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Kaplan(10) **Pub. No.: US 2011/0200600 A1**(43) **Pub. Date: Aug. 18, 2011**(54) **DIAGNOSIS AND PROGNOSIS OF IMMUNE
DISORDERS USING STAT4 EXPRESSION****Publication Classification**(75) Inventor: **Mark H. Kaplan, Fishers, IN (US)**(73) Assignee: **Indiana University Research and
Technology Corporation**(21) Appl. No.: **13/063,094**(22) PCT Filed: **Sep. 9, 2009**(86) PCT No.: **PCT/US09/56332**

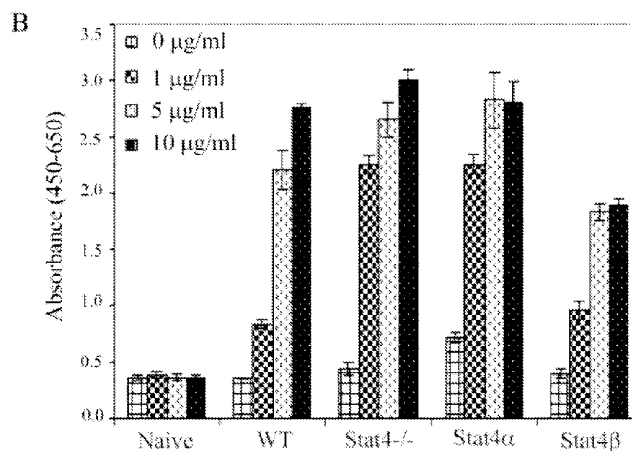
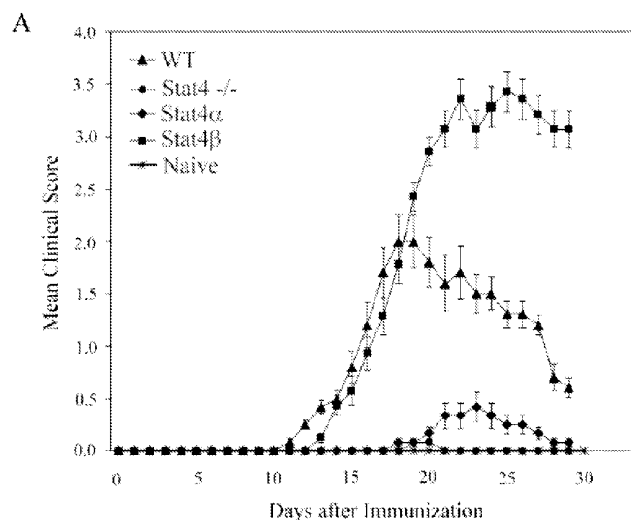
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(2), (4) Date: **Apr. 29, 2011**(51) **Int. Cl.***A61K 39/395* (2006.01)*C12Q 1/68* (2006.01)*G01N 33/68* (2006.01)*G01N 33/53* (2006.01)*A61K 38/02* (2006.01)*C07H 21/00* (2006.01)*C12Q 1/02* (2006.01)*A61P 29/00* (2006.01)*A61P 1/00* (2006.01)*A61P 37/06* (2006.01)*H01J 49/26* (2006.01)(52) **U.S. Cl.** **424/134.1**; 435/6.1; 435/6.11;
435/6.12; 435/7.1; 436/501; 424/133.1; 424/142.1;
424/145.1; 514/1.1; 514/1.4; 536/24.31; 435/29;
435/6.13; 250/282(57) **ABSTRACT**

Methods and compositions that determine the expression levels of Stat4 α and Stat4 β isoforms for therapeutic efficacy of anti-inflammatory treatments, assessing an individual's risk for developing inflammatory diseases including Crohn's disease, ulcerative colitis, rheumatoid arthritis, and multiple sclerosis are disclosed.

Related U.S. Application Data

(60) Provisional application No. 61/095,684, filed on Sep. 10, 2008.



