the IL-15 receptor. This agrees with the data obtained from the proliferation assays using the fully human anti-IL-15 antibodies of the present invention. As described in detail below, neither 404A8 nor 404E4 were able to inhibit IL-15-induced proliferation of CTLL-2 cells and human PBMCs. Both 146B7 and 146H5 were able to inhibit IL-15-induced proliferation. Further, inhibition of proliferation is achieved by blocking the interaction of IL-15 with the γ- subunit of the IL-15 receptor.

Example 5 V_H and V_L – Region Sequences of 146B7

The nucleotide and deduced amino acid sequence of rearranged V_H and V_L–domains of 146B7 were determined using the following procedures. These sequences give information regarding the V_H and V_L germline families used; point mutations in these germline sequences are due to affinity maturation of B-cells during the immunization of the animal.

RNA preparation

Total RNA was prepared from 5 x 10^6 146B7 hybridoma cells with RNAzol (Biogenesis, Poole, England) according to the manufactures protocol.

cDNA preparation

The cDNA of RNA from 146B7 was prepared from 3 μg total RNA with AMV Reverse Transcriptase with buffer (Roche Diagnostics GmbH, Mannheim, Germany), oligo $d(T)_{15}$ (Promega, Madison, WI, USA), dNTP (Boehringer Mannheim corp., USA) and RNAsin (Promega) according to the manufacturer's protocol.

PCR primers used to amplify V_H and V_L regions for cloning

Primer pairs used:

V_H:

FR1 5' primers

(1)	AB62	CAg gTK CAg CTg gTg CAg TC
(2)	AB63	SAg gTg CAg CTg KTg gAg TC
(3)	AB65	gAg gTg CAg CTg gTg CAg TC

V_H leader 5' primers

(4)	AB85	ATg gAC Tgg ACC Tgg AgC ATC
(5)	AB86	ATg gAA TTg ggg CTg AgC Tg
(6)	AB87	ATg gAg TTT ggR CTg AgC Tg
(7)	AB88	ATg AAA CAC CTg Tgg TTC TTC
(8)	AB89	ATg ggg TCA ACC gCC ATC CT

V_H 3' primer

(9) AB90 TgC CAg ggg gAA gAC CgA Tgg

V_K:

FR1 5' primers

(1) AB8 RAC ATC CAg ATg AYC CAg TC
(2) AB9 gYC ATC YRg ATg ACC CAg TC
(3) AB10 gAT ATT gTg ATg ACC CAg AC
(4) AB11 gAA ATT gTg TTg ACR CAg TC
(5) AB12 gAA ATW gTR ATg ACA CAg TC
(6) AB13 gAT gTT gTg ATg ACA CAG TC
(7) AB14 gAA ATT gTg CTg ACT CAg TC

V_K leader 5' primers:

(8) AB123 CCC gCT Cag CTC CTg ggg CTC CTg
(9) AB124 CCC TgC TCA gCT CCT ggg gCT gC
(10) AB125 CCC AgC gCA gCT TCT CTT CCT CCT gC
(11) AB126 ATg gAA CCA Tgg AAg CCC CAg CAC AgC

V_K 3' primer

(12) AB16 Cgg gAA gAT gAA gAC AgA Tg

PCR conditions used to amplify V_H and V_L regions for cloning

PCR Reactions were performed with AmpliTaq polymerase (Perkin Elmer) on a GeneAmp PCR System 9700 (Perkin Elmer Applied Biosystems, Foster City, CA, USA).

PCR cycling protocol:

94° 2'
11cycles 94° 30''
65° 30'', minus 1° per cycle

		72° 30"
	30 cycles	94° 30''
		55° 30"
		72° 30''
		72° 10'
		cool down to 4°

Cloning of V_H and V_L in pGEMT-Vector System I

After analysing the PCR products on an agarose gel, the products were purified with S-400 or S300 microspin columns (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA), or with the QIAEX II Gel Extraction Kit (Qiagen GmbH, Hilden, Germany). For each experiment 2 independently amplified PCR products, using FR1 or leader primers, of each V_H and V_L region were cloned in pGEMT-Vector System I (Promega) according to manufacturers protocol.

After transformation to *E. coli* DH5α, individual colonies were screened by colony PCR using T7 and SP6 primers, 30 cycles at 55°. Plasmid DNA from each individual colony was purified using Qiaprep Spin miniprep kit (Qiagen). To further analyze a *Nco*1/*Not*1 (NE Biolabs, United Kingdom and Roche Diagnostics) digestion was performed and analyzed on agarose gel.

Sequencing

The V-regions were sequenced after cloning in the pGEMT-Vector System I. T7 and Sp6 primers (Eurogentec, Luik, Belgium) were used in combination with the sequence kit: ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Warrington, United Kingdom) according to protocol. The reactions were performed on a ABI PRISM 377 Sequencer (PE Applied Biosystems) and the sequences were analysed with the program DNAStar, SeqmanII. The sequences were then aligned to germline V-gene sequences in VBASE (www.mrc-cpe.cam.ac.uk/imt-doc/public/intro.htm).

Cloning and sequencing of the V_H and V_L-region of 146B7

V_H and V_L-regions from hybridoma 146B7 were amplified by PCR and cloned in pGEMT-Vector System I to determine the cDNA-sequence. The nucleotide and corresponding amino acid sequences are shown in Figure 2 (SEQ ID NOs: 1 and 2) and Figure 3 (SEQ ID NOs: 3 and 4), respectively. The framework (FR) and complementarity determining regions (CDR) are also indicated. The germline family for the V_H-region of 146B7 according to alignment in Vbase: V_H5-51 (V_H5-subgroup), D2-15/D2 (D_H-segment), JH4b (J_H-segment). The germline family for the V_L-region of 146B7 according to alignment in Vbase: A27 (V_KIII-subgroup) and J_K2 (J_K-segment). More information regarding V_H and V_L–domains is shown at the Kabat database http://immuno.bme.nwu.edu/ or at http://www.Vbase.com.

Example 6 Affinity binding characteristics of 146B7

The affinity of 146B7 was analyzed by surface plasmon resonance (SPR) technology using a BIACORE 3000 instrument to determine biomolecular protein interactions according to the following procedures. Changes in the SPR signal on the surface layer caused by biomolecular binding are detected and signify a change in the mass concentration at the surface layer. Affinity is expressed using the following definitions: k_a = association rate constant (M^{-1} sec^{-1}); k_d = dissociation rate constant (sec^{-1}); K_A = association equilibrium constant = k_a/k_d (M^{-1}); and K_D = dissociation equilibrium constant = k_d/k_a (M).

Different procedures were performed to obtain the affinity of 146B7 for human IL-15 (hIL-15). Human recombinant IL-15 from two different suppliers (Immunex corp., Seattle, USA and Peprotech, Rocky Hill, NJ, USA) was coupled to a CM5 sensor chip. The compound coupled to the sensorchip is defined as ligand. In other experiments 146B7 was used as ligand.

In each kinetic analysis, the binding of the analyte, 146B7 or hIL-15 adapted to the ligand coupled to the sensorchip, was compared to the binding to a reference control CM5 sensor chip. Serial dilutions of analyte were tested (0, 3.125, 6.25, 12.5, 25, 50 µg/ml). Association and dissociation curves were fitted for monomeric interaction in the model Langmuir 1:1, to determine k_a and k_d and to calculate K_A and K_D. All data were analyzed using BIA-Evaluation Version 3.1. For a

bivalent interaction the model "bivalent analyte" was used. All analysis were corrected for a drifting baseline.

To determine the antibody affinity of 146B7, the affinity of antibody 146B7 was measured for human recombinant IL-15 derived from two different suppliers, Immunex and Peprotech, at the BIACORE 3000. Using 146B7 as ligand and hIL-15 as analyte, the monovalent interaction was determined (curve fitting Langmuir 1:1).

The affinity of 146B7 for IL-15 (Immunex Corp.) was measured as follows:

The association rate constant k_a:	$1.07 (\pm 0.17) \times 10^5\ M^{-1}\ sec^{-1}$
The dissociation rate constant k_d:	$6.56 (\pm 0.09) \times 10^{-3}\ sec^{-1}$
Association equilibrium constant K_A:	$1.55 (\pm 0.21) \times 10^7\ M^{-1}$
Dissociation equilibrium constant K_D:	$6.59 (\pm 0.88) \times 10^{-8}\ M$

To determine the avidity of 146B7, IL-15 (Immunex Corp.) was used as ligand and 146B7 was used as analyte. When the data obtained were analyzed using Langmuir (1:1) curve fitting the bivalent interaction of the antibody was expressed, the avidity of the antibody was determined.

The avidity of 146B7 for IL-15 (Immunex Corp.) was measured as follows:

The association rate constant k_a:	$7.30 (\pm 0.81) \times 10^5\ M^{-1}\ sec^{-1}$
The dissociation rate constant k_d:	$1.45 (\pm 2.05) \times 10^{-3}\ sec^{-1}$
Association equilibrium constant K_A:	$5.03 (\pm 3.40) \times 10^8\ M^{-1}$
Dissociation equilibrium constant K_D:	$1.55 (\pm 1.24) \times 10^{-9}\ M$

The affinity and avidity of 146B7 for Peprotech derived IL-15 were determined also. No major differences in affinity or avidity for two different sources of IL-15 were seen.

As is described in the example below regarding the inhibition of human interleukin-15 (hIL-15)-induced proliferation of CTLL-2 cells and PBMC by fully human anti-IL-15 antibodies, 146B7 inhibited in a dose dependent manner the IL-15 induced proliferation as was measured by [^{3}H]-thymidine incorporation. The IC50 - concentration at 50 % inhibition, a more functional manner to determine affinity- from these proliferation inhibition experiments was calculated: 3.1 ± 0.91 nM. This IC50 is in agreement with the avidity measured by BIACORE 3000 (K_D 1.5 nM) using 146B7 as ligand and recombinant human IL-15 as analyte and confirmed the affinity and avidity measurements obtained here.

Example 7 Inhibition of hIL-15-induced TNF-α production by fully human anti-IL-15 antibodies

The effect of fully human anti-IL-15 antibodies, 146B7, 146H5, 404E4 and 404A8, on IL-15-induced TNF-α production was studied using peripheral blood derived mononuclear cells (PBMC) from healthy volunteers using the following procedures. To evaluate specificity to IL-15, the effect of these antibodies on IL-2-mediated TNF-α production was also examined.

Cell culture

Cultures were maintained in RPMI-1640 with 2 mM L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin (all derived from Life Technologies, Paisley, Scotland) and 10% heat-inactivated fetal calf serum (HyClone, Utah, USA).

Purification of peripheral blood mononuclear cells (PBMC)

Fresh human blood was drawn from a healthy volunteer after informed consent, heparin was added against coagulation. Purification of PBMC was performed by density gradient centrifugation using Ficoll (Pharmacia, Uppsala, Sweden).

Test Compound

HIL-15, lot no: 6870-011, Immunex corp., Seattle, Washington, USA.

hIL-2, Chiron Benelux BV, Amsterdam, The Netherlands.

Fully human antibodies used: 146B7 (batch: 070101) and 146B7RDJW07, 404A8 (batch: 030101) and 404E4 (batch: 080101) and as isotype control antibody T1 (97-2B11-2B12, batch: 190900).

Inhibition of human IL-15 (hIL-15) or hIL-2-induced TNF-α production by PBMC by anti-IL-15 antibodies

PBMC were cultured in triplicate or quadruplicate in 96-well flat-bottom plate at 1.5×10^5 cells per well in the presence or absence of hIL-2 or hIL-15 and with or without anti-IL-15 antibodies. Isotype control antibody (T1) was included as negative control. Concanavalin A (2.5μg/ml, Calbiochem) was added as a positive control for proliferation. Cells were incubated for 72 hours at 37°C and 5% CO_2. Supernatants were harvested to quantify the amount of human TNF-α by ELISA (U-CyTech, Utrecht, The Netherlands).

The effects of 146B7 and an isotype control antibody were tested on IL-15-mediated TNF-α production by PBMC. 146B7 inhibited hIL-15-mediated TNF-α production in a dose dependent fashion, whereas the isotype control antibody did not inhibit hIL-15-induced TNF-α production (Figure 6). Data of two healthy volunteers are shown. 404E4 and 404A8 were unable to inhibit hIL-15-induced TNF-α production.

To ensure the specificity of the anti-IL-15 antibodies, their effect on hIL-2-mediated TNF-α production was evaluated. No inhibition of IL-2-mediated TNF-α production was induced by 146B7 (Figure 7). No dose dependent inhibition by either 404E4 or 404A8 was seen in hIL-2-mediated TNF-α production.

A dose dependent inhibition of hIL-15-mediated TNF-α production was seen only by 146B7 and not by 404E4 and 404A8. The inhibitory effect was specific for hIL-15; IL-2-mediated TNF-α production was not inhibited.

Example 8 Inhibition of human interleukin-15 (hIL-15)-induced proliferation of CTLL-2 cells and PBMC by fully human anti-IL-15 antibodies

Antibodies 146B7, 146H5, 404E4 and 404A8 were tested for their ability to inhibit T-cell proliferation using CTLL-2 cells (Gillis *et al.*, 1978) and peripheral blood mononuclear cells (PBMC) using the following procedures.

Cell culture

Cultures were maintained in RPMI-1640 with 2 mM L-glutamine, 100 IU/ml penicillin, 100 µg/ml streptomycin (derived from Life Technologies, Paisley, Scotland) and 10% heat-inactivated fetal calf serum (HyClone, Utah, USA). CTLL-2 cells (Gillis *et al.*, 1978) were maintained in the above mentioned medium supplemented with 36 units hIL-2/ml (Chiron Benelux BV, Amsterdam, The Netherlands) and starved for hIL-2 for 3-4 days before the start of the experiment. CTLL-2 cells were washed three times before use.

Purification of peripheral blood mononuclear cells (PBMC)

Fresh human blood was drawn from a healthy volunteer after informed consent, heparin was added against coagulation. Purification of PBMC was performed by density gradient centrifugation using Ficoll (Pharmacia, Uppsala, Sweden).

Test Compound

HIL-15, lot no: 6870-011, Immunex corp., Seattle, Washington, USA.

hIL-2, Chiron Benelux BV, Amsterdam, The Netherlands.

anti-IL-15 antibodies used for CTLL-2 assay in this report shown in Figure 8: 146B7, 146H5, 404A8, 404E4.

anti-IL-15 antibodies used for PBMC assays: 146B7 (batch: 070101), 404A8 (batch: 030101) and 404E4 (batch: 080101).

Inhibition of human IL-15 (hIL-15) or hIL-2 induced CTLL-2 proliferation by anti-IL-15 antibodies

In each experiment, cells were seeded in triplicate in 96-well plate, $5x10^3$ cells per well in the presence or absence of either hIL-2 or hIL-15. To evaluate the effect on proliferation, each of the four anti-IL-15 antibodies were added. Cells were incubated for 16 hours at 37°C and 5% CO_2. [^{3}H]Thymidine (1 μCi/well, Amersham Life Sciences, Little Chalfont, Buckinhamshire, UK) was added 4 hours before harvesting (Harvester 96 Mach II M, Tomtec, Orange CT, USA).

As is shown in Figure 8, IL-15 induced proliferation of CTLL-2 cells was decreased in a dose dependent fashion by 146B7 and 146H5 as was reflected by reduced [^{3}H]-Thymidine incorporation. Both 404E4 and 404A8 were unable to block IL-15 induced proliferation of CTLL-2 cells.

Inhibition of hIL-15 (hIL-15) or hIL-2 induced PBMC proliferation by anti-IL-15 antibodies

PBMC were cultured in triplicate in 96-well U-bottom plate (Nunc, Nalge Nunc International, Denmark), 5×10^4 cells per well in the presence or absence of hIL-2 or hIL-15 and the anti-IL-15 antibodies. Concanavalin A (2.5μg/ml, Calbiochem) was added as a positive control for proliferation. The cells were incubated for 72 hours at 37°C and 5% CO_2. [3H]Thymidine (1 μCi/well, Amersham Life Sciences, Little Chalfont, Buckinhamshire, UK) was added 16 hours before harvesting (Harvester 96, Tomtec, Orange CT, USA).

146B7 was able to inhibit IL-15 induced [^{3}H]-Thymidine incorporation dose dependently and, therefore, inhibited proliferation (IC50 = 3.1 ± 0.91 nM). Both 404E4 and 404A8 were unable to block hIL-15 induced PBMC proliferation. 146H5 was not tested according to data obtained from previously performed experiments. To ensure the specificity of 146B7, 404E4 and 404A8 for IL-15, these antibodies were also evaluated for their effects on IL-2 mediated proliferation. None of the tested anti-IL-15 antibodies exhibited an effect on IL-2 induced proliferation (Figure 9).

Example 9 Human anti-IL-15 antibody 146B7 binds to human IL-15 present on human PBMCs

Test compounds

Human PBMCs were obtained from healthy volunteers after informed consent. Antibody 146B7 (batch no. MDX015), Medarex Inc., Milpitas, CA, USA.

Biotinylation of 146B7 and human IgG

N-hydroxysuccinimido-biotin (Sigma) was first diluted in DMSO (final dilution: 100 mg/ml) and then in 0.1 M $NaHCO_3$ (final dilution: 1 mg/ml, Sigma). Per 1 mg of antibody (diluted in 1 ml), 600 µl of biotin solution was added (dark, 2hrs, RT). Antibody-biotin solution was dialysed in a slide-a-lyzer™ dialysis cassette (10,000 MWCO, Pierce, Perbio Science, Netherlands) (overnight at 4°C) to remove unlabeled biotin. The following day, concentration of biotinylated antibodies was determined by spectrophotometry (Ultrospec 2100pro) at OD 280 nm.

Stimulation of peripheral blood

To induce IL-15, blood was obtained by venapuncture from healthy volunteers. PBMCs were cultured in RPMI 1640 (Biowhittaker Europe) supplemented with penicillin (5 U/ml), streptomycin (50 µg/ml), L-glutamine (2mM) (Biowhittaker Europe), and 10% fetal calf serum (Optimum C241, Multicell, Wisent Inc.) for a maximum of 2 days (37°C), and were stimulated with 500 U/ml IFNγ (Boehringer Ingelheim).

Flow cytometry

Cells were pre-incubated with 10% human AB serum (CLB, Amsterdam, Netherlands) in RPMI 1640 (Biowhittaker Europe) supplemented with penicillin (5 U/ml), streptomycin (50 µg/ml), L-glutamine (2mM) (Biowhittaker Europe) and 10% fetal calf serum (Optimum C241, Multicell, Wisent Inc.). After permeabilization (20 min, 4°C, in Cytofix/Cytoperm™ Kit, Becton Dickinson, San Diego, CA) and washing in Perm/Wash™ buffer (Cytofix/Cytoperm™ Kit), PBMC were subjected to staining of IL-15 by flow cytometry. Continuous permeability was achieved by using

Perm/Wash™ buffer (Cytofix/Cytoperm™ Kit) throughout the staining procedure. After incubating the cells with biotinylated 146B7 or with biotinylated hIgG1 (20 µg/ml, 30 min, 4°C) and washing in Perm/Wash™ buffer, cells were subsequently incubated with streptavidin-phycoerythrin (DAKO) for 30 minutes (4°C). Fluorescence intensity of at least 5000 cells per sample was determined after analysis by flow cytometry (FACS Calibur, Becton Dickinson) and gating on the monocytes, using CellQuest Pro software. Data show the stimulation index (S.I.), which is calculated as follows: S.I. = (mean fluorescence positive staining)/(mean fluorescence background staining)

Immunocytochemistry

To detect IL-15 present in human monocytes, cytospin preparations were made of whole blood samples. After spinning down 5 x 10^4 cells (200 µl) onto Superfrost®-Plus microscope slides (Menzel), slides were air-dried (< 60 min), fixed in 2% paraformaldehyde/PBS (8 min, 4°C), washed with PBS and air-dried again. Before staining, cytospin preparations were permeabilized in PBS (+ 0.1% saponine; PBSS), which was subsequently used throughout the staining procedure. To block endogenous peroxidase activity, cytospin preparations were incubated with 0.05% (v/v) hydrogen peroxide (H_2O_2) diluted in citric acid/phosphate buffer (pH 5.8, 20 min, RT). After washing with PBSS, endogenous biotin activity was blocked according to the manufacturer's instructions (Biotin Blocking Kit, Vector Lab., DAKO). After washing with PBSS, non-specific binding sites were blocked by incubating the cytospin preparations with 10% (v/v) human pooled AB-serum (CLB, Amsterdam, Netherlands) (30 min) in PBSS. Thereafter, cytospin preparations were incubated with biotinylated primary antibody (60 min, RT) and, after washing with PBSS, with streptavidin complexed with biotinylated horseradish peroxidase (streptABComplex/HRP, DAKO; 1:100 in PBSS, containing 2% human AB serum; 30 min, RT). After washing in PBSS, the cytospin preparations were incubated with 3-amino-9-ethylcarbazole (0.5 mg/ml) and H_2O_2 (0.01%), in sodium acetate buffer (50 mM, pH 4.9) for 10 minutes (RT), for the detection of HRP activity. Cytospins were washed with running tap water for 5 minutes, counterstained with haematoxylin (DAKO) for one minute, washed with running tap water for another 5 minutes, and embedded in faramount or glycergel (DAKO).

Flow cytometry

Binding of 146B7 to IFNγ-stimulated human monocytes is shown in Figure 12. Biotinylated 146B7 binds to unstimulated monocytes showing the presence of IL-15 in unstimulated cells. Stimulation of monocytes with IFNγ leads to a increased binding of 146B7 to the cells, with a maximum reached at day one of culture. The control antibody, hIgG1, shows little binding to unstimulated monocytes. Stimulation with IFNγ increases binding of hIgG1 through increased expression of Fcγ receptors on monocytes.

Immunocytochemistry

Figure 13 shows staining of human monocytes with 146B7, or with the control antibody, hIgG1. A clear red staining of the cytoplasm is observed after incubating the cells with 146B7, but not with the control antibody. Accordingly, 146B7 binds hIL-15 in monocytes and this binding is upregulated after stimulation with IFNγ. Figure 13 also shows that IL-15 staining is primarily intracellular.

Example 10 Human anti-IL-15 antibody 146B7 binds IL-15 in tissues by immunohistochemistry

Test compounds

Human psoriatic skin – tissue samples were obtained after informed consent. Louise Villadsen, Department of Dermatology, Gentofte University Hospital, Copenhagen, Denmark.

Antibody 146B7 (batch no. MDX015), Medarex, Milpitas, CA, USA

Biotinylation of 146B7 and human IgG

N-hydroxysuccinimido-biotin (Sigma) was first diluted in DMSO (final dilution: 100 mg/ml) and then in 0.1 M $NaHCO_3$ (final dilution: 1 mg/ml, Sigma). Per 1 mg of antibody (diluted in 1 ml), 600 µl of biotin solution was added (dark, 2hrs, RT). Antibody-biotin solution was dialysed in a slide-a-lyzer™ dialysis cassette (10,000 MWCO, Pierce, Perbio Science, Netherlands) (ON, 4°C) to remove unlabeled biotin.

The following day, concentration of biotinylated antibodies was determined by spectrophotometry (Ultrospec 2100pro) at OD 280 nm.

Immunohistochemistry

Tissues were stored at –80°C until assay. After thawing, tissue sections were fixated in acetone (10 min, RT) and air-dried. To block endogenous peroxidase activity, sections were incubated with 0.05% (v/v) hydrogen peroxide (H_2O_2) diluted in citric acid/phosphate buffer (pH 5.8, 20 min, RT). After washing with PBS-Tween 20 (PBST, 0.05% v/v), endogenous biotin activity was blocked according to the manufacturer's instructions (Biotin Blocking Kit, Vector Lab., DAKO). After washing with PBST, non-specific binding sites were blocked by incubating the tissue sections with 10% (v/v) human pooled AB-serum (CLB, Amsterdam, Netherlands) (30 min) in PBST. Serum was blotted off and sections were subsequently incubated with biotinylated primary antibody (146B7 or hIgG1) diluted in PBS containing 2% human AB serum for 60 minutes (RT). Sections were washed in PBST. After washing in PBST, all tissue sections were incubated with streptABComplex/HRP (DAKO; 1:100 diluted in PBS containing 2% human AB serum; 30 min, RT). After washing in PBST, the sections were incubated with 3-amino-9-ethylcarbazole (0.5 mg/ml) and H_2O_2 (0.01%), in sodium acetate buffer (50 mM, pH 4.9) for 10 minutes (RT), for the detection of HRP activity. Sections were washed with running tap water for 5 minutes, counterstained with haematoxylin (DAKO) for one minute, washed with running tap water for another 5 minutes, and finally embedded in faramount or glycergel (DAKO).

Results

A clear cytoplasmic staining of keratinocytes in psoriatic skin was observed after staining tissue sections with 146B7, but not with the control antibody (Figure 14; 146B7 stains IL-15-positive keratinocytes obtained from psoriatic plaques).

Example 11 Human anti-IL-15 antibody 146B7 blocks IL-15 in SCID mouse-human tissue chimeras: significant inhibition of inflammation in both arthritic and psoriatic tissue

Test compounds

Synovial tissue - obtained form patients with juvenile rheumatoid arthritis, after informed consent; Alexei Grom, division of pediatric rheumatology, Children's Hospital Medical Center, Cincinnati, Ohio, USA.

Keratome biopsies - tissue samples were obtained after informed consent. Louise Villadsen, Department of Dermatology, Gentofte University Hospital, Copenhagen, Denmark.

Antibody 146B7 (batch no. MDX015), Medarex Inc., Milpitas, CA, USA for psoriasis experiments.

Antibody 146B7 (batch no. 15-00RDJW07), Medarex Inc., Milpitas, CA, USA for rheumatoid arthritis experiments.

Blocking IL-15 in SCID mouse – human synovial tissue chimeras

Fresh synovial tissue samples were obtained from patients with juvenile rheumatoid arthritis after joint replacement surgery. Samples were collected in sterile conditions. Minced tissue fragments from the entire synovial tissue sample were thoroughly mixed to ensure homogeneity of each preparation. Minced tissues (2-4 grafts per animal; 100 mg per one site) were engrafted subcutaneously in the back of SCID/NOD mice (Jackson Laboratories). Each animal received 146B7 (500 μg, i.p.) or PBS on the day of graft implantation, and on post-implantation days 7, 14, and 21. Animals were sacrificed on day 28 post-implantation. Synovial grafts were excised and placed on formalin for H&E staining.

Quantification of H&E staining of tissues from SCID mouse – human synovial tissue chimeras (Modified from Lehr *et al.*, J. Histochem. Cytochem. 1997, 45, 1559)

After obtaining digital images (2600x2060, jpg) of sections obtained from SCID mouse – human synovial tissue chimeras using a X10 objective (Zeiss microscope; Axiovision software), data were computer-analysed, by use of Photoshop, version 6.0 (Adobe Systems, Mountain view, CA) and reduced to 1300x1300 pixels.

Within each section six X10 fields were chosen so as to best reflect the overall staining of the tissue on the entire slide. After selection of all stained nuclei (magic wand on dark nucleus with tolerance 10), an optical density plot of the selected area was generated and the mean staining intensity was recorded (after selection of similar/image histogram command). Subsequently, the background was selected and staining was quantified (magic wand on background with tolerance 10). Staining intensity was calculated as the difference between nuclear staining and background staining. This was designated the cytochemical index with arbitrary units. Data are shown as mean and s.e.m. Data were analysed by Student's t-test.

Blocking IL-15 in SCID mouse – human psoriatic tissue chimeras

Keratome biopsies were obtained from psoriatic plaques of two patients, divided and transplanted onto C.B-17 SCID (Jackson Laboratories) mice. Three weeks after transplantation mice received PBS (placebo), CsA (cyclosporine A) (Sandoz) at a dose of 10 mg/kg every second day for 15 days, or 146B7 at a dose of 20 mg/kg on day 1 and 10 mg/kg on days 8 and 15. One week after the last injection, mice were sacrificed, and a 4 mm punch biopsy was taken from each xenograft. Biopsies were fixed in formalin for paraffin embedding and stained in H&E and for Ki-67 nuclear antigen.

Quantification of immunohistochemical staining of tissues from SCID mouse - human psoriatic tissue chimeras

The H&E-stained sections were evaluated for epidermal thickness (μm), grade of parakeratosis (rated from 0-3), and number of inflammatory mononuclear cells in upper dermis. The sections stained for Ki-67 were evaluated for number of cycling keratinocytes/mm^2 section. Mean values for the 4 mice in each treatment group were calculated, and the data from each patient were summarised as mean and s.e.m.

SCID/RA model

Microscopic observation of sections showed that the darkest stained nuclei belong to infiltrating cells. Therefore, the number of nuclei (measured as the relative surface area) are considered as a measure for infiltration. Injection of 146B7 reduces the number of infiltrating cells into inflamed synovial tissue, as compared to

vehicle treatment (Figure 15a, $p<0.05$). Figure 15b illustrates the effects of 146B7 on infiltration of cells into xenografted synovial tissue, and shows a reduction in number of cells with dark nuclei, as compared to vehicle treatment.

SCID/psoriasis model

Figure 16 shows SCID/psoriasis mice treated with 146B7 or control treatment. Compared to the vehicle, PBS, injections of 146B7 reduced the severity of psoriasis evaluated by epidermal thickness when was measured from the stratum corneum to the beginning of the rete pegs (Figure16A)): PBS (177.8 $\pm$ 42.2 μm), CsA (91.0 $\pm$ 15.2 μm), 146B7 (62.5 $\pm$ 9.1 μm). A reduction in thickness was also observed when was measured from the stratum corneum to the deepest part of the rete pegs (Figure 16B): PBS (433.8 $\pm$ 32.1 μm), CsA (303.8 $\pm$ 62.9 μm) and 146B7 (208.0 $\pm$ 33.8 μm). Also, the grade of parakeratosis was reduced by 146B7 treatment (Figure 16C): PBS (1.6 $\pm$ 0.4), CsA (1.3 $\pm$ 0.3), 146B7 (0.5 $\pm$ 0.3). Furthermore, 146B7 reduces the number of inflammatory mononuclear cells in upper dermis (Figure 16D): PBS (33.3 $\pm$ 1.9 mononuclear cells), CsA (19.4 $\pm$ 8.5), 146B7 (16.4 $\pm$ 0.1). The expression of the human Ki-67 protein is strictly associated with cell proliferation. During interphase, the antigen can be exclusively detected within the nucleus, whereas in mitosis most of the protein is relocated to the surface of the chromosomes. The fact that the Ki-67 protein is present during all active phases of the cell cycle (G(1), S, G(2), and mitosis), but is absent from resting cells (G(0)), makes it an excellent marker for determining the so-called growth fraction of a given cell population. 146B7 reduces the number of Ki-67+ cycling keratinocytes (Figure 16E): PBS (247.9 ± 77.0), CsA (116.0 ± 24.1), 146B7 (73.8 ± 9.9).

Treatment with 146B7 inhibited the infiltration of inflammatory cells into inflamed tissue in human SCID models for rheumatoid arthritis. Furthermore, in SCID mice with engrafted human psoriatic plaques, treatment with 146B7 reduced the severity of psoriasis, as compared to treatment with CsA. Indeed, treatment with 146B7 resulted in a major reduction in inflammation, in epidermal thickness, in numbers of dividing keratinocytes, and in severity of parakeratosis in human/SCID mice.

Example 12 Human anti-IL-15 antibody 146B7 recognizes receptor-bound IL-15

Test compounds

hIgG1 - human control antibody (Sigma).

Antibody 146B7 – Medarex Inc., MDX015.