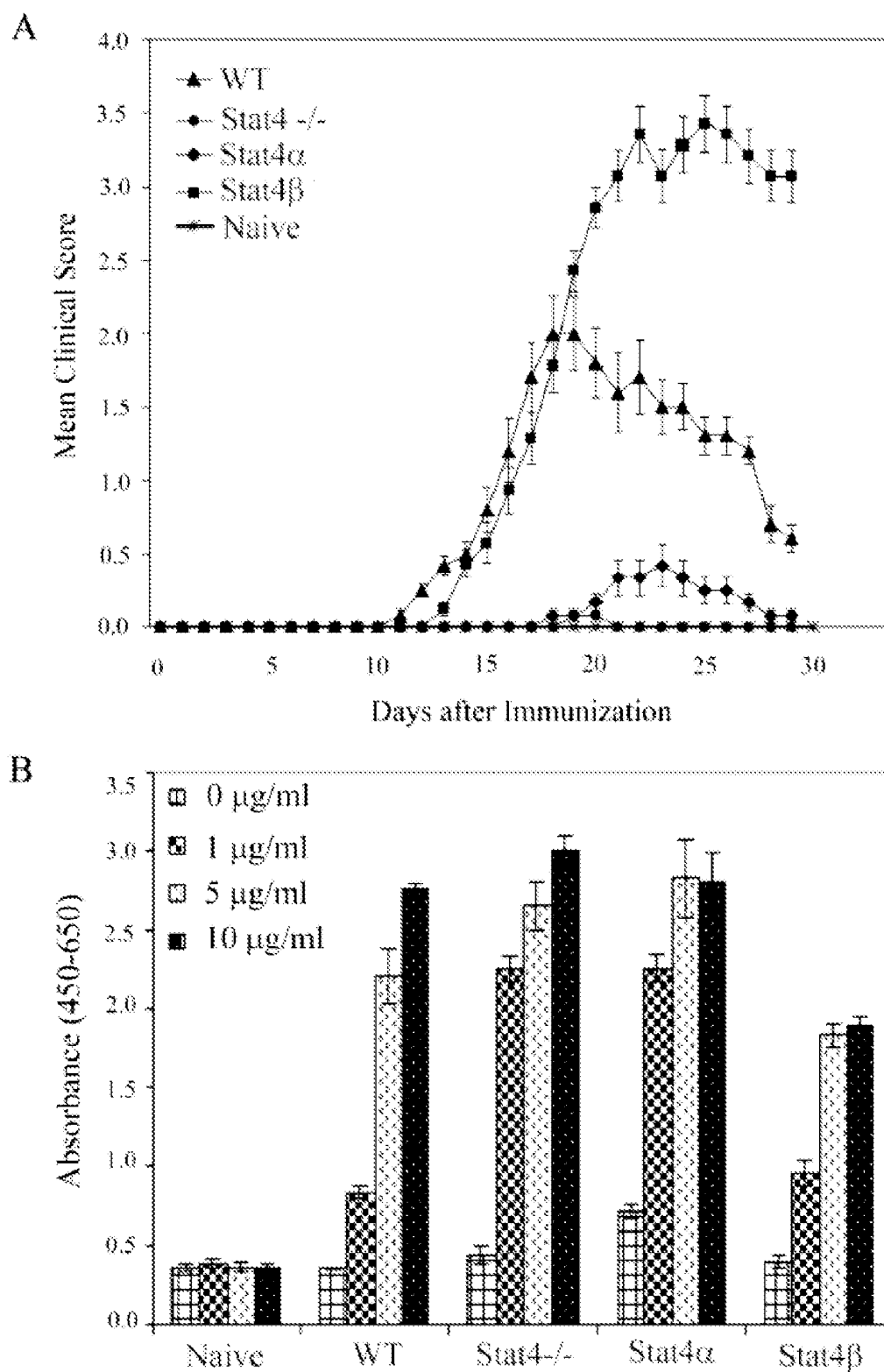


FIG. 1

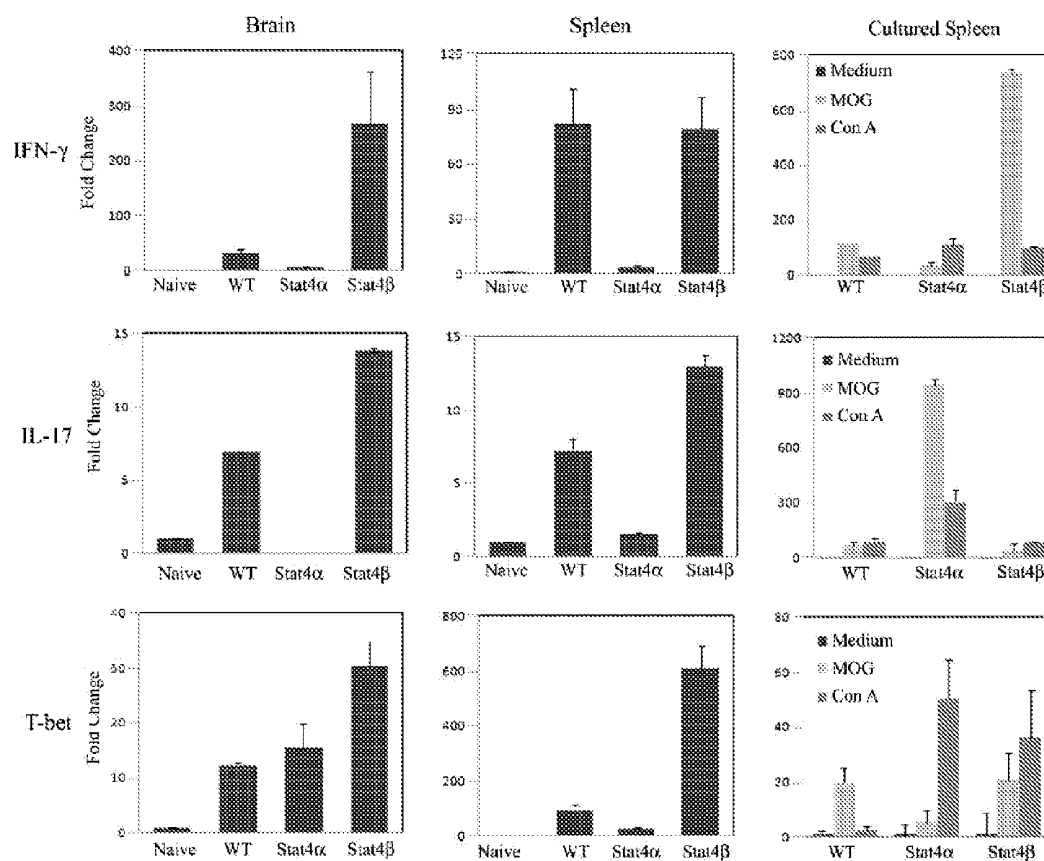


FIG. 2

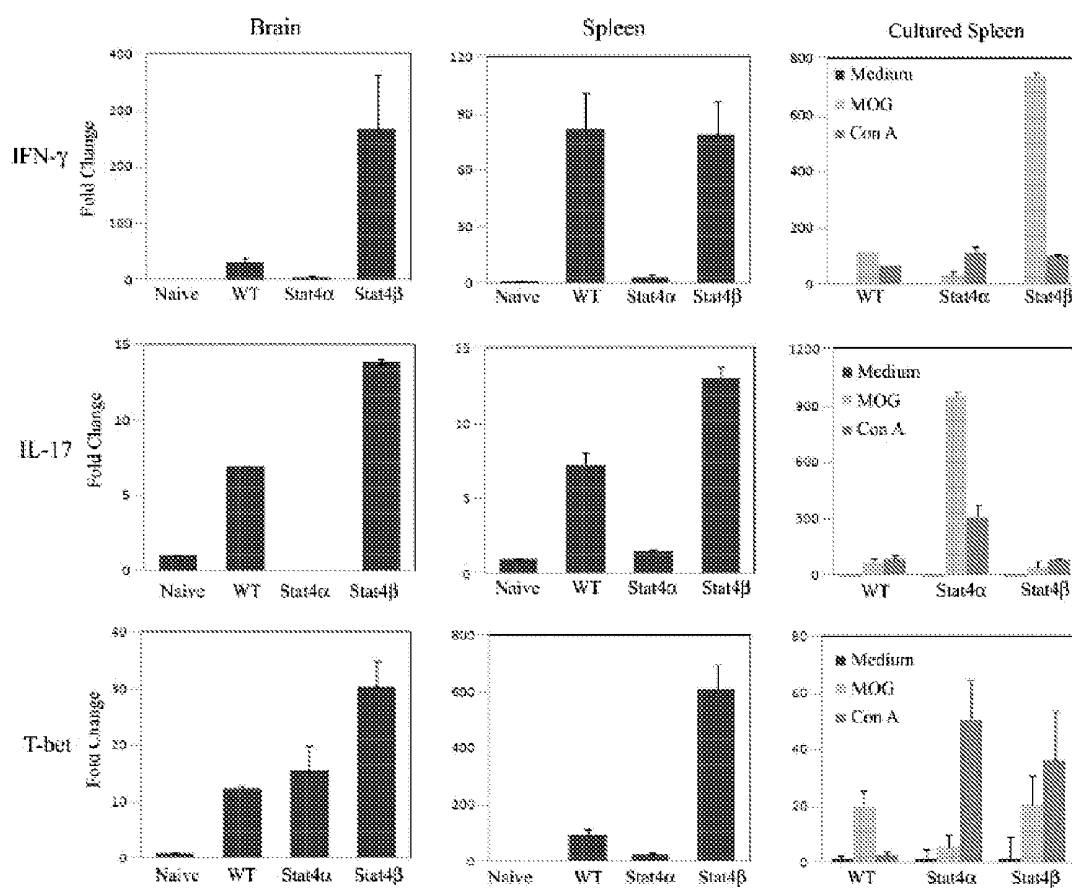


FIG. 3

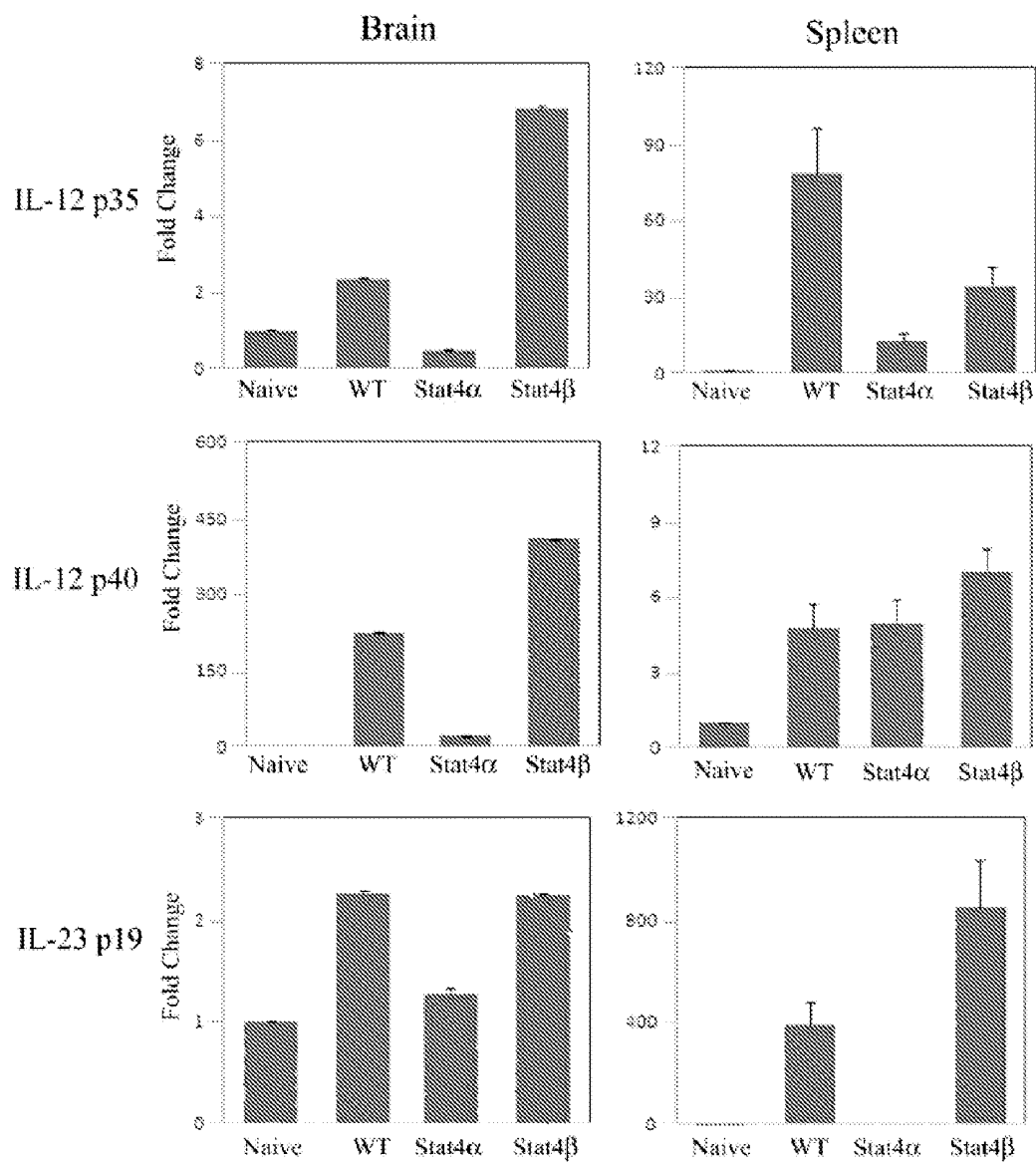


FIG. 4

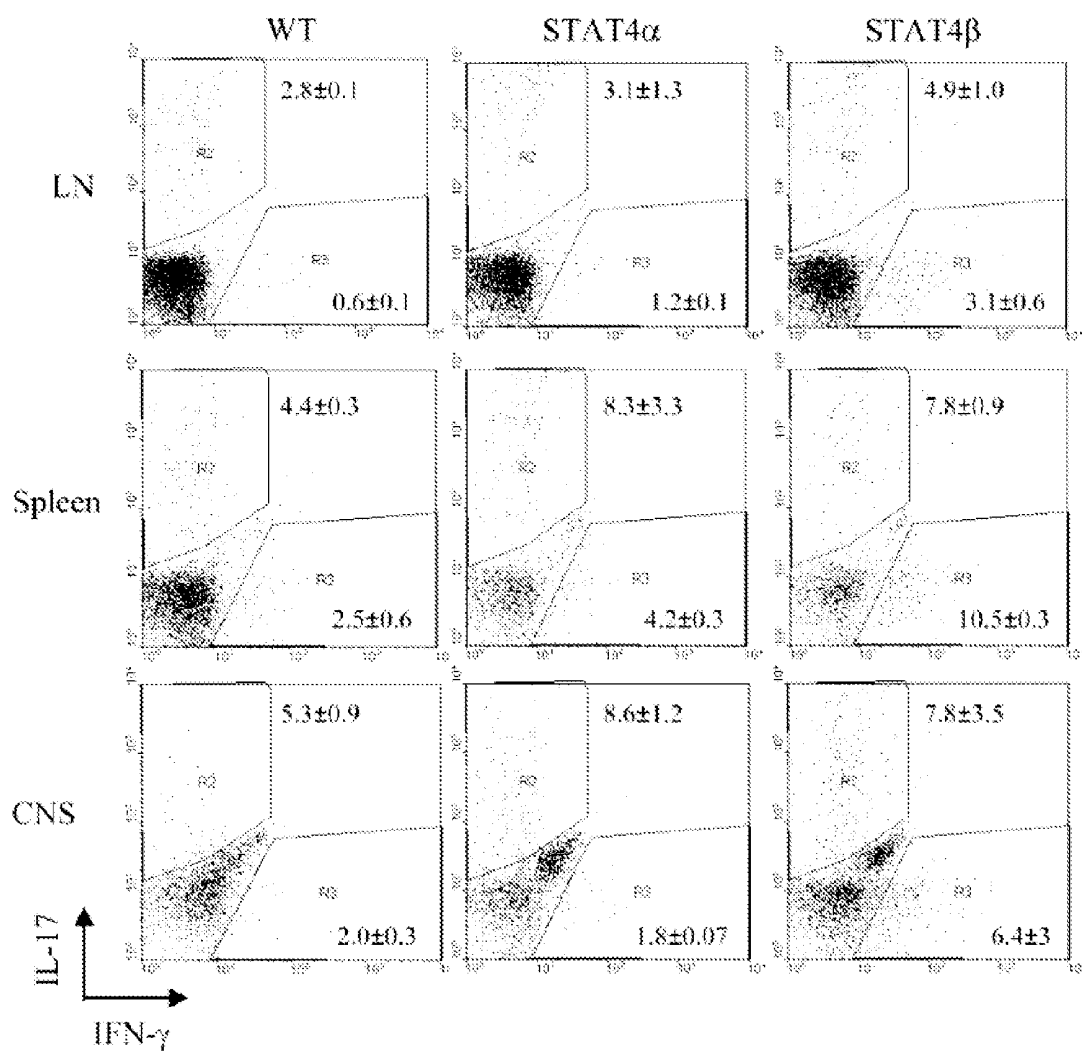
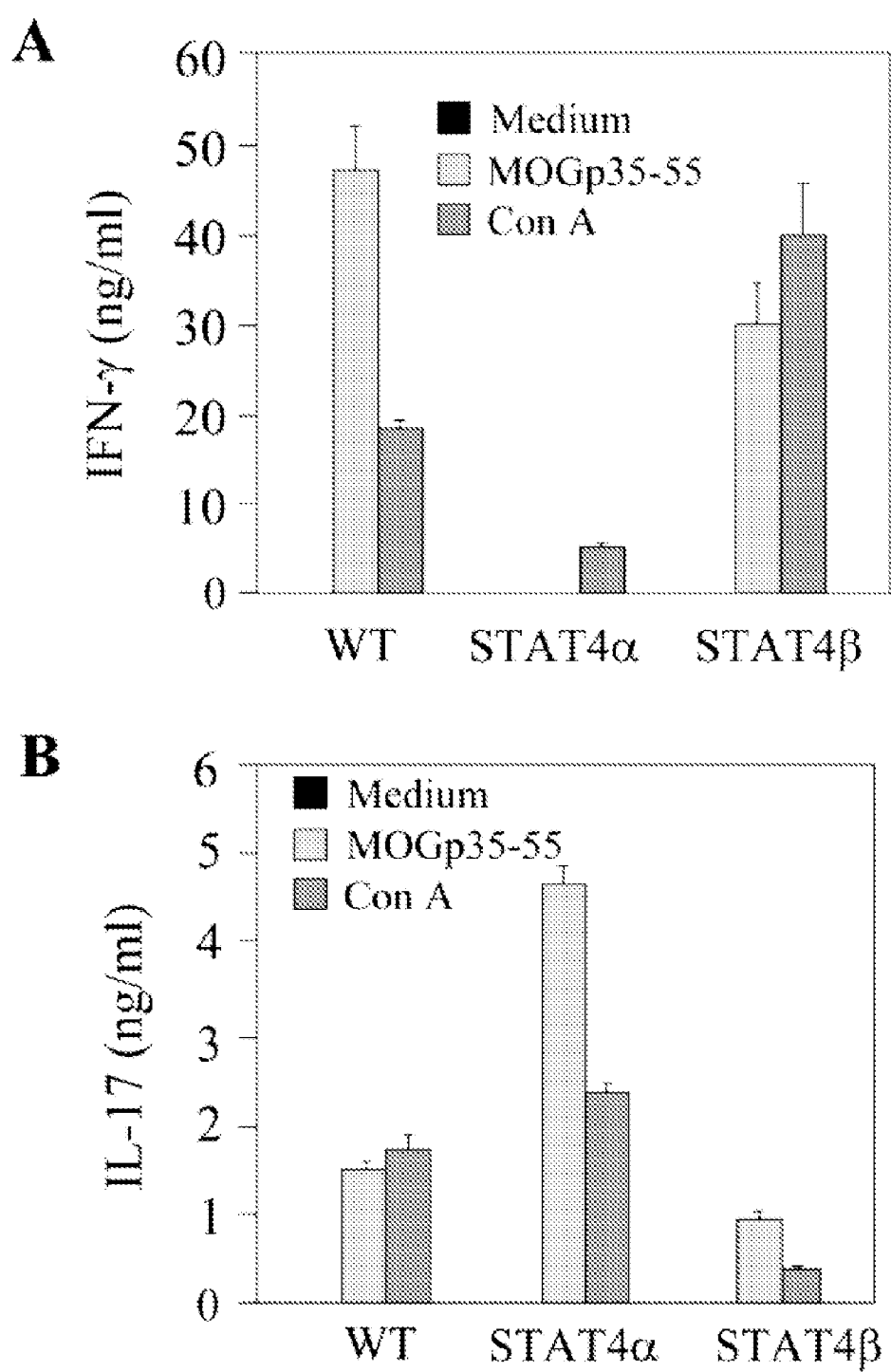


FIG. 5

**FIG. 6**

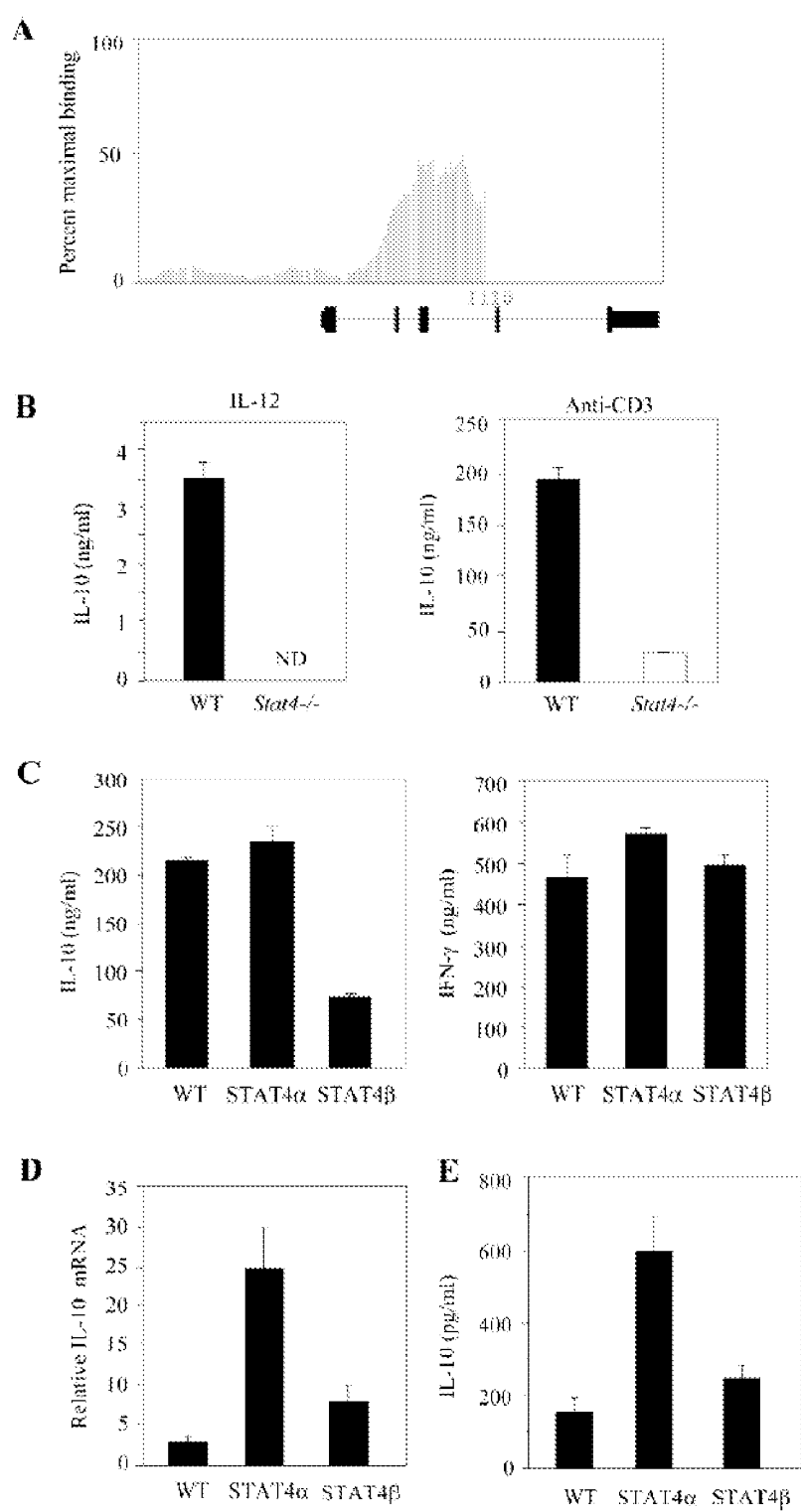


FIG. 7

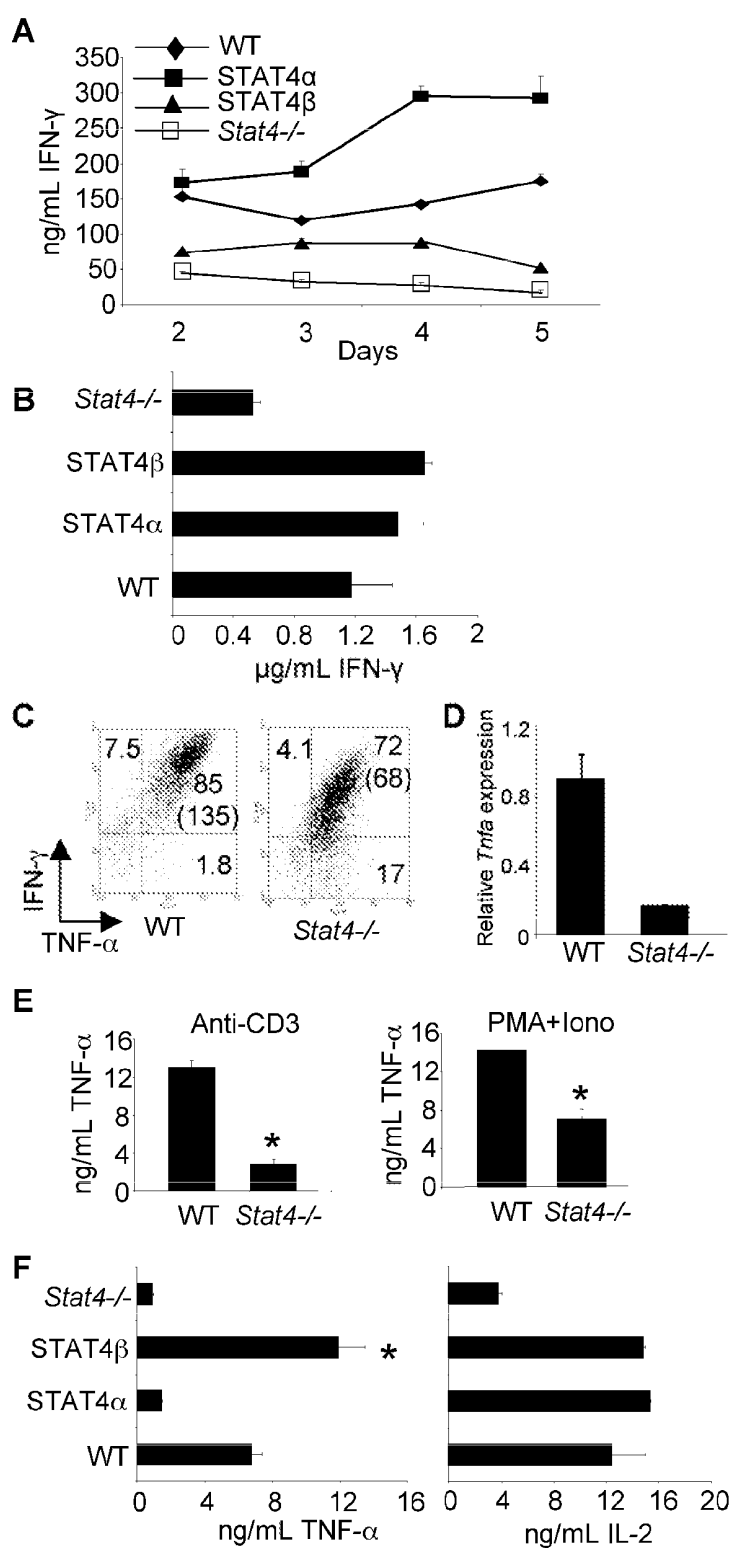


FIG. 8

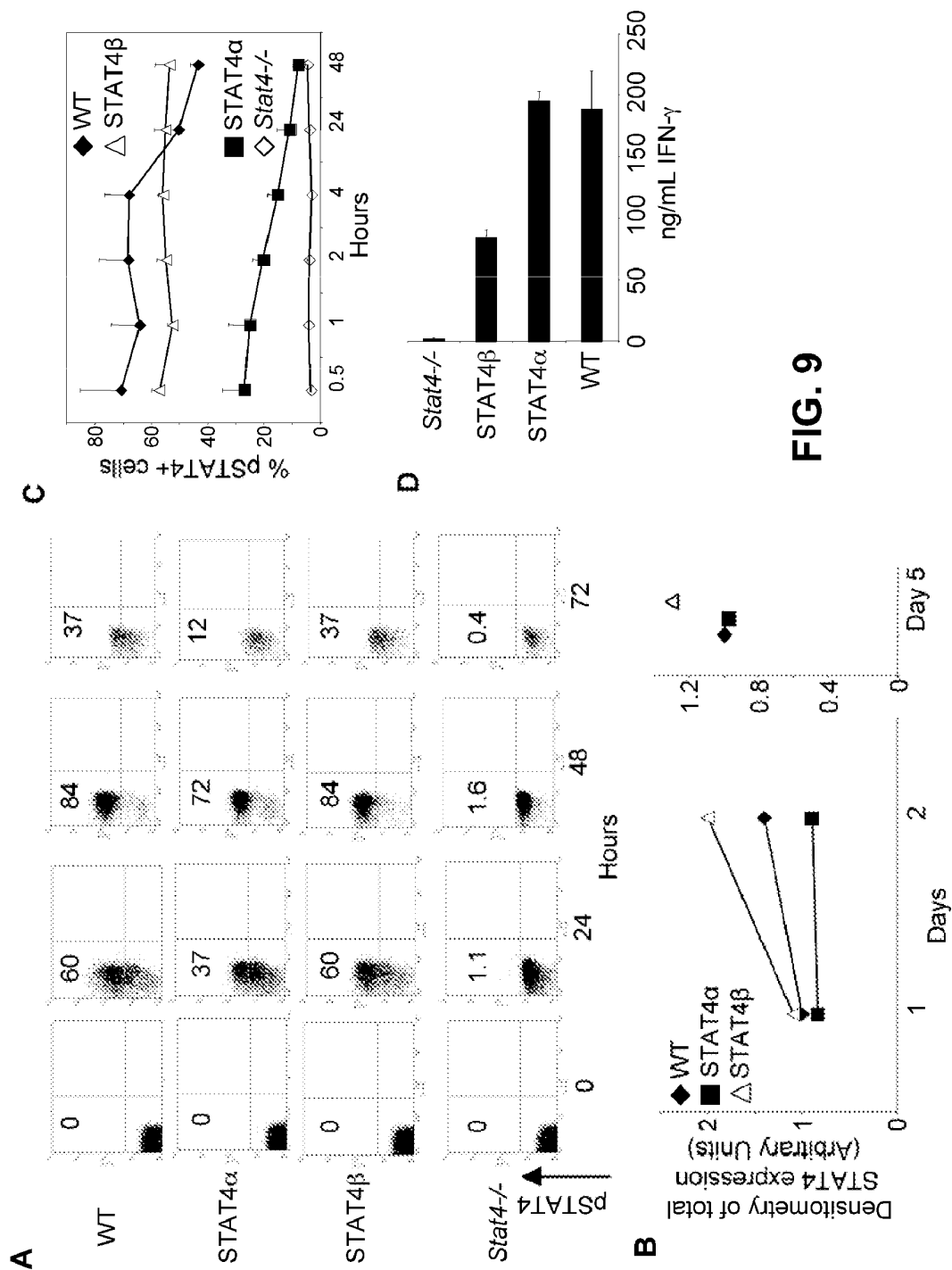


FIG. 9

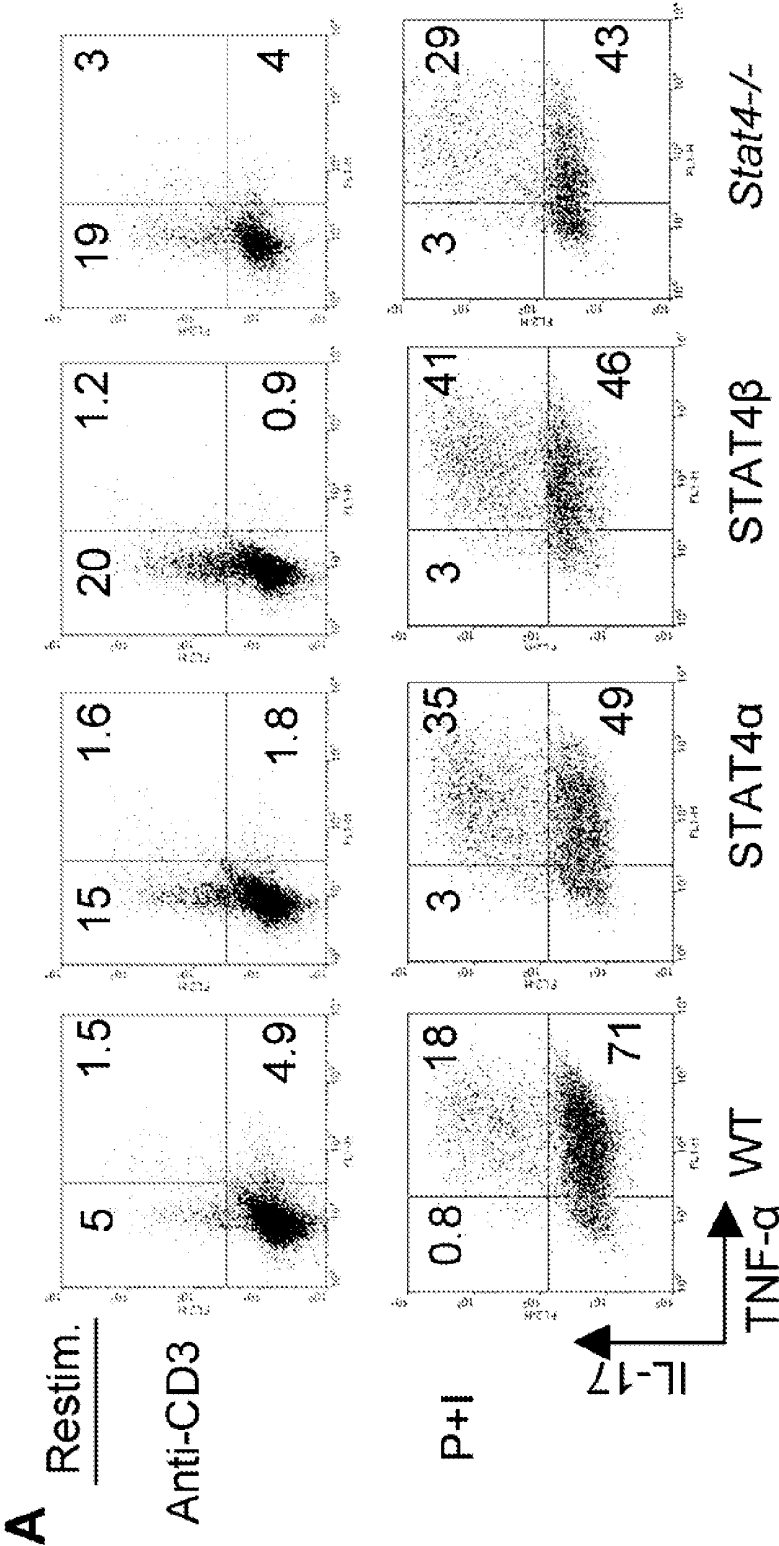


FIG. 10A

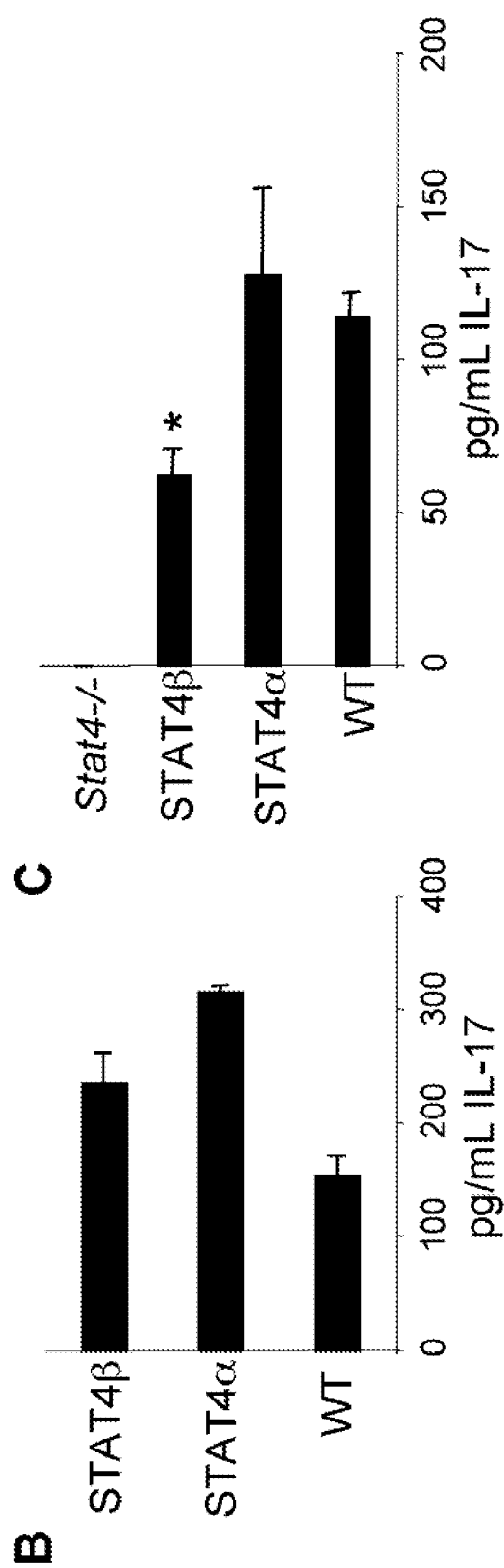


FIG. 10B-C

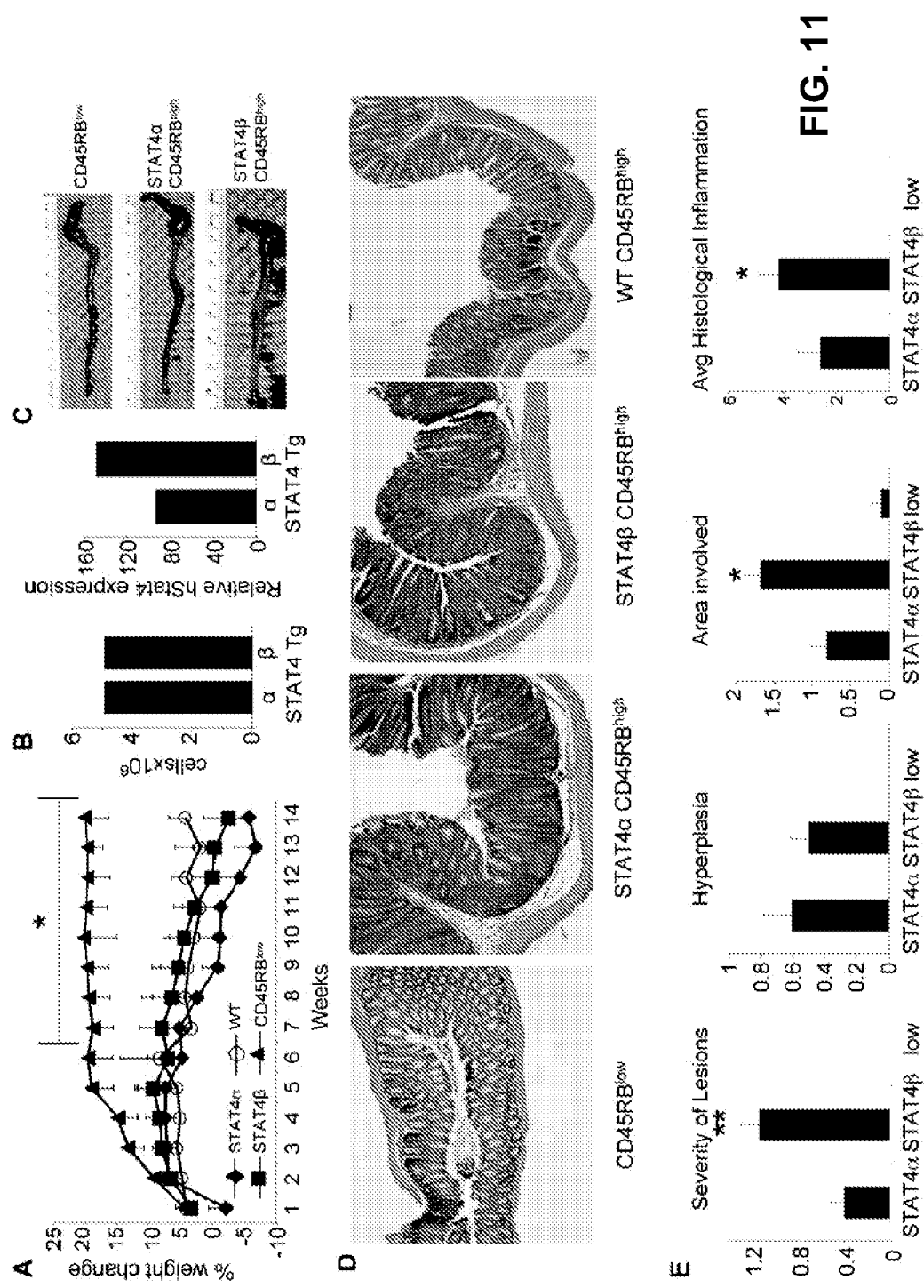


FIG. 11

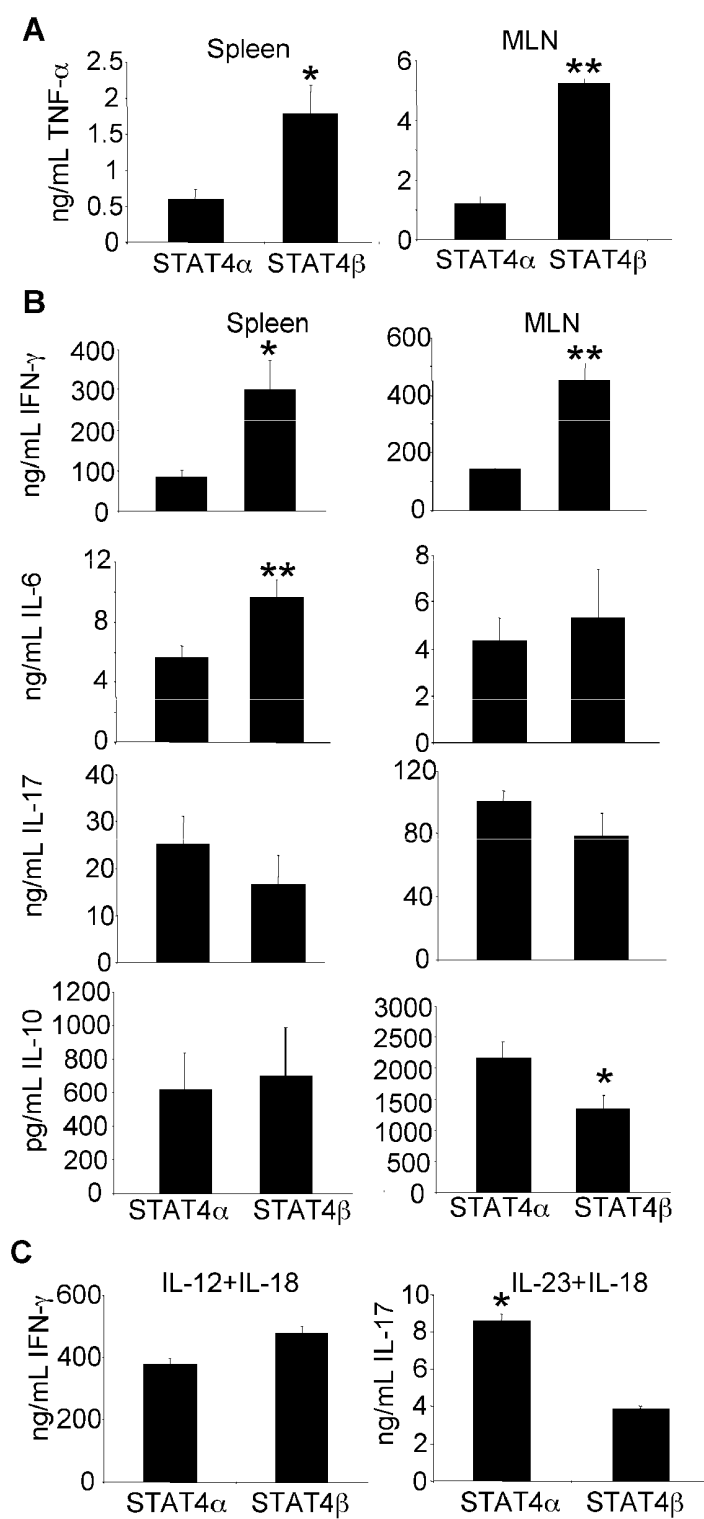


FIG. 12

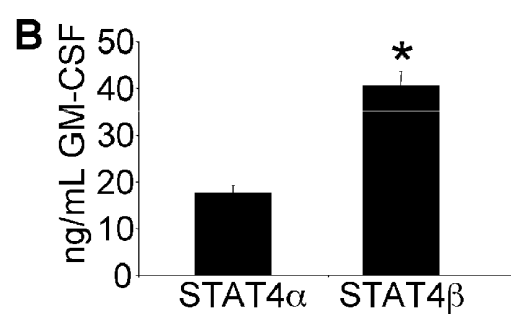
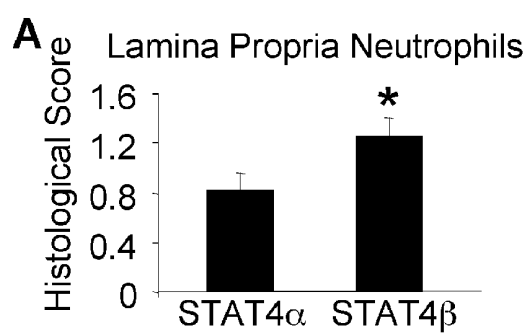


FIG. 13

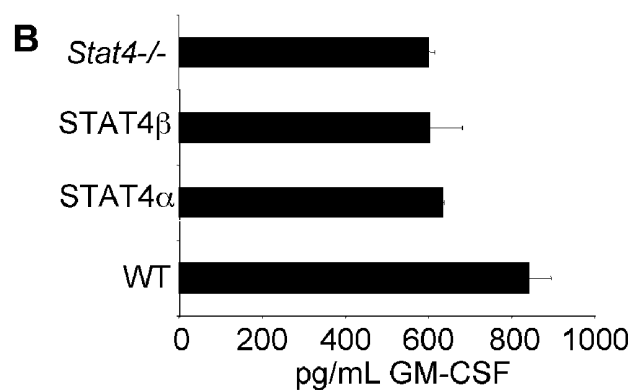
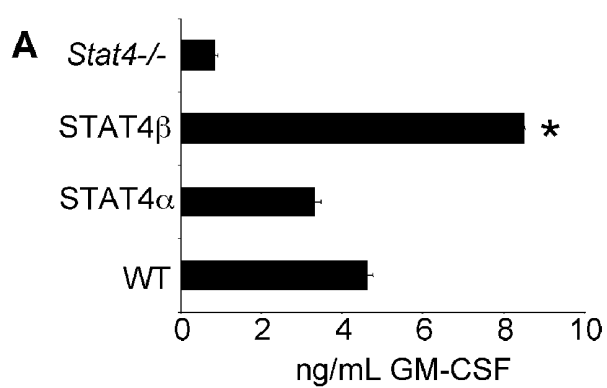


FIG. 14

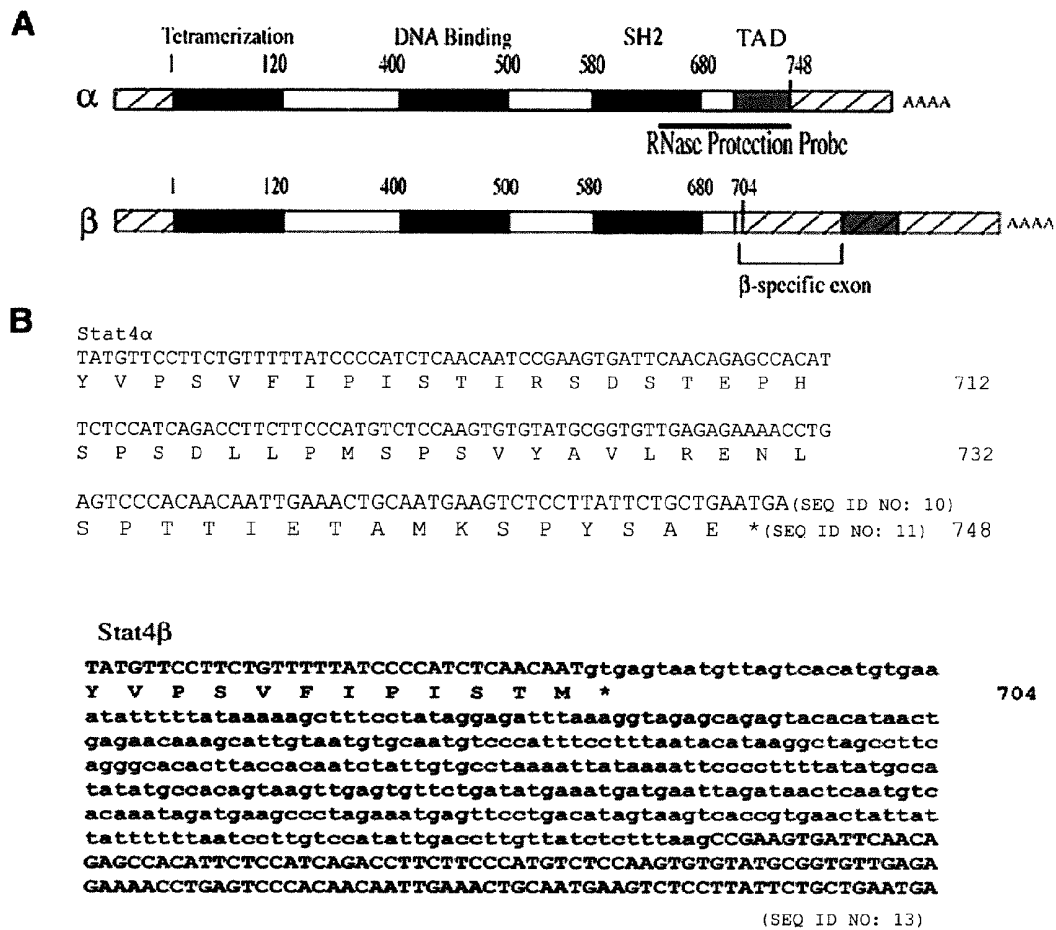


FIG. 15

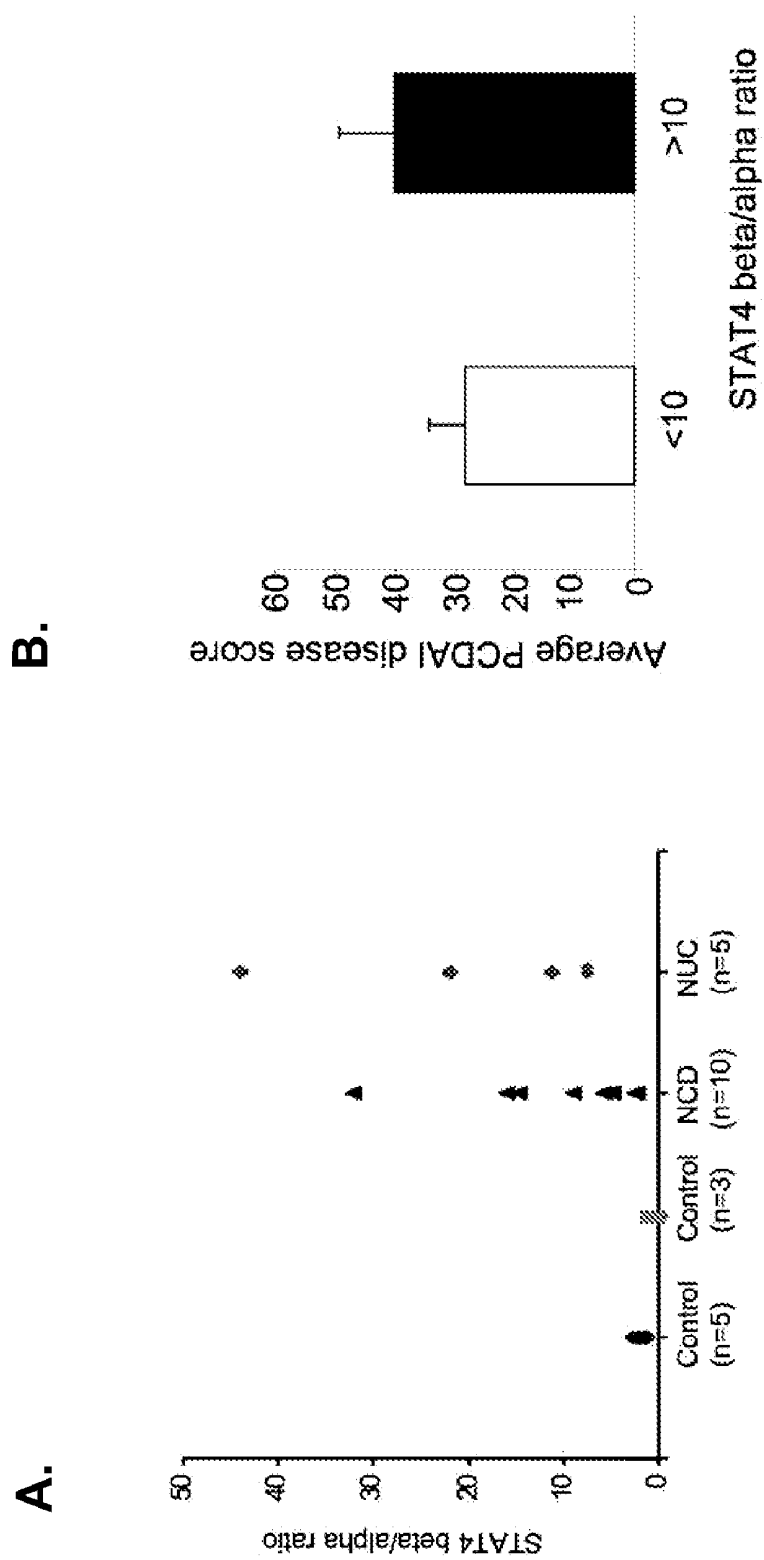


FIG. 16

DIAGNOSIS AND PROGNOSIS OF IMMUNE DISORDERS USING STAT4 EXPRESSION

[0001] This application claims priority to U.S. Ser. No. 61/095,684 filed Sep. 10, 2008, the contents of which is incorporated by reference in its entirety.

[0002] The United States Government has rights in this invention pursuant to funding under National Institutes of Health grant number AI045515.

BACKGROUND

[0003] The present disclosure relates to determining therapeutic efficacy of anti-TNF therapies for treating inflammatory diseases including inflammatory bowel diseases such as Crohn's and ulcerative colitis.