Raji cells with constitutive expression of IL-15Rα (Martin Glennie, Tenovus Research Laboratory, Southampton General Hospital, Southampton, U.K.).

Biotinylation of 146B7 and human IgG

N-hydroxysuccinimido-biotin (Sigma) was first diluted in DMSO (final dilution: 100 mg/ml) and then in 0.1 M $NaHCO_3$ (final dilution: 1 mg/ml, Sigma). Per 1 mg of antibody (diluted in 1 ml), 600 µl of biotin solution was added (dark, 2hrs, RT). Antibody-biotin solution was dialysed in a slide-a-lyzer™ dialysis cassette (10,000 MWCO, Pierce, Perbio Science, Netherlands) (overnight at 4°C) to remove unlabeled biotin. The following day, concentration of biotinylated antibodies was determined by spectrophotometry (Ultrospec 2100pro) at OD 280 nm.

Binding of 146B7 to IL-15 – IL-15Rα complex by ELISA

After coating (overnight at room temperature) flat bottom microtiter plates (Greiner) with IL-15Rα (R&D systems, Minneapolis, MN, USA), plates were incubated with PBS and chicken serum (2%, RT, 60 min). After washing in PBS (+ 0.05% Tween 20: PBST), plates were subsequently incubated with several dilutions of unlabeled IL-15 (50 µl, RT, Immunex, Seattle, USA). After 10 minutes, biotinylated antibodies were added to the wells (50 µl) in different concentrations (90 minutes at room temperature). After washing in PBST, plates were incubated (60 minutes at room temperature) with streptavidin-poly-horseradish peroxidase (CLB, Amsterdam, Netherlands) diluted 1:10,000 in PBST-C (PBST and 2% chicken serum). Finally, plates were washed and subsequently incubated with ABTS (Azinobis-3-ethylbenzthiazoline-sulphonic-acid, Roche Diagnostics, Mannheim, Germany) in ABTS buffer according to the manufacturer's protocol. Color reaction was stopped with 2% oxalic acid (50 µl). Binding was evaluated at 405 nm in an EL808 ELISA-reader (Bio-Tek Instruments, Winooski, VT, USA).

Binding of 146B7 to IL-15 – IL-15R complex on Raji cells

Raji cells are pre-incubated (20 minutes at 4°C) with 10% human pooled AB serum (CLB, Amsterdam, Netherlands)in FACS buffer (PBS, 0.05%BSA, 0.02% $NaNO_3$). Raji cells ($1\text{-}2*10^5$ cells/ml) were put in the wells, and 50 µl of unlabeled IL-15 was added in several concentrations (diluted in FACS buffer with 10% human AB serum). After incubating the cells for 30 minutes (4°C) and washing twice in FACS buffer, 50 µl of biotinylated antibodies (146B7 or hIgG1) was added to the wells (30 minutes at 4°C). After washing twice in FACS buffer, 50 µl of streptavidin-phycoerythrin was added to each well (30 minutes at 4°C). After washing twice in FACS buffer, cells were taken up in 200 µl of FACS buffer, and fluorescence intensity of at least 5000 cells per sample was determined after analysis by flow cytometry (FACS Calibur, Becton Dickinson) using CellQuest software. Data show the stimulation index (S.I.), which is calculated as follows:

S.I. = (mean fluorescence positive staining)/(mean fluorescence background staining)

ELISA

Binding of 146B7 to IL-15/IL-15R complex in ELISA is shown in Figure 19. Binding of 146B7 increases with increasing concentrations of IL-15 binding to its receptor. No effects were observed of binding of control antibody to IL-15 or to IL-15R.

Binding to IL-15R-expressing Raji cells

Binding of 146B7 to IL-15/IL-15R complex on Raji cells is shown in Figure 20. 146B7 binds to the IL-15/IL-15R complex in a dose-dependent manner. No binding of hIgG1 to the IL-15/IL-15R complex on Raji cells was observed (Figure 20).

146B7 is able to bind IL-15 after binding of this cytokine to its receptor. 146B7 binds to an epitope on IL-15 that is not involved in binding to the receptor.

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Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims. Any combination of the embodiments disclosed in the dependent claims are contemplated to be within the scope of the invention.

Incorporation by Reference

All publications, patents, and pending patent applications referred to herein are hereby incorporated by reference in their entirety.

We claim:

1. An isolated human monoclonal antibody which specifically binds to human IL-15 and inhibits IL-15 induced proinflammatory effects.

2. The antibody of claim 1 which inhibits IL-15-induced TNFα production or T cell proliferation.

3. The antibody of claim 2 which inhibits IL-15 induced T cell proliferation with an IC_{50} value of less than approximately 100 nM as determined by proliferation inhibition assay.

4. The antibody of claim 2 which inhibits IL-15 induced T cell proliferation with an IC_{50} value of less than approximately 10 nM as determined by proliferation inhibition assay.

5. The antibody of claim 1 which binds to human IL-15 with a dissociation equilibrium constant (K_D) of below 10^{-7} M as determined by surface plasmon resonance (SPR) technology using recombinant human IL-15 as the analyte and the antibody as the ligand.

6. The antibody of claim 1, wherein the antibody specifically binds to an epitope located on the β-chain or the γ-chain interacting domain of human IL-15.

7. The antibody of claim 6, wherein the antibody specifically binds to an epitope located on the γ-chain interacting domain of human IL-15.

8. The antibody of claim 1, wherein the antibody interferes with the binding of Asp^8 of human IL-15 to the β-unit of the human IL-15 receptor or the binding of Gln^{108} of human IL-15 to the γ-unit of human IL-15 receptor.

9. The antibody of claim 1, wherein the antibody specifically binds to receptor-bound human IL-15.

10. An isolated human monoclonal antibody which specifically binds to human IL-15 encoded by human IgG heavy chain and human kappa light chain nucleic acids comprising nucleotide sequences in their variable regions as set forth in Figure 2 (SEQ ID NO:1) and Figure 3 (SEQ ID NO:3), respectively, and conservative sequence modifications thereof.

11. An isolated human monoclonal antibody which specifically binds to human IL-15 having IgG heavy chain and kappa light chain variable regions which comprise the amino acid sequences shown in Figure 2 (SEQ ID NO:2) and Figure 3 (SEQ ID NO:4), respectively, and conservative sequence modifications thereof.

12. An isolated human monoclonal antibody which specifically binds to human IL-15 comprising a CDR domain selected from the group consisting of

(a) a CDR1 domain comprising the amino acid sequence CDR1 shown in Figure 2 (SEQ ID NO:2) and Figure 3 (SEQ ID NO:4), respectively, and conservative sequence modifications thereof ,

(b) a CDR2 domain comprising the amino acid sequence CDR2 shown in Figure 2 (SEQ ID NO:2) and Figure 3 (SEQ ID NO:4), respectively, and conservative sequence modifications thereof, and

(c) a CDR3 domain comprising the amino acid sequence CDR3 shown in Figure 2 (SEQ ID NO:2) and Figure 3 (SEQ ID NO:4), respectively, and conservative sequence modifications thereof,

wherein the CDR domain is inserted in an antibody framework or joined by a synthetic linker.

13. The antibody of claim 1, wherein the antibody is selected from the group consisting of an IgG1, an IgG2, an IgG3, an IgG4, an IgM, an IgA1, an IgA2, an IgAsec, an IgD, and an IgE antibody.

14. The antibody of claim 1, comprising an IgG1 heavy chain.

15. The antibody of claim 1 which is an antibody fragment or a single chain antibody.

16. The antibody of claim 1 which is a whole antibody.

17. The antibody of claim 1, produced by a hybridoma which includes a B cell obtained from a transgenic non-human animal having a genome comprising a human heavy chain transgene and a human light chain transgene fused to an immortalized cell.

18. An isolated human monoclonal antibody which specifically binds to human IL-15 and inhibits the ability of IL-15 to induce proinflammatory effects upon binding to its receptor.

19. A method of inhibiting IL-15 induced, but not IL-2 induced, TNFα production in T cells or monocytes, comprising contacting IL-15 with an isolated human monoclonal antibody which specifically binds to human IL-15.

20. A method of inhibiting IL-15 induced, but not IL-2 induced, T cell proliferation, comprising contacting IL-15 with an isolated human monoclonal antibody which specifically binds to human IL-15 in the presence of said T cells.

21. The method of claim 20, wherein the T cells are peripheral blood mononuclear cells (PBMCs) or CTLL-2 cells.

22. A hybridoma comprising a B cell obtained from a transgenic non-human animal having a genome comprising a human heavy chain transgene and a light chain transgene, fused to an immortalized cell, wherein the hybridoma produces a human monoclonal antibody which specifically binds to human IL-15.

23. The hybridoma of claim 22 which produces a human monoclonal antibody encoded by human IgG heavy chain and human kappa light chain nucleic acids.

24. A hybridoma which produces a human monoclonal antibody encoded by human IgG heavy chain and human kappa light chain nucleic acids comprising nucleotide sequences in their variable regions as set forth in Figure 2 (SEQ ID NO:1) and Figure 3 (SEQ ID NO:3), respectively, and conservative sequence modifications thereof.

25. A hybridoma which produces a human monoclonal having IgG heavy chain and kappa light chain variable regions which comprise the amino acid sequences shown in Figure 2 (SEQ ID NO:2) and Figure 3 (SEQ ID NO:4), respectively, and conservative sequence modifications thereof.

26. The isolated human antibody of claim 1 produced by a transfectoma comprising nucleic acids encoding a human heavy chain and a human light chain.

27. A transfectoma comprising nucleic acids encoding a human heavy chain and a human light chain, wherein the transfectoma produces a detectable amount of the monoclonal antibody of claim 1.

28. The transfectoma of claim 27, comprising nucleic acids encoding a human heavy chain and a human light chain comprising nucleotide sequences in their variable regions as set forth in SEQ ID NO:1 and SEQ ID NO:3, respectively, or conservative sequence modifications thereof.

29. A transgenic non-human animal which expresses a human monoclonal antibody which specifically binds to human IL-15, wherein the transgenic non-human animal has a genome comprising a human heavy chain transgene and a human light chain transgene.

30. A method of producing a human monoclonal antibody which specifically binds to human IL-15, comprising:

immunizing a transgenic non-human animal having a genome comprising a human heavy chain transgene and a human light chain transgene with human IL-15 or a cell expressing human IL-15, such that antibodies are produced by B cells of the animal;

isolating B cells of the animal;

fusing the B cells with myeloma cells to form immortal, hybridoma cells that secrete human monoclonal antibodies specific for IL-15; and

isolating the human monoclonal antibodies specific for IL-15 from the culture supernatant of the hybridoma.

31. An immunoconjugate comprising the antibody of claim 1 and a therapeutic agent.

32. The immunoconjugate of claim 31, wherein the therapeutic agent is an immunosuppressive agent.

33. The immunoconjugate of claim 31, wherein the therapeutic agent is an anti-inflammatory agent selected from the group consisting of a steroidal anti-inflammatory agent, a nonsteroidal anti-inflammatory agent and a DMARD.

34. The immunoconjugate of claim 31, wherein the therapeutic agent is a cytotoxic agent.

35. A pharmaceutical composition comprising the antibody of claim 1 and a pharmaceutically acceptable carrier.

36. The composition of claim 35 further comprising a therapeutic agent.

37. The composition of claim 36, wherein the agent is an immunosuppressive agent.

38. The composition of claim 37, wherein the immunosuppressive agent is cyclosporine.

39. The composition of claim 36, wherein the agent is an anti-inflammatory agent selected from the group consisting of a steroidal anti-inflammatory agent and a nonsteroidal anti-inflammatory agent.

40. The composition of claim 36, wherein the agent is a DMARD selected from the group consisting of methotrexate, etanercept, and infliximab.

41. The composition of claim 36, wherein the agent is a chemotherapeutic agent selected from the group consisting of doxorubicin, cisplatin, bleomycin, carmustine, cyclophosphamide, and chlorambucil.

42. The composition of claim 36, wherein the agent is an agent for treating psoriasis.

43. The composition of claim 36, wherein the agent is an antibody.

44. The composition of claim 43, wherein the antibody is selected from the group consisting of a CD4 specific antibody and an IL-2 specific antibody.

45. A method of treating or preventing a disorder mediated by human IL-15, comprising administering to a subject the antibody of claim 1 in an amount effective to treat or prevent the disorder.

46. The method of claim 45, wherein the disorder is selected from the group consisting of psoriasis, arthritis, inflammatory bowel disease, cancer, transplant rejection and infectious disease.

47. The method of claim 46, wherein the arthritis is rheumatoid arthritis.

48. The method of claim 45, further comprising co-administration of a therapeutic agent.

49. The method of claim 48, wherein the agent is an immunosuppressive agent. cyclosporine.

50. The method of claim 49, wherein the immunosuppressive agent is cyclosporine.

51. The method of claim 48, wherein the agent is an anti-inflammatory agent selected from the group consisting of a steroidal anti-inflammatory agent and a nonsteroidal anti-inflammatory agent.

52. The method of claim 48, wherein the agent is a DMARD selected from the group consisting of methotrexate, etanercept, and infliximab.

53. The method of claim 48, wherein the agent is a chemotherapeutic selected from the group consisting of doxorubicin, cisplatin, bleomycin, carmustine, cyclophosphamide, and chlorambucil.

54. The method of claim 48, wherein the agent is an agent for treating psoriasis.

55. The method of claim 48, wherein the agent is an antibody.

56. The method of claim 55, wherein the antibody is selected from the group consisting of a CD4 specific antibody and an IL-2 specific antibody.

57. A method of treating or preventing psoriasis, comprising administering to a subject an isolated human monoclonal antibody which specifically binds to human IL-15 and inhibits the ability of IL-15 to induce proinflammatory effects upon binding to its receptor.

58. The method of claim 57, wherein the antibody inhibits the ability of IL-15 to induce parakeratosis.

59. The method of claim 57, wherein the antibody inhibits the ability of IL-15 to induce epidermal thickening.

60. The method of claim 57, wherein the antibody inhibits the ability of IL-15 to induce the proliferation of keratinocytes.

61. A method of treating or preventing rheumatoid arthritis, comprising administering to a subject an isolated human monoclonal antibody which specifically binds to human IL-15 and inhibits the ability of IL-15 to induce proinflammatory effects upon binding to its receptor.

62. The method of claim 61, wherein the antibody inhibits the ability of IL-15 to induce chemotaxis of activated leukocytes.

63. A method of diagnosing an IL-15-mediated disease by detecting the presence of IL-15 antigen, or a cell expressing IL-15, in a sample comprising:

contacting the sample, and a control sample, with the human antibody of claim 1, under conditions that allow for formation of a complex between the antibody or portion thereof and IL-15; and

detecting the formation of a complex,

wherein a difference complex formation between the sample compared to the control sample is indicative of the presence of IL-15 in the sample.

64. A nucleic acid comprising a nucleotide sequence encoding a variable region of a human monoclonal antibody that inhibits the proinflammatory effects of IL-15.

65. A nucleic acid comprising a nucleotide sequence encoding a variable region of a human monoclonal antibody that specifically binds to human IL-15, wherein the nucleotide sequence is selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:3, and conservative sequence modifications thereof.

66. An expression vector comprising a nucleotide sequence encoding the variable region of a light chain, heavy chain, or both light and heavy chains of a human antibody which binds human IL-15.

67. The expression vector of claim 66, further comprising a nucleotide sequence encoding the CDR regions of a light chain, heavy chain or both light and heavy chains of a human antibody which binds IL-15.

68. An expression vector comprising a nucleotide sequence encoding heavy chain and light chain variable regions which comprise the amino acid sequences shown in SEQ ID NO:2 and SEQ ID NO:4, respectively, and conservative sequence modifications thereof.

69. An expression vector comprising a nucleotide sequence encoding heavy and light variable regions which comprise the amino acid sequences shown in SEQ ID NO:2 and SEQ ID NO:4, respectively, and conservative modifications thereof.

70. A transfectoma comprising the expression vector of claim 66.

Figure 1

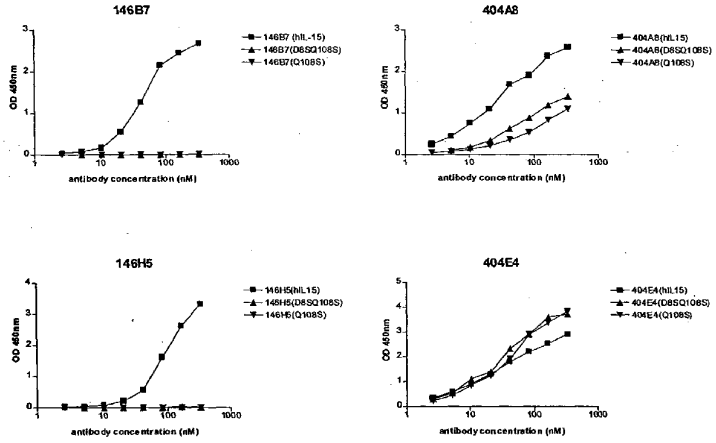

146B7
OD 450nm
antibody concentration (nM)
146B7 (hIL-15)
146B7(D85Q108S)
146B7(Q108S)
404A8
OD 450nm
antibody concentration (nM)
404A8(hIL15)
404A8(D85Q108S)
404A8(Q108S)
146H5
OD 450nm
antibody concentration (nM)
146H5(hIL15)
146H5(D85Q108S)
146H5(Q108S)
404E4
OD 450nm
antibody concentration (nM)
404E4(hIL15)
404E4(D85Q108S)
404E4(Q108S)

Figure 2

146B7 VH

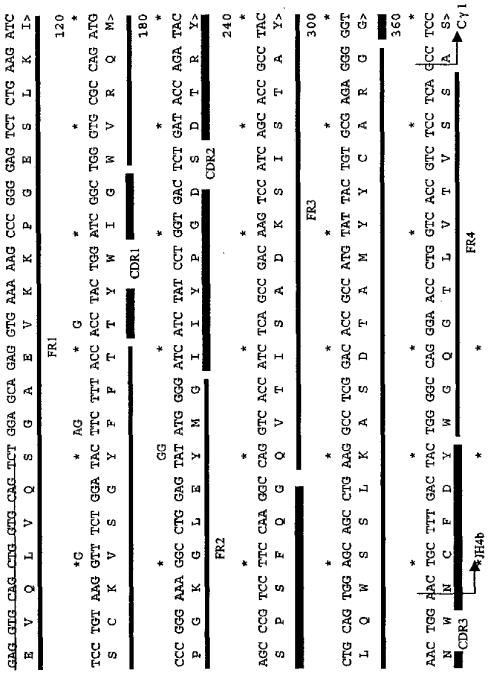
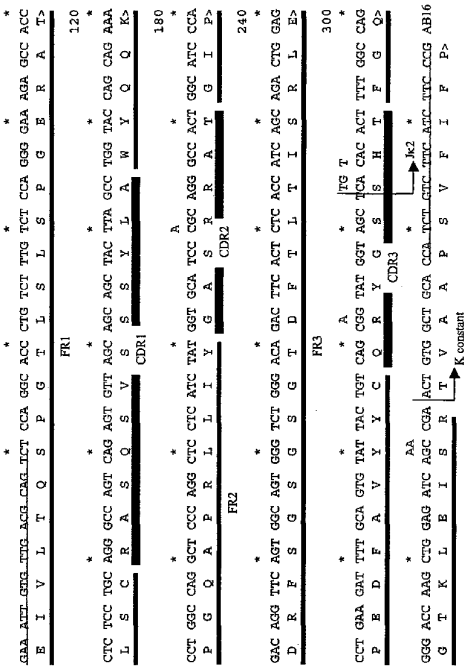


Figure 3

146B7 VK



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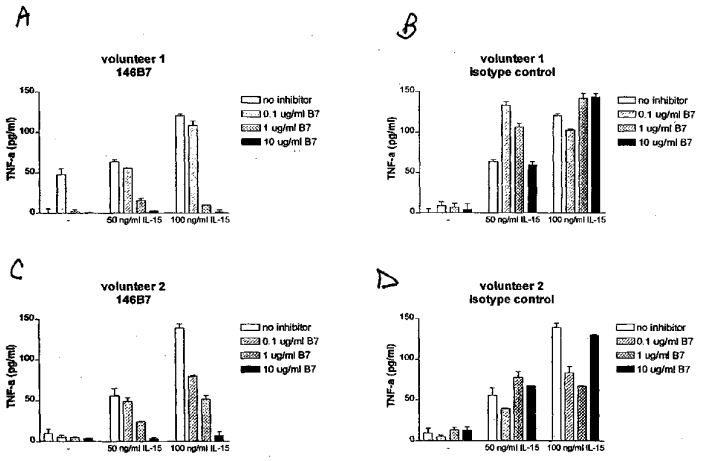
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volunteer 1
146B7
TNF-a (pg/ml)
150
100
50
0
-
50 ng/ml IL-15
100 ng/ml IL-15
no inhibitor
0.1 ug/ml B7
1 ug/ml B7
10 ug/ml B7
B
volunteer 1
isotype control
C
volunteer 2
146B7
D
volunteer 2
isotype control

Figure 5

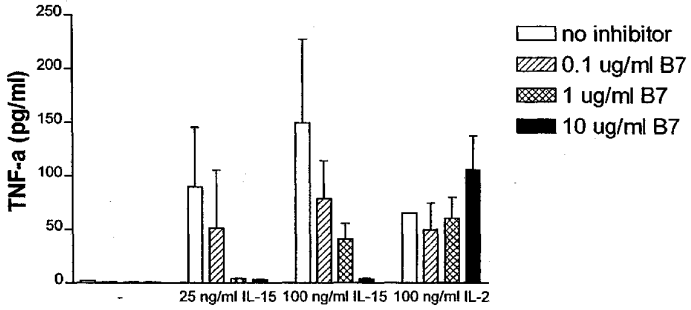

250
200
150
100
50
0
TNF-a (pg/ml)
-
25 ng/ml IL-15
100 ng/ml IL-15
100 ng/ml IL-2
no inhibitor
0.1 ug/ml B7
1 ug/ml B7
10 ug/ml B7

Figure 6

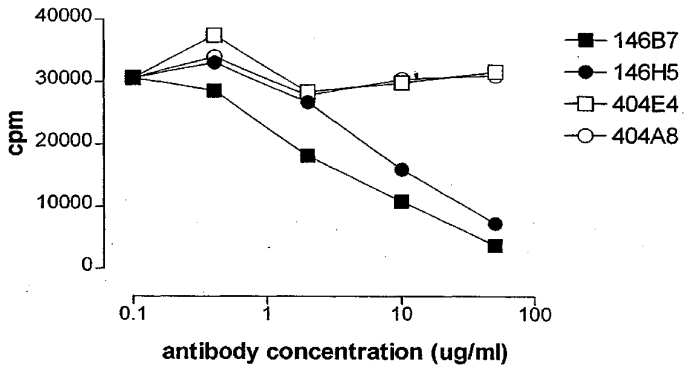

40000
30000
20000
10000
0
cpm
0.1
1
10
100
antibody concentration (ug/ml)
146B7
146H5
404E4
404A8

Figure 7A and B

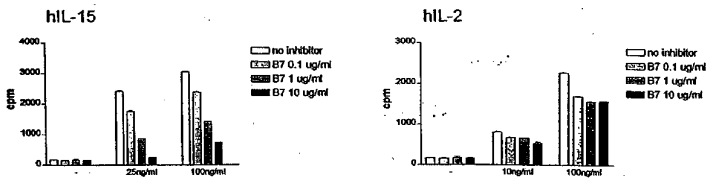


Figure 8A and B

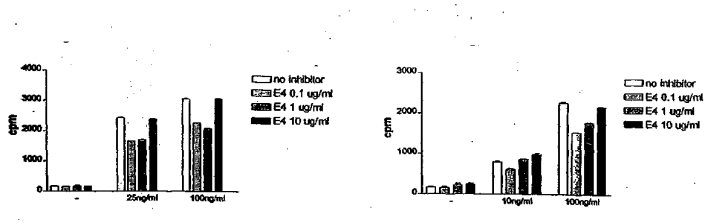
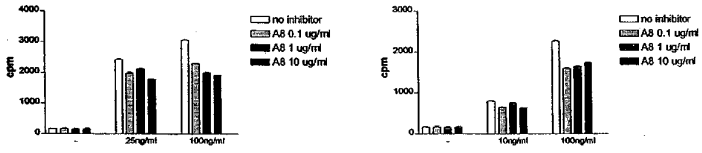


Figure 9A and B



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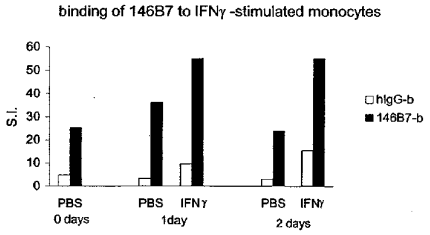
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S.I.
60
50
40
30
20
10
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PBS
0 days
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1day
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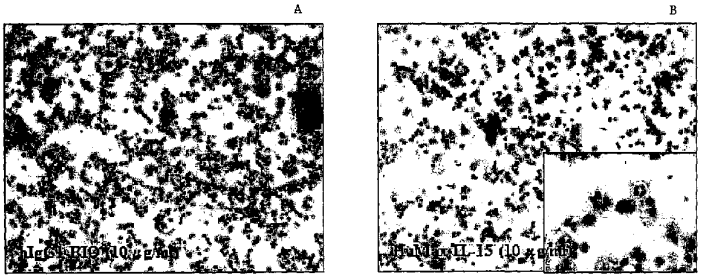
IFNγ
2 days
hIgG-b
146B7-b

Figure 11



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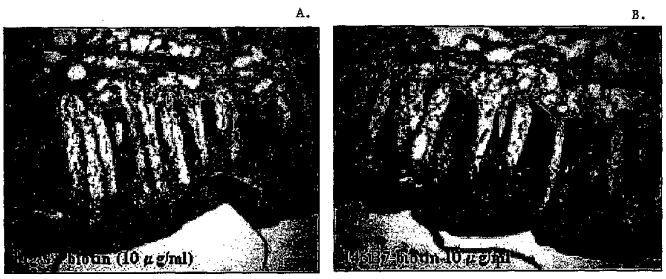
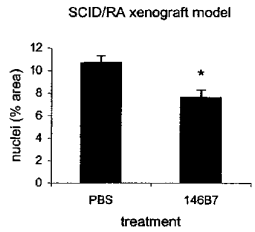


Figure 13A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nuclei (% area)
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10
8
6
4
2
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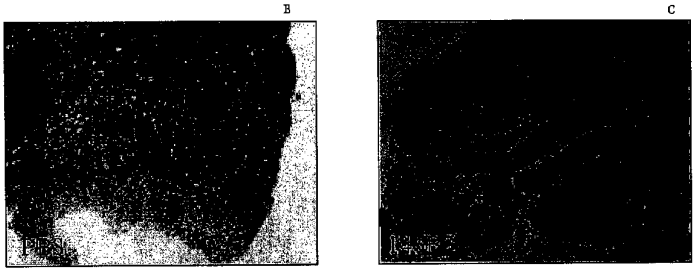
*
PBS
146B7
treatment

Figure 13 (cont.)



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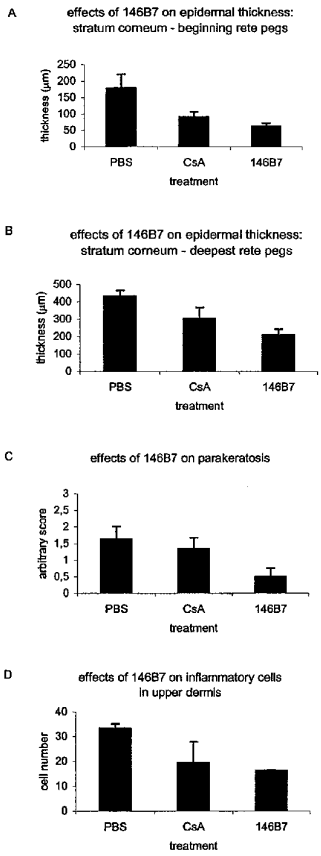
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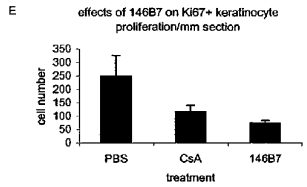
stratum corneum - beginning rete pegs
thickness (µm)
250
200
150
100
50
0
PBS
CsA
146B7
treatment
B
effects of 146B7 on epidermal thickness:
stratum corneum - deepest rete pegs
thickness (µm)
500
400
300
200
100
0
PBS
CsA
146B7
treatment
C
effects of 146B7 on parakeratosis
arbitrary score
3
2,5
2
1,5
1
0,5
0
PBS
CsA
146B7
treatment
D
effects of 146B7 on inflammatory cells
in upper dermis
cell number
40
30
20
10
0
PBS
CsA
146B7
treatment

Figure 14(cont.)



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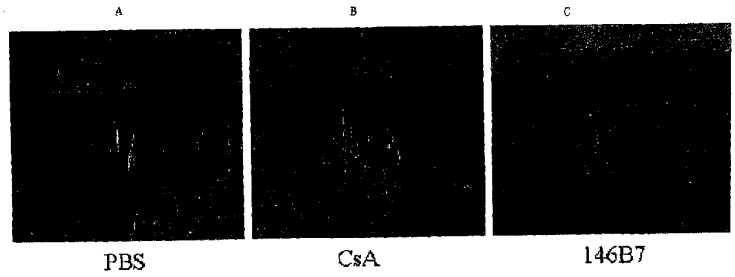

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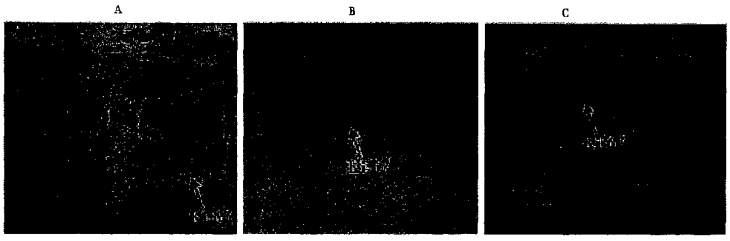

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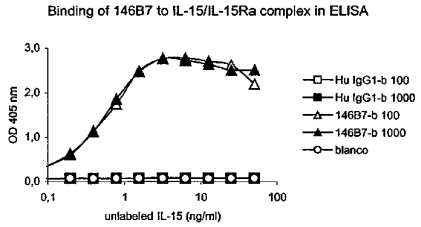
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2,0
1,0
0,0
0,1
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10
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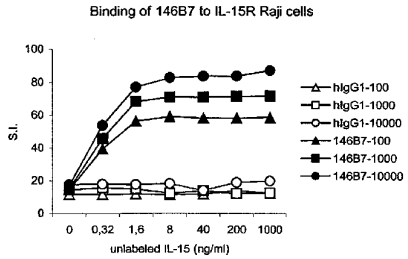
Hu IgG1-b 1000
146B7-b 100
146B7-b 1000
blanco

Figure 18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60
40
20
0
0
0,32
1,6
8
40
200
1000
unlabeled IL-15 (ng/ml)
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Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
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Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
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Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

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Ile Tyr Gly Ala Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

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cct gaa gat ttt gca gtg tat tac tgt cag cgg tat ggt agc tca cac 288
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Arg Tyr Gly Ser Ser His

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                85                  90                  95

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Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Ser Arg Thr Val Ala Ala
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Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
        35                  40                  45
Ile Tyr Gly Ala Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
    50                  55                  60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65                  70                  75                  80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Arg Tyr Gly Ser Ser His
                85                  90                  95
Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Ser Arg Thr Val Ala Ala
            100                 105                 110
Pro Ser Val Phe Ile Phe Pro
        115
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(54) Title: HUMAN ANTIBODIES SPECIFIC FOR INTERLEUKIN 15 (IL-15)

(57) Abstract: Isolated human monoclonal antibodies which specifically bind to IL-15 (e.g., human IL-15), and related antibody-based compositions and molecules, are disclosed. The human antibodies can be produced in a transfectoma or in a non-human transgenic animal, e.g., a transgenic mouse, capable of producing multiple isotypes of human monoclonal antibodies by undergoing V-D-J recombination and isotype switching. Also disclosed are pharmaceutical compositions comprising the human antibodies, non-human transgenic animals, and hybridomas which produce the human antibodies, and therapeutic and diagnostic methods for using the human antibodies.