[0004] Signal Transducer and Activator of Transcription (STAT) proteins are a family of factors implicated in a variety of biological processes. STAT proteins exist as latent monomers within the cytoplasm of cells. Following interaction of a cytokine/growth factor with a cell surface receptor, STATs are recruited to the receptor through specific interactions between the STAT SH2 domain and receptor phosphotyrosines. The STAT then is tyrosine phosphorylated and can form homodimers through reciprocal interactions between phosphotyrosines and SH2 domains of two STAT monomers. The dimers then move to the nucleus, bind DNA and modulate gene transcription. This mechanism provides a direct link between cell surface cytokine/growth factor stimulation and gene activation in the nucleus. Stat4 is a member of the STAT family of proteins.

[0005] Stat4 was first cloned by cross hybridization with other cloned STAT proteins. It is the only STAT protein that shows tissue-restricted expression, with mRNA found mainly in lymphoid and myeloid tissues. The Stat4 monomer is a 90 kDa protein with an N-terminal domain important for the interaction of multiple Stat4 dimers, a coiled coil interaction domain, a DNA binding domain, an SH2 domain and a tyrosine important for dimerization. Stat4 contains a C-terminal transactivation domain (TAD) and phosphorylation of a serine residue within this domain affects transactivation.

[0006] Stat proteins are expressed as multiple isoforms; alpha forms that are full length and beta forms that lack the C-terminal transactivation domain of the alpha form and rather have a novel Cterminal domain resulting from the lack of splicing of the last exon. Although the isoform phenomenon is well documented, the biological role of these isoforms is not entirely clear. For Stat1 and Stat5, the beta isoforms are dominant negatives. The functions of Stat3 are more context dependent, where the beta isoform may interfere with transcription of some genes but activate others. Indeed, Stat3 β can mediate some aspects of liver inflammation and rescue the embryonic lethality of Stat3-deficiency. Stat4 is also expressed as two isoforms, a full length form termed Stat4 α and a β isoform that lacks the TAD, termed Stat4 β (Hoey et al., (2003) Distinct requirements for the naturally occurring splice forms Stat4 α and Stat4 β in IL-12 responses, *The EMBO Journal*, vol. 22:16 pp. 4237-4248). Each Stat4 isoform is able to mediate Th1 differentiation in vitro. However, the role of Stat4 isoforms in the pathogenesis of organ-specific autoimmune diseases in vivo has only recently been examined.

[0007] IL-12 was a hallmark cytokine demonstrated to stimulate the activation of Stat4. Stat4 is also activated by

IFN γ , though differently in human and mouse cells. Stat4 activation by IFN γ may be important in anti-viral responses. IL-23 has also been shown to activate Stat4, though whether Stat4 mediates any IL-23-stimulated biological functions is still unclear. The cytokine binds specifically to two non-covalently linked receptor chains expressed on NK, activated T and B cells. The chains are termed IL-12R β 1 and IL-12R β 2 since both chains have homology to β chains of the gp130 family of receptors. The β 2 chain is tyrosine phosphorylated and is responsible for recruitment and activation of Stat4. The biological effects of IL-12 include induction of IFN γ expression in NK and activated T cells, increasing cytotoxic responses in both T and NK cells, inducing proliferation of activated T cells and stimulating the development of fully functional Th1 cells. IL-12 has also been implicated in many inflammatory diseases.

[0008] IL-23 is a heterodimeric cytokine composed of the IL-12 p40 chain disulfide linked to a novel p19 protein. IL-23 activates similar Janus kinases to IL-12 as well as activating of Stat1, Stat3 and Stat4. In vivo, IL-23 promotes inflammation and is critical for the development of experimental autoimmune encephalomyelitis, supporting a potentially important role for IL-23 in disease.

[0009] In an analysis of mice deficient in Stat4, it was shown that Stat4 is required for all known IL-12 biological functions, including the induction of IFN- γ and the promotion of Th1 differentiation. Despite the deficiencies in IL-12 signaled function, there were no obvious defects in the mature myeloid cell compartment. Furthermore, with the exception of the loss of IL-12 responses, the immune system appeared normal. This demonstrates the exquisite specificity of Stat4 function. It also demonstrates that while other STAT proteins may be activated by IL-12, they are not sufficient to achieve any of the known IL-12 responses.

[0010] The phenotype of disease in Stat4-deficient mice demonstrates the requirement for Stat4 in Type 1 immunity. Stat4-deficient mice are susceptible to infection with *Trypanosoma cruzi*, *Toxoplasma gondii*, *Leishmania major*, *Leishmania mexicana*, *Mycobacterium tuberculosis*, and have decreased DTH responses. In contrast, Stat4-deficient mice are refractory to the induction of inflammatory conditions including colitis, arthritis, diabetes, adhesion formation, myocarditis, cardiac allograft vasculopathy, endotoxemia, renal and hepatic ischemia-reperfusion injury and experimental autoimmune encephalitis. T cell memory responses in Stat4-deficient mice generate little IFN- γ . Thus, the phenotype of the Stat4-deficient model is that of a mouse with greatly impaired Th1 responses in vivo.

[0011] Chronic inflammatory bowel disease (IBD) that affects the intestine (Crohn's Disease, CD) or colon (ulcerative colitis) is increasing in incidence and while mortality is low, symptoms may be debilitating. The need for new treatments and diagnosis tools is great. In mouse models, Stat4 is required for the development of IBD and increased expression of Stat4 results in IBD. It was thought for many years that Th1 cells were critical in the development of Crohn's disease. However, experiments have demonstrated the involvement of Th17 cells and IL-23 in the development of CD. Importantly, Stat4 is a critical factor in the development of Th1 and Th17 cells. In patient samples, constitutively active Stat4 has been observed in intestinal T cells and inflamed mucosal tissue samples from patients with CD. Thus, factors that regulate Stat4 activity are of great interest in understanding disease pathogenesis and may aid in further treatment of disease. To

date, the most effective therapy has been aminosalicylates, sulfasalazine, corticosteroids and anti-TNF- α therapy, all of which either limit the production or activity of proinflammatory cytokines secreted by the leukocytes

[0012] Multiple Sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) that afflicts more than one million people worldwide. The disease usually begins in young adults and affects women more frequently than men. About 30% of MS patients develop clinical paralysis and become wheel chair-bound for the rest of their lives. There is currently no medical treatment available that can cure MS. The destruction of the oligodendrocyte myelin sheath and axonal loss in the CNS are the pathological hallmarks of MS. Although the etiology of MS remains unknown, it is generally viewed as an organ-specific autoimmune disease, mediated by myelin-reactive T cells in the CNS. Activation of immune cells, secretion of inflammatory cytokines and differentiation of encephalitogenic T cells are key processes associated with the pathogenesis of MS. Experimental allergic encephalomyelitis (EAE) is a CD4⁺ Th1/Th17 cell-mediated inflammatory demyelinating autoimmune disease of the CNS. EAE can be induced in susceptible animals by immunization with whole brain homogenate and purified neural antigens such as myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG) or adoptive transfer of neural antigen specific T cells. The clinical and pathological features of EAE show close similarity to human MS and therefore it has commonly been used as an animal model to study the mechanisms of MS pathogenesis and to test the efficacy of potential therapeutic agents for the treatment of MS.

[0013] In the present disclosure, use of the expression ratio of Stat4 α /Stat4 β in evaluating inflammatory inflammatory diseases was analyzed.

SUMMARY

[0014] A method of predicting the likelihood of successful anti-inflammatory therapy for a patient suffering from an inflammatory disease includes: (a) determining the expression level of Stat4 α and Stat4 β isoforms in a biological sample from the patient; and (b) predicting the likelihood of successful anti-TNF therapy for a patient suffering from an inflammatory disease by correlating the relative expression levels of Stat4 α and Stat4 β isoforms in the patient to a control sample or a reference value.

[0015] A method of assessing a patient's risk for developing an inflammatory disease or an inflammatory response includes: (a) quantifying the expression level of Stat4 α and Stat4 β isoforms in a biological sample from the patient; and (b) determining that the patient's risk for the inflammatory disease or the inflammatory response is higher if the patient exhibits a higher Stat4 β :Stat4 α ratio as compared to a control.

[0016] A method of predicting disease severity in a patient's suspected of suffering from an inflammatory disease includes (a) obtaining the expression level of Stat4 α and Stat4 β isoforms in a biological sample from the patient; and (b) determining that the disease severity for the patient suffering from the inflammatory disease is higher if the patient exhibits a higher Stat4 β :Stat4 α ratio as compared to a control.

[0017] A method of preventing or minimizing excessive inflammatory response in an immuno compromised patient includes: (a) determining if the patient exhibits higher risk for the inflammatory response based on the patient's Stat4 β :Stat4 α expression level ratio in a biological sample as com-

pared to a control; and (b) administering an anti-inflammatory therapy to minimize the excessive inflammatory response.

[0018] A suitable biological sample is blood. The expression level of Stat4 α and Stat4 β may be determined by analyzing the expression peripheral blood mononuclear cells (PBMC).

[0019] A suitable inflammatory disease or response is selected from the group consisting of Crohn's disease, ulcerative colitis, rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, lupus, asthma, psoriasis, type I diabetes, carditis, chronic obstructive pulmonary disease (COPD, inflammatory bowel disease (IBD), and multiple sclerosis (MS).

[0020] Suitable ratio of Stat4 β /Stat4 α ranges from about 0.1 to 60.0 or higher. Other ratios (e.g., less than about 0.1 and greater than about 60) are also suitable depending upon the inflammatory condition.

[0021] The expression level of Stat4 α and Stat4 β may be determined by any technique including but not limited to PCR, quantitative PCR or real-time PCR, semi-quantitative PCR, probe-hybridization, and antibody-based quantitation.

[0022] Suitable clinicopathological data, if necessary, may be selected from patient age, previous personal and/or familial history of inflammatory diseases, previous personal and/or familial history of response to anti-inflammatory therapy, and presence of one or more single nucleotide polymorphisms (SNPs) associated with the Stat4 isoforms.

[0023] Suitable anti-inflammatory therapy includes but not limited to infliximab, adalimumab, certolizumab pegol, afelimomab, golimumab, etanercept, abatacept, and anakinra.

[0024] The expression levels of Stat4 α and Stat4 β may be useful for a clinical detection of disease, disease diagnosis, disease prognosis, or treatment outcome or a combination of any two, three or four of these actions.

[0025] In an embodiment, an excessive inflammatory response is associated with sepsis. In an embodiment, the immune compromised patient is treated with an immuno suppressive agent.

[0026] A diagnostic kit to predict the response to anti-inflammatory therapy includes oligonucleotide primers to specifically detect and quantify the expression levels of Stat4 α and Stat4 β isoforms.

[0027] Suitable oligonucleotide primers include for example 5'-TAT CCT GAC ATT CCC AAA GAC-3' (SEQ ID NO: 6), 5'-CTC TCA ACA CCG CAT ACA CAC-3' (SEQ ID NO: 7), and 5' GAC TTA CTA TGT CAG GAA CTC-3' (SEQ ID NO: 8). Other oligo nucleotide primers can be readily designed based on the sequences disclosed herein and as shown in FIG. 15.

[0028] A nucleic acid probe or a primers includes a contiguous region of about 15 nucleotides of SEQ ID NO: 5, wherein the probe is capable of selectively binding to the Stat4 β -specific exon. In an embodiment, a probe includes a reverse complementary strand capable of selectively binding to SEQ ID NO: 5. In other embodiments, a probe or a nucleic acid primer is about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 30, 35, 50 or 100 contiguous nucleotides represented within SEQ ID NO: 5.

[0029] In an embodiment, a probe consists essentially of a sequence of about 15-100 or 15-20 nucleotides capable of selectively binding to SEQ ID NO: 5.

[0030] In an embodiment, the experimental allergic encephalomyelitis (EAE) was used as an exemplary model

system to evaluate the role of stat4 isoforms in regulating autoimmune disorders. EAE is a T cell-mediated autoimmune disease model of multiple sclerosis (MS). Signal transducer and activator of transcription 4 (Stat4) is a transcription factor activated by interleukin 12 (IL-12) and IL-23, two cytokines known to play important roles in the pathogenesis of EAE by inducing T cells to secrete IFN- α and IL-17 respectively. Therapeutic intervention or targeted disruption of Stat4 was effective in ameliorating EAE. A splice variant of Stat4 termed Stat4 β has been characterized that lacks 44 amino acids at the C-terminus of the full length Stat4 α . It was examined herein whether T cells expressing either isoform impacted the pathogenesis of EAE. Transgenic mice expressing Stat4 upon a Stat4-deficient background develop an exacerbated EAE compared to wild-type mice following immunization with MOGp35-55 peptide, while Stat4 α transgenic mice have greatly attenuated disease. The differential development of EAE in transgenic mice correlates with increased IFN γ and IL-17 in Stat4 β -expressing cells in situ, contrasting increased IL-10 production by Stat4 α -expressing cells. It is shown herein that Stat4 isoforms differentially regulate inflammatory cytokines in association with distinct effects on the onset and severity of EAE. Stat4 β transgenic mice developed an exacerbated EAE in association with an increased expression of inflammatory cytokines. The Stat4 α transgenic mice remain resistant to EAE, indicating that Stat4 α is more efficient than Stat4 α in mediating the pathogenesis of EAE.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] FIG. 1 shows development of EAE in Stat4 α and Stat4 β transgenic mice. A, C57BL/6 wild type (WT), Stat4 deficient (Stat4 $^{-/-}$), Stat4 α transgenic (Stat4 α) and Stat4 β transgenic (Stat4 β) mice were induced to develop EAE by immunization with MOGp35-55 antigen. The clinical symptoms were scored every day in a blinded manner. The mean clinical score of all 10 mice from two different experiments is shown. The figure is representative of three independent experiments. B, Neural antigen-induced proliferation of spleen T cells from Stat4 α and R transgenic mice in vitro. Spleen cells were isolated from C57BL/6 wild type (WT), Stat4 deficient (Stat4 $^{-/-}$), Stat4 α transgenic (Stat4 α), and Stat4 β transgenic (Stat4 β) mice on day 14 following induction of EAE. The cells were cultured with MOGp35-55 for 48 hrs and the proliferation measured by WST-1 assay.

[0032] FIG. 2 shows histology of CNS inflammation and demyelination in Stat4 α and Stat4 β transgenic mice with EAE. The spinal cord samples were isolated from C57BL/6 wild type (WT), Stat4 deficient (Stat4 $^{-/-}$), Stat4 α transgenic (Stat4 α) and Stat4 β transgenic (Stat4 β) mice on day 30 following induction of EAE. The transverse sections of cervical, upper thoracic, lower thoracic and lumbar regions of spinal cord were obtained and stained with LFB/PAS (luxol fast blue/periodic acid schiff) along with H&E (hematoxylin and eosin). The pathology of demyelination (left) and inflammation (right) in the spinal cord sections were visualized by microscopy and the representative 10 $\times$ pictures are shown. The number of positive quadrants with inflammation and demyelination were scored and expressed as percentage over the total number of quadrants examined in the histogram.

[0033] FIG. 3 shows expression of effector T cell-derived inflammatory cytokines in the CNS, spleen and cultured spleen cells from Stat4 α and Stat4 β transgenic mice with EAE. Brain and spleen samples were isolated from C57BL/6 wild type (WT), Stat4 α transgenic (Stat4 α), and Stat4 β trans-

genic (Stat4 β) mice on day 14 following induction of EAE by immunization with MOGp3555 antigen. Total RNA was extracted from brain, spleen or spleen cells cultured with neural antigens and the expression of IFN γ , IL-17 and T-bet analyzed by qRT-PCR using GAPDH as internal control. The fold changes in the expression of cytokines in EAE mice were calculated based on naïve mice as control.

[0034] FIG. 4 shows expression of APC-derived inflammatory cytokines in the CNS and spleen of Stat4 α and R transgenic mice with EAE. Brain and spleen were isolated from C57BL/6 wild type (WT), Stat4 α transgenic (Stat4 α) and Stat4 β transgenic (Stat4 β) mice on day 14 following induction of EAE by immunization with MOGp35-55 antigen. Total RNA was extracted from brain and spleen and the expression of IL-12p35, IL-12p40 and IL-23p19 analyzed by qRT-PCR using GAPDH as internal control. The fold changes in the expression of cytokines in EAE mice were calculated based on naïve mice as control.

[0035] FIG. 5 shows intracellular IFN γ and IL-17 in immune cells from Stat4 α and R transgenic mice. Spleen, lymph node and brain cells were isolated from C57BL/6 wild type (WT), Stat4 α transgenic (Stat4 α) and Stat4 β transgenic (Stat4 β) mice on day 14 following induction of EAE. The cells were cultured with PMA+ionomycin for 6 hours before staining with IFN γ and IL-17 specific antibodies and analyzed by flow cytometry.

[0036] FIG. 6 shows neural antigen-induced secretion of IFN γ and IL-17 from Stat4 α and R transgenic spleen cells in culture. Spleen cells were isolated from C57BL/6 wild type (WT), Stat4 α transgenic (Stat4 α) and Stat4 β transgenic (Stat4 β) mice on day 14 following induction of EAE. The cells were cultured with MOGp35-55 or Con A for 36 h, and the release of IFN γ (A) and IL-17 (B) was analyzed by ELISA.

[0037] FIG. 7 shows differential regulation of IL-10 by Stat4 isoforms. A, Affymetrix Integrated Genome Browser analysis of Stat4 binding across the I110 locus using data from a Stat4 ChIP-on-chip dataset. Bars indicate the intensity of Stat4-bound DNA hybridizing to oligonucleotides spanning -7.5 kb to +2.5 kb relative to the I110 transcriptional start. The exon-intron structure of I110 is indicated below the graph. B, Naïve, wild type and Stat4 $^{-/-}$ CD4 $^{+}$ T cells were cultured under Th1 conditions for five days before re-stimulation with IL-12 (left) or anti-CD3 (right) for 24 hrs. IL-10 levels were examined in supernatants using ELISA. C, Naïve, wild type, Stat4 α transgenic and Stat4 β transgenic CD4 $^{+}$ T cells were cultured under Th1 conditions for five days before re-stimulation with anti-CD3 for 24 hours. IL-10 and IFN γ levels were examined in supernatants using ELISA. D, I110 mRNA levels were assessed in total RNA from spleens isolated from C57BL/6 wild type (WT), Stat4 α transgenic (Stat4 α) and Stat4 β transgenic (Stat4 β) mice on day 14 following induction of EAE by immunization with MOGp35-55 antigen by qRT-PCR using GAPDH as internal control. The fold changes in the expression levels were calculated based on naïve spleen. E, Spleen cells were isolated from C57BL/6 wild type (WT), Stat4 α transgenic (Stat4 α) and Stat4 β transgenic (Stat4 β) mice on day 14 following induction of EAE. The cells were cultured as in FIG. 6 and the production of IL-10 was analyzed using ELISA.

[0038] FIG. 8 shows T-cells expressing STAT4 isoforms have differential TNF- α production. A, CD4 $^{+}$ CD62L $^{+}$ T-cells from mice of the indicated genotypes were cultured under Th1 priming conditions (IL-12, anti-IL-4, a-CD3, a-CD28) with irradiated APCs (30 Gy) for five days. Every 24

hours, supernatants of the developing Th1 cells were collected from each genotype. Cell free supernatants were analyzed for IFN- γ production using ELISA. Results are represented as mean $\pm$ SD. B, Cells cultured as in (A) for five days were stimulated for 24 hours and cell-free supernatants were analyzed for IFN- γ using ELISA. Results are represented as mean $\pm$ SD and are representative of 3 independent experiments. C, CD4+CD62L+ T-cells were cultured as in (A) for five days. Cells were collected, washed, and stimulated with PMA and ionomycin in the presence of GolgiPlug before intracellular staining for the indicated cytokines. Data shown are gated on CD4+ cells. Numbers represent % of cells in the respective quadrant while numbers in parentheses represent the MFI of the x-axis. Results are representative of 3 independent experiments. D, RNA was isolated from Th1 cells cultured as in (A) following 4 hours of treatment with anti-CD3. Quantitative PCR was performed for Tnf α mRNA and normalized for P2m expression. Results are relative to WT cells. E, Cells cultured under Th1 priming conditions for five days were stimulated in the indicated condition for 24 hours before cell-free supernatants were collected for analysis of TNF- α . F, Cells cultured as in (A) for five days were stimulated for 24 hours and cell-free supernatants were analyzed by ELISA for TNF- α and IL-2. Results are represented as mean $\pm$ SD and are representative of 2-4 independent experiments. *, significantly different ($p < 0.05$) from wild-type, Stat4 α , and Stat4 α -/- Th1 cultured cells using unpaired Student's T-test.