HUMAN ANTIBODIES SPECIFIC FOR INTERLEUKIN 15 (IL-15)

Background of the Invention

Interleukin-15 (IL-15) is a pro-inflammatory cytokine, a glycoprotein of 14-15 kD. Constitutive expression has been reported in various cells and tissues including monocytes and macrophages, fibroblasts, keratinocytes and dendritic cells (Waldmann and Tagaya, 1999; Fehniger and Caligiuri, 2001). The expression is upregulated under inflammatory conditions, as reported for monocytes stimulated with IFN-γ and LPS or by infection with viruses, bacteria or protozoans (Kirman *et al.*, 1998; Waldmann *et al.*, 1998; Waldmann and Tagaya, 1999; Fehniger and Caligiuri, 2001). Furthermore, in chronic inflammatory diseases such as rheumatoid arthritis, locally produced IL-15 is likely to amplify inflammation by the recruitment and activation of synovial T-cells. This IL-15-induced effect has been suggested to play a pivotal role in disease pathogenesis (Kirman *et al.*, 1998; McInnes *et al.*, 1996; McInnes *et al.*, 1997; McInnes and Liew, 1998; Fehniger and Caligiuri, 2001).

In vitro studies have shown that IL-15 shares several biological activities with IL-2, due to shared receptor components. The IL-15 receptor present on T-cells consists of an unique α-chain, IL-15Rα, but shares the β-chain and the γ-chain with IL-2R. As a consequence, both receptors use the same Jak/STAT-signaling elements. However, based on complex regulation and differential expression of IL-2 and IL-15 and their receptors, critical differences in the *in vivo* functions have been reported (Kirman *et al.*, 1998; Waldmann and Tagaya, 1999; Waldmann *et al.*, 2001). It is also important to note the non-redundant role for IL-15 in natural killer (NK) cell, NK-T cell and intraepithelial lymphocyte development, survival, expansion and function (Kennedy *et al.*, 2000; Liu *et al.*,2000).

McInnes and coworkers (McInnes *et al.*, 1997; McInnes and Liew, 1998) reported the induction of TNF-α production after IL-15 stimulation in T-cells derived from rheumatoid arthritis patients. Furthermore, peripheral blood T cells activated by IL-15 were shown to induce significant TNF-α production by macrophages via a cell-contact-dependent mechanism. Because of the destructive role of TNF-α in rheumatoid arthritis, inhibition of this cytokine decreases disease activity (Bathon *et al.*, 2000; Klippel, 2000; Lovell *et al.*, 2000; Maini and Taylor, 2000).

Summary of the Invention

The present invention is based on the generation and isolation, for the first time, of fully human monoclonal antibodies which specifically bind to human IL-15 and which inhibit the proinflammtory effects induced by IL-15, as well as the characterization of such novel antibodies and the demonstration of their therapeutic value in treating a variety of IL-15 mediated diseases. For example, as described herein, the human antibodies have been shown to inhibit both TNFα production and T cell proliferation, both of which are integrally involved in inflammatory disorders. Accordingly, the human antibodies of the present invention provide an improved means for treating and preventing such disorders (and any other IL-15 mediated disorder), attributable in part to their unique specificity (*e.g.*, epitope and species specificity), affinity, structure, functional activity and the fact that they are fully human, making them significantly less immunogenic and more therapeutically effective and useful when administered to human patients than other IL-15 antibodies previously generated (*e.g.*, murine and humanized antibodies). The present invention is also based on the discovery of new therapeutic applications, including treatment of inflammatory diseases, such as rheumatoid arthritis, psoriasis, transplant rejections and cancers, for IL-15 inhibiting antibodies such as the human antibodies described herein.

Isolated human antibodies of the invention include a variety of antibody isotypes, such as IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgAsec, IgD, and IgE. Typically, they include IgG1 (*e.g.*, IgG1k), IgG3 and IgM isotypes. The antibodies can be full-length (*e.g.*, an IgG1 or IgG3 antibody) or can include only an antigen-binding portion (*e.g.*, a Fab, $F(ab')_2$, Fv, a single chain Fv fragment, an isolated complementarity determining region (CDR) or a combination of two or more isolated CDRs).

In one embodiment, the human antibodies are recombinant antibodies. In a particular embodiment, the human antibody is encoded by human IgG heavy chain and human kappa light chain nucleic acids comprising nucleotide sequences in their variable regions as set forth in SEQ ID NO:1 and SEQ ID NO:3, respectively, and conservative sequence modifications thereof. In another embodiment, the human antibody includes IgG heavy chain and kappa light chain variable regions which comprise the amino acid sequences shown in SEQ ID NO:2 and SEQ ID NO:4, respectively, and conservative sequence modifications thereof.

Human antibodies of the invention can be produced recombinantly in a host cell, such as a transfectoma (*e.g.*, a transfectoma consisting of immortalized CHO cells or lymphocytic cells) containing nucleic acids encoding the heavy and light chains of the antibody, or be obtained directly from a hybridoma which expresses the antibody (*e.g.*, which includes a B cell obtained from a transgenic non-human animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a human light chain transgene that encode the antibody, fused to an immortalized cell). In a particular embodiment, the antibodies are produced by a hybridoma referred to herein as 146B7 or by a host cell (*e.g.*, a CHO cell) transfectoma containing human heavy chain and human light chain nucleic acids which comprise nucleotide sequences in their variable regions as set forth in SEQ ID NOs: 1 and 3, respectively, and conservative modifications thereof. In particular embodiments, the antibodies are produced by hybridomas referred to herein as 146B7, 146H5, 404E4, and 404A8. In a preferred embodiment, the antibody specifically binds to an epitope located on the β- and/or γ-chain interacting domain of IL-15.

In another embodiment, the human antibodies of the present invention specifically bind to human IL-15 and inhibit the ability of IL-15 to induce proinflammatory effects, *e.g.*, inhibit the production of TNFα and/or inhibit the proliferation of T cells, such as PBMC or CTLL-2 T cells, upon IL-15 binding to the IL-15 receptor. Typically, the human antibodies bind to IL-15 with a dissociation equilibrium constant (K_D) of less than approximately 10^{-7} M, such as less than approximately 10^{-8} M, 10^{-9} M or 10^{-10} M or even lower when determined by surface plasmon resonance (SPR) technology in a BIACORE 3000 instrument using recombinant human IL-15 as the analyte and the antibody as the ligand. In a particular embodiment, the antibody binds to human IL-15 with a dissociation equilibrium constant (K_D) of approximately 6.5 x 10^{-8} M.

In another aspect, the invention provides nucleic acid molecules encoding the antibodies, or antigen-binding portions, of the invention. Accordingly, recombinant expression vectors which include the antibody-encoding nucleic acids of the invention, and host cells transfected with such vectors, are also encompassed by the invention, as are methods of making the antibodies of the invention by culturing these host cells.

The invention also relates to an expression vector comprising a nucleotide sequence encoding heavy and light variable regions which comprise the amino acid sequences shown in SEQ ID NO:2 and SEQ ID NO:4, respectively, and conservative modifications thereof. Such expression vectors are well known in the art. Examples hereof include *in vitro* transcription/translation vectors using, for example, reticulocyte lysates.

In yet another aspect, the invention provides isolated B-cells from a transgenic non-human animal, *e.g.*, a transgenic mouse, which are capable of expressing various isotypes (*e.g.*, IgG, IgA and/or IgM) of human monoclonal antibodies that specifically bind to IL-15. Preferably, the isolated B cells are obtained from a transgenic non-human animal, *e.g.*, a transgenic mouse, which has been immunized with a purified or enriched preparation of IL-15 antigen and/or cells expressing IL-15. Preferably, the transgenic non-human animal, *e.g.*, a transgenic mouse, has a genome comprising a human heavy chain transgene and a human light chain transgene. The isolated B-cells are then immortalized to provide a source (*e.g.*, a hybridoma) of human monoclonal antibodies to IL-15.

Accordingly, the present invention also provides a hybridoma capable of producing human monoclonal antibodies that specifically bind to IL-15. In one embodiment, the hybridoma includes a B cell obtained from a transgenic non-human animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a human light chain transgene fused to an immortalized cell. The transgenic non-human animal can be immunized with a purified or enriched preparation of IL-15 antigen and/or cells expressing IL-15 to generate antibody-producing hybridomas. Particular hybridomas provided by the invention include 146B7, 146H5, 404E4, and 404A8.

In yet another aspect, the invention provides a transgenic non-human animal, such as a transgenic mouse, which expresses human monoclonal antibodies that specifically bind to IL-15. In a particular embodiment, the transgenic non-human animal is a transgenic mouse having a genome comprising a human heavy chain transgene and a human light chain transgene. The transgenic non-human animal can be immunized with a purified or enriched preparation of IL-15 antigen and/or cells expressing IL-15. Preferably, the transgenic non-human animal, *e.g.*, the transgenic mouse, is capable of producing multiple isotypes of human monoclonal antibodies to IL-

15 (*e.g.*, IgG, IgA and/or IgM) by undergoing V-D-J recombination and isotype switching. Isotype switching may occur by, *e.g.*, classical or non-classical isotype switching.

In another aspect, the present invention provides methods for producing human monoclonal antibodies which specifically react with IL-15. In one embodiment, the method includes immunizing a transgenic non-human animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a human light chain transgene, with a purified or enriched preparation of IL-15 antigen and/or cells expressing IL-15. B cells (*e.g.*, splenic B cells) of the animal are then obtained and fused with myeloma cells to form immortal, hybridoma cells that secrete human monoclonal antibodies against IL-15.

In another aspect, the present invention features a human anti-IL-15 antibody conjugated to a therapeutic moiety, *e.g.*, a cytotoxic drug, an enzymatically active toxin, or a fragment thereof, a radioisotope, or a small molecule anti-cancer drug.

In another aspect, the present invention provides compositions, *e.g.*, pharmaceutical and diagnostic compositions, comprising a pharmaceutically acceptable carrier and at least one human monoclonal antibody of the invention which specifically binds to IL-15. The composition can further include other therapeutic agents, such as other immunosuppressive agents, or chemotherapeutic agents.

In yet another aspect, the invention provides methods for inhibiting the proinflammatory effects of IL-15, such as inhibiting IL-15 induced TNFα production and/or T cell proliferation, preferably without inhibiting the activity (*e.g.*, TNFα production and/or T cell proliferation) of structurally related proteins/cytokines (*e.g.*, IL-2) using one or more human antibodies of the invention.

Human antibodies of the present invention can be used to treat and/or prevent a variety of IL-15 mediated diseases by administering the antibodies to patients suffering from such diseases.

Exemplary diseases that can be treated (*e.g.*, ameliorated) or prevented using the methods and compositions of the invention include, but are not limited to, inflammatory disorders, such as arthritis (*e.g.*, psoriatic arthritis and rheumatoid arthritis including active rheumatoid arthritis and juvenile rheumatoid arthritis), inflammatory bowel disease. For example, the antibodies have been shown to reduce parakeratosis, reduce epidermal thickness and reduce proliferation of keratinocytes in psoriasis. The

antibodies also have been shown to reduce inflammation and/or prevent chemotaxis of activated leukocytes involved in rheumatoid arthritis. The antibodies also can be used to treat infectious diseases, such as HIV infection. Furthermore, the antibodies can be used to treat transplant rejection. Still further, the antibodies can be used to treat a variety of diseases involving IL-15 mediated neovascularization, such as tumor growth and cancers, *e.g.* T-cell leukaemia.

The human antibodies of the present invention may also be combined with one or more additional therapeutic agents, such as anti-inflammatory agents, DMARDs (disease-modifying anti-rheumatic drugs), immunosuppressive agents, chemotherapeutics, and psoriasis agents.

In one embodiment, the subject can be additionally treated with one or more agents that enhance the inhibition of the proinflammatory effect of the antibodies, *e.g.*, an anti-inflammatory agent, such as a steroidal drug or a NSAID (nonsteroidal anti-inflammatory drug). Preferred agents include, for example, aspirin and other salicylates, Cox-2 inhibitors, such as rofecoxib (Vioxx) and celecoxib (Celebrex), NSAIDs such as ibuprofen (Motrin, Advil), fenoprofen (Nalfon), naproxen (Naprosyn), sulindac (Clinoril), diclofenac (Voltaren), piroxicam (Feldene), ketoprofen (Orudis), diflunisal (Dolobid), nabumetone (Relafen), etodolac (Lodine), oxaprozin (Daypro), and indomethacin (Indocin).

In another embodiment, the human antibodies of the invention can be administered in combination with one or more DMARDs, such as methotrexate (Rheumatrex), hydroxychloroquine (Plaquenil), sulfasalazine (Asulfidine), pyrimidine synthesis inhibitors, *e.g.* leflunomide (Arava), IL-1 receptor blocking agents, *e.g.* anakinra (Kineret), and TNF-α blocking agents, *e.g.* etanercept (Enbrel), infliximab (Remicade) and adalimumab.

In another embodiment, the human antibodies of the invention can be administered in combination with one or more immunosuppressive agents, such as cyclosporine (Sandimmune, Neoral) and azathioprine (Imural).

In another embodiment, the human antibodies of the invention can be administered in combination with one or more chemotherapeutics, such as doxorubicin (Adriamycin), cisplatin (Platinol), bleomycin (Blenoxane), carmustine (Gliadel), cyclophosphamide (Cytoxan, Procytox, Neosar), and chlorambucil (Leukeran). The

human antibodies according to the invention can also be administered in conjuction with radiation therapy.

In another embodiment, the human antibodies of the invention can be administered in combination with one or more agents for treating psoriasis, such as topical medications containing coal tar, A vitamin, cortisone or other corticosteroids, oral or injected medications, such as corticosteroids, methotrexate, retinoids, e.g. acicretin (Neogitason) or cyclosporine (Sandimmune, Neoral). Other treatments may include exposure to sunlight or phototherapy.

In another embodiment, the human antibodies of the invention can be administered in combination with other antibodies, such as CD4 specific antibodies and IL-2 specific antibodies. A combination of the present human antibodies with CD4 specific antibodies or IL-2 specific antibodies are considered particularly useful for treating autoimmune diseases and transplant rejections.

In yet another aspect, the present invention provides a method for detecting *in vitro* or *in vivo* the presence of the IL-15 antigen in a sample, *e.g.*, to diagnose IL-15-mediated diseases. In one embodiment, this is achieved by contacting a sample to be tested, along with a control sample, with a human monoclonal antibody of the invention, or an antigen-binding portion thereof under conditions that allow for formation of a complex between the antibody and IL-15. Complex formation is then detected (*e.g.*, using an ELISA) in both samples, and any statistically significant difference in the formation of complexes between the samples is indicative of the presence of the IL-15 antigen in the test sample.

Other features and advantages of the instant invention will be apparent from the following detailed description and claims.

Brief Description of the Drawings

Figure 1 includes graphs showing the binding of the human IL-15 specific antibodies, 146B7, 147H5, 404A8 and 404E4, to human IL-15 (hIL-15) and to the mutant IL-15 proteins, Q108S and D8SQ108S. Serial dilutions of the antibodies were examined for their binding to hIL-15 or the mutant IL-15 proteins D8SQ108S and Q108S in an ELISA.

Figures 2 and 3 show amino acid (SEQ ID NOs:2 and 4) and nucleotide (SEQ ID NOs:1 and 3) sequences of the V_H and V_L-regions, respectively, from antibody 146B7. The framework (FR) and complementarity determining regions (CDR) are indicated.

Figures 4A-D include graphs showing the inhibition of IL-15-mediated TNF-α release by antibody 146B7. Human PBMC were incubated with hIL-15 (0, 50, 100 ng/ml) in combination with 146B7 antibody or with an isotype control antibody (0.1, 1, 10 μg/ml) for 72 hours. The amount of TNF-α produced was measured by ELISA. Data from two healthy volunteers are shown.

Figure 5 is a graph showing the effect of antibody 146B7 on IL-2 or IL-15-mediated TNF-α production. Human PBMC were incubated with hIL-15 (0, 50, 100 ng/ml) or with hIL-2 (100 ng/ml) in combination with 146B7 (0.1, 1, 10 μg/ml) for 72 hours. The amount of TNF-α produced was measured by ELISA.

Figure 6 is a graph showing the inhibitory activity of antibodies 146B7, 146H5, 404E4 and 404A8 on hIL-15 induced CTLL-2 proliferation. CTLL-2 cells starved for hIL-2 were incubated with hIL-15 (60 pg/ml) combined with serial dilutions of 146B7, 146H5, 404E4 and 404A8 for 48 hours. [^{3}H]-Thymidine incorporation was measured to express proliferation (cpm). The results are presented as mean values.

Figures 7-9 include graphs showing the inhibitory activity of antibodies 146B7 (Figure 7), 404E4 (Figure 8) and 404A8 (Figure 9) on IL-15 induced PBMC proliferation. Human PBMC were incubated with hIL-15 (0, 25, 100 ng/ml; Figures 7A, 8A, and 9A, respectively) or hIL-2 (0, 10, 100 ng/ml; Figures 7B, 8B, and 9B, respectively) in combination with 146B7 (Figure 7), 404E4 (Figure 8) or 404A8 (Figure 9) at 0.1, 1, 10 μg/ml for 72 hours. [3H]-Thymidine incorporation was measured to express proliferation (cpm).

Figure 10 is a graph showing the binding of antibody 146B7 to IFNγ-stimulated monocytes. Human PBMCs were cultured in the presence of IFNγ (500 U/ml) for up to 2 days (37°C). Fluorescence intensity of at least 5000 cells per sample was determined after analysis by flow cytometry and gating on the monocytes. Data show the stimulation index (S.I. = (mean fluorescence positive staining)/(mean fluorescence background staining)).

Figure 11 shows binding of human monocytes with antibody 146B7 (panel B) or with the isotype control antibody (panel A). Human PBMCs were isolated and cytospins were made after culturing the cells with IFNγ (500U/ml). Cells were counterstained with haematoxylin.

Figure 12 shows binding of human psoriatic skin with 146B7 (panel B) or with the isotype control antibody (hIgG1) (panel A). Human psoriatic plaques were obtained from patients after informed consent, and stored at –80°C until assay. Tissues were stained with biotinylated antibodies and visualized after activation of horse radish peroxidase.

Figure 13A is a graph showing the percentage of nucleated cells in rheumatoid arthritic tissue after treatment of SCID mice with 146B7 or with vehicle. Tissues were stained with haematoxilin and eosin (H&E) and analysed with Photo Shop version 6.0. Data are shown as mean and s.e.m. of nuclei (as percentage of total area) of mice after 146B7 treatment (n=4) or vehicle treatment (n=2). *Figure 13B* shows a representative H&E staining of xenografted RA tissue in SCID mice, after treatment with 146B7 (panel B) or with PBS (panel A).

Figure 14 includes graphs showing the effects of antibody 146B7 treatment in SCID/psoriasis mice. Biopsies were fixed in formalin for paraffin embedding and stained in H&E and for Ki-67 nuclear antigen. *Figure 14A* shows the severity of psoriasis evaluated by epidermal thickness which was measured from the stratum corneum to the beginning of the rete pegs. *Figure 14B* shows the epidermal thickness which was measured from the stratum corneum to the deepest part of the rete

pegs. *Figure 14C* shows the grade of parakeratosis. *Figure 14D* shows the number of inflammatory mononuclear cells in upper dermis. *Figure 14E* shows the number of Ki-67+ cycling keratinocytes.

Figure 15 shows H&E staining of human psoriatic skin engrafted in SCID mice, after treatment with antibody 146B7 (panel C), with CsA (panel B), or with vehicle (panel A). Three weeks after transplantation mice received PBS (placebo), CsA (cyclosporine A) (Sandoz) at a dose of 10 mg/kg every second day for 15 days, or 146B7 at a dose of 20 mg/kg on day 1 and 10 mg/kg on days 8 and 15. One week after the last injection, mice were sacrificed, and a 4 mm punch biopsy was taken from each xenograft. Biopsies were fixed in formalin for paraffin embedding and stained in H&E.

Figure 16 shows Ki-67 staining of human psoriatic skin engrafted in SCID mice, after treatment with 146B7 (panel C), with CsA (panel B), or with vehicle (panel A). Three weeks after transplantation mice received PBS (placebo), CsA (cyclosporine A) (Sandoz) at a dose of 10 mg/kg every second day for 15 days, or 146B7 at a dose of 20 mg/kg on day 1 and 10 mg/kg on days 8 and 15. One week after the last injection, mice were sacrificed, and a 4 mm punch biopsy was taken from each xenograft. Biopsies were fixed in formalin for paraffin embedding and stained for Ki-67 nuclear antigen.

Figure 17 is a graph showing the binding of antibody 146B7 to receptor-bound IL-15. Plates were coated with IL-15Rα and incubated with IL-15. After 10 minutes, biotinylated 146B7 was added to the wells. Binding of 146B7 to receptor-bound IL-15 was evaluated at 405 nm in an ELISA-reader.

Figure 18 is a graph showings the binding of antibody 146B7 to IL-15, after binding of IL-15 to its receptor expressed on Raji cells. After incubation of IL-15R-expressing Raji cells with IL-15, biotinylated 146B7 was added to the cells after 10 minutes. Binding of 146B7 to receptor-bound IL-15 was evaluated by FACS analysis.

Detailed Description of the Invention

The present invention provides novel antibody-based therapeutics for treating and diagnosing a variety of disorders mediated by IL-15 (*i.e.*, disorders caused by the proinflammatory effects of IL-15). As used herein, the term "proinflammatory effects of IL-15" includes any humoral or cell-mediated immune response induced by IL-15, such as production of TNFα and other inflammatory mediators, and recruitment/proliferation of T-cells. Therapies of the invention employ isolated human monoclonal antibodies which specifically bind to an epitope present on IL-15.

In one embodiment, the human antibodies are produced in a non-human transgenic animal, *e.g.*, a transgenic mouse, capable of producing multiple isotypes of human monoclonal antibodies to IL-15 (*e.g.*, IgG, IgA and/or IgE) by undergoing V-D-J recombination and isotype switching. Accordingly, various aspects of the invention include antibodies and pharmaceutical compositions thereof, as well as non-human transgenic animals, B-cells, host cell transfectomas, and hybridomas for making such monoclonal antibodies. Methods of using the antibodies of the invention to detect cells to which IL-15 is bound, and/or to inhibit IL-15 mediated functions either *in vitro* or *in vivo*, are also encompassed by the invention. Methods for targeting agents to cells to which IL-15 is bound are also included.

In order that the present invention may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

The terms "IL-15," "IL-15 antigen" and "Interleukin 15" are used interchangeably herein, and include any variants or isoforms which are naturally expressed by cells.

The term "antibody" as referred to herein includes whole antibodies and any antigen binding fragment (*i.e.*, "antigen-binding portion") or single chain thereof. An "antibody" refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The V_H and V_L

regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system.

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, IL-15). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and CH1 domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a V_H domain; and (vi) an isolated complementarity determining region (CDR) or (vii) a combination of two or more isolated CDRs which may optionally be joined by a synthetic linker. Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

The term "monoclonal antibody" as used herein, refers to an antibody which displays a single binding specificity and affinity for a particular epitope. Accordingly, the term "human monoclonal antibody" refers to an antibody which displays a single binding specificity and which has variable and constant regions derived from human germline immunoglobulin sequences. In one embodiment, human monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic non-human animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

The term "recombinant human antibody", as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom (described further in Section I, below), (b) antibodies isolated from a host cell transformed to express the antibody, *e.g.*, from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial human antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

As used herein, a "heterologous antibody" is defined in relation to the transgenic non-human organism producing such an antibody. This term refers to an antibody having an amino acid sequence or an encoding nucleic acid sequence corresponding to that found in an organism not consisting of the transgenic non-human animal, and generally from a species other than that of the transgenic non-human animal.

An "isolated antibody", as used herein, is intended to refer to an antibody which is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds to IL-15 is substantially free of antibodies that specifically bind antigens other than IL-15). An isolated antibody that specifically binds to an epitope of IL-15 may, however, have cross-reactivity to other related cytokines or to other IL-15 proteins from different species. However, the antibody preferably always binds to human IL-15. In addition, an isolated antibody is typically substantially free of other cellular material and/or chemicals. In one embodiment of the invention, a combination of "isolated" monoclonal antibodies having different IL-15 specificities are combined in a well defined composition.

As used herein, "specific binding" refers to antibody binding to a predetermined antigen. Typically, the antibody binds with an affinity (K_D) of approximately less than 10^{-7} M, such as approximately less than 10^{-8} M, 10^{-9} M or 10^{-10} M or even lower when determined by surface plasmon resonance (SPR) technology in a BIACORE 3000 instrument using recombinant human IL-15 as the analyte and the antibody as the ligand, and binds to the predetermined antigen with an affinity that is at least two-fold greater than its affinity for binding to a non-specific antigen (*e.g.*, BSA, casein) other than the predetermined antigen or a closely-related antigen. The phrases "an antibody recognizing an antigen" and " an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen".

The term "K_D", as used herein, is intended to refer to the dissociation equilibrium constant of a particular antibody-antigen interaction.

As used herein, "isotype" refers to the antibody class (*e.g.*, IgM or IgGl) that is encoded by heavy chain constant region genes.

As used herein, "isotype switching" refers to the phenomenon by which the class, or isotype, of an antibody changes from one Ig class to one of the other Ig classes.

As used herein, "nonswitched isotype" refers to the isotypic class of heavy chain that is produced when no isotype switching has taken place; the CH gene encoding the nonswitched isotype is typically the first CH gene immediately downstream from the functionally rearranged VDJ gene. Isotype switching has been classified as classical or non-classical isotype switching. Classical isotype switching

occurs by recombination events which involve at least one switch sequence region in the transgene. Non-classical isotype switching may occur by, for example, homologous recombination between human σ_μ and human Σ_μ (δ-associated deletion). Alternative non-classical switching mechanisms, such as intertransgene and/or interchromosomal recombination, among others, may occur and effectuate isotype switching.

As used herein, the term "switch sequence" refers to those DNA sequences responsible for switch recombination. A "switch donor" sequence, typically a μ switch region, will be 5' (*i.e.*, upstream) of the construct region to be deleted during the switch recombination. The "switch acceptor" region will be between the construct region to be deleted and the replacement constant region (*e.g.*, γ, ε, etc.). As there is no specific site where recombination always occurs, the final gene sequence will typically not be predictable from the construct.

As used herein, "glycosylation pattern" is defined as the pattern of carbohydrate units that are covalently attached to a protein, more specifically to an immunoglobulin protein. A glycosylation pattern of a heterologous antibody can be characterized as being substantially similar to glycosylation patterns which occur naturally on antibodies produced by the species of the nonhuman transgenic animal, when one of ordinary skill in the art would recognize the glycosylation pattern of the heterologous antibody as being more similar to said pattern of glycosylation in the species of the nonhuman transgenic animal than to the species from which the CH genes of the transgene were derived.

The term "naturally-occurring" as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally-occurring.

The term "rearranged" as used herein refers to a configuration of a heavy chain or light chain immunoglobulin locus wherein a V segment is positioned immediately adjacent to a D-J or J segment in a conformation encoding essentially a complete V_H or V_L domain, respectively. A rearranged immunoglobulin gene locus can be identified by comparison to germline DNA; a rearranged locus will have at least one recombined heptamer/nonamer homology element.

The term "unrearranged" or "germline configuration" as used herein in reference to a V segment refers to the configuration wherein the V segment is not recombined so as to be immediately adjacent to a D or J segment.

The term "nucleic acid molecule", as used herein, is intended to include DNA molecules and RNA molecules. A nucleic acid molecule may be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term "isolated nucleic acid molecule", as used herein in reference to nucleic acids encoding antibodies or antibody portions (*e.g.*, V_H, V_L, CDR3) that bind to IL-15, is intended to refer to a nucleic acid molecule in which the nucleotide sequences encoding the antibody or antibody portion are free of other nucleotide sequences encoding antibodies or antibody portions that bind antigens other than IL-15, which other sequences may naturally flank the nucleic acid in human genomic DNA. SEQ ID NOS: 1-4 correspond to the nucleotide and amino acid sequences comprising the heavy chain (V_H) and light chain (V_L) variable regions of the human anti-IL-15 antibody 146B7 of the invention. In particular, SEQ ID NO:1 and 2 correspond to the V_H of the 146B7 antibody, SEQ ID NO:3 and 4 correspond to the V_L of the 146B7 antibody.

The present invention also encompasses "conservative sequence modifications" of the sequences set forth in SEQ ID NOs: 1-4, *i.e.*, nucleotide and amino acid sequence modifications which do not significantly affect or alter the binding characteristics of the antibody encoded by the nucleotide sequence or containing the amino acid sequence. Such conservative sequence modifications include nucleotide and amino acid substitutions, additions and deletions. Modifications can be introduced into SEQ ID NOs:1-4 by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions include ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a human

anti-IL-15 antibody is preferably replaced with another amino acid residue from the same side chain family.

Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a anti-IL-15 antibody coding sequence, such as by saturation mutagenesis, and the resulting modified anti-IL-15 antibodies can be screened for binding activity.

Accordingly, antibodies encoded by the (heavy and light chain variable region) nucleotide sequences disclosed herein and/or containing the (heavy and light chain variable region) amino acid sequences disclosed herein (*i.e.*, SEQ ID NOs: 1-4) include substantially similar antibodies encoded by or containing similar sequences which have been conservatively modified. Further discussion as to how such substantially similar antibodies can be generated based on the partial (*i.e.*, heavy and light chain variable regions) sequences disclosed herein as SEQ ID Nos:1-4 is provided below.

For nucleic acids, the term "substantial homology" indicates that two nucleic acids, or designated sequences thereof, when optimally aligned and compared, are identical, with appropriate nucleotide insertions or deletions, in at least about 80% of the nucleotides, usually at least about 90% to 95%, and more preferably at least about 98% to 99.5% of the nucleotides. Alternatively, substantial homology exists when the segments will hybridize under selective hybridization conditions, to the complement of the strand.

The percent identity between two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % homology = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

The percent identity between two nucleotide sequences can be determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. The percent identity between two nucleotide or amino acid sequences can also be determined using the algorithm of E.

Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to the nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to the protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov.

The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. A nucleic acid is "isolated" or "rendered substantially pure" when purified away from other cellular components or other contaminants, *e.g.*, other cellular nucleic acids or proteins, by standard techniques, including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis and others well known in the art. *See*, F. Ausubel, *et al.*, ed. Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York (1987).

The nucleic acid compositions of the present invention, while often in a native sequence (except for modified restriction sites and the like), from either cDNA, genomic or mixtures thereof may be mutated, in accordance with standard techniques to provide gene sequences. For coding sequences, these mutations, may affect amino acid

sequence as desired. In particular, DNA sequences substantially homologous to or derived from native V, D, J, constant, switches and other such sequences described herein are contemplated (where "derived" indicates that a sequence is identical or modified from another sequence).

A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence. With respect to transcription regulatory sequences, operably linked means that the DNA sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame. For switch sequences, operably linked indicates that the sequences are capable of effecting switch recombination.

The term "vector", as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The term "recombinant host cell" (or simply "host cell"), as used herein, is intended to refer to a cell into which a recombinant expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications

may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

As used herein, the term "subject" includes any human or non-human animal. For example, the methods and compositions of the present invention can be used to treat a subject with an inflammatory disease, such as arthritis, *e.g.*, rheumatoid arthritis. The term "non-human animal" includes all vertebrates, *e.g.*, mammals and non-mammals, such as non-human primates, sheep, dog, cow, chickens, amphibians, reptiles, etc.

Various aspects of the invention are described in further detail in the following subsections.

I. Production of Human Antibodies to IL-15

Human monoclonal antibodies of the invention can be produced using a variety of known techniques, such as the standard somatic cell hybridization technique described by Kohler and Milstein, Nature 256: 495 (1975). Although somatic cell hybridization procedures are preferred, in principle, other techniques for producing monoclonal antibodies also can be employed, *e.g.*, viral or oncogenic transformation of B lymphocytes, phage display technique using libraries of human antibody genes.

The preferred animal system for generating hybridomas which produce human monoclonal antibodies of the invention is the murine system. Hybridoma production in the mouse is well known in the art, including immunization protocols and techniques for isolating and fusing immunized splenocytes.

In one embodiment, human monoclonal antibodies directed against IL-15 are generated using transgenic or transchromosomal mice carrying parts of the human immune system rather than the mouse system. In one embodiment, the invention employs transgenic mice, referred to herein as "HuMAb mice" which contain a human immunoglobulin gene miniloci that encodes unrearranged human heavy (μ and γ) and κ light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and κ chain loci (Lonberg, N. *et al.* (1994) *Nature* 368(6474): 856-859). Accordingly, the mice exhibit reduced expression of mouse IgM or κ, and in response to immunization, the introduced human heavy and light chain transgenes

undergo class switching and somatic mutation to generate high affinity human IgGκ monoclonal antibodies (Lonberg, N. *et al.* (1994), *supra*; reviewed in Lonberg, N. (1994) *Handbook of Experimental Pharmacology* 113:49-101; Lonberg, N. and Huszar, D. (1995) *Intern. Rev. Immunol.* Vol. 13: 65-93, and Harding, F. and Lonberg, N. (1995) *Ann. N.Y. Acad. Sci* 764:536-546). The preparation of HuMAb mice is described in detail in Section II below and in Taylor, L. *et al.* (1992) *Nucleic Acids Research* 20:6287-6295; Chen, J. *et al.* (1993) *International Immunology* 5: 647-656; Tuaillon *et al.* (1993) *Proc. Natl. Acad. Sci USA* 90:3720-3724; Choi *et al.* (1993) *Nature Genetics* 4:117-123; Chen, J. *et al.* (1993) *EMBO J.* 12: 821-830; Tuaillon *et al.* (1994) *J. Immunol.* 152:2912-2920; Lonberg *et al.*, (1994) *Nature* 368(6474): 856-859; Lonberg, N. (1994) *Handbook of Experimental Pharmacology* 113:49-101; Taylor, L. *et al.* (1994) *International Immunology* 6: 579-591; Lonberg, N. and Huszar, D. (1995) *Intern. Rev. Immunol.* Vol. 13: 65-93; Harding, F. and Lonberg, N. (1995) *Ann. N.Y. Acad. Sci* 764:536-546; Fishwild, D. *et al.* (1996) *Nature Biotechnology* 14: 845-851. See further, U.S. Patent Nos. 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,877,397; 5,661,016; 5,814,318; 5,874,299; and 5,770,429; all to Lonberg and Kay, and GenPharm International; U.S. Patent No. 5,545,807 to Surani *et al.*; International Publication Nos. WO 98/24884, published on June 11, 1998; WO 94/25585, published November 10, 1994; WO 93/1227, published June 24, 1993; WO 92/22645, published December 23, 1992; WO 92/03918, published March 19, 1992. The preparation of HCO12 transgenic HuMAb mice, in particular, is described in Example 2.

Immunizations

To generate fully human monoclonal antibodies to IL-15, transgenic or transchromosomal mice containing human immunoglobulin genes (*e.g.*, HCo12, HCo7 or KM mice) can be immunized with a purified or enriched preparation of the IL-15 antigen and/or cells expressing IL-15, as described, for example, by Lonberg *et al.* (1994) *Nature* 368(6474): 856-859; Fishwild *et al.* (1996) *Nature Biotechnology* 14: 845-851 and WO 98/24884. Alternatively, mice can be immunized with DNA encoding human IL-15. Preferably, the mice will be 6-16 weeks of age upon the first infusion. For example, a purified or enriched preparation (5-50 μg) of the IL-15 antigen can be used to immunize the HuMAb mice intraperitoneally. In the event that immunizations using a purified or enriched preparation of the IL-15 antigen do not result in antibodies,

mice can also be immunized with cells expressing IL-15, *e.g.*, a cell line, to promote immune responses.

Cumulative experience with various antigens has shown that the HuMAb transgenic mice respond best when initially immunized intraperitoneally (IP) or subcutaneously (SC) with antigen in complete Freund's adjuvant, followed by every other week IP/SC immunizations (up to a total of 10) with antigen in incomplete Freund's adjuvant. The immune response can be monitored over the course of the immunization protocol with plasma samples being obtained by retroorbital bleeds. The plasma can be screened by ELISA (as described below), and mice with sufficient titers of anti-IL-15 human immunoglobulin can be used for fusions. Mice can be boosted intravenously with antigen 3 days before sacrifice and removal of the spleen.

Generation of Hybridomas Producing Human Monoclonal Antibodies to IL-15

To generate hybridomas producing human monoclonal antibodies to IL-15, splenocytes and lymph node cells from immunized mice can be isolated and fused to an appropriate immortalized cell line, such as a mouse myeloma cell line. The resulting hybridomas can then be screened for the production of antigen-specific antibodies. For example, single cell suspensions of splenic lymphocytes from immunized mice can be fused to SP2/0-Ag8.653 nonsecreting mouse myeloma cells (ATCC, CRL 1580) with 50% PEG (w/v). Cells can be plated at approximately 1 x 10^5 in flat bottom microtiter plate, followed by a two week incubation in selective medium containing besides usual reagents 10% fetal Clone Serum, 5-10% origen hybridoma cloning factor (IGEN) and 1X HAT (Sigma). After approximately two weeks, cells can be cultured in medium in which the HAT is replaced with HT. Individual wells can then be screened by ELISA for human anti-IL-15 monoclonal IgM and IgG antibodies. Once extensive hybridoma growth occurs, medium can be observed usually after 10-14 days. The antibody secreting hybridomas can be replated, screened again, and if still positive for human IgG, anti-IL-15 monoclonal antibodies can be subcloned at least twice by limiting dilution. The stable subclones can then be cultured *in vitro* to generate antibody in tissue culture medium for characterization.

Generation of Transfectomas Producing Human Monoclonal Antibodies to IL-15

Human antibodies of the invention also can be produced in a host cell transfectoma using, for example, a combination of recombinant DNA techniques and gene transfection methods as is well known in the art (Morrison, S. (1985) Science 229:1202).

For example, in one embodiment, the gene(s) of interest, *e.g.*, human antibody genes, can be ligated into an expression vector such as a eukaryotic expression plasmid such as used by GS gene expression system disclosed in WO 87/04462, WO 89/01036 and EP 338 841 or other expression systems well known in the art. The purified plasmid with the cloned antibody genes can be introduced in eukaryotic host cells such as CHO-cells or NSO-cells or alternatively other eukaryotic cells like a plant derived cells, fungi or yeast cells. The method used to introduce these genes could be methods described in the art such as electroporation, lipofectine, lipofectamine or other. After introducing these antibody genes in the host cells, cells expressing the antibody can be identified and selected. These cells represent the transfectomas which can then be amplified for their expression level and upscaled to produce antibodies. Recombinant antibodies can be isolated and purified from these culture supernatants and/or cells.

Alternatively these cloned antibody genes can be expressed in other expression systems such as *E. coli* or in complete organisms or can be synthetically expressed.

Use of Partial Antibody Sequences to Express Intact Antibodies

Antibodies interact with target antigens predominantly through amino acid residues that are located in the six heavy and light chain complementarity determining regions (CDRs). For this reason, the amino acid sequences within CDRs are more diverse between individual antibodies than sequences outside of CDRs. Because CDR sequences are responsible for most antibody-antigen interactions, it is possible to express recombinant antibodies that mimic the properties of specific naturally occurring antibodies by constructing expression vectors that include CDR sequences from the specific naturally occurring antibody grafted onto framework sequences from a different antibody with different properties (see, *e.g.*, Riechmann, L. *et al.*, 1998, *Nature* 332:323-327; Jones, P. *et al.*, 1986, *Nature* 321:522-525; and Queen, C. *et al.*, 1989, *Proc. Natl. Acad. See.* U.S.A. 86:10029-10033). Such framework

sequences can be obtained from public DNA databases that include germline antibody gene sequences. These germline sequences will differ from mature antibody gene sequences because they will not include completely assembled variable genes, which are formed by V(D)J joining during B cell maturation. Germline gene sequences will also differ from the sequences of a high affinity secondary repertoire antibody at individual evenly across the variable region. For example, somatic mutations are relatively infrequent in the amino-terminal portion of framework region. For example, somatic mutations are relatively infrequent in the amino terminal portion of framework region 1 and in the carboxy-terminal portion of framework region 4. Furthermore, many somatic mutations do not significantly alter the binding properties of the antibody. For this reason, it is not necessary to obtain the entire DNA sequence of a particular antibody in order to recreate an intact recombinant antibody having binding properties similar to those of the original antibody (see PCT/US99/05535 filed on March 12, 1999). Partial heavy and light chain sequence spanning the CDR regions is typically sufficient for this purpose. The partial sequence is used to determine which germline variable and joining gene segments contributed to the recombined antibody variable genes. The germline sequence is then used to fill in missing portions of the variable regions. Heavy and light chain leader sequences are cleaved during protein maturation and do not contribute to the properties of the final antibody. To add missing sequences, cloned cDNA sequences can be combined with synthetic oligonucleotides by ligation or PCR amplification. Alternatively, the entire variable region can be synthesized as a set of short, overlapping, oligonucleotides and combined by PCR amplification to create an entirely synthetic variable region clone. This process has certain advantages such as elimination or inclusion or particular restriction sites, or optimization of particular codons.

The nucleotide sequences of heavy and light chain transcripts from a hybridoma are used to design an overlapping set of synthetic oligonucleotides to create synthetic V sequences with identical amino acid coding capacities as the natural sequences. The synthetic heavy and kappa chain sequences can differ from the natural sequences in three ways: strings of repeated nucleotide bases are interrupted to facilitate oligonucleotide synthesis and PCR amplification; optimal translation initiation sites are incorporated according to Kozak's rules (Kozak, 1991, J. Biol. Chem. 266L19867019870); and, HindIII sites are engineered upstream of the translation initiation sites.

For both the heavy and light chain variable regions, the optimized coding, and corresponding non-coding, strand sequences are broken down into 30 – 50 nucleotide approximately the midpoint of the corresponding non-coding oligonucleotide. Thus, for each chain, the oligonucleotides can be assembled into overlapping double stranded sets that span segments of 150 – 400 nucleotides. The pools are then used as templates to produce PCR amplification products of 150 – 400 nucleotides. Typically, a single variable region oligonucleotide set will be broken down into two pools which are separately amplified to generate two overlapping PCR products. These overlapping products are then combined by PCR amplification to form the complete variable region. It may also be desirable to include an overlapping fragment of the heavy or light chain constant region (including the BbsI site of the kappa light chain, or the AgeI site if the gamma heavy chain) in the PCR amplification to generate fragments that can easily be cloned into the expression vector constructs.

The reconstructed heavy and light chain variable regions are then combined with cloned promoter, leader sequence, translation initiation, leader sequence, constant region, 3' untranslated, polyadenylation, and transcription termination, sequences to form expression vector constructs. The heavy and light chain expression constructs can be combined into a single vector, co-transfected, serially transfected, or separately transfected into host cells which are then fused to form a host cell expressing both chains.

Plasmids for use in construction of expression vectors for human IgGκ are described below (Example 1). The plasmids were constructed so that PCR amplified V heavy and V kappa light chain cDNA sequences could be used to reconstruct complete heavy and light chain minigenes. These plasmids can be used to express completely human $IgG_1\kappa$ or $IgG_4\kappa$ antibodies. Fully human and chimeric antibodies of the present invention also include IgG2, IgG3, IgE, IgA, IgM, and IgD antibodies. Similar plasmids can be constructed for expression of other heavy chain isotypes, or for expression of antibodies comprising lambda light chains.

Thus, in another aspect of the invention, the structural features of an human anti-IL-15 antibodies of the invention, 146B7, 147H5, 404A8 and 404E4, are used to create structurally related human anti-IL-15 antibodies that retain at least one functional property of the antibodies of the invention, such as binding to IL-15. More specifically, one or more CDR regions of 146B7, 147H5, 404A8 and 404E4 can be

combined recombinantly with known human framework regions and CDRs to create additional, recombinantly-engineered, human anti-IL-15 antibodies of the invention.

Accordingly, in another embodiment, the invention provides a method for preparing an anti-IL-15 antibody comprising:

preparing an antibody comprising (1) human heavy chain framework regions and human heavy chain CDRs, wherein at least one of the human heavy chain CDRs comprises an amino acid sequence selected from the amino acid sequences of CDRs shown in Figure 2 (or corresponding amino acid residues in SEQ ID NO: 2); and (2) human light chain framework regions and human light chain CDRs, wherein at least one of the human heavy chain CDRs comprises an amino acid sequence selected from the amino acid sequences of CDRs shown in Figure 3 (or corresponding amino acid residues in SEQ ID NO: 4);

wherein the antibody retains the ability to bind to IL-15.

The ability of the antibody to bind IL-15 can be determined using standard binding assays, such as those set forth in the Examples (*e.g.*, an ELISA).

Since it is well known in the art that antibody heavy and light chain CDR3 domains play a particularly important role in the binding specificity/affinity of an antibody for an antigen, the recombinant antibodies of the invention prepared as set forth above preferably comprise the heavy and light chain CDR3s of 146B7, 147H5, 404A8 and 404E4. The antibodies further can comprise the CDR2s of 146B7, 147H5, 404A8 and 404E4. The antibodies further can comprise the CDR1s 146B7, 147H5, 404A8 and 404E4. The antibodies can further comprise any combinations of the CDRs.

Accordingly, in another embodiment, the invention further provides anti-IL-15 antibodies comprising: (1) human heavy chain framework regions, a human heavy chain CDR1 region, a human heavy chain CDR2 region, and a human heavy chain CDR3 region, wherein the human heavy chain CDR3 region is selected from the CDR3s of 146B7, 147H5, 404A8 and 404E4, for example, a human heavy chain CDR region of 146B7 as shown in Figure 2 (or corresponding amino acid residues in SEQ ID NO: 2); and (2) human light chain framework regions, a human light chain CDR1 region, a human light chain CDR2 region, and a human light chain CDR3 region, wherein the human light chain CDR3 region is selected from the CDR3s of 146B7, 147H5, 404A8 and 404E4, for example, a human light chain CDR region of 146B7 as shown in Figure 3 (or corresponding amino acid residues in SEQ ID NO: 4), wherein the antibody binds

IL-15. The antibody may further comprise the heavy chain CDR2 and/or the light chain CDR2 of 146B7, 147H5, 404A8 and 404E4. The antibody may further comprise the heavy chain CDR1 and/or the light chain CDR1 of 146B7, 147H5, 404A8 and 404E4.

The CDR1, 2, and/or 3 regions of the engineered antibodies described above can comprise the exact amino acid sequence(s) as those of 146B7, 147H5, 404A8 and 404E4 disclosed herein. However, the ordinarily skilled artisan will appreciate that some deviation from the exact CDR sequences of 146B7, 147H5, 404A8 and 404E4 may be possible while still retaining the ability of the antibody to bind IL-15 effectively (*e.g.*, conservative sequence modifications). Accordingly, in another embodiment, the engineered antibody may be composed of one or more CDRs that are, for example, 90%, 95%, 98% or 99.5% identical to one or more CDRs of 146B7, 147H5, 404A8 and 404E4.

In addition to simply binding IL-15, engineered antibodies such as those described above may be selected for their retention of other functional properties of antibodies of the invention, such as:

(1) binding to human IL-15 and inhibiting IL-15 induced proinflammatory effects;

(2) inhibiting IL-15 induced TNFα production or T cell proliferation;

(3) binding to human IL-15 with a dissociation equilibrium constant (K_D) of less than approximately 10^{-7} M when determined by surface plasmon resonance (SPR) technology in a BIACORE 3000 instrument using recombinant human IL-15 as the analyte and the antibody as the ligand;

(4) binding to an epitope located on the β- and/or γ-chain interacting domain of human IL-15;

(5) interfering with the binding of Asp^8 of human IL-15 to the β-unit of the human IL-15 receptor and/or of Gln^{108} of human IL-15 to the γ-unit of human IL-15 receptor;

(6) binding to receptor-bound human IL-15;

(7) binding to human IL-15 and inhibiting the ability of human IL-15 to induce parakeratosis;

(8) binding to human IL-15 and inhibiting the ability of human IL-15 to induce epidermal thickening;

(9) binding to human IL-15 and inhibiting the ability of human IL-15 to induce proliferation of keratinocytes; and/or

(10) binding to human IL-15 and inhibiting the ability of human IL-15 to induce chemotaxis of activated leukocytes.

Characterization of Human Monoclonal Antibodies to IL-15

Human monoclonal antibodies of the invention can be characterized for binding to IL-15 using a variety of known techniques. Generally, the antibodies are initially characterized by ELISA. Briefly, microtiter plates can be coated with purified IL-15 in PBS, and then blocked with irrelevant proteins such as bovine serum albumin (BSA) diluted in PBS. Dilutions of plasma from IL-15-immunized mice are added to each well and incubated for 1-2 hours at 37°C. The plates are washed with PBS/Tween 20 and then incubated with a goat-anti-human IgG Fc-specific polyclonal reagent conjugated to alkaline phosphatase for 1 hour at 37°C. After washing, the plates are developed with ABTS substrate, and analyzed at OD of 405. Preferably, mice which develop the highest titers will be used for fusions.

An ELISA assay as described above can be used to screen for antibodies and, thus, hybridomas that produce antibodies that show positive reactivity with the IL-15 immunogen. Hybridomas that bind, preferably with high affinity, to IL-15 can than be subcloned and further characterized. One clone from each hybridoma, which retains the reactivity of the parent cells (by ELISA), can then be chosen for making a cell bank, and for antibody purification.

To purify human anti-IL-15 antibodies, selected hybridomas can be grown in roller bottles, two-liter spinner-flasks or other culture systems. Supernatants can be filtered and concentrated before affinity chromatography with protein A-sepharose (Pharmacia, Piscataway, NJ) to purify the protein. After buffer exchange to PBS, the concentration can be determined by OD_{280} using 1.43 extinction coefficient or preferably by nephelometric analysis. IgG can be checked by gel electrophoresis and by antigen specific method..

To determine if the selected human anti-IL-15 monoclonal antibodies bind to unique epitopes, each antibody can be biotinylated using commercially available reagents (Pierce, Rockford, IL).. Biotinylated MAb binding can be detected with a streptavidin labeled probe. To determine the isotype of purified antibodies, isotype

ELISAs can be performed using art recognized techniques. For example, wells of microtiter plates can be coated with 10 μg/ml of anti-human Ig overnight at 4°C. After blocking with 5% BSA, the plates are reacted with 10 μg/ml of monoclonal antibodies or purified isotype controls, at ambient temperature for two hours. The wells can then be reacted with either human IgGl or other human isotype specific conjugated probes. Plates are developed and analyzed as described above.

To test the binding of monoclonal antibodies to live cells expressing IL-15, flow cytometry can be used. Briefly, cell lines and/or human PBMCs expressing membrane-bound IL-15 (grown under standard growth conditions) are mixed with various concentrations of monoclonal antibodies in PBS containing 0.1% BSA and 0.01% NaN3 at 4°C for 1 hour. After washing, the cells are reacted with Fluorescein-labeled anti-human IgG antibody under the same conditions as the primary antibody staining. The samples can be analyzed by FACScan instrument using light and side scatter properties to gate on single cells and binding of the labeled antibodies is determined. An alternative assay using fluorescence microscopy may be used (in addition to or instead of) the flow cytometry assay. Cells can be stained exactly as described above and examined by fluorescence microscopy. This method allows visualization of individual cells, but may have diminished sensitivity depending on the density of the antigen.

Anti-IL-15 human IgGs can be further tested for reactivity with the IL-15 antigen by Western blotting. Briefly, cell extracts from cells expressing IL-15 can be prepared and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis. After electrophoresis, the separated antigens will be transferred to nitrocellulose membranes, blocked with 20% mouse serum, and probed with the monoclonal antibodies to be tested. Human IgG binding can be detected using anti-human IgG alkaline phosphatase and developed with BCIP/NBT substrate tablets (Sigma Chem. Co., St. Louis, MO).

II. Production of Transgenic and Transchromosomal Nonhuman Animals Which Generate Human Monoclonal Anti-IL-15 Antibodies

In yet another aspect, the invention provides transgenic and transchromosomal non-human animals, such as transgenic or transchromosomal mice, which are capable of expressing human monoclonal antibodies that specifically bind to

IL-15. In a particular embodiment, the invention provides a transgenic or transchromosomal mouse having a genome comprising a human heavy chain transgene, such that the mouse produces human anti-IL-15 antibodies when immunized with IL-15 antigen and/or cells expressing IL-15. The human heavy chain transgene can be integrated into the chromosomal DNA of the mouse, as is the case for transgenic, *e.g.*, HuMAb mice as described in detail herein and exemplified. Alternatively, the human heavy chain transgene can be maintained extrachromosomally, as is the case for transchromosomal (*e.g.*, KM) mice as described in WO 02/43478 (published June 6, 2002). Such transgenic and transchromosomal mice are capable of producing multiple isotypes of human monoclonal antibodies to IL-15 (*e.g.*, IgG, IgA and/or IgE) by undergoing V-D-J recombination and isotype switching. Isotype switching may occur by, *e.g.*, classical or non-classical isotype switching.

The design of a transgenic or transchromsomal non-human animal that responds to foreign antigen stimulation with a heterologous antibody repertoire, requires that the heterologous immunoglobulin transgenes contained within the transgenic animal function correctly throughout the pathway of B-cell development. This includes, for example, isotype switching of the heterologous heavy chain transgene. Accordingly, transgenes are constructed so as to produce isotype switching and one or more of the following of antibodies: (1) high level and cell-type specific expression, (2) functional gene rearrangement, (3) activation of and response to allelic exclusion, (4) expression of a sufficient primary repertoire, (5) signal transduction, (6) somatic hypermutation, and (7) domination of the transgene antibody locus during the immune response.

Not all of the foregoing criteria need be met. For example, in those embodiments wherein the endogenous immunoglobulin loci of the transgenic animal are functionally disrupted, the transgene need not activate allelic exclusion. Further, in those embodiments wherein the transgene comprises a functionally rearranged heavy and/or light chain immunoglobulin gene, the second criteria of functional gene rearrangement is unnecessary, at least for that transgene which is already rearranged. For background on molecular immunology, see, Fundamental Immunology, 2nd edition (1989), Paul William E., ed. Raven Press, N.Y.

In certain embodiments, the transgenic or transchromosomal non-human animals used to generate the human monoclonal antibodies of the invention contain rearranged, unrearranged or a combination of rearranged and unrearranged heterologous

immunoglobulin heavy and light chain transgenes in the germline of the transgenic animal. Each of the heavy chain transgenes comprises at least one C_H gene. In addition, the heavy chain transgene may contain functional isotype switch sequences, which are capable of supporting isotype switching of a heterologous transgene encoding multiple C_H genes in the B-cells of the transgenic animal. Such switch sequences may be those which occur naturally in the germline immunoglobulin locus from the species that serves as the source of the transgene C_H genes, or such switch sequences may be derived from those which occur in the species that is to receive the transgene construct (the transgenic animal). For example, a human transgene construct that is used to produce a transgenic mouse may produce a higher frequency of isotype switching events if it incorporates switch sequences similar to those that occur naturally in the mouse heavy chain locus, as presumably the mouse switch sequences are optimized to function with the mouse switch recombinase enzyme system, whereas the human switch sequences are not. Switch sequences may be isolated and cloned by conventional cloning methods, or may be synthesized *de novo* from overlapping synthetic oligonucleotides designed on the basis of published sequence information relating to immunoglobulin switch region sequences (Mills *et al.*, *Nucl. Acids Res.* 15:7305-7316 (1991); Sideras *et al.*, *Intl. Immunol.* 1:631-642 (1989)). For each of the foregoing transgenic animals, functionally rearranged heterologous heavy and light chain immunoglobulin transgenes are found in a significant fraction of the B-cells of the transgenic animal (at least 10 percent).

The transgenes used to generate the transgenic animals of the invention include a heavy chain transgene comprising DNA encoding at least one variable gene segment, one diversity gene segment, one joining gene segment and at least one constant region gene segment. The immunoglobulin light chain transgene comprises DNA encoding at least one variable gene segment, one joining gene segment and at least one constant region gene segment. The gene segments encoding the light and heavy chain gene segments are heterologous to the transgenic non-human animal in that they are derived from, or correspond to, DNA encoding immunoglobulin heavy and light chain gene segments from a species not consisting of the transgenic non-human animal. In one aspect of the invention, the transgene is constructed such that the individual gene segments are unrearranged, *i.e.*, not rearranged so as to encode a functional immunoglobulin light or heavy chain. Such unrearranged transgenes support recombination of the V, D, and J gene segments (functional rearrangement) and

preferably support incorporation of all or a portion of a D region gene segment in the resultant rearranged immunoglobulin heavy chain within the transgenic non-human animal when exposed to the IL-15 antigen.

In an alternate embodiment, the transgenes comprise an unrearranged "mini-locus". Such transgenes typically comprise a substantial portion of the C, D, and J segments as well as a subset of the V gene segments. In such transgene constructs, the various regulatory sequences, *e.g.* promoters, enhancers, class switch regions, splice-donor and splice-acceptor sequences for RNA processing, recombination signals and the like, comprise corresponding sequences derived from the heterologous DNA. Such regulatory sequences may be incorporated into the transgene from the same or a related species of the non-human animal used in the invention. For example, human immunoglobulin gene segments may be combined in a transgene with a rodent immunoglobulin enhancer sequence for use in a transgenic mouse. Alternatively, synthetic regulatory sequences may be incorporated into the transgene, wherein such synthetic regulatory sequences are not homologous to a functional DNA sequence that is known to occur naturally in the genomes of mammals. Synthetic regulatory sequences are designed according to consensus rules, such as, for example, those specifying the permissible sequences of a splice-acceptor site or a promoter/enhancer motif. For example, a minilocus comprises a portion of the genomic immunoglobulin locus having at least one internal (*i.e.*, not at a terminus of the portion) deletion of a non-essential DNA portion (*e.g.*, intervening sequence; intron or portion thereof) as compared to the naturally-occurring germline Ig locus.

In a preferred embodiment of the invention, the transgenic or transchromosomal animal used to generate human antibodies to IL-15 contains at least one, typically 2-10, and sometimes 25-50 or more copies of the transgene described in Example 12 of WO 98/24884 (*e.g.*, pHC1 or pHC2) bred with an animal containing a single copy of a light chain transgene described in Examples 5, 6, 8, or 14 of WO 98/24884, and the offspring bred with the J_H deleted animal described in Example 10 of WO 98/24884. Animals are bred to homozygosity for each of these three traits. Such animals have the following genotype: a single copy (per haploid set of chromosomes) of a human heavy chain unrearranged mini-locus (described in Example 12 of WO 98/24884), a single copy (per haploid set of chromosomes) of a rearranged human K light chain construct (described in Example 14 of WO 98/24884), and a deletion at each

endogenous mouse heavy chain locus that removes all of the functional J_H segments (described in Example 10 of WO 98/24884). Such animals are bred with mice that are homozygous for the deletion of the J_H segments (Examples 10 of WO 98/24884) to produce offspring that are homozygous for the J_H deletion and hemizygous for the human heavy and light chain constructs. The resultant animals are injected with antigens and used for production of human monoclonal antibodies against these antigens.

B cells isolated from such an animal are monospecific with regard to the human heavy and light chains because they contain only a single copy of each gene. Furthermore, they will be monospecific with regards to human or mouse heavy chains because both endogenous mouse heavy chain gene copies are nonfunctional by virtue of the deletion spanning the J_H region introduced as described in Example 9 and 12 of WO 98/24884. Furthermore, a substantial fraction of the B cells will be monospecific with regards to the human or mouse light chains because expression of the single copy of the rearranged human κ light chain gene will allelically and isotypically exclude the rearrangement of the endogenous mouse κ and lambda chain genes in a significant fraction of B-cells.

Transgenic and transchromsomal mice employed in the present invention exhibit immunoglobulin production with a significant repertoire, ideally substantially similar to that of a native mouse. Thus, for example, in embodiments where the endogenous Ig genes have been inactivated, the total immunoglobulin levels will range from about 0.1 to 10 mg/ml of serum, preferably 0.5 to 5 mg/ml, ideally at least about 1.0 mg/ml. When a transgene capable of effecting a switch to IgG from IgM has been introduced into the transgenic mouse, the adult mouse ratio of serum IgG to IgM is preferably about 10:1. The IgG to IgM ratio will be much lower in the immature mouse. In general, greater than about 10%, preferably 40 to 80% of the spleen and lymph node B cells express exclusively human IgG protein.

The repertoire will ideally approximate that shown in a native mouse, usually at least about 10% as high, preferably 25 to 50% or more. Generally, at least about a thousand different immunoglobulins (ideally IgG), preferably 10^4 to 10^6 or more, will be produced, depending primarily on the number of different V, J and D regions introduced into the mouse genome. These immunoglobulins will typically recognize about one-half or more of highly antigenic proteins, e.g., staphylococcus

protein A. Typically, the immunoglobulins will exhibit an affinity (K_D) for preselected antigens of below 10^{-7} M, such as of below 10^{-8} M, 10^{-9} M or 10^{-10} M or even lower.

In some embodiments, it may be preferable to generate mice with predetermined repertoires to limit the selection of V genes represented in the antibody response to a predetermined antigen type. A heavy chain transgene having a predetermined repertoire may comprise, for example, human V_H genes which are preferentially used in antibody responses to the predetermined antigen type in humans. Alternatively, some V_H genes may be excluded from a defined repertoire for various reasons (*e.g.*, have a low likelihood of encoding high affinity V regions for the predetermined antigen; have a low propensity to undergo somatic mutation and affinity sharpening; or are immunogenic to certain humans). Thus, prior to rearrangement of a transgene containing various heavy or light chain gene segments, such gene segments may be readily identified, *e.g.* by hybridization or DNA sequencing, as being from a species of organism other than the transgenic animal.