[0039] FIG. 9 shows activation kinetics of the STAT4 isoforms during Th1 differentiation. A, Naïve CD4+ T cells freshly isolated (0 time point) or cultured in Th1 conditions for 24, 48, or 72 hours were collected for intracellular staining with anti-pStat4. Results are representative of 2 independent experiments. Numbers in quadrants represent % of pSTAT4+ T cells. B, Total cell extracts from WT and STAT4 transgenic T cells were immunoblotted for STAT4 protein levels at day 1, 2 and 5 of Th1 differentiation. Data is presented as arbitrary units of densitometry normalized to actin expression and relative to WT day 1 in the left panel or WT day 5 in the right panel. C, Th1 cells cultured for 5 days were washed and stimulated with IL-12 for the indicated time points before being intracellularly stained for pSTAT4. Data are shown for the averages of duplicate samples of representative data. D, Th1 cells were stimulated with IL-12 and IL-18 for 24 hours and cell-free supernatants were analyzed for IFN- γ using ELISA. Results are shown as mean $\pm$ SD.

[0040] FIG. 10 shows effects of STAT4 α and STAT4 β expression on Th17 differentiation. A, CD4+CD62L+ T-cells were cultured in the presence of TGF- β 1, IL-6, IL-23, anti-IL-4, and anti-IFN- γ for 5 days. Cells were collected, washed, and stimulated with plate-bound a-CD3 or PMA and Ionomycin (P+I) in the presence of Golgi-Plug before intracellular staining for the indicated cytokines. CD4+ cells were gated and the results were plotted as indicated. Numbers represent % of cells in the respective quadrant. Results are representative of 2 independent experiments. B, Total CD4 T cells were cultured for five days in the presence of IL-23 before restimulation with anti-CD3 and assessing production of IL-17A using ELISA. Results are shown as mean $\pm$ SD and are representative of 3 independent experiments. C, T cells cultured as in (B) were stimulated with IL-23 and IL-18 for 24 hours and cell-free supernatants were analyzed by ELISA for IL-17A. Results are shown as mean $\pm$ SD of results from 2-4 independent experiments.

[0041] FIG. 11 shows that STAT4 α and STAT4 β mediate inflammatory bowel disease. A, The change of weight over time is expressed as percent of the original weight. Data represent the mean $\pm$ SEM of each group (7-10 mice per group). Mice were sacrificed 14 weeks after T-cell reconstitution. *, CD45RB^{low} cells are significantly different ($p < 0.05$) from CD45RB^{high} WT, STAT4 α or STAT4 β using 2-way ANOVA and unpaired Student's Ttest post-hoc. B, MLN single-cell suspensions were counted and surface stained for CD4 and analyzed by FACS. Absolute cell numbers were calculated from % of CD4+ cells and cell counts (left panel). QPCR was performed for STAT4 using cDNA made from MLN RNA (right panel). C, Gross appearance of representative colon from each group as indicated. D, Representative photomicrographs (100 $\times$) of colon from mice of the indicated group were stained with H&E. E, The mean histological scores $\pm$ SEM for the SCID mice reconstituted with the CD4+ T-cells as indicated with STAT4 α or STAT4 β signifying histological scores from the SCID mice reconstituted with the CD45RB^{high} subset and the low signifying histological scores from the SCID mice reconstituted with the CD45RB^{low} subset. *, $p < 0.05$ where STAT4 β is significantly different from both STAT4 α and the CD45RB^{low} subset using the Mann-Whitney U-test.

[0042] FIG. 12 shows cytokine production from STAT4 α - and STAT4 β -expressing T cells ex vivo. (A and B) Cells were isolated and stimulated as described in Materials and Methods and concentration of cytokines were determined by ELISA and are displayed as mean $\pm$ SEM (Stat4 α n=9; Stat4 β n=10). *, $p < 0.05$, ** $p < 0.02$ using Unpaired Student's T-test. C, Cells were isolated and stimulated as described in Materials and Methods. The concentration of cytokines were determined by ELISA and are displayed as mean $\pm$ SD of pooled MLNs from the SCID mice reconstituted with the CD45RB^{high} subset of the indicated STAT4 isoform. *, $p < 0.05$ using unpaired Student's T-test.

[0043] FIG. 13 shows increased lamina propria neutrophil infiltration correlates with increased GM-CSF levels seen in the SCID mice reconstituted with the STAT4 β isoform. A, PMN scores were determined as described in Materials and Methods. Data are presented as mean $\pm$ SEM. *, $p < 0.05$ using Mann-Whitney U test. B, Single cell suspensions from MLNs were pooled from the indicated mice, stimulated with anti-CD3 for 72 hours and cell-free supernatants were analyzed using ELISA for GM-CSF. Data are presented as mean $\pm$ SD. *, $p < 0.05$ using unpaired Student's T-test.

[0044] FIG. 14 shows that STAT4 β Th1 cells are programmed to secrete more GM-CSF than STAT4 α Th1 cells. A, CD4+CD62L+ T-cells were primed for Th1 differentiation using the same conditions as in FIG. 1. After five days, cells were stimulated for 24 hours and cell-free supernatants were analyzed by ELISA for GM-CSF. Results are represented as mean $\pm$ SD and are representative of 3 independent experiments. *, $p < 0.05$ using unpaired Student's T-test. B, Cells cultured under Th17 conditions as in FIG. 3A for five days were stimulated for 24 hours and analyzed by ELISA for GM-CSF production. Results are presented as mean $\pm$ SD and are representative of 2 independent experiments.

[0045] FIG. 15 shows nucleic acid and amino acid sequences for Stat4 α and Stat4 β isoforms and the different exons.

[0046] FIG. 16 shows the STAT4 β /STAT4 α ratio in two different control groups (the left infants; the right a group that is age-matched to the patients), CD and UC patient groups.

The ratio is consistently elevated in the patient samples. The right graph shows the Crohn's disease severity score with the beta/alpha ratio divided into groups higher or lower than 10. The data indicates that patients with higher ratios have more severe disease symptoms.

DETAILED DESCRIPTION

[0047] In the present disclosure the ability of Stat4 α and Stat4 β transgenic T cells to mediate inflammatory disease was tested. In a model of colitis, the Stat4 β isoform appeared to be more potent in generating inflammation than the Stat4 α isoform. This correlates with an increased production of inflammatory cytokines, particularly TNF α . Similarly, in an Experimental Autoimmune Encephalomyelitis (EAE) model of central demyelination, the Stat4 β transgenic mice get worse disease than wild type mice while the Stat4 α transgenic mice get less severe disease than wild type mice. Stat4-deficient mice are relatively protected from disease in each model.

[0048] Knowledge of Stat4 alpha/beta ratios may provide clinicians with information to decide a patient's risk for developing inflammatory diseases, predicting the severity (and for taking precautionary steps to avoid undesirable outcomes), and/or determining potential response/non-response to immunosuppressive treatments such as anti-TNF therapy for diseases including rheumatoid arthritis, lupus, inflammatory bowel disease, multiple sclerosis and the like. Because immunosuppression therapy often increases an individual's risk to sepsis, tuberculosis and other infectious diseases, predictive evidence for efficacy of an immunosuppressive treatment such as anti-TNF therapy helps reduce the overall incidence of life-threatening infections.

[0049] Stat4 α and Stat4 β transgenic mice were used to define the ability of Stat4 isoforms to mediate CNS inflammation and demyelination in the EAE model of MS. Stat4 β transgenic mice develop exacerbated EAE compared to wild type mice, while the Stat4 α transgenic mice developed mild EAE. The exacerbation of EAE in Stat4 β transgenic mice associated with lower levels of IL-10 production and increased expression of inflammatory cytokines including IFN γ and IL-17 compared to Stat4 α transgenic mice. These findings highlight the fact that Stat4 isoforms play distinct roles in the pathogenesis of EAE.

[0050] Each isoform of Stat4 is sufficient to program Th1 development through both common and distinct subsets of target genes. The ability of these isoforms to mediate inflammation in vivo has not been examined. Using a model of colitis that develops following transfer of CD4⁺ CD45RBhi T-cells expressing either the STAT4 α or STAT4 β isoform into SCID mice, it was determined that while both isoforms mediate inflammation and weight loss, STAT4 β promotes greater colonic inflammation and tissue destruction. This correlates with STAT4 isoform-dependent expression of TNF- α and GM-CSF in vitro and in vivo, but not Th1 expression of IFN- γ or Th17 expression of IL17, which were similar in STAT4 α - and STAT4 β -expressing T cells. Thus, higher expression of a subset of inflammatory cytokines from STAT4 β -expressing T cells correlates with the ability of STAT4 β -expressing T cells to mediate more severe inflammatory disease.

[0051] To test the ability of Stat4 isoforms to mediate inflammatory disease, a model wherein CD4⁺CD45RBhi T cells expressing either Stat4 α or Stat4 β were transferred into SCID recipients to induce colitis was used. Stat4 β mediated more severe inflammation and this correlated with the ability

of Stat4 β -expressing T cells to secrete higher levels of a subset of Th1 inflammatory cytokines in vitro and in vivo. Thus, Stat4 β , an isoform that lacks the C-terminal transactivation domain, is more efficient than Stat4 α in promoting inflammation in vivo.

[0052] Data provided herein demonstrate that T cells expressing Stat4 β are much more efficient in mediating inflammation than T cells expressing the Stat4 α isoform. Stat4 β transgenic mice develop much more severe disease with greater levels of demyelination than those observed in Stat4 α transgenic mice or wild type mice. The mechanism for this increased disease may include the altered cytokine environments observed in the transgenic mice. While Stat4 α and Stat4 β transgenic cells are equally capable of becoming IFN γ - or IL-17-secreting cells in vitro, Stat4 α transgenic cells have increased levels of IL-10 production (FIG. 7). Similar increased IL-10 production was observed in Stat4 α transgenic mice with EAE in vivo and ex vivo (FIG. 7). The lower levels of IL-10 produced in Stat4 β transgenic mice are associated with increased IL-12 and IL-23 mRNA levels in CNS and spleen tissue, and increased IFN γ expression in tissue and from antigen-stimulated Stat4 β -expressing cells compared to those observed in Stat4 α -expressing cells (FIGS. 3-7). The lower levels of IFN γ produced by Stat4 α transgenic cells compared to Stat4 β transgenic cells may, at least in part, be responsible for the observed increases in IL-17 from Stat4 α transgenic cells in the periphery (FIGS. 4 and 6). Data from a ChIP-on-chip assay are provided herein that Stat4 directly binds IL-10, and show that acute stimulation of Th1 cells with IL-12 results in IL-10 production from wild type but not Stat4 α cells (FIG. 7). Moreover, Stat4 α , but not Stat4 β , can mediate the programming of the IL-10 gene for increased expression in Th1 cultures. Thus, while Stat4 α can rescue Stat4-deficiency in vitro and compensates for some in vivo Stat4 functions, altered cytokine profiles from these cells limit their ability to promote the development of EAE. As IL-10 is critical regulator of inflammation in EAE, the increased IL-10 production in Stat4 α transgenic mice (FIG. 7) provides a mechanism how Stat4 isoforms differentially regulate the pathogenesis of EAE. These results also indicate that modulating the splicing between the alpha and beta isoforms of Stat4 has therapeutic value for inflammatory diseases.

[0053] It is also possible that other Stat4 α - or Stat4 β -specific functions might be important for the pathogenesis of EAE. While both isoforms could mediate Th1 development in vitro, Stat4 β -expressing cells produced slightly less IFN γ in response to IL-12. Since there was more severe disease in Stat4 β transgenic mice, it seems unlikely this contributes to the level of disease. Stat4 β transgenic cells had much higher proliferation than Stat4 α or wild type cells in a pattern that paralleled the severity of disease. However, MOGp35-55-specific proliferation indicates that there is no significant increase in the overall number of antigen-reactive T cells in EAE (FIG. 1). Similarly, intracellular cytokine staining did not show dramatic differences among the percentages of cytokine-positive cells, though IFN γ was increased in the Stat4 β transgenic cells. As noted above, this is more likely to result from changes in the balance of IL-10 and IFN γ production, and their resulting effects on IL-17 production. However, there may be additional genes that are differentially regulated by Stat4 isoforms which may also contribute to the development of inflammatory diseases.

[0054] Data provided herein further demonstrates that Stat4 expression in T cells may be sufficient to mediate inflammatory immunity. The Stat4 α and Stat4 β transgenes are expressed from a CD2 locus control region that promotes transcription primarily in T cells, with considerably lower expression in other lymphoid cells. The transgenic mice have been backcrossed to the Stat4 $^{-/-}$ background so that the Stat4 isoforms are expressed in T cells but not other cells in the mouse. As the transgenic mice in this study lack Stat4 in any myeloid compartment, results indicate that Stat4 expression in non-lymphoid cells may not be required for the development of EAE. It may be possible to alter Stat4 function by modulating the splicing of Stat4 isoforms and thus altering the ability of immune cells to mediate disease.

[0055] TNF α and GM-CSF production are Stat4-dependent in Th1 cells and that Stat4 β more effectively programs the secretion of these cytokines following subsequent antigen receptor stimulation. Thus, Stat4 isoforms may have differing roles in the development of inflammation.

[0056] As Stat4 has been implicated as a pathogenic factor in Th1 and Th17-mediated autoinflammatory diseases, including IBD, an IBD model system where colitis is induced in SCID mice upon reconstitution with CD4 $^{+}$ CD45RBhigh T cells was chosen to test the roles of Stat4 isoforms in disease. This model system has the advantage of being able to directly test the ability of T-cells expressing the Stat4 isoforms to mediate pathogenesis with minimal manipulation after reconstitution.

[0057] Although Crohn's disease and ulcerative colitis (UC) are the most common forms of inflammatory bowel disease (IBD), other forms include for example, collagenous colitis, lymphocytic colitis, ischaemic colitis, diversion colitis, Behçet's syndrome, infective colitis, and indeterminate colitis. In young adults that include pediatric patients of ages under 17, one or more forms of the above-listed IBD may be more common.

[0058] The ratio of Stat4 β /stat4 α expression levels may vary depending upon the disease or tissue or the inflammatory response being investigated. For example, the ratio (β/α) may range from about 0.1 to about 10 or about 0.2, 0.3, 0.4, 0.5, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 15.0, 20.0, 25.0, 30.0, 40.0, 50.0 or higher. Depending on the conditions, the ratio may also range less than about 5.0 or 2.0 or 3.0. It may also range about 0.1-2.00. Depending on the sensitivity of the detection system and the relative units, the ratio may vary over a broader range. In other words, the scope of the disclosure is not limited to the explicit ratios disclosed herein. A skilled artisan, based on the disclosure and guidance herein may readily evaluate the ratio by determining the relative expression levels of α and β isoforms of Stat4 in a cell, plurality of cells, tissue, for a disease of interest. In some instances, one of the isoforms e.g., α or β may not be expressed to a detectable level or expressed to an extremely low level. Accordingly, appropriate ratios or the mere absence of a particular isoform may be used as predictive or diagnostic markers for an inflammatory response of interest. In an embodiment, the relative expression status of the α or the β isoform of Stat4 is a marker useful for evaluating, diagnosing or predicting the efficacy of anti-inflammatory therapies.

[0059] The term correlating in reference to a parameter, e.g., expression levels of Stat4 α/β in a sample generally includes any method of relating levels of expression of markers to a standard or a reference value for the assessment of the diagnosis, prediction of an immune disorder including

Crohn's disease, ulcerative colitis, IBD and/or assessment of efficacy of clinical treatment, e.g., anti-TNF therapy, identification of a patient group that responds to a particular therapy, selection of a subject for a particular treatment, and monitoring of the progress of treatment with an anti-TNF therapy.

[0060] In an embodiment, the Stat4 β :Stat4 α expression ratio are correlated with a well-known scoring metric, e.g., Pediatric Crohn's Disease Activity Index (PCDAI), Hyams et al. (1991), Development and validation of a pediatric Crohn's disease activity index. *J Pediatr Gastroenterol Nutr*; 12:439-447, incorporated herein by reference. See also, Harvey R, Bradshaw J (1980). A simple index of Crohn's-disease activity. *Lancet* 1 (8167): 514; Yoshida et al., (1999), The Crohn's Disease Activity Index, its derivatives and the Inflammatory Bowel Disease Questionnaire: a review of instruments to assess Crohn's disease. *Can J. Gastroenterol.*; 13(1):65-73; and Turner et al., (2007) Development, Validation, and Evaluation of a Pediatric Ulcerative Colitis Activity Index: A Prospective Multicenter Study, *Gastroenterology*; 133(2): 423-432, all incorporated by reference herein.

[0061] Anti-tumor necrosis factor (TNF) strategies for immune disorders, include for example chimeric monoclonal (infliximab), humanized monoclonal (CDP571 and the PEGylated CDP870) and fully human monoclonal (adalimumab) antibodies against TNF, p75 fusion protein (etanercept), p55 soluble receptor (onercept) and small molecules such as MAPkinase inhibitors. Infliximab is often prescribed in treating active Crohn's disease patients that do not respond to or intolerant of conventional therapies (e.g., steroidal or other non-steroidal treatments). In patients who fail to respond therapy with corticosteroids and immunosuppressive therapy and are poor candidates for surgery, and patients with fistulizing disease, where infliximab therapy is chosen, regular maintenance therapy with infliximab may be required. Afelimomab (also known as Fab2 or MAK 195F) is an anti-TNF- α monoclonal antibody. Certolizumab pegol is a monoclonal antibody directed against tumor necrosis factor alpha. It is a PEGylated Fab' fragment of a humanized TNF inhibitor monoclonal antibody.

[0062] Nucleic acid or nucleic acid sequence or polynucleotide or polynucleotide sequence refers to the sequence of a single- or double-stranded DNA or RNA molecule of genomic or synthetic origin, i.e., a polymer of deoxyribonucleotide or ribonucleotide bases, respectively.

[0063] Detection of expression of Stat4 isoforms or fragments thereof and any other genes include detecting the expression of RNA, its reverse transcribed cDNA and the resulting protein products thereof. For example, Stat4 isoform RNA can be detected using in situ RT-PCR or in vitro RT-PCR or standard PCR or through any hybridization techniques that involve Stat4 α and β isoform specific probes or primers. RNA expression can also be quantified by any known quantification PCR (qPCR) and competitive PCR technology (see e.g., Nolan T, Hands R E, Bustin S A (2006). "Quantification of mRNA using real-time RT-PCR." *Nat. Protoc.* 1: 1559-1582). Microarrays are also useful in quantifying gene expression. RNA can be extracted from a suitable tissue or a cell population, converted into cDNA and quantified using any known method. RNA can also be directly quantified in situ, within the tissue or the cell population itself. Probes specific to Stat4 isoforms may display high

stringency hybridization. Similar techniques are also useful in determining the expression pattern and quantity of any other genes.

[0064] High stringency hybridization conditions or highly stringent hybridization include at least about 6×SSC and 1% SDS at 65° C., with a first wash for 10 minutes at about 42° C. with about 20% (v/v) formamide in 0.1×SSC, and with a subsequent wash with 0.2×SSC and 0.1% SDS at 65° C. These conditions are used for detecting expression levels of Stat4α/β isoforms using the probes disclosed herein or to identify additional specific probes for expression detection. Moderately stringent conditions may be obtained by varying the temperature at which the hybridization reaction occurs and/or the wash conditions as set forth above.