Transgenic and transchromosomal mice as described above can be immunized with, for example, a purified or enriched preparation of IL-15 antigen and/or cells expressing IL-15. Alternatively, the transgenic mice can be immunized with DNA encoding human IL-15. The mice will then produce B cells which undergo class-switching via intratransgene switch recombination (cis-switching) and express immunoglobulins reactive with IL-15. The immunoglobulins can be human antibodies (also referred to as "human sequence antibodies"), wherein the heavy and light chain polypeptides are encoded by human transgene sequences, which may include sequences derived by somatic mutation and V region recombinatorial joints, as well as germline-encoded sequences; these human antibodies can be referred to as being substantially identical to a polypeptide sequence encoded by a human V_L or V_H gene segment and a human J_L or D_H and J_H segment, even though other non-germline sequences may be present as a result of somatic mutation and differential V-J and V-D-J recombination joints. The variable regions of each antibody chain are typically at least 80 percent encoded by human germline V, J, and, in the case of heavy chains, D, gene segments; frequently at least 85 percent of the variable regions are encoded by human germline sequences present on the transgene; often 90 or 95 percent or more of the variable region sequences are encoded by human germline sequences present on the transgene. However, since non-germline sequences are introduced by somatic mutation and VJ and

VDJ joining, the human sequence antibodies will frequently have some variable region sequences (and less frequently constant region sequences) which are not encoded by human V, D, or J gene segments as found in the human transgene(s) in the germline of the mice. Typically, such non-germline sequences (or individual nucleotide positions) will cluster in or near CDRs, or in regions where somatic mutations are known to cluster.

Human antibodies which bind to the predetermined antigen can result from isotype switching, such that human antibodies comprising a human sequence γ chain (such as γl, γ2a, γ2B, or γ3) and a human sequence light chain (such as kappa) are produced. Such isotype-switched human antibodies often contain one or more somatic mutation(s), typically in the variable region and often in or within about 10 residues of a CDR) as a result of affinity maturation and selection of B cells by antigen, particularly subsequent to secondary (or subsequent) antigen challenge. These high affinity human antibodies may have binding affinities (K_D) of below 10^{-7} M, such as of below 10^{-8} M, 10^{-9} M or 10^{-10} M or even lower.

Another aspect of the invention includes B cells derived from transgenic or transchromosomal mice as described herein. The B cells can be used to generate hybridomas expressing human monoclonal antibodies which bind with high affinity (*e.g.*, lower than 10^{-7} M) to human IL-15. Thus, in another embodiment, the invention provides a hybridoma which produces a human antibody having an affinity (K_D) of below 10^{-7} M, such as of below 10^{-8} M, 10^{-9} M or 10^{-10} M or even lower when determined by surface plasmon resonance (SPR) technology in a BIACORE 3000 instrument using recombinant human IL-15 as the analyte and the antibody as the ligand for binding human IL-15, wherein the antibody comprises:

a human sequence light chain composed of (1) a light chain variable region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human V_L gene segment and a human J_L segment, and (2) a light chain constant region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human C_L gene segment; and

a human sequence heavy chain composed of a (1) a heavy chain variable region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human V_H gene segment, optionally a D region, and a human J_H

segment, and (2) a constant region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human C_H gene segment.

The development of high affinity human monoclonal antibodies against IL-15 can be facilitated by a method for expanding the repertoire of human variable region gene segments in a transgenic mouse having a genome comprising an integrated human immunoglobulin transgene, said method comprising introducing into the genome a V gene transgene comprising V region gene segments which are not present in said integrated human immunoglobulin transgene. Often, the V region transgene is a yeast artificial chromosome comprising a portion of a human V_H or V_L (V_K) gene segment array, as may naturally occur in a human genome or as may be spliced together separately by recombinant methods, which may include out-of-order or omitted V gene segments. Often at least five or more functional V gene segments are contained on the YAC. In this variation, it is possible to make a transgenic mouse produced by the V repertoire expansion method, wherein the mouse expresses an immunoglobulin chain comprising a variable region sequence encoded by a V region gene segment present on the V region transgene and a C region encoded on the human Ig transgene. By means of the V repertoire expansion method, transgenic mice having at least 5 distinct V genes can be generated; as can mice containing at least about 24 V genes or more. Some V gene segments may be non-functional (*e.g.*, pseudogenes and the like); these segments may be retained or may be selectively deleted by recombinant methods available to the skilled artisan, if desired.

Once the mouse germline has been engineered to contain a functional YAC having an expanded V segment repertoire, substantially not present in the human Ig transgene containing the J and C gene segments, the trait can be propagated and bred into other genetic backgrounds, including backgrounds where the functional YAC having an expanded V segment repertoire is bred into a mouse germline having a different human Ig transgene. Multiple functional YACs having an expanded V segment repertoire may be bred into a germline to work with a human Ig transgene (or multiple human Ig transgenes). Although referred to herein as YAC transgenes, such transgenes when integrated into the genome may substantially lack yeast sequences, such as sequences required for autonomous replication in yeast; such sequences may optionally be removed by genetic engineering (*e.g.*, restriction digestion and pulsed-field gel electrophoresis or other suitable method) after replication in yeast is no longer

necessary (*i.e.*, prior to introduction into a mouse ES cell or mouse prozygote). Methods of propagating the trait of human sequence immunoglobulin expression, include breeding a transgenic mouse having the human Ig transgene(s), and optionally also having a functional YAC having an expanded V segment repertoire. Both V_H and V_L gene segments may be present on the YAC. The transgenic mouse may be bred into any background desired by the practitioner, including backgrounds harboring other human transgenes, including human Ig transgenes and/or transgenes encoding other human lymphocyte proteins. The invention also provides a high affinity human sequence immunoglobulin produced by a transgenic mouse having an expanded V region repertoire YAC transgene. Although the foregoing describes a preferred embodiment of the transgenic animal of the invention, other embodiments are contemplated which have been classified in four categories:

I. Transgenic animals containing an unrearranged heavy and rearranged light immunoglobulin transgene;

II. Transgenic animals containing an unrearranged heavy and unrearranged light immunoglobulin transgene;

III. Transgenic animal containing rearranged heavy and an unrearranged light immunoglobulin transgene; and

IV. Transgenic animals containing rearranged heavy and rearranged light immunoglobulin transgenes.

Of these categories of transgenic animal, the preferred order of preference is as follows II > I > III > IV where the endogenous light chain genes (or at least the K gene) have been knocked out by homologous recombination (or other method) and I > II > III >IV where the endogenous light chain genes have not been knocked out and must be dominated by allelic exclusion.

III. Antibody Conjugates/Immunotoxins

In another aspect, the present invention features a human anti-IL-15 monoclonal antibody conjugated to a therapeutic moiety, such as a cytotoxin, a drug (*e.g.*, an immunosuppressant) or a radioisotope. When conjugated to a cytotoxin, these antibody conjugates are referred to as "immunotoxins." A cytotoxin or cytotoxic agent includes any agent that is detrimental to (*e.g.*, kills) cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide,

tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine). An antibody of the present invention can be conjugated to a radioisotope, *e.g.*, radioactive iodine, to generate cytotoxic radiopharmaceuticals for treating a IL-15-related disorder, such as a cancer.

The antibody conjugates of the invention can be used to modify a given biological response. The therapeutic moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, an enzymatically active toxin, or active fragment thereof, such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor or interferon-γ; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other cytokines or growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in

Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982).

IV. Pharmaceutical Compositions

In another aspect, the present invention provides a composition, *e.g.*, a pharmaceutical composition, containing one or a combination of human monoclonal antibodies, or antigen-binding portion(s) thereof, of the present invention, formulated together with a pharmaceutically acceptable carrier. In a preferred embodiment, the compositions include a combination of multiple (*e.g.*, two or more) isolated human antibodies of the invention. Preferably, each of the antibodies of the composition binds to a distinct, pre-selected epitope of IL-15.

Pharmaceutical compositions of the invention also can be administered in combination therapy, *i.e.*, combined with other agents. For example, the combination therapy can include a composition of the present invention with at least one or more additional therapeutic agents, such as anti-inflammatory agents, DMARDs (disease-modifying anti-rheumatic drugs), immunosuppressive agents, chemotherapeutics, and psoriasis agents. The pharmaceutical compositions of the invention can also be administered in conjunction with radiation therapy. Co-administration with other antibodies, such as CD4 specific antibodies and IL-2 specific antibodies, are also encompassed by the invention. Such combinations with CD4 specific antibodies or IL-2 specific antibodies are considered particularly useful for treating autoimmune diseases and transplant rejections.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (*e.g.*, by injection or infusion). Depending on the route of administration, the active compound, *i.e.*, antibody, bispecific and multispecific molecule, may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

A "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired

toxicological effects (see *e.g.*, Berge, S.M., *et al.* (1977) J. Pharm. Sci. 66:1-19). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

A composition of the present invention can be administered by a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. *See, e.g., Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

To administer a compound of the invention by certain routes of administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation. For example, the compound may be administered to a subject in an appropriate carrier, for example, liposomes, or a diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes (Strejan *et al.* (1984) *J. Neuroimmunol.* 7:27).

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is

incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Dosage regimens are adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. For example, the human antibodies of the invention may be administered once or twice weekly by subcutaneous injection or once or twice monthly by subcutaneous injection.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein

refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

For the therapeutic compositions, formulations of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated, and the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 0.001 per cent to about ninety percent of active ingredient, preferably from about 0.005 per cent to about 70 per cent, most preferably from about 0.01 per cent to about 30 per cent.

Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate. Dosage forms for the topical or transdermal administration of compositions of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a

pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, supra, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

When the compounds of the present invention are administered as pharmaceuticals, to humans and animals, they can be given alone or as a pharmaceutical composition containing, for example, 0.001 to 90% (more preferably, 0.005 to 70%, such as 0.01 to 30%) of active ingredient in combination with a pharmaceutically acceptable carrier.

Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into

pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts. A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, a suitable daily dose of a compositions of the invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. It is preferred that administration be intravenous, intramuscular, intraperitoneal, or subcutaneous, preferably administered proximal to the site of the target. If desired, the effective daily dose of a therapeutic compositions may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. While it is possible for a compound of the present invention to be administered alone, it is preferable to administer the compound as a pharmaceutical formulation (composition).

Therapeutic compositions can be administered with medical devices known in the art. For example, in a preferred embodiment, a therapeutic composition of the invention can be administered with a needleless hypodermic injection device, such as the devices disclosed in U.S. Patent Nos. 5,399,163, 5,383,851, 5,312,335, 5,064,413,

4,941,880, 4,790,824, or 4,596,556. Examples of well-known implants and modules useful in the present invention include: U.S. Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4.,486,194, which discloses a therapeutic device for administering medicants through the skin; U.S. Patent No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Patent No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and U.S. Patent No. 4,475,196, which discloses an osmotic drug delivery system. Many other such implants, delivery systems, and modules are known to those skilled in the art.

In certain embodiments, the human monoclonal antibodies of the invention can be formulated to ensure proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of the invention cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, *e.g.*, U.S. Patents 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (*see, e.g.*, V.V. Ranade (1989) *J. Clin. Pharmacol.* 29:685). Exemplary targeting moieties include folate or biotin (see, *e.g.*, U.S. Patent 5,416,016 to Low *et al.*); mannosides (Umezawa *et al.*, (1988) *Biochem. Biophys. Res. Commun.* 153:1038); antibodies (P.G. Bloeman *et al.* (1995) *FEBS Lett.* 357:140; M. Owais *et al.* (1995) *Antimicrob. Agents Chemother.* 39:180); surfactant protein A receptor (Briscoe *et al.* (1995) *Am. J. Physiol.* 1233:134), different species of which may comprise the formulations of the inventions, as well as components of the invented molecules; p120 (Schreier *et al.* (1994) *J. Biol. Chem.* 269:9090); see also K. Keinanen; M.L. Laukkanen (1994) *FEBS Lett.* 346:123; J.J. Killion; I.J. Fidler (1994) *Immunomethods* 4:273. In one embodiment of the invention, the therapeutic compounds of the invention are formulated in liposomes; in a more preferred embodiment, the liposomes include a targeting moiety. In a most preferred embodiment, the therapeutic compounds in the liposomes are delivered by bolus injection to a site proximal to the tumor or infection. The composition must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be

preserved against the contaminating action of microorganisms such as bacteria and fungi.

A "therapeutically effective dosage" for rheumatoid arthritis preferably will result in an ACR20 Preliminary Definition of Improvement in the patients, more preferred in an ACR50 Preliminary Definition of Improvement and even more preferred in an ARCD70 Preliminary Definition of Improvement.

ACR20 Preliminary Definition of Improvement is defined as:
≥ 20% improvement in: Tender Joint Count (TCJ) and Swollen Joint Count (SWJ) and ≥ 20% improvement in 3 of following 5 assessments: Patient Pain Assessment (VAS), Patient Global assessment (VAS), Physician Global Assessment (VAS), Patent Self-Assessed Disability (HAQ), Acute Phase Reactant (CRP or ESR).

ACR50 and ACR70 are defined in the same way with ≥ 50% and ≥ 70% improvements, respectively. For further details see Felson *et al.* in American College of Rheumatology Preliminary Definition of Improvement in Rheumatoid Arthritis; Arthritis Rheumatism (1995) 38: 727-735.

The ability of a compound to inhibit cancer can be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit, such inhibition *in vitro* by assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound can decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

The ability of the antibodies to treat or prevent psoriasis can also be evaluated according to methods well known in the art.

The composition must be sterile and fluid to the extent that the composition is deliverable by syringe. In addition to water, the carrier can be an isotonic buffered saline solution, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. Proper fluidity can be maintained, for example, by use of coating such as lecithin, by maintenance of required particle size in the case of dispersion and by use of surfactants. In many cases, it is preferable to include isotonic agents, for example, sugars,

polyalcohols such as mannitol or sorbitol, and sodium chloride in the composition. Long-term absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

When the active compound is suitably protected, as described above, the compound may be orally administered, for example, with an inert diluent or an assimilable edible carrier.

V. Uses and Methods of the Invention

Human anti-IL-15 antibodies to IL-15 of the present invention (including derivatives and conjugates of the antibodies) and compositions containing the antibodies can be used in a variety of *in vitro* and *in vivo* diagnostic and therapeutic applications.

In one embodiment, human antibodies of the invention are used to inhibit IL-15 induced TNFα production by T cells and/or monocytes/macrophages, preferably without inhibiting TNFα production induced by other cytokines, such as IL-2. By contacting the antibody with IL-15 (*e.g.*, by administering the antibody to a subject), the ability of IL-15 to signal through the IL-15 receptor is inhibited and, thus, the production of TNFα by T-cells and/or monocytes/macrophages also is inhibited. Preferred antibodies bind to epitopes (*e.g.*, particular subunits, such as the gamma subunit) which are specific to IL-15 and, thus, advantageously inhibit IL-15-induced TNFα production, but do not interfere with TNFα production by structurally related cytokines, such as IL-2.

In another embodiment, human antibodies of the invention are used to inhibit IL-15 induced T cell recruitment and/or proliferation, preferably without inhibiting T cell proliferation induced by other structurally related cytokines, such as IL-2. As with TNFα production, by contacting the antibody with IL-15 (*e.g.*, by administering the antibody to a subject), the ability of IL-15 to signal through the IL-15 receptor is inhibited and, thus, T cell stimulation by IL-15 is inhibited.

Accordingly, in yet another embodiment, the present invention provides a method for treating or preventing a disorder mediated by IL-15 (*e.g.*, an autoimmune disease, such as psoriasis, rheumatoid arthritis, or inflammatory bowel disease, or an infectious disease, such as HIV), by administering to a subject a human antibody of the

invention in an amount effective to treat or prevent the disorder. The antibody can be administered alone or along with another therapeutic agent, such as an anti-inflammatory agent, *e.g.*, a steroidal or nonsteroidal inflammatory agent, or a cytotoxin which acts in conjunction with or synergistically with the antibody to treat or prevent the IL-15 mediated disease.

In a particular embodiment, human antibodies of the present invention are used to treat or to prevent rheumatoid arthritis (RA). The antibodies limit the role that IL-15 plays in the progression of inflammation associated with diseases such as RA. T cells, particularly CD4+ T-helper cells, are involved in the initiation and maintenance of inflammatory processes in RA. TNF-α, another cytokine, is also involved in the inflammatory pathways which ultimately lead to joint destruction and incapacitation of the patient with RA. Local synthesis of IL-15 plays a key role both in the activation and recruitment of T cells and in the induction of TNF-α and other inflammatory cytokines. The role of IL-15 in the progression of RA involves a process whereby IL-15, which is synthesized by macrophages, induces T cell recruitment. The activated T cells then: (1) maintain macrophage activation; and (2) induce TNF-α production. Stimulated macrophages promote the synthesis of more IL-15 and T cell activation, thus, continuing the cycle. In addition to its effects on TNF-α and macrophages, IL-15 also activates neutrophils and affects local B cell immunoglobulin secretion, particularly rheumatoid factor synthesis.

Accordingly, anti-IL-15 antibodies of the invention can be used to prevent or block the foregoing effects of IL-15 which cause RA and, thus, can be used to prevent or treat this disease. For example, anti-IL-15 antibodies of the invention can be used to inhibit inflammation and/or prevent chemotaxis of activated leukocytes involved in RA.

The human antibodies of the present invention may be used for inhibition of progression of structural damage in patients with rheumatoid arthritis who have had an inadequate response to methotrexate or for reducing sign and symptoms and delaying structural damage in patients with moderately to severely active rheumatoid arthritis, including those who have not previously failed treatment with a DMARD.

Human antibodies of the present invention also can be used to block or inhibit other effects of IL-15. IL-15 is expressed in various cells and tissues including

monocytes and macrophages, fibroblasts, dendritic cells, and keratinocytes. Keratinocytes are major constituents of the epidermis and the epithelial lining of mucosal tissue. Control of keratinocyte growth is mediated by a complex network of cytokines and growth factors, some of which are produced by keratinocytes themselves. Keratinocyte-derived IL-15 contributes to T cell accumulation, proliferation, and survival in psoriatic plaques. Many diseases are known wherein the number of keratinocytes is increased which leads to epidermal hyperplasia which is responsible for at least some of the related disease symptoms. These diseases include chronic diseases such as psoriasis and atopic dermatitis, as well as conditions like chronic hand eczema, contact dermatitis, viral warts (HPV associated), cutaneous T cell lymphoma, impaired wound healing, such as impaired wound healing due to diabetes. Accordingly, the invention provides methods for treating or preventing such disorders by administering to patients a human anti-IL-15 antibody of the invention in an amount effective to treat or prevent the disorder. For example, anti-IL-15 antibodies of the invention can be used to block or inhibit parakeratosis in psoriasis, reduce epidermal thickness in psoriasis, and reduce proliferation of keratinocytes in psoriasis.

IL-15 also modulates the function of intestinal epithelial cells (Reinecker, *et al.* (1996) Gastroenterology 111:1706-13). Specifically, IL-15 can cause modifications on mucosal epithelial cells and on intestinal epithelial cell lines and, therefore, is involved in the pathogenesis of inflammatory bowel disease, *e.g.*, celiac disease. The role of IL-15 in such diseases is shown by the selective over-representation of IL-15+ cells in the small intestine of untreated patients with celiac disease (WO 00/02582). Thus, it has been shown that IL-15 is directly involved in the initiation and maintenance of celiac disease. Accordingly, in another embodiment, anti-IL-15 human antibodies of the present invention (*i.e.*, which inhibit the proinflammatory effects of IL-15) can be used to treat and/or to prevent celiac disease by administering the antibody to a patient in an amount effective to treat or prevent the disorder.

In addition, it has been found by the inventors of the present invention that IL-15 also promotes the formation of new blood vessels, a process called neovascularization or angiogenesis. Accordingly, yet another use for the antibodies of the invention includes the prevention or treatment of diseases involving neovascularization. These diseases include a variety of cancers which rely on or are characterized by neovascularization, in addition to inflammatory diseases.

Human antibodies of the present invention also can be used to block or inhibit the effects of IL-15 associated with infectious diseases, such as HIV. Accordingly, another use for the antibodies of the invention includes the prevention or treatment of infectious diseases, *e.g.*, HIV-1.

For example, the antibodies can be used *in vitro* or *in vivo* to diagnose a variety of diseases mediated by IL-15. Specifically, the antibodies can be used to detect levels of IL-15, or levels of cells which contain IL-15 on their membrane surface or linked to their receptors (receptor-bound human IL-15). The detection of such levels of IL-15 can then be correlated to certain disease symptoms. Alternatively, the antibodies can be used to inhibit or block IL-15 function which, in turn, can prevent or ameliorate disease symptoms caused by IL-15 function.

As previously described, human anti-IL-15 antibodies of the invention can be co-administered with one or other more therapeutic agents, *e.g.*, an immunosuppressive agent or an anti-inflammatory agent to increase the overall anti-inflammatory effect. The antibody can be linked to the agent (as an immunocomplex) or can be administered separate from the agent. In the latter case (separate administration), the antibody can be administered before, after or concurrently with the agent. Suitable therapeutic agents include, among others, anti-inflammatory agents, DMARDs (disease-modifying anti-rheumatic drugs), immunosuppressive agents, chemotherapeutics, and psoriasis agents. The human antibodies according to the invention can also be administered in conjunction with radiation therapy.

In another embodiment, the human antibodies of the invention can be administered in combination with other antibodies, such as CD4 specific antibodies and IL-2 specific antibodies. A combination of the present human antibodies with CD4 specific antibodies or IL-2 specific antibodies are considered particularly useful for treating autoimmune diseases and transplant rejections.

Also within the scope of the present invention are kits comprising human anti-IL-15 antibodies of the invention and, optionally, instructions for use. The kit can further contain one ore more additional reagents, such as an immunosuppressive reagent, or one or more additional human antibodies of the invention (*e.g.*, a human antibody having a complementary activity which binds to an epitope in the IL-15 antigen distinct from the first human antibody).

Accordingly, patients treated with antibodies of the invention can be additionally administered (prior to, simultaneously with, or following administration of a human antibody of the invention) with another therapeutic agent, such as an anti-inflammatory agent, which enhances or augments the therapeutic effect of the human antibodies.

In yet another embodiment, human antibodies of the invention can be used to target compounds (*e.g.*, therapeutic agents, labels, cytotoxins, immunosuppressants etc.) to cells which have IL-15 bound to their surface (*e.g.*, membrane bound or bound to IL-15 receptor by linking such compounds to the antibody. Thus, the invention also provides methods for localizing *ex vivo*, *in vivo* or *in vitro* cells expressing IL-15 and IL-15 receptor (*e.g.*, with a detectable label, such as a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor).

Other embodiments of the present invention are described in the following Examples.

The present invention is further illustrated by the following examples which should not be construed as further limiting. The contents of Sequence Listing, figures and all references, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

EXAMPLES

Example 1 Generation of Cmu targeted mice

Construction of a CMD targeting vector

The plasmid pICEmu contains an EcoRI/XhoI fragment of the murine Ig heavy chain locus, spanning the mu gene, that was obtained from a Balb/C genomic lambda phage library (Marcu *et al.* Cell 22: 187, 1980). This genomic fragment was subcloned into the XhoI/EcoRI sites of the plasmid pICEMI9H (Marsh et al; Gene 32, 481-485, 1984). The heavy chain sequences included in pICEmu extend downstream of the EcoRI site located just 3' of the mu intronic enhancer, to the XhoI site located approximately 1 kb downstream of the last transmembrane exon of the mu gene; however, much of the mu switch repeat region has been deleted by passage in *E. coli*.

The targeting vector was constructed as follows. A 1.3 kb HindIII/SmaI fragment was excised from pICEmu and subcloned into HindIII/SmaI digested pBluescript (Stratagene, La Jolla, CA). This pICEmu fragment extends from the HindIII site located approximately 1 kb 5' of Cmu1 to the SmaI site located within Cmu1. The resulting plasmid was digested with SmaI/SpeI and the approximately 4 kb SmaI/XbaI fragment from pICEmu, extending from the Sma I site in Cmu1 3' to the XbaI site located just downstream of the last Cmu exon, was inserted. The resulting plasmid, pTAR1, was linearized at the SmaI site, and a neo expression cassette inserted. This cassette consists of the neo gene under the transcriptional control of the mouse phosphoglycerate kinase (pgk) promoter (XbaI/TaqI fragment; Adra *et al.* (1987) Gene 60: 65-74) and containing the pgk polyadenylation site (PvuII/HindIII fragment; Boer *et al.* (1990) Biochemical Genetics 28: 299-308). This cassette was obtained from the plasmid pKJ1 (described by Tybulewicz *et al.* (1991) Cell 65: 1153-1163) from which the neo cassette was excised as an EcoRI/HindIII fragment and subcloned into EcoRI/HindIII digested pGEM-7Zf (+) to generate pGEM-7 (KJ1). The neo cassette was excised from pGEM-7 (KJ1) by EcoRI/SalI digestion, blunt ended and subcloned into the SmaI site of the plasmid pTAR1, in the opposite orientation of the genomic Cmu sequences. The resulting plasmid was linearized with Not I, and a herpes simplex virus thymidine kinase (tk) cassette was inserted to allow for enrichment of ES clones bearing homologous recombinants, as described by Mansour *et al.* (1988) Nature 336: 348-352. This cassette consists of the coding sequences of the tk gene bracketed by the mouse pgk promoter and polyadenylation site, as described by Tybulewicz *et al.* (1991) Cell 65: 1153-1163. The resulting CMD targeting vector contains a total of approximately 5.3 kb of homology to the heavy chain locus and is designed to generate a mutant mu gene into which has been inserted a neo expression cassette in the unique SmaI site of the first Cmu exon. The targeting vector was linearized with PvuI, which cuts within plasmid sequences, prior to electroporation into ES cells.

Generation and analysis of targeted ES cells

AB-1 ES cells (McMahon, A. P. and Bradley, A., (1990) Cell 62: 1073-1085) were grown on mitotically inactive SNL76/7 cell feeder layers (ibid.) essentially as described (Robertson, E. J. (1987) in Teratocarcinomas and Embryonic Stem Cells: a Practical Approach (E. J. Robertson, ed.) Oxford: IRL Press, p. 71-112). The linearized

CMD targeting vector was electroporated into AB-1 cells by the methods described Hasty *et al.* (Hasty, P. R. *et al.* (1991) Nature 350: 243-246). Electroporated cells were plated into 100 mm dishes at a density of 1-2 x 10^6 cells/dish. After 24 hours, G418 (200 micrograms/ml of active component) and FIAU (5 x 10^{-7} M) were added to the medium, and drug-resistant clones were allowed to develop over 8-9 days. Clones were picked, trypsinized, divided into two portions, and further expanded. Half of the cells derived from each clone were then frozen and the other half analyzed for homologous recombination between vector and target sequences.

DNA analysis was carried out by Southern blot hybridization. DNA was isolated from the clones as described by Laird *et al.* (Laird, P. W. *et al.*, (1991) Nucleic Acids Res. 19 : 4293). Isolated genomic DNA was digested with SpeI and probed with a 915 bp SacI fragment, probe A (see Figure 1), which hybridizes to a sequence between the mu intronic enhancer and the mu switch region. Probe A detects a 9.9 kb SpeI fragment from the wild type locus, and a diagnostic 7.6 kb band from a mu locus which has homologously recombined with the CMD targeting vector (the neo expression cassette contains a SpeI site). Of 1132 G418 and FIAU resistant clones screened by Southern blot analysis, 3 displayed the 7.6 kb Spe I band indicative of homologous recombination at the mu locus. These 3 clones were further digested with the enzymes BglI, BstXI, and EcoRI to verify that the vector integrated homologously into the mu gene. When hybridized with probe A, Southern blots of wild type DNA digested with BglI, BstXI, or EcoRI produce fragments of 15.7, 7.3, and 12.5 kb, respectively, whereas the presence of a targeted mu allele is indicated by fragments of 7.7, 6.6, and 14.3 kb, respectively. All 3 positive clones detected by the SpeI digest showed the expected BglI, BstXI, and EcoRI restriction fragments diagnostic of insertion of the neo cassette into the Cmu1 exon.

Generation of mice bearing the mutated mu gene

The three targeted ES clones, designated number 264, 272, and 408, were thawed and injected into C57BL/6J blastocysts as described by Bradley (Bradley, A. (1987) in Teratocarcinomas and Embryonic Stem Cells: a Practical Approach. (E. J. Robertson, ed.) Oxford: IRL Press, p. 113-151). Injected blastocysts were transferred into the uteri of pseudopregnant females to generate chimeric mice representing a mixture of cells derived from the input ES cells and the host blastocyst. The extent of

ES cell contribution to the chimera can be visually estimated by the amount of agouti coat coloration, derived from the ES cell line, on the black C57BL/6J background. Clones 272 and 408 produced only low percentage chimeras (*i.e.* low percentage of agouti pigmentation) but clone 264 produced high percentage male chimeras. These chimeras were bred with C57BL/6J females and agouti offspring were generated, indicative of germline transmission of the ES cell genome. Screening for the targeted mu gene was carried out by Southern blot analysis of BglI digested DNA from tail biopsies (as described above for analysis of ES cell DNA). Approximately 50% of the agouti offspring showed a hybridizing BglI band of 7.7 kb in addition to the wild type band of 15.7 kb, demonstrating a germline transmission of the targeted mu gene.

Analysis of transgenic mice for functional inactivation of mu gene

To determine whether the insertion of the neo cassette into Cmu1 has inactivated the Ig heavy chain gene, a clone 264 chimera was bred with a mouse homozygous for the JHD mutation, which inactivates heavy chain expression as a result of deletion of the JH gene segments (Chen et al, (1993) Immunol. 5: 647-656). Four agouti offspring were generated. Serum was obtained from these animals at the age of 1 month and assayed by ELISA for the presence of murine IgM. Two of the four offspring were completely lacking IgM (see Table 1). Genotyping of the four animals by Southern blot analysis of DNA from tail biopsies by BglI digestion and hybridization with probe A (see Figure 1), and by StuI digestion and hybridization with a 475 bp EcoRI/StuI fragment (ibid.) demonstrated that the animals which fail to express serum IgM are those in which one allele of the heavy chain locus carries the JHD mutation, the other allele the Cmu1 mutation. Mice heterozygous for the JHD mutation display wild type levels of serum Ig. These data demonstrate that the Cmu1 mutation inactivates expression of the mu gene.

TABLE 1

Mouse	Serum IgM (micrograms/ml)	Ig H chain genotype
42	<0.002	CMD/JHD
43	196	+/JHD
44	<0.002	CMD/JHD
45	174	+/JHD
129 x BL6 F1	153	+/+
JHD	<0.002	JHD/JHD

Table 1 shows the levels of serum IgM, detected by ELISA, for mice carrying both the CMD and JHD mutations (CMD/JHD), for mice heterozygous for the JHD mutation (+/JHD), for wild type (129Sv x C57BL/6J)F1 mice (+/+), and for B cell deficient mice homozygous for the JHD mutation (JHD/JHD).

Example 2 Generation of HCO12 transgenic mice

The HCO12 human heavy chain transgene

The HCO12 transgene was generated by coinjection of the 80 kb insert of pHC2 (Taylor *et al.*, 1994, Int. Immunol., 6: 579-591) and the 25 kb insert of pVx6. The plasmid pVx6 was constructed as described below.

An 8.5 kb HindIII/SalI DNA fragment, comprising the germline human V_H 1-18 (DP-14) gene together with approximately 2.5 kb of 5' flanking, and 5 kb of 3' flanking genomic sequence was subcloned into the plasmid vector pSP72 (Promega, Madison, WI) to generate the plasmid p343.7.16. A 7 kb BamHI/HindIII DNA fragment, comprising the germline human V_H 5-51 (DP-73) gene together with approximately 5 kb of 5' flanking and 1 kb of 3' flanking genomic sequence, was cloned into the pBR322 based plasmid cloning vector pGP1f (Taylor *et al.* 1992, Nucleic Acids Res. 20: 6287-6295), to generate the plasmid p251f. A new cloning vector derived from pGP1f, pGP1k (SEQ ID NO:13), was digested with EcoRV/BamHI, and ligated to a 10 kb EcoRV/BamHI DNA fragment, comprising the germline human V_H 3-23 (DP47) gene together with approximately 4 kb of 5' flanking and 5 kb of 3' flanking genomic

sequence. The resulting plasmid, p112.2RR.7, was digested with BamHI/SalI and ligated with the 7 kb purified BamHI/SalI insert of p251f. The resulting plasmid, pVx4, was digested with XhoI and ligated with the 8.5 kb XhoI/SalI insert of p343.7.16.