[0065] Nucleic acid probes are used to detect and/or quantify the presence of Stat4 transcript in a sample, e.g., as hybridization probes, or to amplify Stat4 transcript or partial regions thereof in a sample, e.g., as a primer. Probes have a complementary nucleic acid sequence that selectively hybridizes to the desired target nucleic acid sequence. The hybridization probe must have sufficient identity with the target sequence, i.e., at least 70%, e.g., 80%, 90%, 95%, 98%, or 99% or more identity to the target sequence. The probe sequence is also sufficiently long so that the probe exhibits selectivity for the target sequence over non-target sequences. For example, the probe will be at least about 20, e.g., 25, 30, 35, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or more, nucleotides in length. Probes include primers that refer to a single-stranded oligonucleotide probe that can act as a point of initiation of template-directed DNA synthesis using methods such as PCR (polymerase chain reaction), LCR (ligase chain reaction), etc., for amplification of a target sequence.

[0066] Nucleic acid probes and oligonucleotide primers may also contain modified nucleotides to enhance stability and/or specificity.

[0067] Stat4 gene expression may be measured using any suitable method such as for example, western/immunoblot methods that detect the protein product. Western/immunoblot uses gel electrophoresis to separate native or denatured Stat4 proteins by the length of the polypeptide (i.e., α and the β isoforms) or by the 3-D structure of the protein (native/non-denaturing conditions). The proteins are transferred to a membrane (e.g., nitrocellulose), where they are detected using antibodies specific to the target protein or polypeptides.

[0068] Stat4 gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence Stat4 α and β polypeptides or against a synthetic peptide of Stat4α or β based on the DNA sequences provided herein.

[0069] Stat4 isoforms (proteins) or fragments or polypeptides thereof detection can also be carried in situ or after extraction. Antibodies including monoclonal antibodies against a specific Stat4 isoform are useful in quantifying Stat4α and Stat4β protein levels. Standard immunohistochemistry techniques are capable of detecting the presence and the amount of Stat4α/β antibodies either directed to the full length Stat4 isoforms or a peptide fragment thereof. The antibodies include monoclonal or polyclonal antibodies.

Anti-Stat4 antibodies are available. See e.g., Hoey et al., (2003), incorporated by reference in its entirety, including Stat4α and β specific sequences and antibodies that recognize both the isoforms.

[0070] The term prognosis generally refers to a forecast or prediction of the probable course or outcome of an immune disorder.

[0071] The term predictive marker generally refers to a factor that indicates sensitivity or resistance to a specific treatment. Thus, a predictive marker provides a measure of likelihood of response or resistance to a particular therapy.

[0072] For example, ratio of Stat4α/Stat4β expression is used as both a prognostic marker (e.g., better response for anti-TNF therapy) and as a predictive marker (greater likelihood of response to anti-TNF therapy) for one or more of the immune disorders.

[0073] A sample (also used as a biological sample or tissue or cell sample) generally includes a variety of sample types obtained from an individual and can be used in a diagnostic or monitoring assay. The term includes blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures or cells derived therefrom, and the progeny thereof. A suitable tissue sample is for example, a sample of tissue from the lining of the intestine or biopsy of the gastric antrum. The term also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components, such as proteins or polynucleotides, or embedding in a semi-solid or solid matrix for sectioning purposes. The term biological sample encompasses a clinical sample, and also includes cells in culture, cell supernatants, cell lysates, serum, plasma, biological fluid, and tissue samples. The source of the biological sample may be solid tissue as from a fresh, frozen and/or preserved organ or tissue sample or biopsy or aspirate; blood or any blood constituents; amniotic fluid, peritoneal fluid, or interstitial fluid. The biological sample may contain compounds which are not naturally intermixed with the tissue in nature such as preservatives, anticoagulants, buffers, fixatives, nutrients, antibiotics, or the like. In an embodiment, a suitable sample includes T-cells.

[0074] The term gene generally refers to any polynucleotide sequence or portion thereof with a functional role in encoding or transcribing a protein or regulating other gene expression. The gene may include all the nucleic acids responsible for encoding a functional protein or only a portion of the nucleic acids responsible for encoding or expressing a protein. The polynucleotide sequence may contain a genetic abnormality within exons, introns, initiation or termination regions, promoter sequences, other regulatory sequences or unique adjacent regions to the gene.

[0075] The term antibody generally refers to intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies) formed from at least two intact antibodies, and antibody fragments that are capable of recognizing Stat4 expression products.

[0076] The term treatment or therapy generally relates to an approach for obtaining beneficial or desired clinical results. This includes: inhibiting and/or relieving to some extent one or more of the symptoms associated with the disorder, decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease,

delaying the progression of the disease, and/or mitigating the side-effects associated with the therapies.

[0077] Some of the symptoms for Crohn's disease include for example, abdominal pain, often in the right lower quadrant, and diarrhea. Rectal bleeding, weight loss, arthritis, skin disorders, eye inflammation and fever may also occur. Bleeding may be serious and persistent, leading to anemia. Children with Crohn's disease may have delayed development and stunted growth.

[0078] Symptoms for ulcerative colitis include for example, include rectal bleeding and diarrhea. Symptoms may vary depending on the amount of the colon and rectum that are inflamed and the intensity of inflammation. Ulcerative colitis are generally classified according to the location and the extent of inflammation, including for example, ulcerative proctitis (inflammation that is limited to the rectum), proctosigmoiditis (inflammation of the rectum and the sigmoid colon), pancolitis or universal colitis (inflammation affecting the entire colon), and fulminant colitis (severe form of pancolitis).

[0079] The identification and analysis of molecular markers (expression levels of Stat4 α and β), have numerous therapeutic and diagnostic purposes. Clinical applications include, for example, selection of therapy including dosage, prediction of a therapeutic response; prediction of efficacy of therapy; monitoring of patients' response trajectories (e.g., prior to onset of disease) and/or after the onset of disease; prediction of adverse response; monitoring of therapy associated efficacy and toxicity; prediction of probability of occurrence; recommendation for prophylactic measures; and detection of recurrence.

[0080] The molecular markers disclosed herein may be detected using any suitable conventional analytical technique including but not limited to, immunoassays, protein chips, multiplexed immunoassays, complex detection with aptamers, chromatographic separation with spectrophotometric detection, mass spectroscopy, cDNA microarrays, and nucleic acid probe hybridization.

[0081] Stat4 α /Stat4 β expression in the test biological sample (i.e., the biological sample from a patient having an immune disorder or suspected of having the immune disorder) may be compared to a suitable control sample, as is well known in the art. Exemplary controls include comparable normal samples (e.g., normal tissue or cells of the same type as present in the test biological sample), matched normal samples from a similar patient, universal control samples, or a normal reference value (also termed a control reference value). The term control or control sample may also encompass a normal reference value. Methods for comparison of expression levels (such as presence or absence of or amount of expression) are known in the art.

[0082] As discussed herein, expression in a biological sample can be detected by a number of methods which are well-known in the art, including but not limited to, immuno-histochemical and/or Western analysis, biochemical enzymatic activity assays, in situ hybridization, Northern analysis and/or PCR analysis of mRNAs, and genomic Southern analysis (to examine, for example, gene deletion or amplification), as well as any one of the wide variety of assays that can be performed by gene, protein, and/or tissue array analysis.

[0083] Consists essentially or consisting essentially of refers to a portion of Stat4 α or Stat4 β nucleic acid or amino acid sequences that are specific to each of the isoforms and

may contain other non-specific sequences that do not alter the specific detection capabilities.

EXAMPLES

[0084] The following examples are to be considered as exemplary and not restrictive or limiting in character and that all changes and modifications that come within the spirit of the disclosure are desired to be protected.

Example 1

Determining Expression Levels of Stat4 α and Stat4 β

[0085] Samples from patients exhibiting inflammatory bowel disease-like symptoms were analyzed for the expression levels of Stat4 isoforms. The primer sequences used to amplify and quantify the Stat4 isoforms are as follows:

```
Stat4ex17f                                     (SEQ ID NO: 6)
(5'- TAT CCT GAC ATT CCC AAA GAC -3')
```