A clone was obtained with the V_H 1-18 gene in the same orientation as the other two V genes. This clone, designated pVx6, was then digested with NotI and the purified 26 kb insert coinjected--together with the purified 80 kb NotI insert of pHC2 at a 1:1 molar ratio--into the pronuclei of one-half day (C57BL/6J x DBA/2J)F2 embryos as described by Hogan *et al.* (B. Hogan *et al.*, Manipulating the Mouse Embryo, A Laboratory Manual, 2nd edition, 1994, Cold Spring Harbor Laboratory Press, Plainview NY). Three independent lines of transgenic mice comprising sequences from both Vx6 and HC2 were established from mice that developed from the injected embryos. These lines are designated (HCO12)14881, (HCO12)15083, and (HCO12)15087. Each of the three lines were then bred with mice comprising the CMD mutation described in Example 1, the JKD mutation (Chen *et al.* 1993, EMBO J. 12: 811-820), and the (KCo5)9272 transgene (Fishwild *et al.* 1996, Nature Biotechnology 14: 845-851). The resulting mice express human heavy and kappa light chain transgenes in a background homozygous for disruption of the endogenous mouse heavy and kappa light chain loci.

Example 3 Production of Human Monoclonal Antibodies Against IL-15

HCo12 and HCo7 transgenic mice, generated as described above and supplied from Medarex, San José, CA, USA, were immunised with human recombinant IL-15 (hIL-15, Immunex corp., Seattle, USA) supplemented with either Complete Freunds Adjuvant (CFA, lot no. 121024LA, Difco Laboratories, Detroit, Michigan, USA) or with Incomplete Freunds Adjuvant (ICFA, lot no. 121195LA, Difco, subcutaneously (SC) intraperitoneally (IP) or intravenously (IV). In several instances hIL-15 coupled to KLH was used for immunisation. After several boosts with hIL-15 supplemented with either Complete or Incomplete Freunds Adjuvant, the serum of the mice was tested for the presence of human antibodies directed against IL-15.

Immunisation schemes of the transgenic mice which resulted in final clones 146B7, 146H5, 404E4 and 404A8

Mouse no. 146 (HCo12), ID 995-146, Female

170699	SC	12 µg hIL-15 in CFA (Difco, Lot no. 121024LA)
010799	SC	12 µg hIL-15 in ICFA (Difco, Lot no. 121195LA)
150799	SC	12 µg hIL-15 in ICFA
020899	SC	12 µg hIL-15-KLH in ICFA
070999	SC	12 µg hIL-15-KLH in ICFA
280999	SC	12 µg hIL-15-KLH in CFA
111099	IV	30 µg hIL-15 in PBS
121099	IV	30 µg hIL-15 in PBS
151099		fusion of lymph node and spleen cells of this mouse with SP2/0

Mouse no. 404 (HCo7), ID 997-404, Female

201099	IP	25 µg hIL-15-KLH in CFA (Difco, lot no. 121024LA)
031199	IP	12.5 µg hIL-15, 12.5 µg hIL-15-KLH, 25 µg in ICFA (Difco, lot no. 121195LA)
101199	IV	12.5 µg hIL-15, 12.5 µg hIL-15-KLH
121199	IV	12.5 µg hIL-15, 12.5 µg hIL-15-KLH
191199		fusion of lymph node and spleen cells of this mouse with SP2/0

Culture media

Fusion Partner Medium (FPM):

Iscoves Modified Dulbecco's Medium was supplemented with 100 IU/ml penicillin, 100 µg/ml streptomycin, 1 mM Na-Pyruvate, 0.5 mM β-mercaptoethanol (Life Technologies, Paisley, Scotland) and 10% heat-inactivated fetal calf serum (HyClone, Utah, USA).

Fusion Selection Medium (FSM):

FPM supplemented with 30 ml Origen Hybridoma Cloning Factor (IGEN, Gaithersburg, MD, USA), HAT (1 vial, manufacturer's recommended concentration, Sigma Chemical Co., St. Louis, MO, USA) and 0.5 mg/ml kanamycin (Life Technologies, Paisley, Scotland).

Fusion Cloning Medium (FCM):

FPM supplemented with 20 ml Origen Hybridoma Cloning Factor (IGEN, Gaithersburg, MD, USA), HT (1 vial, manufacturer's recommended concentration, Sigma Chemical Co., St. Louis, MO, USA) and 0.5 mg/ml kanamycin (Life Technologies, Paisley, Scotland).

Hybridoma preparation: fusion of spleen and lymph node cells with SP2/0 myeloma cells

To obtain hybridomas, spleen, inguinal and para-aortic lymph nodes were removed from the mice. Single cells suspensions of spleen and lymph node cells were mixed with SP2/0 myeloma cells in a cell ratio 1:2. Cells were spun down and the pellet was resuspended gently in 1 ml polyethyleneglycol (50% w/v in PBS, Sigma-Aldrich, Irvine, UK) at 37°C. After swirling the cells for 60 seconds, 25 ml FPM-2 was added and cells were incubated at 37°C for 30-60 minutes. After incubation, cells were cultured at a cell concentration of $0,75 \times 10^5$ cells per well (in 100 µl) in 96-wells plates in FSM. After 3 days, 100 µl FSM was added to each well.

Fusion of spleen and lymph nodes of HCo7 and HCo12 mice immunised with hIL-15 resulted in the generation of several hybridomas producing antibodies directed against IL-15. The following four stable clones producing fully human anti-IL-15 antibodies were isolated: (1) 146LyD7F7B7 renamed: 146B7; (2) 146DE2E12A3H5 renamed: 146H5; (3) 404CG11B7E4 renamed: 404E4; and (4) 404FB12E7A8 renamed: 404A8. These clones were all of the human IgG1/k subclass.

Screening of the hybridomas

Between day 7 and 11 after the fusion, the wells were screened for the presence of human antibodies using the following ELISAs:

ELISA to screen for the presence of human IgG in the culture supernatants

To perform the ELISA to detect the presence of human IgG antibodies, 100 µl/well of 0.9 µg/ml rabbit-α-k-light chains antibodies (DAKO, Glostrup, Denmark) was added in phosphate buffered saline (PBS) to Nunc Maxisorp ELISA-plate (incubation overnight at room temperature). After blocking the plate with PBS supplemented with chicken serum (2 %; Life Technologies, Paisley, Scotland) and Tween-20 (0.05 %; PBSTC), culture supernatants were added. After incubation for 1.5 hour the plates were washed and rabbit-α-Human IgG (Fab2-fragments) conjugated with horse radish peroxidase (DAKO, Glostrup, Denmark) 0.5 µg/ml diluted in PBSTC was added. After incubation for 1 hour, the wells were washed and substrate, ABTS (2,2'-Azinobis-3-ethylbenzthiazoline-sulphonic-acid, Roche Diagnostics, Mannheim, Germany) was added according to the manufacturer's protocol and antibody binding was evaluated at 405 nm in an EL808 ELISA-reader (Bio-tek Instruments, Winooski, VT, USA).

ELISA to screen for the presence of IL-15 specific antibodies

Wells containing human IgG/k antibodies were further tested for the presence of human anti-IL-15 antibodies in an IL-15-specific ELISA. To perform the ELISA, 100 µl/well of 1 µg/ml IL-15 was added in phosphate buffered saline (PBS) to Nunc Maxisorp ELISA-plate (incubation overnight at room temperature). After blocking the plate with PBS supplemented with chicken serum (2 %; Life Technologies, Paisley, Scotland) and Tween-20 (0.05 %; PBSTC), culture supernatants were added. After incubation for 1.5 hours the plates were washed and α-Human IgG Fc conjugated with horse radish peroxidase (Jackson Immuno research, West Grove, Pennsylvania, USA) 1/5000 diluted in PBSTC was added. After incubation for 1 hour, the wells were washed and substrate, ABTS (2,2'-Azinobis-3-ethylbenzthiazoline-sulphonic-acid, Roche Diagnostics, Mannheim, Germany) was added according to the manufacturer's protocol and antibody binding was evaluated at 405 nm in an EL808 ELISA-reader (Bio-tek Instruments, Winooski, VT, USA).

Subcloning of the hybridomas

To obtain stable anti-IL-15 cell lines, the hybridomas were subcloned by a limiting dilution of the cells (to 0.5 cell/well) in 96-wells plates.

The subclones were tested after approximately 10 days with the above mentioned IL-15 ELISA. During the several subcloning procedures, FSM was changed in phases via FCM to FPM. The isotype of the subclones was determined with the ELISA described below.

Isotype determination of the anti-IL-15 antibodies by ELISA

To perform the isotype ELISA, 100 µl/well of 1 µg/ml anti-human Fc (Jackson Immuno research) was added in phosphate buffered saline (PBS) to Nunc Maxisorp ELISA-plate (incubation overnight at room temperature). After blocking the plate with PBS supplemented with chicken serum (2 %; Life Technologies, Paisley, Scotland) and Tween-20 (0.05 %; PBSTC), culture supernatants were added. After incubation for 1.5 hours the plates were washed and mouse-α-HuIgG1 conjugated with alkaline phosphatase (Zymed, plaats, land), or mouse-α-HuIgG3 conjugated with horse radish peroxidase (Zymed) was added. After incubation for 1 hour the wells were washed and substrate, ABTS (2,2'-Azinobis-3-ethylbenzthiazoline-sulphonic-acid, Roche Diagnostics, Mannheim, Germany) was added according to the manufacturer's protocol. Antibody binding was evaluated at 405 nm in an EL808 ELISA-reader (Bio-tek Instruments, Winooski, VT, USA.

Example 4 Epitope Specificity of Fully Human Anti-IL-15 Antibodies

To function therapeutically and to inhibit IL-15-induced proinflammatory effects, IL-15 specific antibodies need to recognize the IL-15 epitopes involved in interaction with the IL-2Rβ-chain and/or the γ-chain of IL-15 receptor.

Mutant proteins (described by Pettit *et al.*)were used to evaluate the epitope specificity of the fully human anti-IL-15 antibodies, 146B7, 146H5, 404A8 and 404E4. The IL-15 mutants used include IL-15 mutant Q108S (Gln at residue 108 was replaced by Ser; a mutation in the γ-chain interaction site) and mutant D8SQ108S (Gln

at residue 108 was replaced by Ser and Asp at position 8 was substituted for Ser; mutations in both the β and γ-chain interaction sites of IL-15).

ELISA to determine binding of the hIL-15 specific antibodies, 146B7, 147H5, 404A8 and 404E4, to hIL-15 and to mutant IL-15 proteins

To perform the ELISA, 100 µl of 1 µg/ml IL-15 or hIL-15 mutant protein, in phosphate buffered saline (PBS), was added to Nunc Maxisorp ELISA-plate for coating. After blocking the plate with PBS supplemented with chicken serum (2 %; Life Technologies, Paisley, Scotland) and Tween-20 (0.05 %; PBSTC), serial dilutions of the hIL-15 specific antibodies were incubated. After washing, α-Human IgG Fc conjugated with peroxidase (Jackson Immuno research, West Grove, Pennsylvania, USA) 1/5000 diluted in PBSTC was added. After washing substrate, ABTS (2,2'-Azinobis-3-ethylbenzthiazoline-sulphonic-acid, Roche Diagnostics, Mannheim, Germany) was added according to the manufacturer's protocol and antibody binding was evaluated at 405 nm in an EL808 ELISA-reader (Bio-tek Instruments, Winooski, VT, USA).

The binding of the fully human IL-15 specific antibodies 146B7, 146H5, 404A8 and 404E4 to hIL-15 and to the IL-15 mutant proteins Q108S and D8SQ108S is shown in Figure 1. Neither 146B7 nor 146H5 were able to bind to these mutant IL-15 proteins. Since both mutants carry the Q108S mutation, the epitope recognized by 146B7 and 146H5 is within the critical domains of IL-15 which interact with the γ-chain of the IL-15 receptor. 404A8 and 404E4 were both able to bind the mutant proteins, therefore, these antibodies recognize an epitope outside the β- and γ-chain interacting domains of IL-15. Both 146B7 and 146H5 bind to IL-15 at the region that interacts with the γ-chain of the IL-15 receptor. This agrees with the data obtained from the proliferation assays using the fully human anti-IL-15 antibodies of the present invention. As described in detail below, neither 404A8 nor 404E4 were able to inhibit IL-15-induced proliferation of CTLL-2 cells and human PBMCs. Both 146B7 and 146H5 were able to inhibit IL-15-induced proliferation. Further, inhibition of proliferation is achieved by blocking the interaction of IL-15 with the γ- subunit of the IL-15 receptor.

Example 5 V_H and V_L – Region Sequences of 146B7

The nucleotide and deduced amino acid sequence of rearranged V_H and V_L–domains of 146B7 were determined using the following procedures. These sequences give information regarding the V_H and V_L germline families used; point mutations in these germline sequences are due to affinity maturation of B-cells during the immunization of the animal.

RNA preparation

Total RNA was prepared from 5 x 10^6 146B7 hybridoma cells with RNAzol (Biogenesis, Poole, England) according to the manufactures protocol.

cDNA preparation

The cDNA of RNA from 146B7 was prepared from 3 μg total RNA with AMV Reverse Transcriptase with buffer (Roche Diagnostics GmbH, Mannheim, Germany), oligo $d(T)_{15}$ (Promega, Madison, WI, USA), dNTP (Boehringer Mannheim corp., USA) and RNAsin (Promega) according to the manufacturer's protocol.

PCR primers used to amplify V_H and V_L regions for cloning

Primer pairs used:

V_H:

FR1 5' primers

(1)	AB62	CAg gTK CAg CTg gTg CAg TC
(2)	AB63	SAg gTg CAg CTg KTg gAg TC
(3)	AB65	gAg gTg CAg CTg gTg CAg TC

V_H leader 5' primers

(4)	AB85	ATg gAC Tgg ACC Tgg AgC ATC
(5)	AB86	ATg gAA TTg ggg CTg AgC Tg
(6)	AB87	ATg gAg TTT ggR CTg AgC Tg
(7)	AB88	ATg AAA CAC CTg Tgg TTC TTC
(8)	AB89	ATg ggg TCA ACC gCC ATC CT

V_H 3' primer

(9) AB90 TgC CAg ggg gAA gAC CgA Tgg

V_K:

FR1 5' primers

(1) AB8 RAC ATC CAg ATg AYC CAg TC
(2) AB9 gYC ATC YRg ATg ACC CAg TC
(3) AB10 gAT ATT gTg ATg ACC CAg AC
(4) AB11 gAA ATT gTg TTg ACR CAg TC
(5) AB12 gAA ATW gTR ATg ACA CAg TC
(6) AB13 gAT gTT gTg ATg ACA CAG TC
(7) AB14 gAA ATT gTg CTg ACT CAg TC

V_K leader 5' primers:

(8) AB123 CCC gCT Cag CTC CTg ggg CTC CTg
(9) AB124 CCC TgC TCA gCT CCT ggg gCT gC
(10) AB125 CCC AgC gCA gCT TCT CTT CCT CCT gC
(11) AB126 ATg gAA CCA Tgg AAg CCC CAg CAC AgC

V_K 3' primer

(12) AB16 Cgg gAA gAT gAA gAC AgA Tg

PCR conditions used to amplify V_H and V_L regions for cloning

PCR Reactions were performed with AmpliTaq polymerase (Perkin Elmer) on a GeneAmp PCR System 9700 (Perkin Elmer Applied Biosystems, Foster City, CA, USA).

PCR cycling protocol:

94° 2'
11cycles 94° 30''
65° 30'', minus 1° per cycle

	72° 30"
30 cycles	94° 30''
	55° 30"
	72° 30''
	72° 10'
	cool down to 4°

Cloning of V_H and V_L in pGEMT-Vector System I

After analysing the PCR products on an agarose gel, the products were purified with S-400 or S300 microspin columns (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA), or with the QIAEX II Gel Extraction Kit (Qiagen GmbH, Hilden, Germany). For each experiment 2 independently amplified PCR products, using FR1 or leader primers, of each V_H and V_L region were cloned in pGEMT-Vector System I (Promega) according to manufacturers protocol.

After transformation to *E. coli* DH5α, individual colonies were screened by colony PCR using T7 and SP6 primers, 30 cycles at 55°. Plasmid DNA from each individual colony was purified using Qiaprep Spin miniprep kit (Qiagen). To further analyze a *Nco*1/*Not*1 (NE Biolabs, United Kingdom and Roche Diagnostics) digestion was performed and analyzed on agarose gel.

Sequencing

The V-regions were sequenced after cloning in the pGEMT-Vector System I. T7 and Sp6 primers (Eurogentec, Luik, Belgium) were used in combination with the sequence kit: ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Warrington, United Kingdom) according to protocol. The reactions were performed on a ABI PRISM 377 Sequencer (PE Applied Biosystems) and the sequences were analysed with the program DNAStar, SeqmanII. The sequences were then aligned to germline V-gene sequences in VBASE (www.mrc-cpe.cam.ac.uk/imt-doc/public/intro.htm).

Cloning and sequencing of the V_H and V_L-region of 146B7

V_H and V_L-regions from hybridoma 146B7 were amplified by PCR and cloned in pGEMT-Vector System I to determine the cDNA-sequence. The nucleotide and corresponding amino acid sequences are shown in Figure 2 (SEQ ID NOs: 1 and 2) and Figure 3 (SEQ ID NOs: 3 and 4), respectively. The framework (FR) and complementarity determining regions (CDR) are also indicated. The germline family for the V_H-region of 146B7 according to alignment in Vbase: V_H5-51 (V_H5-subgroup), D2-15/D2 (D_H-segment), JH4b (J_H-segment). The germline family for the V_L-region of 146B7 according to alignment in Vbase: A27 (V_KIII-subgroup) and J_K2 (J_K-segment). More information regarding V_H and V_L-domains is shown at the Kabat database http://immuno.bme.nwu.edu/ or at http://www.Vbase.com.

Example 6 Affinity binding characteristics of 146B7

The affinity of 146B7 was analyzed by surface plasmon resonance (SPR) technology using a BIACORE 3000 instrument to determine biomolecular protein interactions according to the following procedures. Changes in the SPR signal on the surface layer caused by biomolecular binding are detected and signify a change in the mass concentration at the surface layer. Affinity is expressed using the following definitions: k_a = association rate constant (M^{-1} sec^{-1}); k_d = dissociation rate constant (sec^{-1}); K_A = association equilibrium constant = k_a/k_d (M^{-1}); and K_D = dissociation equilibrium constant = k_d/k_a (M).

Different procedures were performed to obtain the affinity of 146B7 for human IL-15 (hIL-15). Human recombinant IL-15 from two different suppliers (Immunex corp., Seattle, USA and Peprotech, Rocky Hill, NJ, USA) was coupled to a CM5 sensor chip. The compound coupled to the sensorchip is defined as ligand. In other experiments 146B7 was used as ligand.

In each kinetic analysis, the binding of the analyte, 146B7 or hIL-15 adapted to the ligand coupled to the sensorchip, was compared to the binding to a reference control CM5 sensor chip. Serial dilutions of analyte were tested (0, 3.125, 6.25, 12.5, 25, 50 μg/ml). Association and dissociation curves were fitted for monomeric interaction in the model Langmuir 1:1, to determine k_a and k_d and to calculate K_A and K_D. All data were analyzed using BIA-Evaluation Version 3.1. For a

bivalent interaction the model "bivalent analyte" was used. All analysis were corrected for a drifting baseline.

To determine the antibody affinity of 146B7, the affinity of antibody 146B7 was measured for human recombinant IL-15 derived from two different suppliers, Immunex and Peprotech, at the BIACORE 3000. Using 146B7 as ligand and hIL-15 as analyte, the monovalent interaction was determined (curve fitting Langmuir 1:1).

The affinity of 146B7 for IL-15 (Immunex Corp.) was measured as follows:

The association rate constant k_a:	$1.07 (\pm 0.17) \times 10^5\ M^{-1}\ sec^{-1}$
The dissociation rate constant k_d:	$6.56 (\pm 0.09) \times 10^{-3}\ sec^{-1}$
Association equilibrium constant K_A:	$1.55 (\pm 0.21) \times 10^7\ M^{-1}$
Dissociation equilibrium constant K_D:	$6.59 (\pm 0.88) \times 10^{-8}\ M$

To determine the avidity of 146B7, IL-15 (Immunex Corp.) was used as ligand and 146B7 was used as analyte. When the data obtained were analyzed using Langmuir (1:1) curve fitting the bivalent interaction of the antibody was expressed, the avidity of the antibody was determined.

The avidity of 146B7 for IL-15 (Immunex Corp.) was measured as follows:

The association rate constant k_a:	$7.30 (\pm 0.81) \times 10^5\ M^{-1}\ sec^{-1}$
The dissociation rate constant k_d:	$1.45 (\pm 2.05) \times 10^{-3}\ sec^{-1}$
Association equilibrium constant K_A:	$5.03 (\pm 3.40) \times 10^8\ M^{-1}$
Dissociation equilibrium constant K_D:	$1.55 (\pm 1.24) \times 10^{-9}\ M$

The affinity and avidity of 146B7 for Peprotech derived IL-15 were determined also. No major differences in affinity or avidity for two different sources of IL-15 were seen.

As is described in the example below regarding the inhibition of human interleukin-15 (hIL-15)-induced proliferation of CTLL-2 cells and PBMC by fully human anti-IL-15 antibodies, 146B7 inhibited in a dose dependent manner the IL-15 induced proliferation as was measured by [^{3}H]-thymidine incorporation. The IC50 - concentration at 50 % inhibition, a more functional manner to determine affinity- from these proliferation inhibition experiments was calculated: 3.1 ± 0.91 nM. This IC50 is in agreement with the avidity measured by BIACORE 3000 (K_D 1.5 nM) using 146B7 as ligand and recombinant human IL-15 as analyte and confirmed the affinity and avidity measurements obtained here.

Example 7 Inhibition of hIL-15-induced TNF-α production by fully human anti-IL-15 antibodies

The effect of fully human anti-IL-15 antibodies, 146B7, 146H5, 404E4 and 404A8, on IL-15-induced TNF-α production was studied using peripheral blood derived mononuclear cells (PBMC) from healthy volunteers using the following procedures. To evaluate specificity to IL-15, the effect of these antibodies on IL-2-mediated TNF-α production was also examined.

Cell culture

Cultures were maintained in RPMI-1640 with 2 mM L-glutamine, 100 IU/ml penicillin, 100 µg/ml streptomycin (all derived from Life Technologies, Paisley, Scotland) and 10% heat-inactivated fetal calf serum (HyClone, Utah, USA).

Purification of peripheral blood mononuclear cells (PBMC)

Fresh human blood was drawn from a healthy volunteer after informed consent, heparin was added against coagulation. Purification of PBMC was performed by density gradient centrifugation using Ficoll (Pharmacia, Uppsala, Sweden).

Test Compound

HIL-15, lot no: 6870-011, Immunex corp., Seattle, Washington, USA.

hIL-2, Chiron Benelux BV, Amsterdam, The Netherlands.

Fully human antibodies used: 146B7 (batch: 070101) and 146B7RDJW07, 404A8 (batch: 030101) and 404E4 (batch: 080101) and as isotype control antibody T1 (97-2B11-2B12, batch: 190900).

Inhibition of human IL-15 (hIL-15) or hIL-2-induced TNF-α production by PBMC by anti-IL-15 antibodies

PBMC were cultured in triplicate or quadruplicate in 96-well flat-bottom plate at 1.5×10^5 cells per well in the presence or absence of hIL-2 or hIL-15 and with or without anti-IL-15 antibodies. Isotype control antibody (T1) was included as negative control. Concanavalin A (2.5μg/ml, Calbiochem) was added as a positive control for proliferation. Cells were incubated for 72 hours at 37°C and 5% CO_2. Supernatants were harvested to quantify the amount of human TNF-α by ELISA (U-CyTech, Utrecht, The Netherlands).

The effects of 146B7 and an isotype control antibody were tested on IL-15-mediated TNF-α production by PBMC. 146B7 inhibited hIL-15-mediated TNF-α production in a dose dependent fashion, whereas the isotype control antibody did not inhibit hIL-15-induced TNF-α production (Figure 6). Data of two healthy volunteers are shown. 404E4 and 404A8 were unable to inhibit hIL-15-induced TNF-α production.

To ensure the specificity of the anti-IL-15 antibodies, their effect on hIL-2-mediated TNF-α production was evaluated. No inhibition of IL-2-mediated TNF-α production was induced by 146B7 (Figure 7). No dose dependent inhibition by either 404E4 or 404A8 was seen in hIL-2-mediated TNF-α production.

A dose dependent inhibition of hIL-15-mediated TNF-α production was seen only by 146B7 and not by 404E4 and 404A8. The inhibitory effect was specific for hIL-15; IL-2-mediated TNF-α production was not inhibited.

Example 8 Inhibition of human interleukin-15 (hIL-15)-induced proliferation of CTLL-2 cells and PBMC by fully human anti-IL-15 antibodies

Antibodies 146B7, 146H5, 404E4 and 404A8 were tested for their ability to inhibit T-cell proliferation using CTLL-2 cells (Gillis *et al.*, 1978) and peripheral blood mononuclear cells (PBMC) using the following procedures.

Cell culture

Cultures were maintained in RPMI-1640 with 2 mM L-glutamine, 100 IU/ml penicillin, 100 µg/ml streptomycin (derived from Life Technologies, Paisley, Scotland) and 10% heat-inactivated fetal calf serum (HyClone, Utah, USA). CTLL-2 cells (Gillis *et al.*, 1978) were maintained in the above mentioned medium supplemented with 36 units hIL-2/ml (Chiron Benelux BV, Amsterdam, The Netherlands) and starved for hIL-2 for 3-4 days before the start of the experiment. CTLL-2 cells were washed three times before use.

Purification of peripheral blood mononuclear cells (PBMC)

Fresh human blood was drawn from a healthy volunteer after informed consent, heparin was added against coagulation. Purification of PBMC was performed by density gradient centrifugation using Ficoll (Pharmacia, Uppsala, Sweden).

Test Compound

HIL-15, lot no: 6870-011, Immunex corp., Seattle, Washington, USA.

hIL-2, Chiron Benelux BV, Amsterdam, The Netherlands.

anti-IL-15 antibodies used for CTLL-2 assay in this report shown in Figure 8: 146B7, 146H5, 404A8, 404E4.

anti-IL-15 antibodies used for PBMC assays: 146B7 (batch: 070101), 404A8 (batch: 030101) and 404E4 (batch: 080101).

Inhibition of human IL-15 (hIL-15) or hIL-2 induced CTLL-2 proliferation by anti-IL-15 antibodies

In each experiment, cells were seeded in triplicate in 96-well plate, $5x10^3$ cells per well in the presence or absence of either hIL-2 or hIL-15. To evaluate the effect on proliferation, each of the four anti-IL-15 antibodies were added. Cells were incubated for 16 hours at 37°C and 5% CO_2. [^{3}H]Thymidine (1 µCi/well, Amersham Life Sciences, Little Chalfont, Buckinhamshire, UK) was added 4 hours before harvesting (Harvester 96 Mach II M, Tomtec, Orange CT, USA).

As is shown in Figure 8, IL-15 induced proliferation of CTLL-2 cells was decreased in a dose dependent fashion by 146B7 and 146H5 as was reflected by reduced [^{3}H]-Thymidine incorporation. Both 404E4 and 404A8 were unable to block IL-15 induced proliferation of CTLL-2 cells.

Inhibition of hIL-15 (hIL-15) or hIL-2 induced PBMC proliferation by anti-IL-15 antibodies

PBMC were cultured in triplicate in 96-well U-bottom plate (Nunc, Nalge Nunc International, Denmark), 5×10^4 cells per well in the presence or absence of hIL-2 or hIL-15 and the anti-IL-15 antibodies. Concanavalin A (2.5µg/ml, Calbiochem) was added as a positive control for proliferation. The cells were incubated for 72 hours at 37°C and 5% CO_2. [3H]Thymidine (1 µCi/well, Amersham Life Sciences, Little Chalfont, Buckinhamshire, UK) was added 16 hours before harvesting (Harvester 96, Tomtec, Orange CT, USA).

146B7 was able to inhibit IL-15 induced [^{3}H]-Thymidine incorporation dose dependently and, therefore, inhibited proliferation (IC50 = 3.1 ± 0.91 nM). Both 404E4 and 404A8 were unable to block hIL-15 induced PBMC proliferation. 146H5 was not tested according to data obtained from previously performed experiments. To ensure the specificity of 146B7, 404E4 and 404A8 for IL-15, these antibodies were also evaluated for their effects on IL-2 mediated proliferation. None of the tested anti-IL-15 antibodies exhibited an effect on IL-2 induced proliferation (Figure 9).

Example 9 Human anti-IL-15 antibody 146B7 binds to human IL-15 present on human PBMCs

Test compounds

Human PBMCs were obtained from healthy volunteers after informed consent. Antibody 146B7 (batch no. MDX015), Medarex Inc., Milpitas, CA, USA.

Biotinylation of 146B7 and human IgG

N-hydroxysuccinimido-biotin (Sigma) was first diluted in DMSO (final dilution: 100 mg/ml) and then in 0.1 M $NaHCO_3$ (final dilution: 1 mg/ml, Sigma). Per 1 mg of antibody (diluted in 1 ml), 600 µl of biotin solution was added (dark, 2hrs, RT). Antibody-biotin solution was dialysed in a slide-a-lyzer™ dialysis cassette (10,000 MWCO, Pierce, Perbio Science, Netherlands) (overnight at 4°C) to remove unlabeled biotin. The following day, concentration of biotinylated antibodies was determined by spectrophotometry (Ultrospec 2100pro) at OD 280 nm.

Stimulation of peripheral blood

To induce IL-15, blood was obtained by venapuncture from healthy volunteers. PBMCs were cultured in RPMI 1640 (Biowhittaker Europe) supplemented with penicillin (5 U/ml), streptomycin (50 µg/ml), L-glutamine (2mM) (Biowhittaker Europe), and 10% fetal calf serum (Optimum C241, Multicell, Wisent Inc.) for a maximum of 2 days (37°C), and were stimulated with 500 U/ml IFNγ (Boehringer Ingelheim).