(Common forward primer)

```
Stat4ex19r                                     (SEQ ID NO: 7)
(5'- CTC TCA ACA CCG CAT ACA CAC -3')
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(α -specific reverse primer)

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Stat4 $\beta$ r                                       (SEQ ID NO: 8)
(5' GAC TTA CTA TGT CAG GAA CTC -3')
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(β -specific reverse primer).

[0086] This example demonstrates that tested samples from patients suffering from Crohn's disease of ulcerative colitis exhibit different Stat4 β /Stat4 α ratio compared to the control group. Stat4 β /Stat4 α ratios are useful in predicting efficacy of anti-TNF therapy for immune disorders.

Example 2

Clinical Investigation of Stat4 Beta/Alpha Ratios in Patients

[0087] This example illustrates protocols and experimental design to collect data for testing that patients with active Crohn's Disease, and/or ulcerative colitis have a higher β/α ratio than children who are healthy and there will be a change in the β/α ratio in response to corticosteroids or Infliximab treatment.

[0088] For example, in an embodiment, peripheral blood samples are purified over Ficoll to isolate mononuclear cells. Cells are initially frozen in liquid nitrogen aliquoted among 2-4 vials depending on cell number isolated. Once a sufficient number of samples has been collected, 5-10 samples are thawed and RNA is isolated using Trizol. RNA is then used as a template to make cDNA. The cDNA is used in a PCR reaction using primers specific for either the alpha or beta isoform of Stat4 (see Example 1 for an exemplary set of primers). PCR may be performed either semi-quantitatively, using dilutions of the cDNA to quantify mRNA levels, or using real time PCR for quantitative analysis. Relative levels are recorded and when sufficient numbers of samples have been examined, data are used for statistical analysis.

[0089] Peripheral blood samples may also be obtained for IL-12, IL-17 and TNF α levels, Sed rate (ESR) and Hematocrit. If the patients involve children, for example, the pediatric Crohn's disease activity index, the Rachmilewitz Clini-

cal Activity index and the Colitis Symptom Score may also be assessed. See also D'Haens et al. (2007), A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. *Gastroenterology*; 132(4763-786. The above mentioned scores and indices may also be assessed at suitable intervals, e.g., 2-weeks. A signed consent/assent statement and completed eligibility checklist to include the subjects age, gender, and listing of prescription and over the counter medications are obtained from the subject/subject's family prior to analysis.

[0090] Descriptive statistics are calculated including medians, means and standard deviations of the measurements as appropriate for each of the three groups. No parametric distribution may be assumed for β/α ratio, the primary outcome of interest. A two sample Wilcoxon Rank Sum test may be used to assess if patients with active Crohn's Disease, and/or ulcerative colitis have a higher β/α ratio than patients who are healthy. To evaluate the change in the β/α ratio at two weeks post treatment from baseline in response to for example, corticosteroids or Infliximab treatment, Wilcoxon Signed Rank test may be performed. Graphical presentation may also be used to show the patient specific change in β/α ratio. The point estimate and 95% confidence interval for the mean of the β/α ratio may be reported on the log scale by group.

Example 3

Analysis of Stat4 Beta/Alpha Isoform Ratio in IBD

[0091] The expression of Stat4 α and β isoforms in patients suffering from IBD was investigated. As shown in FIG. 16, the graph (FIG. 16A) shows the STAT4 β /STAT4 α ratio in two different control groups (the left infants; the right a group that is age-matched to the patients), Crohn's disease (CD) and ulcerative colitis (UC) patient groups. The STAT4 β /STAT4 α ratio is consistently elevated in the patient samples. The graph (FIG. 16B) shows the Crohn's disease severity score with the β/α ratio divided into groups higher or lower than 10. This example indicates that higher STAT4 β /STAT4 α ratios correlate to the disease symptoms, e.g., Crohn's disease.

Example 4

Stat4 β Transgenic Mice Develop an Exacerbated EAE

[0092] To study the distinct roles played by Stat4 isoforms in autoimmune disease, the development of EAE in transgenic mice was examined that express Stat4 α and Stat4 β directed by the CD2 locus control region backcrossed to a Stat4-deficient background and compared with wild type and Stat4 $^{-/-}$ mice. As shown in FIG. 1A, Stat4 β transgenic mice developed an exacerbated EAE compared to wild type mice. The day of onset and MCS in Stat4 β transgenic mice was similar to wild type mice in the early phase of EAE, but the MCS continued to worsen in Stat4 β transgenic mice in the later phase of EAE. In contrast, Stat4 deficient mice remained resistant to EAE and Stat4 α transgenic mice developed mild EAE with delayed onset and earlier remission than the Stat4 β transgenic and wild type mice (FIG. 1A). The Stat4 β transgenic mice also showed a significant increase in AUC, MMCS and AMCS than the wild type and Stat4 α transgenic mice. These results show that Stat4 β transgenic mice develop an exacerbated EAE compared to Stat4 α transgenic or wild type mice and suggest the distinct abilities of Stat4 isoforms to mediate the pathogenesis of EAE.

[0093] To confirm that Stat4 α transgenic and Stat4 $^{-/-}$ mice were sensitized to MOGp35-55 peptide, the antigen-induced T cell proliferation ex vivo was measured. As shown in FIG. 1B, in vitro culture of spleen cells from wild type, Stat4-deficient, Stat4 α transgenic and Stat4 β transgenic mice showed a dose-dependent proliferation in response to MOGp35-55 antigen with the Stat4 β transgenic mice displaying a slightly decreased proliferation compared to cells from mice of the other genotypes. These results indicate that the lack of disease in Stat4 α transgenic and Stat4 $^{-/-}$ mice is not due to the lack of development of MOG-specific T cell responses.

Example 5

Stat4 β Transgenic Mice Develop Severe Inflammation and Demyelination in the CNS

[0094] To further establish the differential regulation of EAE by Stat4 isoforms, the pathology of CNS inflammation and demyelination were examined. As shown in FIG. 2, the wild type mice with EAE showed extensive myelin loss (demyelination) associated with infiltration of immune cells (inflammation) in the spinal cord. When compared with wild type, the Stat4 β transgenic mice with EAE showed a significant increase in the extent of inflammation and demyelination in the spinal cord. However, the Stat4 $^{-/-}$ and Stat4 α transgenic mice induced to develop EAE showed no sign of inflammation or demyelination in the CNS. Therefore, T cells expressing Stat4 β caused more CNS pathology compared to T cells lacking Stat4 or those expressing Stat4 α .

[0095] Histological analyses revealed that wild type mice with EAE display 18% and 21% spinal cord quadrants positive for demyelination and inflammation, respectively (FIG. 2). When compared with wild type mice, the Stat4 β transgenic mice developed severe pathology with 71% (4 fold increase; $p < 0.01$) and 61% (3 fold increase; $p < 0.01$) spinal cord quadrants positive for demyelination and inflammation, respectively. The Stat4 α transgenic mice developed very mild CNS pathology with 3% and 1.47% spinal cord quadrants positive for demyelination and inflammation respectively. The Stat4 $^{-/-}$ mice failed to show any symptoms of CNS pathology (FIG. 2). These results indicate that the clinical symptoms of EAE correlate with the pathology of CNS inflammation and demyelination in Stat4 α and Stat4 β transgenic mice.

Example 6

Stat4 β Transgenic Mice with EAE Express Elevated Levels of Effector T Cell-Derived Inflammatory Cytokines in the CNS and Lymphoid Organs

[0096] The mechanism in the differential regulation of EAE in Stat4 α and Stat4 β transgenic mice was analyzed. As mice that are deficient in Stat4 have multiple defects in Th1 differentiation, Th17 function, migration and adhesion of T cells to inflamed sites, the analysis was focused on comparing the Stat4 α and Stat4 β transgenic immune cells where differences likely reflect specific effects of the isoforms. Th1 differentiation in vitro is largely similar in Stat4 α and Stat4 β transgenic cells. The expression of effector T cell-derived inflammatory cytokines in the CNS, spleen and spleen cells cultured with antigen was measured. The levels of IFN γ and IL-17 mRNA detected in the brain and spleen of mice with EAE were significantly increased over unimmunized naïve

mice and largely correlated to disease severity with tissues from wild type or Stat4 β transgenic mice having the highest levels (FIG. 3). Expression of the Th1 transcription factor T-bet also correlated with IFN γ expression in tissues from Stat4 β transgenic mice though less well in tissues from wild type mice. The mRNA levels of cytokines from antigen stimulated spleen cells were somewhat different with higher levels of IFN γ observed in wild type and Stat4 β transgenic cultures but higher IL-17 in Stat4 α transgenic cultures (FIG. 3). Thus, while the expression of IFN γ and IL-17 in the CNS correlated with disease severity, differences in cytokine profile between Stat4 α and Stat4 β transgenic mice indicate that Stat4 isoforms may differentially regulate cytokine production in EAE.

Example 7

Stat4 β Transgenic Mice with EAE Express Elevated Levels of APC-Derived Inflammatory Cytokines in the CNS and Lymphoid Organs

[0097] To further define the mechanism in the differential regulation of EAE in Stat4 α and Stat4 β transgenic mice, the expression of antigen presenting cell-derived inflammatory cytokines in the CNS and lymphoid organs was examined. As shown in FIG. 4, the wild type and Stat4 β transgenic mice with EAE showed an increased expression of IL-12p35, IL-12p40 and IL-23p19 in the brain and spleen compared to naïve mice. Stat4 α transgenic mice with EAE showed little or no increase in the expression of IL-12p35, IL-12p40 or IL-23p19 in the brain and spleen. Interestingly, the levels of IL-12p35 mRNA correlated well with IFN γ mRNA levels and the levels of IL-12p40 and IL-23p19 mRNA correlated with IL-17 mRNA levels in both brain and spleen (compare FIG. 4 to FIG. 3) and with the clinical and pathological symptoms of EAE in Stat4 α and Stat4 β transgenic mice.

Example 8

IFN γ Production in the Periphery Correlates with the Severity of EAE in Stat4 β Transgenic Mice

[0098] To determine if the differences observed in mRNA expression in FIGS. 3-4 results in differential cytokine production, IFN γ and IL-17 production by intracellular cytokine staining (FIG. 5) and ELISA (FIG. 6) were examined. Cells isolated from CNS, spleen or draining LN were stimulated with PMA and ionomycin before intracellular staining with anti-IL-17 and anti IFN γ antibodies in CD4 $^{+}$ cells. Early cytokine production in WT, Stat4 α and Stat4 β transgenic cells were not substantially different, with Stat4 β transgenic cells having a slightly greater propensity for IFN γ production (FIG. 5). Moreover, while there was decreased inflammation in the Stat4 α transgenic CNS (FIG. 2), Stat4 α transgenic T cells in the CNS were capable of producing IL-17 and IFN γ at levels similar to wild-type cells (FIG. 5). In response to antigen stimulation, spleen cells from wild type and Stat4 β transgenic mice produced higher levels of IFN γ than Stat4 α transgenic cells, while, Stat4 α transgenic cells produced more IL-17 than either wild type or Stat4 β transgenic cells (FIG. 6). IL-12 or IL-23 production from antigen-stimulated spleen cells was not detected. These results highlight that the decreased disease in Stat4 α transgenic mice is not due to an inability to develop inflammatory cell types in vivo.

[0099] To identify other genes that demonstrate Stat4-dependence, a Stat4 ChIP-on-chip experiment was performed.

IL-10 was identified in this analysis and bound by Stat4 in the second and third introns (FIG. 7A). Wild type and Stat4 $^{-/-}$ Th1 culture stimulated with either IL-12 or anti-CD3 demonstrated Stat4-dependence in the induction of IL10 production (FIG. 7B). As IL-10 is an important regulatory cytokine that inhibits the development of EAE, the regulation of IL-10 was examined by Stat4 isoforms. Wild type, Stat4 α transgenic and Stat4 β transgenic cells re-stimulated with anti-CD3 after culture under Th1 conditions demonstrated similar production of IFN γ (FIG. 7C). In contrast, Stat4 β transgenic cells had decreased IL-10 production in these cultures (FIG. 7C). To test if this phenotype was reflected in vivo during disease, RNA from wild type, Stat4 α transgenic and Stat4 β transgenic mice were tested for IL10 expression in situ. Three-five fold more IL10 mRNA was detected in Stat4 α transgenic samples than in wild type or Stat4 β transgenic samples (FIG. 7D). Splenic cultures from wild type, Stat4 α transgenic and Stat4 β transgenic were examined to assay for IL-10 production following stimulation as in FIG. 6. While wild type and Stat4 β transgenic cells had similar IL-10 production, Stat4 α transgenic cells produced 2-3-fold higher levels of IL-10 (FIG. 7E). Thus, Stat4 α -expressing T cells have an increased propensity for IL-10 production and this is associated with decreased CNS inflammation and pathology in Stat4 α transgenic mice, compared to wild type or Stat4 β transgenic mice.

Example 9

Th1 Cells Expressing Stat4 β Secrete Significantly More TNF- α Upon TCR Stimulation than Stat4 α Expressing Th1 Cells

[0100] T cells expressing either Stat4 α or STAT4 β could differentiate into Th1 cells, Stat4 α was more efficient than STAT4 β in the induction of IFN- γ following IL-12 stimulation. Supernatants from naïve CD4 $^{+}$ T-cells undergoing Th1 differentiation in the presence of IL-12 for IFN- γ production (FIG. 8A) were examined. There was significantly less IFN- γ present in the supernatant throughout the differentiation period in Stat4 β -expressing and Stat4-deficient cultures. Despite these differences, upon antiCD3 stimulation of differentiated Th1 cells, there were no significant differences in IFN γ production between the isoforms (FIG. 8B). These results indicate that the differences in endogenous IFN- γ production stimulated by the Stat4 isoforms during the differentiation period, did not affect the process of differentiation.

[0101] Although IFN- γ levels were not different between Stat4 α - and Stat4 β -expressing Th1 cells, the levels of other cytokines were examined. The dependence of TNF- α production on Stat4 either in vitro or in vivo during the development of disease is not clear. To examine Stat4-dependent TNF- α production, wild type and Stat4-naïve CD4 $^{+}$ T-cells were cultured in Th1 priming conditions for five days. At the end of the five-day culture, the cells were stimulated with IL-12, IL-12+IL-18, anti-CD3 or PMA+Ionomycin and analyzed for TNF- α and IFN- γ production. Maximal TNF- α production, as assessed by intracellular cytokine staining and mRNA levels, was dependent upon STAT4 (FIGS. 8C and D). While the percentage of TNF- α positive CD4 $^{+}$ T-cells did not differ drastically between wild type and Stat4 $^{-/-}$ cells, the mean fluorescence intensity (MFI) at 4 hours and the secretion of TNF- α over a 24-hour time period showed TNF- α production significantly reduced in the absence of Stat4 (FIGS. 8C and E). In contrast, TNF- α production was not detected following stimulation with IL-12, in the presence or absence of IL-18.

[0102] Having demonstrated the STAT4-dependence in TNF- α production, the ability of the STAT4 isoforms to prime Th1 cells to secrete TNF- α was examined. Naïve CD4⁺ T-cells expressing either STAT4 α or STAT4 β were cultured under Th1 culture conditions for five days and stimulated with anti-CD3 before examining the levels of TNF- α and IL-2 using ELISA. The Th1 cells expressing STAT4 β consistently secreted significantly more TNF- α compared to the CD4⁺ T-cells expressing STAT4 α while IL-2 levels between cells expressing the STAT4 isoforms were similar (FIG. 8E). Similar to the data for Stat4^{-/-} cells, decreased TNF- α production from STAT4 α -expressing Th1 cells was due to decreased TNF- α per cell compared to STAT4 β cultures, with only minor differences in the percentage of TNF- α ⁺ cells, as assessed by intracellular cytokine staining. These results indicate that IL-12 stimulation of STAT4 β differentially programs the developing Th1 cells to secrete more TNF- α and that this programming is specific and independent of the concentration of IFN- γ throughout the culture period. Thus, these data indicate that STAT4 isoforms dictate differential cytokine expression in Th1 cells.

[0103] To determine if differential activation of STAT4 contributed to the production of distinct Th cytokines, developing Th1 cultures were stained for phospho-STAT4 (pSTAT4) levels over the first three days of culture. Wild type and STAT4 β -expressing cells showed similar percentages of pSTAT4⁺ cells at all of the time points examined (FIG. 9A). In contrast, there was less pSTAT4 in STAT4 α transgenic cells than in wild type cells or STAT4 β transgenic cells at all of the time points (FIG. 9A). During this time period there were modest changes in the expression of total STAT4 in each of the cell types (FIG. 9B). After five days of differentiation, IL-12 stimulation resulted in greater induction of pSTAT4 in wild type and STAT4 β -expressing cells than in STAT4 α -expressing cells, despite similar levels of total STAT4 expression (FIGS. 9B and C). Moreover, STAT4 expression did not change over the course of the stimulation. STAT4 α phosphorylation decreased over time while STAT4 β phosphorylation stayed constant over the 48-hour assay period (FIG. 9C). Despite lower levels of pSTAT4 α during Th1 differentiation and following IL-12 restimulation, STAT4 α was still more potent than STAT4 β in the acute production of IFN- γ (FIG. 9D). These data indicate that the differential activation of the isoforms in response to IL-12 can contribute to differential gene expression but that the amount of activated STAT4 does not directly correlate with IFN- γ gene transcription.

Example 10

STAT4 Isoforms are Equally Efficient in Promoting Th17 Differentiation

[0104] IL-23 also activates STAT4 and induces Th17 cells to secrete IL-17. The ability of Th17 cells expressing STAT4 isoforms to secrete IL-17 and TNF- α was examined. Naïve T-cells were differentiated with TGF- β 1, IL-6, and IL-23 for five days and stimulated cells with anti-CD3 or PMA+Ionomycin (FIG. 10A). There were no significant differences between the percentage of TNF- α positive cells in Th17 cells expressing either isoform although the percentage of TNF- α positive cells was considerably higher following PMA+ionomycin stimulation, compared to anti-CD3 (FIG. 10A). The Th17 cells expressing either isoform had similar capabilities to produce IL-17. Because generation of Th17 cells by TGF β +IL-6 is independent of STAT4, the effects of culture

with IL-23 on IL-17 production from STAT4 isoform expressing T cells were examined. After a week of culture in IL-23 cells were restimulated with anti-CD3 and IL-17 production was analyzed using ELISA. There was also no defect in IL-17 production from T cells expressing either STAT4 isoform, and production was increased compared to wild type cells (FIG. 10B). To assess the responsiveness of the STAT4 isoforms to IL-23-induced cytokine production, IL-17 levels were examined by ELISA after 24 hours of stimulating the cells with IL-23 and IL-18 (FIG. 10C). T cells expressing the STAT4 α isoform secreted similar amounts to wild type cells and significantly more IL-17 than cells expressing the STAT4 β isoform. Thus, while either STAT4 isoform is sufficient for the generation of Th17 cells, activation of STAT4 α by IL-23 can more efficiently induce IL-17 than the STAT4 β isoform.

Example 11

STAT4 β Promotes More Severe Colitic Inflammation than STAT4 α

[0105] Since some differences were observed in the ability of T cells expressing STAT4 α or STAT4 β to secrete inflammatory cytokines, the ability of the T-cells expressing each isoform was tested to mediate inflammation. Therefore, SCID mice were reconstituted with CD4⁺CD45RB^{high} or CD4⁺CD45RB^{low} T-cells that expressed either STAT4 α or STAT4 β and examined the weight loss kinetics of the mice. There was no significant difference in the kinetics of weight loss or the end point weight loss between the SCID mice reconstituted with either isoform or wild type mice (FIG. 11A). However, there was a significant difference between the weight loss of mice reconstituted with the CD4⁺CD45RB^{high} cells compared to the mice reconstituted with CD4⁺CD45RB^{low} cells, indicating that the CD4⁺CD45RB^{high} T-cells expressing either isoform were sufficient to induce colitis (FIG. 11A). As wild type mice had the same overall disease course as STAT4 isoform-expressing cells, the comparison between cells expressing the transgenic STAT4 isoforms was analyzed. To determine if the differences in T cell proliferation between the STAT4 isoforms resulted in differences in cell reconstitution in vivo, the absolute CD4⁺ cell numbers were determined in MLN cells and the percentage of CD4⁺ T-cells in the splenocytes and observed no significant difference between the repopulation efficiency of the CD4⁺ T-cells expressing either isoform (FIG. 11B). Similar to protein levels seen in FIG. 9B, STAT4 mRNA expression was slightly higher in STAT4 β -expressing cells than STAT4 α -expressing cells in vivo (FIG. 11B).

[0106] Although weight loss was not significantly different between the SCID mice reconstituted with either STAT4 isoform, gross examination of the colon and scoring of the slides showed that the SCID mice reconstituted with the CD4⁺CD45RB^{high} cells expressing the STAT4 β isoform had more significant mucosal inflammation than the SCID mice reconstituted with the STAT4 α as assessed by area and severity of the lesion (FIG. 4C-E). There was no difference in mucosal hyperplasia between the mice reconstituted with STAT4 α or STAT4 β expressing T cells. Importantly, SCID mice reconstituted with the CD4⁺CD45RB^{low} cells had essentially no inflammatory infiltrates into the tissues (FIG. 11E).

Example 12

STAT4 β Expressing T-Cells from Colitic Mice have Increased Inflammatory Cytokine Production Compared to Mice Reconstituted with STAT4 α T-Cells

[0107] To examine whether the increased histological inflammation seen in the SCID mice reconstituted with the

STAT4 β expressing T-cells correlated with increased TNF- α production, isolated splenocytes and MLN cells were stimulated with anti-CD3 to assess ex vivo TNF- α production (FIG. 12A). The SCID mice reconstituted with the STAT4 β expressing T-cells had significantly more TNF- α compared to the mice adoptively transferred with the STAT4 α T-cells upon stimulation with anti-CD3. SCID mice reconstituted with CD4+CD45RB^{low} from either isoform had barely detectable TNF- α that was significantly less than the cells isolated from the SCID mice reconstituted with the CD45RB^{high} subset of cells.

[10108] To determine if the STAT4 isoforms differentially regulated other cytokines in vivo, T-cell produced cytokines were examined that have been implicated in the pathogenesis of colitis, including IFN- γ , IL-6, IL-10, and IL-17 (FIG. 12B). Corresponding to the level of inflammation, SCID mice reconstituted with the STAT4 β expressing T-cells had more inflammatory cytokine production. IFN- γ production was significantly increased from STAT4 β expressing cells compared to STAT4 α expressing cells from either spleen or MLNs. IL-6 production in the spleen was also increased but not in the MLNs of mice reconstituted with the STAT4 β T-cells. IL-17 did not significantly differ between SCID mice reconstituted with T-cells expressing either isoform, and IL-10 was detected at higher levels from MLNs in mice reconstituted with STAT4 α -expressing T cells but there was no significant difference in production detected from spleen cells (FIG. 12B). There was no significant difference in TGFP1 expression in MLN.