Flow cytometry

Cells were pre-incubated with 10% human AB serum (CLB, Amsterdam, Netherlands) in RPMI 1640 (Biowhittaker Europe) supplemented with penicillin (5 U/ml), streptomycin (50 µg/ml), L-glutamine (2mM) (Biowhittaker Europe) and 10% fetal calf serum (Optimum C241, Multicell, Wisent Inc.). After permeabilization (20 min, 4°C, in Cytofix/Cytoperm™ Kit, Becton Dickinson, San Diego, CA) and washing in Perm/Wash™ buffer (Cytofix/Cytoperm™ Kit), PBMC were subjected to staining of IL-15 by flow cytometry. Continuous permeability was achieved by using

Perm/Wash™ buffer (Cytofix/Cytoperm™ Kit) throughout the staining procedure. After incubating the cells with biotinylated 146B7 or with biotinylated hIgG1 (20 µg/ml, 30 min, 4°C) and washing in Perm/Wash™ buffer, cells were subsequently incubated with streptavidin-phycoerythrin (DAKO) for 30 minutes (4°C). Fluorescence intensity of at least 5000 cells per sample was determined after analysis by flow cytometry (FACS Calibur, Becton Dickinson) and gating on the monocytes, using CellQuest Pro software. Data show the stimulation index (S.I.), which is calculated as follows: S.I. = (mean fluorescence positive staining)/(mean fluorescence background staining)

Immunocytochemistry

To detect IL-15 present in human monocytes, cytospin preparations were made of whole blood samples. After spinning down 5 x 10^4 cells (200 µl) onto Superfrost®-Plus microscope slides (Menzel), slides were air-dried (< 60 min), fixed in 2% paraformaldehyde/PBS (8 min, 4°C), washed with PBS and air-dried again. Before staining, cytospin preparations were permeabilized in PBS (+ 0.1% saponine; PBSS), which was subsequently used throughout the staining procedure. To block endogenous peroxidase activity, cytospin preparations were incubated with 0.05% (v/v) hydrogen peroxide (H_2O_2) diluted in citric acid/phosphate buffer (pH 5.8, 20 min, RT). After washing with PBSS, endogenous biotin activity was blocked according to the manufacturer's instructions (Biotin Blocking Kit, Vector Lab., DAKO). After washing with PBSS, non-specific binding sites were blocked by incubating the cytospin preparations with 10% (v/v) human pooled AB-serum (CLB, Amsterdam, Netherlands) (30 min) in PBSS. Thereafter, cytospin preparations were incubated with biotinylated primary antibody (60 min, RT) and, after washing with PBSS, with streptavidin complexed with biotinylated horseradish peroxidase (streptABComplex/HRP, DAKO; 1:100 in PBSS, containing 2% human AB serum; 30 min, RT). After washing in PBSS, the cytospin preparations were incubated with 3-amino-9-ethylcarbazole (0.5 mg/ml) and H_2O_2 (0.01%), in sodium acetate buffer (50 mM, pH 4.9) for 10 minutes (RT), for the detection of HRP activity. Cytospins were washed with running tap water for 5 minutes, counterstained with haematoxylin (DAKO) for one minute, washed with running tap water for another 5 minutes, and embedded in faramount or glycergel (DAKO).

Flow cytometry

Binding of 146B7 to IFNγ-stimulated human monocytes is shown in Figure 12. Biotinylated 146B7 binds to unstimulated monocytes showing the presence of IL-15 in unstimulated cells. Stimulation of monocytes with IFNγ leads to a increased binding of 146B7 to the cells, with a maximum reached at day one of culture. The control antibody, hIgG1, shows little binding to unstimulated monocytes. Stimulation with IFNγ increases binding of hIgG1 through increased expression of Fcγ receptors on monocytes.

Immunocytochemistry

Figure 13 shows staining of human monocytes with 146B7, or with the control antibody, hIgG1. A clear red staining of the cytoplasm is observed after incubating the cells with 146B7, but not with the control antibody. Accordingly, 146B7 binds hIL-15 in monocytes and this binding is upregulated after stimulation with IFNγ. Figure 13 also shows that IL-15 staining is primarily intracellular.

Example 10 Human anti-IL-15 antibody 146B7 binds IL-15 in tissues by immunohistochemistry

Test compounds

Human psoriatic skin – tissue samples were obtained after informed consent. Louise Villadsen, Department of Dermatology, Gentofte University Hospital, Copenhagen, Denmark.

Antibody 146B7 (batch no. MDX015), Medarex, Milpitas, CA, USA

Biotinylation of 146B7 and human IgG

N-hydroxysuccinimido-biotin (Sigma) was first diluted in DMSO (final dilution: 100 mg/ml) and then in 0.1 M $NaHCO_3$ (final dilution: 1 mg/ml, Sigma). Per 1 mg of antibody (diluted in 1 ml), 600 µl of biotin solution was added (dark, 2hrs, RT). Antibody-biotin solution was dialysed in a slide-a-lyzer™ dialysis cassette (10,000 MWCO, Pierce, Perbio Science, Netherlands) (ON, 4°C) to remove unlabeled biotin.

The following day, concentration of biotinylated antibodies was determined by spectrophotometry (Ultrospec 2100pro) at OD 280 nm.

Immunohistochemistry

Tissues were stored at –80°C until assay. After thawing, tissue sections were fixated in acetone (10 min, RT) and air-dried. To block endogenous peroxidase activity, sections were incubated with 0.05% (v/v) hydrogen peroxide (H_2O_2) diluted in citric acid/phosphate buffer (pH 5.8, 20 min, RT). After washing with PBS-Tween 20 (PBST, 0.05% v/v), endogenous biotin activity was blocked according to the manufacturer's instructions (Biotin Blocking Kit, Vector Lab., DAKO). After washing with PBST, non-specific binding sites were blocked by incubating the tissue sections with 10% (v/v) human pooled AB-serum (CLB, Amsterdam, Netherlands) (30 min) in PBST. Serum was blotted off and sections were subsequently incubated with biotinylated primary antibody (146B7 or hIgG1) diluted in PBS containing 2% human AB serum for 60 minutes (RT). Sections were washed in PBST. After washing in PBST, all tissue sections were incubated with streptABComplex/HRP (DAKO; 1:100 diluted in PBS containing 2% human AB serum; 30 min, RT). After washing in PBST, the sections were incubated with 3-amino-9-ethylcarbazole (0.5 mg/ml) and H_2O_2 (0.01%), in sodium acetate buffer (50 mM, pH 4.9) for 10 minutes (RT), for the detection of HRP activity. Sections were washed with running tap water for 5 minutes, counterstained with haematoxylin (DAKO) for one minute, washed with running tap water for another 5 minutes, and finally embedded in faramount or glycergel (DAKO).

Results

A clear cytoplasmic staining of keratinocytes in psoriatic skin was observed after staining tissue sections with 146B7, but not with the control antibody (Figure 14; 146B7 stains IL-15-positive keratinocytes obtained from psoriatic plaques).

Example 11 Human anti-IL-15 antibody 146B7 blocks IL-15 in SCID mouse-human tissue chimeras: significant inhibition of inflammation in both arthritic and psoriatic tissue

Test compounds

Synovial tissue - obtained form patients with juvenile rheumatoid arthritis, after informed consent; Alexei Grom, division of pediatric rheumatology, Children's Hospital Medical Center, Cincinnati, Ohio, USA.

Keratome biopsies - tissue samples were obtained after informed consent. Louise Villadsen, Department of Dermatology, Gentofte University Hospital, Copenhagen, Denmark.

Antibody 146B7 (batch no. MDX015), Medarex Inc., Milpitas, CA, USA for psoriasis experiments.

Antibody 146B7 (batch no. 15-00RDJW07), Medarex Inc., Milpitas, CA, USA for rheumatoid arthritis experiments.

Blocking IL-15 in SCID mouse – human synovial tissue chimeras

Fresh synovial tissue samples were obtained from patients with juvenile rheumatoid arthritis after joint replacement surgery. Samples were collected in sterile conditions. Minced tissue fragments from the entire synovial tissue sample were thoroughly mixed to ensure homogeneity of each preparation. Minced tissues (2-4 grafts per animal; 100 mg per one site) were engrafted subcutaneously in the back of SCID/NOD mice (Jackson Laboratories). Each animal received 146B7 (500 μg, i.p.) or PBS on the day of graft implantation, and on post-implantation days 7, 14, and 21. Animals were sacrificed on day 28 post-implantation. Synovial grafts were excised and placed on formalin for H&E staining.

Quantification of H&E staining of tissues from SCID mouse – human synovial tissue chimeras (Modified from Lehr *et al.*, J. Histochem. Cytochem. 1997, 45, 1559)

After obtaining digital images (2600x2060, jpg) of sections obtained from SCID mouse – human synovial tissue chimeras using a X10 objective (Zeiss microscope; Axiovision software), data were computer-analysed, by use of Photoshop, version 6.0 (Adobe Systems, Mountain view, CA) and reduced to 1300x1300 pixels.

Within each section six X10 fields were chosen so as to best reflect the overall staining of the tissue on the entire slide. After selection of all stained nuclei (magic wand on dark nucleus with tolerance 10), an optical density plot of the selected area was generated and the mean staining intensity was recorded (after selection of similar/image histogram command). Subsequently, the background was selected and staining was quantified (magic wand on background with tolerance 10). Staining intensity was calculated as the difference between nuclear staining and background staining. This was designated the cytochemical index with arbitrary units. Data are shown as mean and s.e.m. Data were analysed by Student's t-test.

Blocking IL-15 in SCID mouse – human psoriatic tissue chimeras

Keratome biopsies were obtained from psoriatic plaques of two patients, divided and transplanted onto C.B-17 SCID (Jackson Laboratories) mice. Three weeks after transplantation mice received PBS (placebo), CsA (cyclosporine A) (Sandoz) at a dose of 10 mg/kg every second day for 15 days, or 146B7 at a dose of 20 mg/kg on day 1 and 10 mg/kg on days 8 and 15. One week after the last injection, mice were sacrificed, and a 4 mm punch biopsy was taken from each xenograft. Biopsies were fixed in formalin for paraffin embedding and stained in H&E and for Ki-67 nuclear antigen.

Quantification of immunohistochemical staining of tissues from SCID mouse - human psoriatic tissue chimeras

The H&E-stained sections were evaluated for epidermal thickness (μm), grade of parakeratosis (rated from 0-3), and number of inflammatory mononuclear cells in upper dermis. The sections stained for Ki-67 were evaluated for number of cycling keratinocytes/mm^2 section. Mean values for the 4 mice in each treatment group were calculated, and the data from each patient were summarised as mean and s.e.m.

SCID/RA model

Microscopic observation of sections showed that the darkest stained nuclei belong to infiltrating cells. Therefore, the number of nuclei (measured as the relative surface area) are considered as a measure for infiltration. Injection of 146B7 reduces the number of infiltrating cells into inflamed synovial tissue, as compared to

vehicle treatment (Figure 15a, $p<0.05$). Figure 15b illustrates the effects of 146B7 on infiltration of cells into xenografted synovial tissue, and shows a reduction in number of cells with dark nuclei, as compared to vehicle treatment.

SCID/psoriasis model

Figure 16 shows SCID/psoriasis mice treated with 146B7 or control treatment. Compared to the vehicle, PBS, injections of 146B7 reduced the severity of psoriasis evaluated by epidermal thickness when was measured from the stratum corneum to the beginning of the rete pegs (Figure16A)): PBS (177.8 $\pm$ 42.2 μm), CsA (91.0 $\pm$ 15.2 μm), 146B7 (62.5 $\pm$ 9.1 μm). A reduction in thickness was also observed when was measured from the stratum corneum to the deepest part of the rete pegs (Figure 16B): PBS (433.8 $\pm$ 32.1 μm), CsA (303.8 $\pm$ 62.9 μm) and 146B7 (208.0 $\pm$ 33.8 μm). Also, the grade of parakeratosis was reduced by 146B7 treatment (Figure 16C): PBS (1.6 $\pm$ 0.4), CsA (1.3 $\pm$ 0.3), 146B7 (0.5 $\pm$ 0.3). Furthermore, 146B7 reduces the number of inflammatory mononuclear cells in upper dermis (Figure 16D): PBS (33.3 $\pm$ 1.9 mononuclear cells), CsA (19.4 $\pm$ 8.5), 146B7 (16.4 $\pm$ 0.1). The expression of the human Ki-67 protein is strictly associated with cell proliferation. During interphase, the antigen can be exclusively detected within the nucleus, whereas in mitosis most of the protein is relocated to the surface of the chromosomes. The fact that the Ki-67 protein is present during all active phases of the cell cycle (G(1), S, G(2), and mitosis), but is absent from resting cells (G(0)), makes it an excellent marker for determining the so-called growth fraction of a given cell population. 146B7 reduces the number of Ki-67+ cycling keratinocytes (Figure 16E): PBS (247.9 ± 77.0), CsA (116.0 ± 24.1), 146B7 (73.8 ± 9.9).

Treatment with 146B7 inhibited the infiltration of inflammatory cells into inflamed tissue in human SCID models for rheumatoid arthritis. Furthermore, in SCID mice with engrafted human psoriatic plaques, treatment with 146B7 reduced the severity of psoriasis, as compared to treatment with CsA. Indeed, treatment with 146B7 resulted in a major reduction in inflammation, in epidermal thickness, in numbers of dividing keratinocytes, and in severity of parakeratosis in human/SCID mice.

Example 12 Human anti-IL-15 antibody 146B7 recognizes receptor-bound IL-15

Test compounds

hIgG1 - human control antibody (Sigma).

Antibody 146B7 – Medarex Inc., MDX015.

Raji cells with constitutive expression of IL-15Rα (Martin Glennie, Tenovus Research Laboratory, Southampton General Hospital, Southampton, U.K.).

Biotinylation of 146B7 and human IgG

N-hydroxysuccinimido-biotin (Sigma) was first diluted in DMSO (final dilution: 100 mg/ml) and then in 0.1 M $NaHCO_3$ (final dilution: 1 mg/ml, Sigma). Per 1 mg of antibody (diluted in 1 ml), 600 µl of biotin solution was added (dark, 2hrs, RT). Antibody-biotin solution was dialysed in a slide-a-lyzer™ dialysis cassette (10,000 MWCO, Pierce, Perbio Science, Netherlands) (overnight at 4°C) to remove unlabeled biotin. The following day, concentration of biotinylated antibodies was determined by spectrophotometry (Ultrospec 2100pro) at OD 280 nm.

Binding of 146B7 to IL-15 – IL-15Rα complex by ELISA

After coating (overnight at room temperature) flat bottom microtiter plates (Greiner) with IL-15Rα (R&D systems, Minneapolis, MN, USA), plates were incubated with PBS and chicken serum (2%, RT, 60 min). After washing in PBS (+ 0.05% Tween 20: PBST), plates were subsequently incubated with several dilutions of unlabeled IL-15 (50 µl, RT, Immunex, Seattle, USA). After 10 minutes, biotinylated antibodies were added to the wells (50 µl) in different concentrations (90 minutes at room temperature). After washing in PBST, plates were incubated (60 minutes at room temperature) with streptavidin-poly-horseradish peroxidase (CLB, Amsterdam, Netherlands) diluted 1:10,000 in PBST-C (PBST and 2% chicken serum). Finally, plates were washed and subsequently incubated with ABTS (Azinobis-3-ethylbenzthiazoline-sulphonic-acid, Roche Diagnostics, Mannheim, Germany) in ABTS buffer according to the manufacturer's protocol. Color reaction was stopped with 2% oxalic acid (50 µl). Binding was evaluated at 405 nm in an EL808 ELISA-reader (Bio-Tek Instruments, Winooski, VT, USA).

Binding of 146B7 to IL-15 – IL-15R complex on Raji cells

Raji cells are pre-incubated (20 minutes at 4°C) with 10% human pooled AB serum (CLB, Amsterdam, Netherlands)in FACS buffer (PBS, 0.05%BSA, 0.02% $NaNO_3$). Raji cells (1-2*10^5 cells/ml) were put in the wells, and 50 µl of unlabeled IL-15 was added in several concentrations (diluted in FACS buffer with 10% human AB serum). After incubating the cells for 30 minutes (4°C) and washing twice in FACS buffer, 50 µl of biotinylated antibodies (146B7 or hIgG1) was added to the wells (30 minutes at 4°C). After washing twice in FACS buffer, 50 µl of streptavidin-phycoerythrin was added to each well (30 minutes at 4°C). After washing twice in FACS buffer, cells were taken up in 200 µl of FACS buffer, and fluorescence intensity of at least 5000 cells per sample was determined after analysis by flow cytometry (FACS Calibur, Becton Dickinson) using CellQuest software. Data show the stimulation index (S.I.), which is calculated as follows:

S.I. = (mean fluorescence positive staining)/(mean fluorescence background staining)

ELISA

Binding of 146B7 to IL-15/IL-15R complex in ELISA is shown in Figure 19. Binding of 146B7 increases with increasing concentrations of IL-15 binding to its receptor. No effects were observed of binding of control antibody to IL-15 or to IL-15R.

Binding to IL-15R-expressing Raji cells

Binding of 146B7 to IL-15/IL-15R complex on Raji cells is shown in Figure 20. 146B7 binds to the IL-15/IL-15R complex in a dose-dependent manner. No binding of hIgG1 to the IL-15/IL-15R complex on Raji cells was observed (Figure 20).

146B7 is able to bind IL-15 after binding of this cytokine to its receptor. 146B7 binds to an epitope on IL-15 that is not involved in binding to the receptor.

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Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims. Any combination of the embodiments disclosed in the dependent claims are contemplated to be within the scope of the invention.

Incorporation by Reference

All publications, patents, and pending patent applications referred to herein are hereby incorporated by reference in their entirety.

We claim:

1. An isolated human monoclonal antibody which specifically binds to human IL-15 and inhibits IL-15 induced proinflammatory effects.

2. The antibody of claim 1 which inhibits IL-15-induced TNFα production or T cell proliferation.

3. The antibody of claim 2 which inhibits IL-15 induced T cell proliferation with an IC_{50} value of less than approximately 100 nM as determined by proliferation inhibition assay.

4. The antibody of claim 2 which inhibits IL-15 induced T cell proliferation with an IC_{50} value of less than approximately 10 nM as determined by proliferation inhibition assay.

5. The antibody of claim 1 which binds to human IL-15 with a dissociation equilibrium constant (K_D) of below 10^{-7} M as determined by surface plasmon resonance (SPR) technology using recombinant human IL-15 as the analyte and the antibody as the ligand.

6. The antibody of claim 1, wherein the antibody specifically binds to an epitope located on the β-chain or the γ-chain interacting domain of human IL-15.

7. The antibody of claim 6, wherein the antibody specifically binds to an epitope located on the γ-chain interacting domain of human IL-15.

8. The antibody of claim 1, wherein the antibody interferes with the binding of Asp^{8} of human IL-15 to the β-unit of the human IL-15 receptor or the binding of Gln^{108} of human IL-15 to the γ-unit of human IL-15 receptor.

9. The antibody of claim 1, wherein the antibody specifically binds to receptor-bound human IL-15.

10. An isolated human monoclonal antibody which specifically binds to human IL-15 encoded by human IgG heavy chain and human kappa light chain nucleic acids comprising nucleotide sequences in their variable regions as set forth in Figure 2 (SEQ ID NO:1) and Figure 3 (SEQ ID NO:3), respectively, and conservative sequence modifications thereof.

11. An isolated human monoclonal antibody which specifically binds to human IL-15 having IgG heavy chain and kappa light chain variable regions which comprise the amino acid sequences shown in Figure 2 (SEQ ID NO:2) and Figure 3 (SEQ ID NO:4), respectively, and conservative sequence modifications thereof.

12. An isolated human monoclonal antibody which specifically binds to human IL-15 comprising a CDR domain selected from the group consisting of

(a) a CDR1 domain comprising the amino acid sequence CDR1 shown in Figure 2 (SEQ ID NO:2) and Figure 3 (SEQ ID NO:4), respectively, and conservative sequence modifications thereof ,

(b) a CDR2 domain comprising the amino acid sequence CDR2 shown in Figure 2 (SEQ ID NO:2) and Figure 3 (SEQ ID NO:4), respectively, and conservative sequence modifications thereof, and

(c) a CDR3 domain comprising the amino acid sequence CDR3 shown in Figure 2 (SEQ ID NO:2) and Figure 3 (SEQ ID NO:4), respectively, and conservative sequence modifications thereof,

wherein the CDR domain is inserted in an antibody framework or joined by a synthetic linker.

13. The antibody of claim 1, wherein the antibody is selected from the group consisting of an IgG1, an IgG2, an IgG3, an IgG4, an IgM, an IgA1, an IgA2, an IgAsec, an IgD, and an IgE antibody.

14. The antibody of claim 1, comprising an IgG1 heavy chain.

15. The antibody of claim 1 which is an antibody fragment or a single chain antibody.

16. The antibody of claim 1 which is a whole antibody.

17. The antibody of claim 1, produced by a hybridoma which includes a B cell obtained from a transgenic non-human animal having a genome comprising a human heavy chain transgene and a human light chain transgene fused to an immortalized cell.

18. An isolated human monoclonal antibody which specifically binds to human IL-15 and inhibits the ability of IL-15 to induce proinflammatory effects upon binding to its receptor.

19. A method of inhibiting IL-15 induced, but not IL-2 induced, TNFα production in T cells or monocytes, comprising contacting IL-15 with an isolated human monoclonal antibody which specifically binds to human IL-15.

20. A method of inhibiting IL-15 induced, but not IL-2 induced, T cell proliferation, comprising contacting IL-15 with an isolated human monoclonal antibody which specifically binds to human IL-15 in the presence of said T cells.

21. The method of claim 20, wherein the T cells are peripheral blood mononuclear cells (PBMCs) or CTLL-2 cells.

22. A hybridoma comprising a B cell obtained from a transgenic non-human animal having a genome comprising a human heavy chain transgene and a light chain transgene, fused to an immortalized cell, wherein the hybridoma produces a human monoclonal antibody which specifically binds to human IL-15.

23. The hybridoma of claim 22 which produces a human monoclonal antibody encoded by human IgG heavy chain and human kappa light chain nucleic acids.

24. A hybridoma which produces a human monoclonal antibody encoded by human IgG heavy chain and human kappa light chain nucleic acids comprising nucleotide sequences in their variable regions as set forth in Figure 2 (SEQ ID NO:1) and Figure 3 (SEQ ID NO:3), respectively, and conservative sequence modifications thereof.

25. A hybridoma which produces a human monoclonal having IgG heavy chain and kappa light chain variable regions which comprise the amino acid sequences shown in Figure 2 (SEQ ID NO:2) and Figure 3 (SEQ ID NO:4), respectively, and conservative sequence modifications thereof.

26. The isolated human antibody of claim 1 produced by a transfectoma comprising nucleic acids encoding a human heavy chain and a human light chain.

27. A transfectoma comprising nucleic acids encoding a human heavy chain and a human light chain, wherein the transfectoma produces a detectable amount of the monoclonal antibody of claim 1.

28. The transfectoma of claim 27, comprising nucleic acids encoding a human heavy chain and a human light chain comprising nucleotide sequences in their variable regions as set forth in SEQ ID NO:1 and SEQ ID NO:3, respectively, or conservative sequence modifications thereof.

29. A transgenic non-human animal which expresses a human monoclonal antibody which specifically binds to human IL-15, wherein the transgenic non-human animal has a genome comprising a human heavy chain transgene and a human light chain transgene.

30. A method of producing a human monoclonal antibody which specifically binds to human IL-15, comprising:

immunizing a transgenic non-human animal having a genome comprising a human heavy chain transgene and a human light chain transgene with human IL-15 or a cell expressing human IL-15, such that antibodies are produced by B cells of the animal;

isolating B cells of the animal;

fusing the B cells with myeloma cells to form immortal, hybridoma cells that secrete human monoclonal antibodies specific for IL-15; and

isolating the human monoclonal antibodies specific for IL-15 from the culture supernatant of the hybridoma.

31. An immunoconjugate comprising the antibody of claim 1 and a therapeutic agent.

32. The immunoconjugate of claim 31, wherein the therapeutic agent is an immunosuppressive agent.

33. The immunoconjugate of claim 31, wherein the therapeutic agent is an anti-inflammatory agent selected from the group consisting of a steroidal anti-inflammatory agent, a nonsteroidal anti-inflammatory agent and a DMARD.

34. The immunoconjugate of claim 31, wherein the therapeutic agent is a cytotoxic agent.

35. A pharmaceutical composition comprising the antibody of claim 1 and a pharmaceutically acceptable carrier.

36. The composition of claim 35 further comprising a therapeutic agent.

37. The composition of claim 36, wherein the agent is an immunosuppressive agent.

38. The composition of claim 37, wherein the immunosuppressive agent is cyclosporine.

39. The composition of claim 36, wherein the agent is an anti-inflammatory agent selected from the group consisting of a steroidal anti-inflammatory agent and a nonsteroidal anti-inflammatory agent.

40. The composition of claim 36, wherein the agent is a DMARD selected from the group consisting of methotrexate, etanercept, and infliximab.

41. The composition of claim 36, wherein the agent is a chemotherapeutic agent selected from the group consisting of doxorubicin, cisplatin, bleomycin, carmustine, cyclophosphamide, and chlorambucil.

42. The composition of claim 36, wherein the agent is an agent for treating psoriasis.

43. The composition of claim 36, wherein the agent is an antibody.

44. The composition of claim 43, wherein the antibody is selected from the group consisting of a CD4 specific antibody and an IL-2 specific antibody.

45. A method of treating or preventing a disorder mediated by human IL-15, comprising administering to a subject the antibody of claim 1 in an amount effective to treat or prevent the disorder.

46. The method of claim 45, wherein the disorder is selected from the group consisting of psoriasis, arthritis, inflammatory bowel disease, cancer, transplant rejection and infectious disease.

47. The method of claim 46, wherein the arthritis is rheumatoid arthritis.

48. The method of claim 45, further comprising co-administration of a therapeutic agent.

49. The method of claim 48, wherein the agent is an immunosuppressive agent. cyclosporine.

50. The method of claim 49, wherein the immunosuppressive agent is cyclosporine.

51. The method of claim 48, wherein the agent is an anti-inflammatory agent selected from the group consisting of a steroidal anti-inflammatory agent and a nonsteroidal anti-inflammatory agent.

52. The method of claim 48, wherein the agent is a DMARD selected from the group consisting of methotrexate, etanercept, and infliximab.

53. The method of claim 48, wherein the agent is a chemotherapeutic selected from the group consisting of doxorubicin, cisplatin, bleomycin, carmustine, cyclophosphamide, and chlorambucil.

54. The method of claim 48, wherein the agent is an agent for treating psoriasis.

55. The method of claim 48, wherein the agent is an antibody.

56. The method of claim 55, wherein the antibody is selected from the group consisting of a CD4 specific antibody and an IL-2 specific antibody.

57. A method of treating or preventing psoriasis, comprising administering to a subject an isolated human monoclonal antibody which specifically binds to human IL-15 and inhibits the ability of IL-15 to induce proinflammatory effects upon binding to its receptor.

58. The method of claim 57, wherein the antibody inhibits the ability of IL-15 to induce parakeratosis.

59. The method of claim 57, wherein the antibody inhibits the ability of IL-15 to induce epidermal thickening.

60. The method of claim 57, wherein the antibody inhibits the ability of IL-15 to induce the proliferation of keratinocytes.

61. A method of treating or preventing rheumatoid arthritis, comprising administering to a subject an isolated human monoclonal antibody which specifically binds to human IL-15 and inhibits the ability of IL-15 to induce proinflammatory effects upon binding to its receptor.

62. The method of claim 61, wherein the antibody inhibits the ability of IL-15 to induce chemotaxis of activated leukocytes.

63. A method of diagnosing an IL-15-mediated disease by detecting the presence of IL-15 antigen, or a cell expressing IL-15, in a sample comprising:

contacting the sample, and a control sample, with the human antibody of claim 1, under conditions that allow for formation of a complex between the antibody or portion thereof and IL-15; and

detecting the formation of a complex,

wherein a difference complex formation between the sample compared to the control sample is indicative of the presence of IL-15 in the sample.

64. A nucleic acid comprising a nucleotide sequence encoding a variable region of a human monoclonal antibody that inhibits the proinflammatory effects of IL-15.

65. A nucleic acid comprising a nucleotide sequence encoding a variable region of a human monoclonal antibody that specifically binds to human IL-15, wherein the nucleotide sequence is selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:3, and conservative sequence modifications thereof.

66. An expression vector comprising a nucleotide sequence encoding the variable region of a light chain, heavy chain, or both light and heavy chains of a human antibody which binds human IL-15.

67. The expression vector of claim 66, further comprising a nucleotide sequence encoding the CDR regions of a light chain, heavy chain or both light and heavy chains of a human antibody which binds IL-15.

68. An expression vector comprising a nucleotide sequence encoding heavy chain and light chain variable regions which comprise the amino acid sequences shown in SEQ ID NO:2 and SEQ ID NO:4, respectively, and conservative sequence modifications thereof.

69. An expression vector comprising a nucleotide sequence encoding heavy and light variable regions which comprise the amino acid sequences shown in SEQ ID NO:2 and SEQ ID NO:4, respectively, and conservative modifications thereof.

70. A transfectoma comprising the expression vector of claim 66.

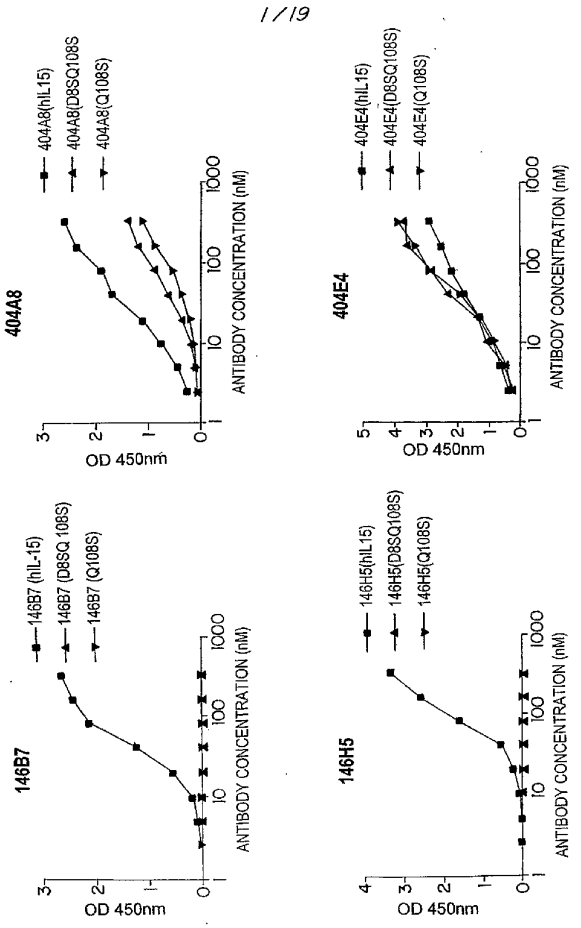
404A8
404A8(hIL15)
404A8(D8SQ108S
404A8(Q108S)
OD 450nm
ANTIBODY CONCENTRATION (nM)
404E4
404E4(hIL15)
404E4(D8SQ108S)
404E4(Q108S)
OD 450nm
ANTIBODY CONCENTRATION (nM)
146B7
146B7 (hIL-15)
146B7 (D8SQ 108S)
146B7 (Q108S)
OD 450nm
ANTIBODY CONCENTRATION (nM)
146H5
146H5(hIL15)
146H5(D8SQ108S)
146H5(Q108S)
OD 450nm
ANTIBODY CONCENTRATION (nM)

FIG. 1

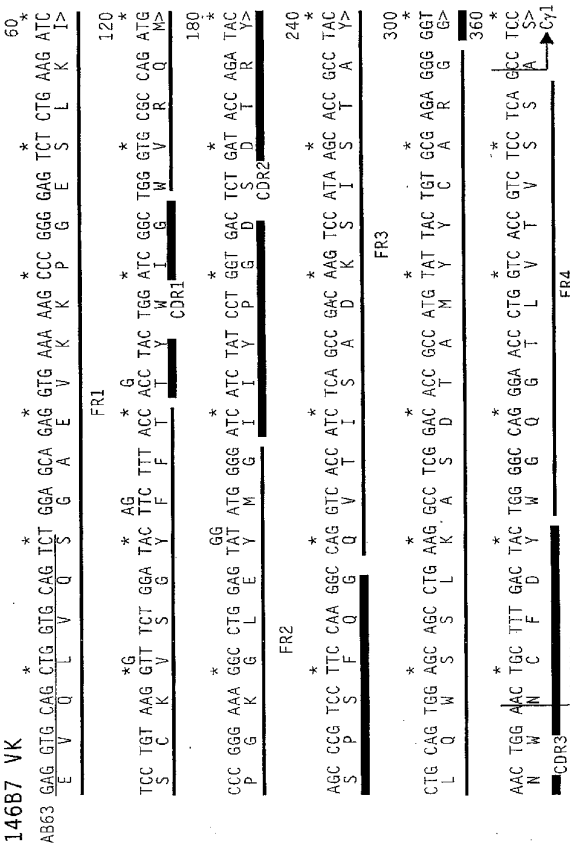

FIG. 2

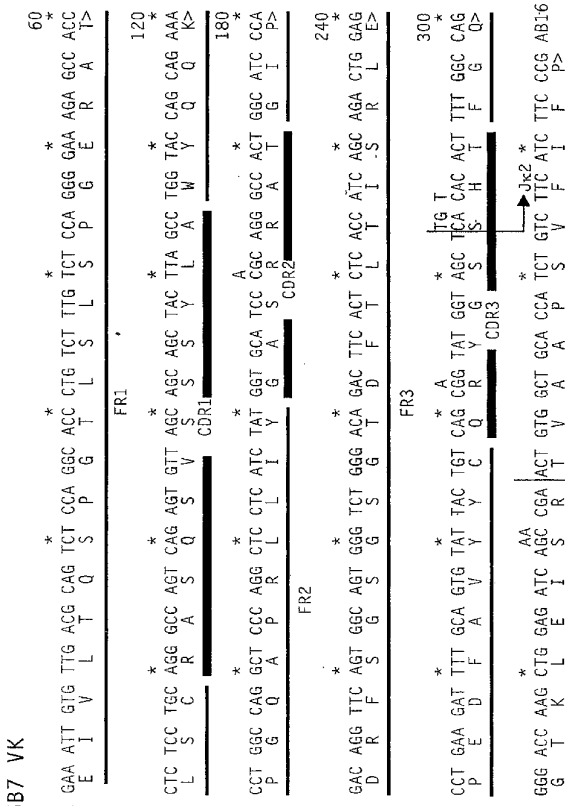

FIG. 3

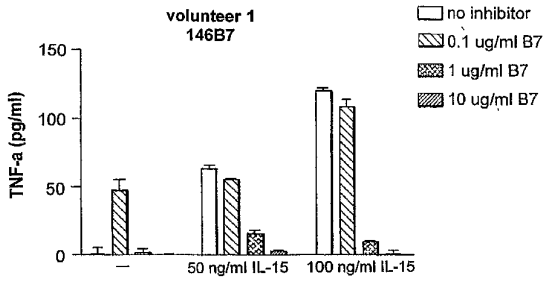
volunteer 1
146B7
no inhibitor
0.1 ug/ml B7
1 ug/ml B7
10 ug/ml B7
TNF-a (pg/ml)
150
100
50
0
—
50 ng/ml IL-15
100 ng/ml IL-15

FIG. 4A

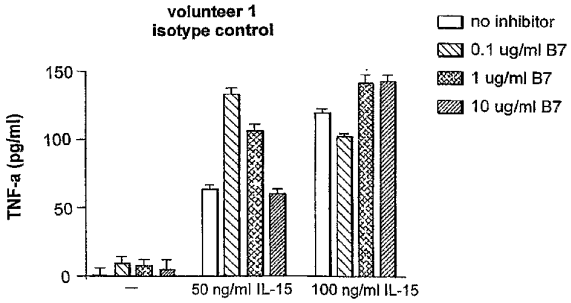
volunteer 1
isotype control
no inhibitor
0.1 ug/ml B7
1 ug/ml B7
10 ug/ml B7
TNF-a (pg/ml)
150
100
50
0
—
50 ng/ml IL-15
100 ng/ml IL-15

FIG. 4B

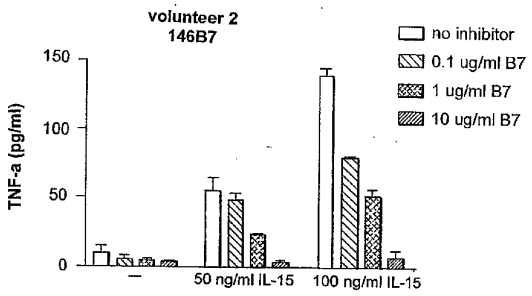
volunteer 2
146B7
no inhibitor
0.1 ug/ml B7
1 ug/ml B7
10 ug/ml B7
TNF-a (pg/ml)
150
100
50
0
–
50 ng/ml IL-15
100 ng/ml IL-15

FIG. 4C

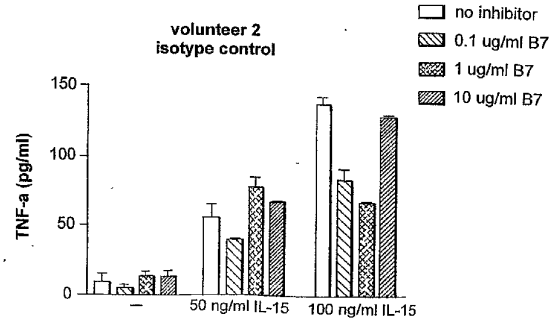
volunteer 2
isotype control
no inhibitor
0.1 ug/ml B7
1 ug/ml B7
10 ug/ml B7
TNF-a (pg/ml)
150
100
50
0
–
50 ng/ml IL-15
100 ng/ml IL-15

FIG. 4D

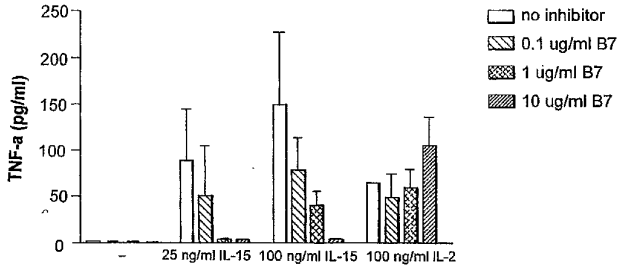
250
200
150
100
50
0
TNF-a (pg/ml)
no inhibitor
0.1 ug/ml B7
1 ug/ml B7
10 ug/ml B7
-
25 ng/ml IL-15
100 ng/ml IL-15
100 ng/ml IL-2

FIG. 5

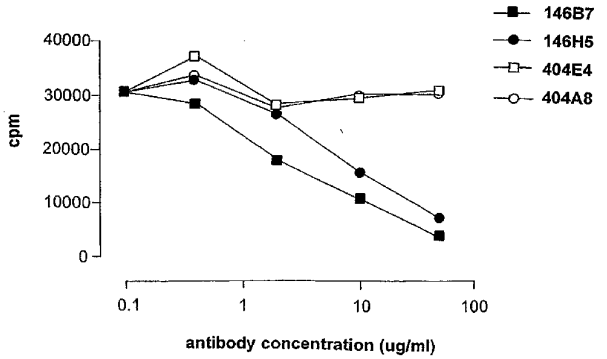
40000
30000
20000
10000
0
cpm
146B7
146H5
404E4
404A8
0.1
1
10
100
antibody concentration (ug/ml)

FIG. 6

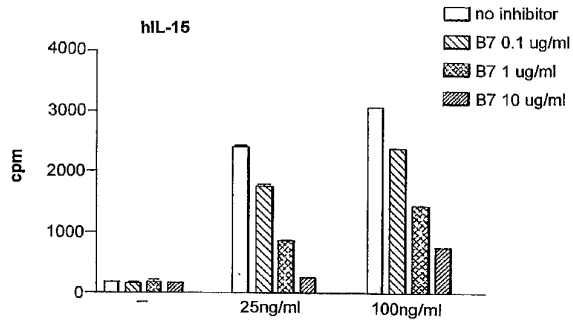
hIL-15
no inhibitor
B7 0.1 ug/ml
B7 1 ug/ml
B7 10 ug/ml
cpm
4000
3000
2000
1000
0
–
25ng/ml
100ng/ml

FIG. 7A

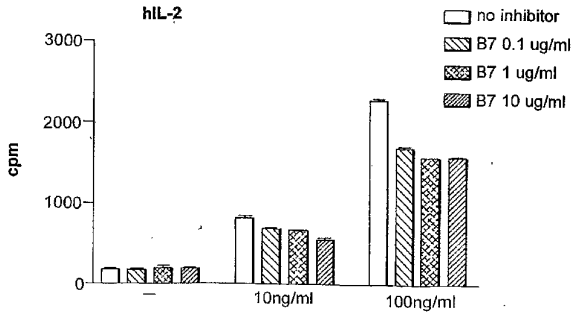
hIL-2
no inhibitor
B7 0.1 ug/ml
B7 1 ug/ml
B7 10 ug/ml
cpm
3000
2000
1000
0
–
10ng/ml
100ng/ml

FIG. 7B

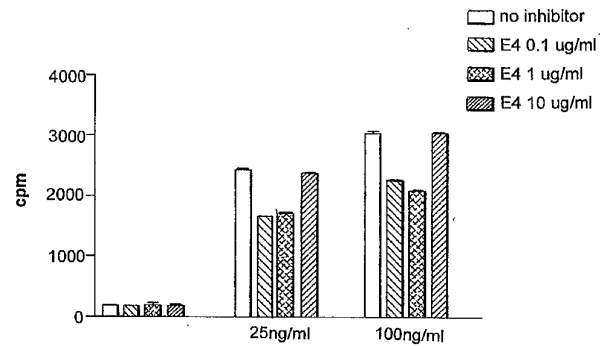
no inhibitor
E4 0.1 ug/ml
E4 1 ug/ml
E4 10 ug/ml
4000
3000
2000
1000
0
cpm
25ng/ml
100ng/ml

FIG. 8A

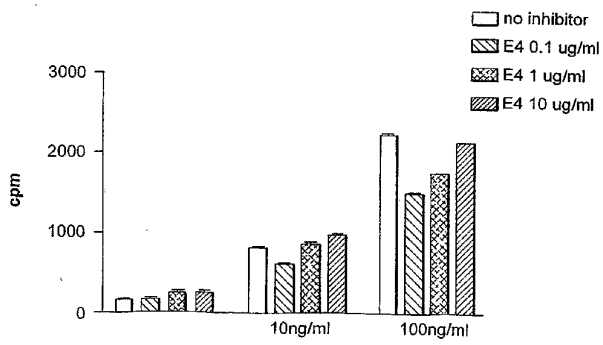
no inhibitor
E4 0.1 ug/ml
E4 1 ug/ml
E4 10 ug/ml
3000
2000
1000
0
cpm
10ng/ml
100ng/ml

FIG. 8B

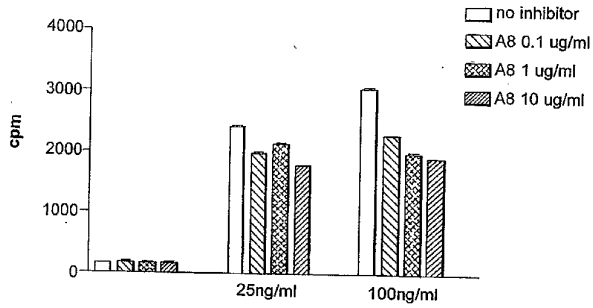
4000
3000
2000
1000
0
cpm
25ng/ml
100ng/ml
no inhibitor
A8 0.1 ug/ml
A8 1 ug/ml
A8 10 ug/ml

FIG. 9A

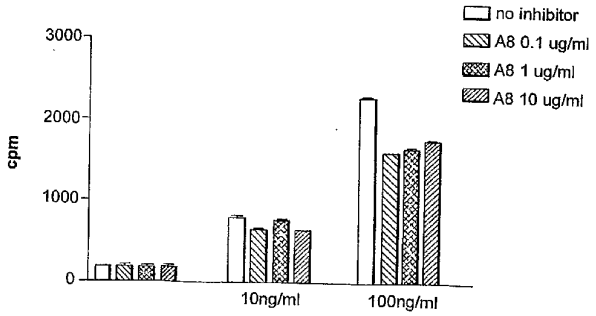
3000
2000
1000
0
cpm
10ng/ml
100ng/ml
no inhibitor
A8 0.1 ug/ml
A8 1 ug/ml
A8 10 ug/ml

FIG. 9B

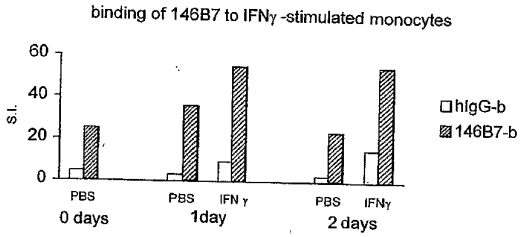
binding of 146B7 to IFNγ -stimulated monocytes
60
40
20
0
S.I.
PBS
PBS
IFN γ
PBS
IFNγ
0 days
1day
2 days
hIgG-b
146B7-b

FIG. 10

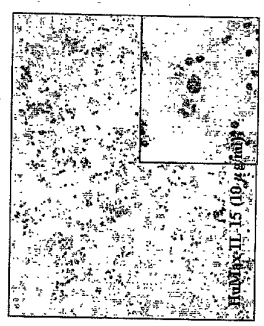

FIG. 11B

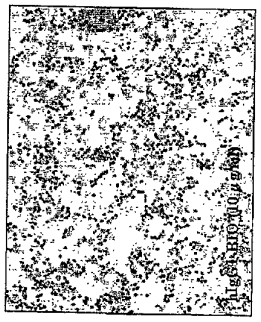

FIG. 11A

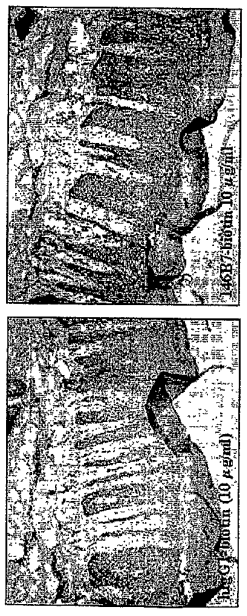

FIG. 12A FIG. 12B

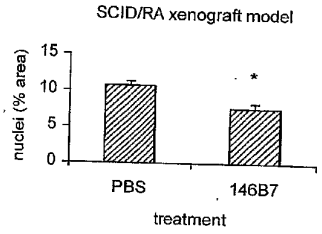
SCID/RA xenograft model
nuclei (% area)
15
10
5
0
*
PBS
146B7
treatment

FIG. 13A

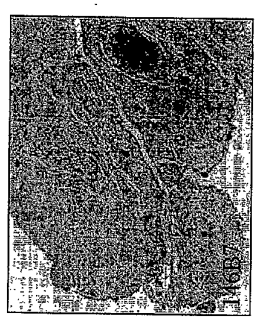

FIG. 13C

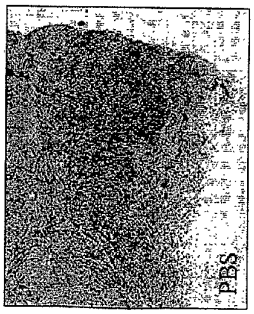

FIG. 13B

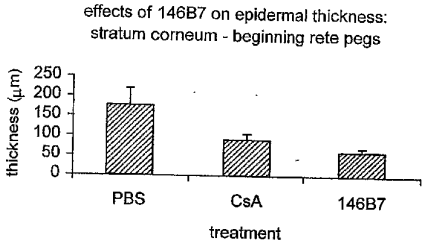

FIG. 14A

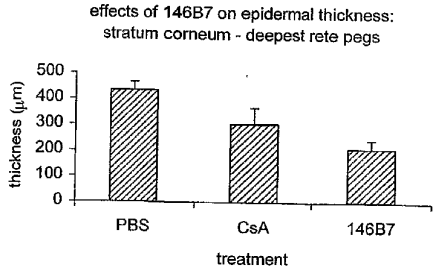

FIG. 14B

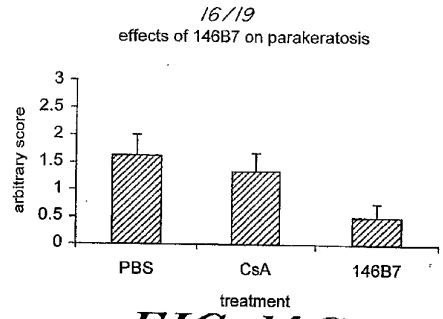

FIG. 14C

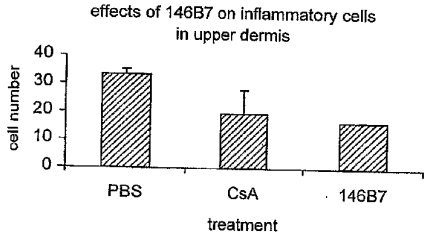

FIG. 14D

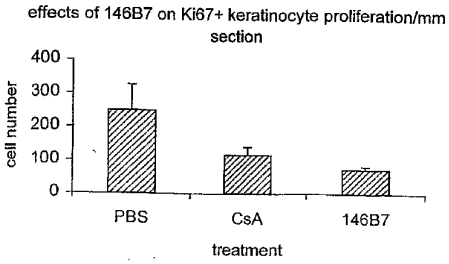

FIG. 14E

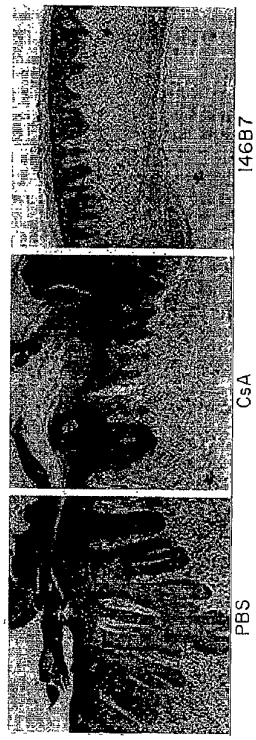

FIG. 15A FIG. 15B FIG. 15C

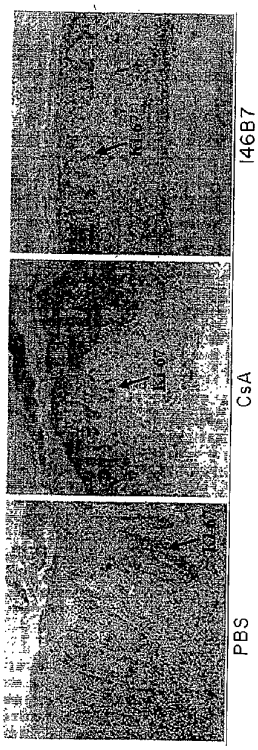

FIG. 16A FIG. 16B FIG. 16C

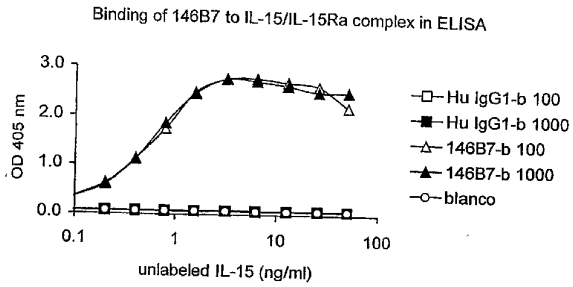
Binding of 146B7 to IL-15/IL-15Ra complex in ELISA
OD 405 nm
3.0
2.0
1.0
0.0
0.1
1
10
100
unlabeled IL-15 (ng/ml)
Hu IgG1-b 100
Hu IgG1-b 1000
146B7-b 100
146B7-b 1000
blanco

FIG. 17

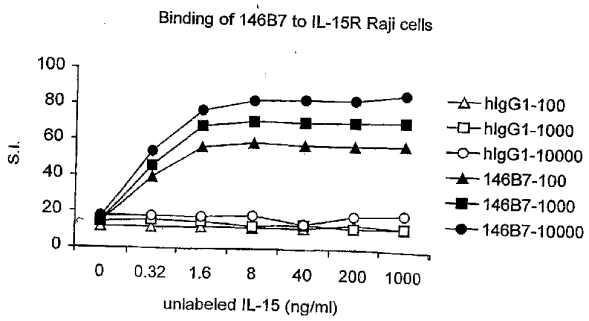
Binding of 146B7 to IL-15R Raji cells
S.I.
100
80
60
40
20
0
0
0.32
1.6
8
40
200
1000
unlabeled IL-15 (ng/ml)
hIgG1-100
hIgG1-1000
hIgG1-10000
146B7-100
146B7-1000
146B7-10000

FIG. 18

SEQUENCE LISTING

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<120> HUMAN ANTIBODIES SPECIFIC FOR INTERLEUKIN 15 (IL-15)

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<150> US 60/314,731
<151> 2001-08-23

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<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)...(390)

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Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
 1               5                  10                  15

tct ctg aag atc tcc tgt aag gtt tct gga tac ttc ttt acc acc tac   96
Ser Leu Lys Ile Ser Cys Lys Val Ser Gly Tyr Phe Phe Thr Thr Tyr
            20                  25                  30

tgg atc ggc tgg gtg cgc cag atg ccc ggg aaa ggc ctg gag tat atg   144
Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Tyr Met
        35                  40                  45

ggg atc atc tat cct ggt gac tct gat acc aga tac agc ccg tcc ttc   192
Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe
    50                  55                  60

caa ggc cag gtc acc atc tca gcc gac aag tcc atc agc acc gcc tac   240
Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

ctg cag tgg agc agc ctg aag gcc tcg gac acc gcc atg tat tac tgt   288
Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
                85                  90                  95

gcg aga ggg ggt aac tgg aac tgc ttt gac tac tgg ggc cag gga acc   336
Ala Arg Gly Gly Asn Trp Asn Cys Phe Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110

ctg gtc acc gtc tcc tca gcc tcc acc aag ggc cca tcg gtc ttc ccc   384
Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
        115                 120                 125

ctg gca                                                          390
Leu Ala
    130
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<210> 2
<211> 130
<212> PRT
<213> Homo sapiens

<400> 2
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1 5 10 15
Ser Leu Lys Ile Ser Cys Lys Val Ser Gly Tyr Phe Phe Thr Thr Tyr
20 25 30
Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Tyr Met
35 40 45
Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe
50 55 60
Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
65 70 75 80
Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
85 90 95
Ala Arg Gly Gly Asn Trp Asn Cys Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125
Leu Ala
130

<210> 3
<211> 357
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)...(357)

<400> 3
gaa att gtg ttg acg cag tct cca ggc acc ctg tct ttg tct cca ggg 48
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

gaa aga gcc acc ctc tcc tgc agg gcc agt cag agt gtt agc agc agc 96
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

tac tta gcc tgg tac cag cag aaa cct ggc cag gct ccc agg ctc ctc 144
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

atc tat ggt gca tcc cgc agg gcc act ggc atc cca gac agg ttc agt 192
Ile Tyr Gly Ala Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

ggc agt ggg tct ggg aca gac ttc act ctc acc atc agc aga ctg gag 240
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

cct gaa gat ttt gca gtg tat tac tgt cag cgg tat ggt agc tca cac 288
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Arg Tyr Gly Ser Ser His

```
                85                  90                  95

act ttt ggc cag ggg acc aag ctg gag atc agc cga act gtg gct gca   336
Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Ser Arg Thr Val Ala Ala
            100                 105                 110

cca tct gtc ttc atc ttc ccg                                     357
Pro Ser Val Phe Ile Phe Pro
        115
```

<210> 4
<211> 119
<212> PRT
<213> Homo sapiens

<400> 4

```
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1               5                  10                  15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
            20                  25                  30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
        35                  40                  45
Ile Tyr Gly Ala Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
    50                  55                  60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65                  70                  75                  80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Arg Tyr Gly Ser Ser His
                85                  90                  95
Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Ser Arg Thr Val Ala Ala
            100                 105                 110
Pro Ser Val Phe Ile Phe Pro
        115
```

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(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **VAN DE WINKEL, Jan, G., J.** [NL/NL]; Verlengde Slotlaan 80, NL-3707 CK Zeist (NL). **VAN DIJK, Marcus, Antonius** [NL/NL]; Gaailaan 16, NL-3722 ZJ Bilthoven (NL). **SCHUURMAN, Janine** [NL/NL]; Avogadrostraat 11-13, NL-1098 SP Amsterdam (NL). **GERRITSEN, Arnout, F.** [NL/NL]; Hoenderkopershoek 31, NL-3981 TB Bunnik (NL). **BAADSGAARD, Ole** [DK/SE]; Kyrkogatan, S-21122 Malmo (SE).

(74) Agents: **REMILLARD, Jane, E.** et al.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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WO 03/017935 A3

(54) Title: HUMAN ANTIBODIES SPECIFIC FOR INTERLEUKIN 15 (IL-15)

(57) Abstract: Isolated human monoclonal antibodies which specifically bind to IL-15 (e.g., human IL-15), and related antibody-based compositions and molecules, are disclosed. The human antibodies can be produced in a transfectoma or in a non-human transgenic animal, e.g., a transgenic mouse, capable of producing multiple isotypes of human monoclonal antibodies by undergoing V-D-J recombination and isotype switching. Also disclosed are pharmaceutical compositions comprising the human antibodies, non-human transgenic animals, and hybridomas which produce the human antibodies, and therapeutic and diagnostic methods for using the human antibodies.

【国際調査報告】

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/26769

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12N 5/10, 5/12, 5/16, 5/18, 15/12, 15/63, 15/64; C07K 14/47, 16/24; A61K 39/395, 39/44
US CL : 536/23.1, 23.5, 23.53; 530/387.1, 388.1, 388.23; 424/130.1, 139.1, 141.1, 145.1, 178.1; 435/69.1, 325, 326, 331, 320.1

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 536/23.1, 23.5, 23.53; 530/387.1, 388.1, 388.23; 424/130.1, 139.1, 141.1, 145.1, 178.1; 435/69.1, 325, 326, 331, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,795,966 A (GRABSTEIN et al) 18 August 1998 (18.08.1998), column 3, lines 5-20, column 15-16.	1-2, 9, 13, 14, 16, 35, 45-47, 61-62 --- 57-60
A	US 5,591,630 A (ANDERSON et al) 07 January 1997 (07.01.1997), see entire document.	3-8, 10-12, 15, 17-34, 36-44, 48-56

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
22 November 2002 (22.11.2002)

Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

PCT/US02/26769

Continuation of B. FIELDS SEARCHED Item 3:
CAS ONLINE, MEDLINE, WEST, CAPLUS, USPATFULL
search terms: IL-15 antibody, monoclonal, compositions, hybridoma, transfectoma, immunoconjugate, method of production, therapy, treatment, method, administer, diagnosis, assay, nucleic acid, expresson vector

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Ａ６１Ｐ 35/00	Ａ６１Ｐ	31/00		
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	Ｃ１２Ｎ	5/00	Ａ	

(81)指定国 AP(GH,GM,KE,LS,MW,MZ,SD,SL,SZ,TZ,UG,ZM,ZW),EA(AM,AZ,BY,KG,KZ,MD,RU,TJ,TM),EP(AT,BE,BG,CH,CY,CZ,DE,DK,EE,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE,SK,TR),OA(BF,BJ,CF,CG,CI,CM,GA,GN,GQ,GW,ML,MR,NE,SN,TD,TG),AE,AG,AL,AM,AT,AU,AZ,BA,BB,BG,BR,BY,BZ,CA,CH,CN,CO,CR,CU,CZ,DE,DK,DM,DZ,EC,EE,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KP,KR,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,MZ,NO,NZ,OM,PH,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TN,TR,TT,TZ,UA,UG,US,UZ,VN,YU,ZA,ZM,ZW

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4B064 AG27 CA10 CA20 CC24 DA01
4B065 AA91X AA92X AA93Y AB01 AB05 BA08 CA25 CA44 CA46
4C084 AA19 MA02 NA14 ZA681 ZA682 ZA891 ZA892 ZA961 ZA962 ZB071
ZB072 ZB081 ZB082 ZB111 ZB112 ZB151 ZB152 ZB261 ZB262 ZB311
ZB312 ZC551 ZC552
4C085 AA14 BB33 BB34 BB35 BB36 BB37 DD63 EE03
4H045 AA11 AA20 AA30 CA40 DA76 EA22 FA74

专利名称(译)	特异于白细胞介素15（IL-15）的人抗体		
公开(公告)号	JP2005503151A	公开(公告)日	2005-02-03
申请号	JP2003522458	申请日	2002-08-23
[标]申请(专利权)人(译)	根马布股份公司		
申请(专利权)人(译)	Genmabu ER / ES		
[标]发明人	ファンデウィンケルヤンヘーイェイ ファンダイクマルクスアントニウス スフィールマンジャニンヌ ヘェリッツェンアルナウツエフ バーズガードオリー		
发明人	ファン デ ウィンケル, ヤン, ヘー.,イェイ. ファン ダイク, マルクス, アントニウス スフィールマン, ジャニンヌ ヘェリッツェン, アルナウツ エフ. バーズガード, オリー		
IPC分类号	A01K67/027 A61K39/395 A61K45/00 A61P1/04 A61P17/06 A61P19/02 A61P29/00 A61P31/00 A61P35/00 A61P37/02 A61P37/06 C07K16/24 C12N1/15 C12N1/19 C12N1/21 C12N5/10 C12N5/18 C12N15/09 C12N15/12 C12P21/08 G01N33/53 G01N33/543		
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F-TERM分类号	4B024/AA01 4B024/BA63 4B024/CA02 4B024/CA05 4B024/DA02 4B024/DA06 4B024/HA17 4B064/AG27 4B064/CA10 4B064/CA20 4B064/CC24 4B064/DA01 4B065/AA91X 4B065/AA92X 4B065/AA93Y 4B065/AB01 4B065/AB05 4B065/BA08 4B065/CA25 4B065/CA44 4B065/CA46 4C084/AA19 4C084/MA02 4C084/NA14 4C084/ZA681 4C084/ZA682 4C084/ZA891 4C084/ZA892 4C084/ZA961 4C084/ZA962 4C084/ZB071 4C084/ZB072 4C084/ZB081 4C084/ZB082 4C084/ZB111 4C084/ZB112 4C084/ZB151 4C084/ZB152 4C084/ZB261 4C084/ZB262 4C084/ZB311 4C084/ZB312 4C084/ZC551 4C084/ZC552 4C085/AA14 4C085/BB33 4C085/BB34 4C085/BB35 4C085/BB36 4C085/BB37 4C085/DD63 4C085/EE03 4H045/AA11 4H045/AA20 4H045/AA30 4H045/CA40 4H045/DA76 4H045/EA22 4H045/FA74		
代理人(译)	远藤顺治		
优先权	60/314731 2001-08-23 US		
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外部链接	Espacenet		

摘要(译)

公开了与IL-15特异性结合的分离的人单克隆抗体和相关的基于抗体的组合物和分子（例如人IL-15）。人抗体可以通过引起V-D-J重组和同种型转换，在能够产生多种同型人单克隆抗体的非人转基因动物如转染瘤或转基因小鼠中产生。此外，还公开了含有本发明人抗体，非人转基因动物和产生本发明人抗体的杂交瘤的药物组合物，以及使用本发明人抗体的治疗方法和诊断方法。

マウス	血清IgM (マイクログラム/ml)	Ig H鎖遺伝子型
42	<0.002	CMD/JHD
43	196	+/JHD
44	<0.002	CMD/JHD
45	174	+/JHD
129 x BL6 F1	153	+/+
JHD	<0.002	JHD/JHD