[10109] Since data in FIGS. 2-3 show that STAT4 α is more efficient than STAT4 β in cytokine stimulated production of IFN- γ and IL-17, the MLN cells from colitic mice were examined for their ability to produce these cytokines following treatment with IL-12 and IL-18 or IL-23 and IL-18 for 72 hours. While the IL-23 and IL18 stimulated cells from the SCID mice reconstituted with STAT4 α secreted more IL-17, similar to results from in vitro differentiated cells, there was no significant difference in the amount of IFN- γ secreted from the cells isolated from the SCID mice reconstituted with either isoform (FIG. 12C). Overall, these data indicate that the increased inflammatory disease caused by STAT4 β -expressing T cells correlates with increased inflammatory cytokine production.

[10110] TNF- α and GM-CSF are important in neutrophil chemotaxis to inflamed tissues. To examine whether the increased TNF- α secretion from STAT4 β expressing T-cells correlated with increased neutrophils in the lamina propria, microscopic sections of the colon for PMN infiltration were examined. Consistent with the increased TNF- α seen in the SCID mice reconstituted with the STAT4 β isoform, there were also increased neutrophils present in the lamina propria compared to the SCID mice reconstituted with STAT4 α (FIG. 13A). Since anti-TNF therapies have been shown to inhibit GM-CSF production, GM-CSF levels in the mice with colitis were analyzed. Supernatants from stimulated MLN cell cultures were examined to assess GM-CSF production (FIG. 13B). Consistent with the increased neutrophil infiltration, GM-CSF was significantly increased from STAT4 β -expressing T cells, further supporting the ability of T-cells expressing the STAT4 β isoform to mediate potent inflammatory responses.

[10111] Since there was increased GM-CSF production from STAT4 β -expressing cells ex vivo, it was examined whether this reflected an increased propensity for STAT4 β

expressing T cells to produce GM-CSF or whether it was a result of the in vivo inflammatory environment. To test this, naïve T-cells were isolated expressing either isoform and differentiated them in Th1 or Th17 conditions for five days and stimulated them with anti-CD3 to examine their ability to secrete GM-CSF. Production of GM-CSF in Th1 cultures was dependent upon STAT4 (FIG. 14a). The STAT4 β expressing Th1 cells secreted significantly more GM-CSF than STAT4 α expressing Th1 cells. In contrast, there was no STAT4 dependence for GM-CSF production from Th17 cells and no significant difference in the amount of GM-CSF produced by Th17 cells expressing either Stat4 isoform (FIG. 14B). No detectable GM-CSF was secreted upon acute stimulation with IL-12 or IL-23 with or without IL-18 suggesting that STAT4 does not directly induce transcription of GM-CSF. Together, these data demonstrate the increased inflammatory propensity of T cells expressing STAT4 β and suggest that the increased inflammatory cytokine production by STAT4 β -expressing T cells results in greater inflammatory disease in vivo.

Materials and Methods

[10112] Animals: The C57BL/6 mice were purchased from Harlan (Indianapolis, Ind.). The Stat4 $^{-/-}$ mouse in C57BL/6 background was generated as described earlier (26, 30). The transgenic mice expressing Stat4 α or Stat4 β genes were generated as described earlier (26). The mice were maintained in the animal care facility at Methodist Research Institute. All animal protocols used in the experiments were approved by the Institutional Animal Care and Use Committee.

[10113] Reagents: The 21 amino acid peptide [MEVGWYRSPFSRVVHLYRNGK] (SEQ ID NO: 9) corresponding to mouse MOGp35-55 was obtained from Genemed Synthesis, Inc. (San Francisco, Calif.). Murine recombinant IL-17, IFN γ and IL-10 were purchased from R&D Systems, Inc. (Minneapolis, Minn.). The biotin/FITC-conjugated anti-IL-17, anti-IFN γ and anti-IL-10 antibodies were purchased from e-Bioscience. All reagents for qRT-PCR were purchased from Applied Biosystems (Foster City, Calif.).

[10114] Induction of EAE: To induce EAE, 4 to 6 weeks old female mice (5 per group) were immunized (s.c.) with 100 μ g of MOGp35-55 peptide antigen in 150 μ l emulsion of Incomplete Freund's Adjuvant containing 50 μ g/ml heat-killed *Mycobacterium tuberculosis* (H37Ra, Difco Laboratories, Detroit, Mich.) in the lower dorsum on days 0 and 7. The mice also received (i.p) 100 ng of pertussis toxin (Sigma Chemicals, St Louis, Mo.) on days 0 and 2. The clinical symptoms were scored every day from day 0 to 30 in a blinded manner as follows; 0, normal; 0.5, stiff tail; 1, limp tail; 1.5, limp tail with inability to right; 2, paralysis of one limb; 2.5, paralysis of one limb and weakness of one other limb; 3, complete paralysis of both hind limbs; 4, moribund; 5, death. Mean clinical score (MCS) was calculated by adding every day clinical score for all mice in a group and then divided by total number of mice. Mean maximum clinical score (MMCS) was the MCS at the peak of disease. Average mean clinical score (AMCS) was calculated by adding the MCS for all days (from day 0 to 30) and then divided by number of days. The mean clinical score more than one (MCS>1) was obtained by counting the number of days with MCS more than one. The area under the curve (AUC) was calculated using GraphPad Prism 5.0 Software.

[10115] Histological analysis: The mice induced to develop EAE were euthanized on day 30 by CO₂ asphyxiation and

perfused by intracardiac infusion of 4% paraformaldehyde and 1% glutaraldehyde in PBS. Brain and spinal cord samples were removed and fixed in 10% formalin in PBS. Tissues were processed and transverse sections from cervical, upper thoracic, lower thoracic and lumbar regions of the spinal cord were stained with Luxol Fast Blue or hematoxylin and eosin. Inflammation and demyelination in the CNS were assessed under microscope in a blinded manner. The spinal cord sections were viewed as anterior, posterior and two lateral columns (4 quadrants). Each quadrant displaying the infiltration of mononuclear cells or loss of myelin was assigned a score of one inflammation or one demyelination, respectively. Thus, each animal has a potential maximum score of 16 and this study represents the analysis of spinal cords from 5 mice per group. The pathological score from each group is expressed as percent positive over total number of quadrants examined.

[0116] Quantitative real-time polymerase chain reaction: The quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) was performed using the ABI Prism 7900 Fast Sequence Detection System (Applied Biosystems, Foster City, Calif.) according to manufacturer's instructions. The brain and spleen samples were isolated on day 14 following induction of EAE. Spleen cells were cultured in 24 well tissue culture plates in RPMI medium with 10 μ g/ml MOGp35-55 antigen or 5 μ g/ml Con A for 24 hrs. Total RNA was extracted from brain, spleen, and cultured spleen cells using TRIzol reagent (Invitrogen, Carlsbad, Calif.) according to manufacturer's instructions. The RNA samples (5 μ g/100 μ l reaction) were reverse transcribed into cDNA (RT-PCR) using random hexamer primers and TaqMan reverse transcription kit (Applied Biosystems, Foster City, Calif.). The cDNA (2 μ l/sample) was subjected to qPCR analysis in triplicate using forward and reverse primers, TaqMan Universal Master Mix and probe (10 μ l/reaction) in fast optical 96-well plate. Controls include RT-PCR in the absence of RNA and real-time PCR in the absence of cDNA. The data were analyzed using the ABI Prism 7900 relative quantification (delta-delta-Ct) study software (Applied Biosystems, Foster City, Calif.). In this study primer sets for 10 selected inflammatory genes were used with GAPDH (Applied Biosystems, Foster City, Calif.) as internal controls. The expression levels of inflammatory genes normalized to GAPDH are presented as arbitrary fold changes compared to control samples.

[0117] T cell proliferation assay: T cell proliferation was measured by WST-1 assay (Roche, Indianapolis, Ind.). The spleen cells were isolated on day 14 following induction of EAE and cultured in 96 well tissue culture plates in RPMI medium (1×10^5 /200 μ l/well) with 0, 1, 5 and 10 μ g/ml MOGp35-55 peptide. WST-1 reagent (10 μ l/well) was added after 72 hrs of culture and the absorbance was measured at 450 nm using 2100 microplate reader (Alpha Diagnostics Inc., San Antonio, Tex.) as a measure of viable cell count.

[0118] Intracellular cytokine staining: Spleen, lymph node and brain cells isolated on day 14 following induction of EAE were cultured in 24 well tissue culture plates in RPMI medium (1×10^6 /ml) in the presence of 10 μ g/ml MOGp35-55 antigen or 5 μ g/ml Con A for 24 hrs. Monensin (2 μ M) was added during the last 4 hrs to block protein secretion. The cells were isolated fixed and permeabilized by incubating in PBS containing 1% paraformaldehyde and 0.02% Triton X-100 at 4° C. for 15 min. After washing in PBS, the cells were stained with fluorochrome conjugated IL-17 and IFN γ

antibodies at 4° C. for 30 min and analyzed using a three color FACSCanto flow cytometer to determine the percentage of cells expressing cytokines.

[0119] ELISA for IFN γ , IL-17 and IL-10: To determine the cytokine response, spleen cells from MOGp35-55-sensitized mice were cultured in 24 well plates in RPMI medium (1×10^6 /ml) in the presence of 0 and 10 μ g/ml MOGp35-55 or 5 μ g/ml Con A. The culture supernatants were collected after 48 hrs and the levels of IFN γ , IL-17 and IL-10 measured by ELISA. Briefly, 96-well ELISA plates were coated with 2 μ g/ml of anti-IL-17 or anti-IFN γ capture antibody in 100 μ l/well of 0.06 M Carbonate buffer, pH 9.6. After overnight incubation at 4° C., the excess Abs were washed off and the residual binding sites blocked by the addition of 100 μ l/well of 1% BSA in PBS for 1 h. The test samples (culture supernatants) and standards (rIL-17, rIFN γ , rIL-10) were added and incubated at 4° C. overnight. Plates were washed with PBS containing 0.05% Tween-20 and 0.2 μ g/ml of biotin-conjugated anti-IL-17, anti-IFN γ or anti-IL-10 added as detection antibody. After incubation at room temperature for 1 h, the plates were washed three times and avidin-alkaline phosphatase added followed by 1 mg/ml of p-nitrophenyl phosphate. After 30 min incubation at room temperature, the absorbance was read at 405 nm and the concentrations of IL-17, IFN γ and IL-10 in the culture supernatants were calculated from the standard curve. For some experiments, CD4+ T cells were cultured under Th1 conditions.

[0120] Statistical analysis: All the experiments were repeated two or three times and the values are expressed as mean $\pm$ SD. The differences between groups were analyzed by ANOVA and the values of $p < 0.05$ were considered significant.

[0121] A two sample Wilcoxon Rank Sum test is used to assess if patient samples with active Crohn's Disease, and/or ulcerative colitis have a higher α/β ratio than controls who are healthy. To evaluate the change in the α/β ratio at e.g., two weeks post treatment from baseline in response to e.g., corticosteroids or Infliximab treatment, Wilcoxon Signed Rank test is performed. Graphical presentation is also be used to show the patient specific change in α/β ratio. The point estimate and 95% confidence interval for the mean of the α/β ratio is reported on the log scale by group.

[0122] Isolation of CD45RB^{hi} and CD45RB^{low}CD4+ T-cells and Induction of Colitis by Cell Transfer: Spleen and lymph node cells were used as a source of CD4+ cells for reconstitution of B6 SCID recipient mice. CD4+ T-cells were isolated as previously described (28). The enriched CD4+ T-cells were then labeled for cell sorting with FITC-conjugated CD4 and PE-conjugated CD45RB (BD Pharmingen). Subsequently, cells were sorted under sterile conditions by flow cytometry for CD4+CD45RB^{hi} on a FACS Vantage machine (Becton Dickinson). The CD45RB^{high} and CD45RB^{low} populations were defined as the brightest staining 10-15% and the dimmest staining 15-20% CD4+ T cells, respectively. Intermediate staining populations were discarded. All populations were >99% pure on re-analysis. The purified CD45RB^{hi}CD4+ (4×10^5) cells diluted in 200 μ l of PBS were injected intraperitoneally into B6 SCID recipient mice. A separate group of B6 SCID mice received CD45RB^{low}CD4+ (4×10^5) cells as a negative control. The recipient mice were weighed initially, then weekly thereafter. The animals were sacrificed 14 weeks after transfer.

[0123] Macroscopic and microscopic assessment of Colon Appearance: Once the animals were sacrificed, tissue

samples were taken from each segment of the colon (cecum, ascending, transverse, and descending colon and rectum) and fixed in 10% neutral buffered formalin. The samples were routinely processed, sectioned at 5 μ m thickness, and stained with hematoxylin and eosin (H&E) for light microscopic examination. The slides were evaluated by light microscopy in a blind fashion using a semi-quantitative scoring system. In brief, four general criteria were evaluated in all sections: (1) severity, (2) degree of mucosal hyperplasia, (3) degree of ulceration, if present, and (4) percentage of area involved. The score was then determined from each slide by the following mathematical formula: ((inflammation score+ulceration score+hyperplasia score) $\times$ (Area involved score)) for a score

range of 0-27. Scores from each section of the colon were averaged to determine the overall histological score per experimental group. Histological grades were assigned in a blinded fashion. For scoring the lamina propria neutrophils, the following scoring system was used and scores were averaged from 5-10 high-powered fields: 0-0-5 PMNs, 1-6-10 PMNs, 2-11-20 PMNs, 3-21 PMNs and above.

[0124] Blood samples: Peripheral blood samples is also obtained for IL-12, IL-23, IL-17 and TNF α levels, Sed rate (ESR) and Hematocrit. The pediatric Crohn's disease activity index, the Rachmilewitz Clinical Activity index and the Colitis Symptom Score is also assessed.

Stat4 α isoform nucleotide sequence (2247 nt)

(SEQ ID NO: 1)

ATGTCTCAGTGGAAATCAAGTCCAACAGTTAGAAATCAAGTTTTTGGAGCAGGTGGATCAATCTATGATG
 ACAACTTTCCCATGGAAATTCGGCATCTGTTGGCCCAATGGATTGAAAATCAAGACTGGGAGGCAGCTTC
 TAACAATGAAACCATGGCAACGATCTTCTTCAAACTTGTTAATACAACTGGATGAACAGTTAGGTCGT
 GTTTCCAAAGAGAAAAACCTACTCTTGATACACAATCTAAAAAGAATTAGGAAGGTCCTTCAGGGAAGAA
 TTTCATGGAAATCCAATGCATGTAGCTGTGGTTATTTCAAACGTGTTAAGGGAAGAGAGAGAATATTGGC
 TGCAGCCAAACATGCCTGTCCAGGGGCCTCTAGAGAAATCCTTACAAGTCTTCAGTTTCAGAAAGACAG
 AGGAATGTGGAGCACAAAGTGGCTGCCATTAAAAACAGTGTGCAGATGACAGAACAGATACCAAATACT
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 GGCTGTTGCTAAAGGATAAAATGCCTGGCACCTTTTATTAAGATTCAAGTGAAGCCATCTCGGAGGAAT
 AACTTTCACCTGGGTGGACATTCTGAAAGTGGGAAGTGAGATTCCACTCTGTAGAACCCTACAATAAA
 GGCCGGTTGTCTGCTCTGCCATTGCTGACATCCTGCGAGACTACAAAGTTATTATGGCTGAAAACATTC
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STAT4 β Nucleotide Sequence

(SEQ ID NO: 2)

ATGTCCTCAGTGGAAATCAAGTCCAACAGTCTAGAAATCAAGTTTGTGGAGCAGGTGGATCAATTCTATGATC
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 TGAAGTCTCCTTATTCTGTGAATGA

STAT413-specific exon sequence

(SEQ ID NO: 3)

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 aaggctagccttcagggcacacttaccacaatctattgtgcctaaaattataaaatcccccttta
 tatgccatatatgccacagtaagttgagtggtctgatatgaaatgatgaattagataactcaatgt
 cacaatatagatgaagccctagaaatgagttcctgacatagtaagtcaccgtgaactattattattt
 tttaatccttgccatattgaccttggttatctctttaag

STAT4 α Amino Acid Sequence

(SEQ ID NO: 4)

MSQWNQVQQL EIKFLEQVDQ FYDDNFPMEI RHLLAQWIEN QDWEAASNNE TMTILLQNL
 70 80 90 100 110 120
 LIQLDEQLGR VSKEKNLLLI HNLKRIRKVL QGKFHGNPMH VAVVISNCLR EERRILAAAN
 130 140 150 160 170 180
 MPVQGPLEKS LQSSSVSERQ RNVEHKVA AI KNSVQMT EQD TKYLEDLQDE FDYRYKTIQT
 190 200 210 220 230 240
 MDQSDKNSAM VNQEVLTLQE MLNSLDFK RK EALSKMTQII HETDLLMNTM LIEELQDWKR
 250 260 270 280 290 300
 RQQIACIGGP LHNGLDQLQN CFTLLAESLF QLRRQLEKLE EQSTKMTYEG DPIPQMORTHM
 310 320 330 340 350 360
 LERVTFLLIYN LFKNSPVVER QPCMPHTPQR PLVLKTLIQF TVKLRLLLIKL PELNYQVKVK
 370 380 390 400 410 420
 ASIDKNVSTL SNRRFVLCGT NVKAMSIEES SNGSLSV EFR HLQPKEMKSS AGGKGNEGCH
 430 440 450 460 470 480
 MVTEELHSIT FETQICLYGL TIDLETSSLP VVMISNVSQL PNAWASIIWY NVSTNDSQNL
 490 500 510 520 530 540
 VFFNPPPAT LSQLEVMW QFSSVGRGL NSDQLHMLAE KLTQSSYS D GHLTWAKFCK
 550 560 570 580 590 600
 EHLPGKSFTF WTWLEAILDL IKKHILPLWI DGYVMGFVSK EKERLLKDK MPGTFLLRFS
 610 620 630 640 650 660
 ESHLGGITFT WVDHSESGEV RFHSVEPYNK GRLSALPFAD ILRDYKVIMA ENIPENPLKY
 670 680 690 700 710 720
 LYPDIPKDKA FGKHYSSQPC EVSRPTERGD KGYVPSVFIP ISTIRSDSTE PHSPSDL LPM
 730 740
 SPSVYAVLRE NLSPTTIETA MKSPYSAE

STAT4 β -Amino Acid Sequence

(SEQ ID NO: 5)

MSQWNQVQQL EIKFLEQVDQ FYDDNFPMEI RHLLAQWIEN QDWEAASNNE TMTILLQNL
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 LIQLDEQLGR VSKEKNLLLI HNLKRIRKVL QGKFHGNPMH VAVVISNCLR EERRILAAAN
 130 140 150 160 170 180
 MPVQGPLEKS LQSSSVSERQ RNVEHKVA AI KNSVQMT EQD TKYLEDLQDE FDYRYKTIQT
 190 200 210 220 230 240
 MDQSDKNSAM VNQEVLTLQE MLNSLDFK RK EALSKMTQII HETDLLMNTM LIEELQDWKR

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      310      320      330      340      350      360
LERVTFLIYN LFKNSFVVER QPCMPHPQR PLVLKTLIQF TVKLRLLIK L PELNYQVKVK

      370      380      390      400      410      420
ASIDKNVSTL SNRRFVLCGT NVKAMSIEES SNGSLSVFVR HLQPKEMKSS AGGKGNEGCH

      430      440      450      460      470      480
MVTEELHSIT FETQICLYGL TIDLETSSLP VVMISNVSQL PNAWASIIWY NVSTNDSQNL

      490      500      510      520      530      540
VFFNNPPPAT LSQLEVMWSV QFSSYVGRGL NSDQLHMLAE KLTVQSSYSD GHLTWAKFCK

      550      560      570      580      590      600
EHLPGKSFTF WTWLEAILDL IKKHILPLWI DGYVMGFVSK EKERLLKDK MPGTFLLRFS

      610      620      630      640      650      660
ESHLGGITFT WVDHSESGEV RFHSVEPYNK GRSLALPFAD ILRDYKVIMA ENIPENPLKY

      670      680      690      700
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SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 13

<210> SEQ ID NO 1

<211> LENGTH: 2247

<212> TYPE: DNA

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Description of Unknown: STAT4-alpha isoform sequence

<400> SEQUENCE: 1

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ttaatacaac tggatgaaca gttaggctgt gtttccaaag agaaaaacct actcttgata      240
cacaatctaa aaagaattag gaaggtcctt cagggaaaat ttcattgaaa tccaatgcat      300
gtagctgtgg ttatttcaaa ctgtttaagg gaagagagga gaattattggc tgcagccaac      360
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atgcttaaca gcctcgattt caagagaaaag gaggtcttca gtaaaatgac ccaaatcatc      660
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ctagaaaagag tcaccttctt gatctacaac cttttcaaga actcatttgt ggttgagcga      960
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<210> SEQ ID NO 2
<211> LENGTH: 2616
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Description of Unknown: STAT4-beta
sequence

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<400> SEQUENCE: 2

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caagactggg aggcagcttc taacaatgaa accatggcaa cgattcttct tcaaaacttg 180
ttaatacaac tggatgaaca gttaggtcgt gtttccaaag agaaaaacct actcttgata 240
cacaatctaa aaagaattag gaaggtcctt cagggaaaat ttcattgaaa tccaatgcat 300
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accaaatact tagaagatct gcaagacgaa ttgactaca ggtataaaac aattcagaca 540
atggatcaga gtgacaagaa tagtgccatg gtgaatcagg aagttttgac actgcaggaa 600
atgcttaaca gctcgtattt caagagaaaag gaggtctctc gtaaaatgac ccaaatcatc 660

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tgctttacac tattggcaga aagtcttttc caactgagaa ggcaattgga gaaactagag	840
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ctagaaagag tcacctcttt gatctacaac cttttcaaga actcatttgt ggttgagcga	960
cagccatgta tgccaaccca cctcagagg ccgttggtac ttaaaacct aattcagttc	1020
actgtaaac taaggctact aataaaattg ccagaactaa actatcaggt aaaggttaag	1080
gcataaattg acaagaatgt ttcaactcta agcaaccgaa gatttgact ttgtggaact	1140
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catttgcaac caaaggaaat gaagtccagt gctggaggta aaggaaatga gggctgtcac	1260
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accatagatt tgagaccag ctcatcgct gtggtgatga tttccaatgt cagtcagtta	1380
cctaagtctt gggcatccat catttggtac aacgtgtcaa ccaacgattc ccagaacttg	1440
gttttcttta ataatectcc acctgccaca ttgagtcaac tactggagggt gatgagctgg	1500
cagttttcat cgtacgttgg tcgtggtctt aactcagatc aactccatat gctggcagag	1560
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gaccttgta tctctttaag ccgaagtgat tcaacagagc cacattctcc atcagacctt	2520
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<210> SEQ ID NO 3

<211> LENGTH: 369

<212> TYPE: DNA

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Description of Unknown: STAT4-beta specific exon sequence

<400> SEQUENCE: 3

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cctttaatac ataaggctag ccttcagggc acacttacca caatctattg tgcctaaaaat      180
tataaaattc cctttttata tgccatatat gccacagtaa gttgagtgtt ctgatatgaa      240
atgatgaatt agataactca atgtcacaaa tagatgaagc cctagaaatg agttcctgac      300
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<210> SEQ ID NO 4
<211> LENGTH: 748
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Description of Unknown: STAT4-alpha
sequence

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<400> SEQUENCE: 4

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Gln Val Asp Gln Phe Tyr Asp Asp Asn Phe Pro Met Glu Ile Arg His
20          25          30
Leu Leu Ala Gln Trp Ile Glu Asn Gln Asp Trp Glu Ala Ala Ser Asn
35          40          45
Asn Glu Thr Met Ala Thr Ile Leu Leu Gln Asn Leu Leu Ile Gln Leu
50          55          60
Asp Glu Gln Leu Gly Arg Val Ser Lys Glu Lys Asn Leu Leu Leu Ile
65          70          75          80
His Asn Leu Lys Arg Ile Arg Lys Val Leu Gln Gly Lys Phe His Gly
85          90          95
Asn Pro Met His Val Ala Val Val Ile Ser Asn Cys Leu Arg Glu Glu
100         105         110
Arg Arg Ile Leu Ala Ala Ala Asn Met Pro Val Gln Gly Pro Leu Glu
115         120         125
Lys Ser Leu Gln Ser Ser Ser Val Ser Glu Arg Gln Arg Asn Val Glu
130         135         140
His Lys Val Ala Ala Ile Lys Asn Ser Val Gln Met Thr Glu Gln Asp
145         150         155         160
Thr Lys Tyr Leu Glu Asp Leu Gln Asp Glu Phe Asp Tyr Arg Tyr Lys
165         170         175
Thr Ile Gln Thr Met Asp Gln Ser Asp Lys Asn Ser Ala Met Val Asn
180         185         190
Gln Glu Val Leu Thr Leu Gln Glu Met Leu Asn Ser Leu Asp Phe Lys
195         200         205
Arg Lys Glu Ala Leu Ser Lys Met Thr Gln Ile Ile His Glu Thr Asp
210         215         220
Leu Leu Met Asn Thr Met Leu Ile Glu Glu Leu Gln Asp Trp Lys Arg
225         230         235         240
Arg Gln Gln Ile Ala Cys Ile Gly Gly Pro Leu His Asn Gly Leu Asp
245         250         255
Gln Leu Gln Asn Cys Phe Thr Leu Leu Ala Glu Ser Leu Phe Gln Leu
260         265         270

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Arg	Arg	Gln	Leu	Glu	Lys	Leu	Glu	Glu	Gln	Ser	Thr	Lys	Met	Thr	Tyr	275	280	285
Glu	Gly	Asp	Pro	Ile	Pro	Met	Gln	Arg	Thr	His	Met	Leu	Glu	Arg	Val	290	295	300
Thr	Phe	Leu	Ile	Tyr	Asn	Leu	Phe	Lys	Asn	Ser	Phe	Val	Val	Glu	Arg	305	310	315
Gln	Pro	Cys	Met	Pro	Thr	His	Pro	Gln	Arg	Pro	Leu	Val	Leu	Lys	Thr	325	330	335
Leu	Ile	Gln	Phe	Thr	Val	Lys	Leu	Arg	Leu	Leu	Ile	Lys	Leu	Pro	Glu	340	345	350
Leu	Asn	Tyr	Gln	Val	Lys	Val	Lys	Ala	Ser	Ile	Asp	Lys	Asn	Val	Ser	355	360	365
Thr	Leu	Ser	Asn	Arg	Arg	Phe	Val	Leu	Cys	Gly	Thr	Asn	Val	Lys	Ala	370	375	380
Met	Ser	Ile	Glu	Glu	Ser	Ser	Asn	Gly	Ser	Leu	Ser	Val	Glu	Phe	Arg	385	390	395
His	Leu	Gln	Pro	Lys	Glu	Met	Lys	Ser	Ser	Ala	Gly	Gly	Lys	Gly	Asn	405	410	415
Glu	Gly	Cys	His	Met	Val	Thr	Glu	Glu	Leu	His	Ser	Ile	Thr	Phe	Glu	420	425	430
Thr	Gln	Ile	Cys	Leu	Tyr	Gly	Leu	Thr	Ile	Asp	Leu	Glu	Thr	Ser	Ser	435	440	445
Leu	Pro	Val	Val	Met	Ile	Ser	Asn	Val	Ser	Gln	Leu	Pro	Asn	Ala	Trp	450	455	460
Ala	Ser	Ile	Ile	Trp	Tyr	Asn	Val	Ser	Thr	Asn	Asp	Ser	Gln	Asn	Leu	465	470	475
Val	Phe	Phe	Asn	Asn	Pro	Pro	Pro	Ala	Thr	Leu	Ser	Gln	Leu	Leu	Glu	485	490	495
Val	Met	Ser	Trp	Gln	Phe	Ser	Ser	Tyr	Val	Gly	Arg	Gly	Leu	Asn	Ser	500	505	510
Asp	Gln	Leu	His	Met	Leu	Ala	Glu	Lys	Leu	Thr	Val	Gln	Ser	Ser	Tyr	515	520	525
Ser	Asp	Gly	His	Leu	Thr	Trp	Ala	Lys	Phe	Cys	Lys	Glu	His	Leu	Pro	530	535	540
Gly	Lys	Ser	Phe	Thr	Phe	Trp	Thr	Trp	Leu	Glu	Ala	Ile	Leu	Asp	Leu	545	550	555
Ile	Lys	Lys	His	Ile	Leu	Pro	Leu	Trp	Ile	Asp	Gly	Tyr	Val	Met	Gly	565	570	575
Phe	Val	Ser	Lys	Glu	Lys	Glu	Arg	Leu	Leu	Leu	Lys	Asp	Lys	Met	Pro	580	585	590
Gly	Thr	Phe	Leu	Leu	Arg	Phe	Ser	Glu	Ser	His	Leu	Gly	Gly	Ile	Thr	595	600	605
Phe	Thr	Trp	Val	Asp	His	Ser	Glu	Ser	Gly	Glu	Val	Arg	Phe	His	Ser	610	615	620
Val	Glu	Pro	Tyr	Asn	Lys	Gly	Arg	Leu	Ser	Ala	Leu	Pro	Phe	Ala	Asp	625	630	635
Ile	Leu	Arg	Asp	Tyr	Lys	Val	Ile	Met	Ala	Glu	Asn	Ile	Pro	Glu	Asn	645	650	655
Pro	Leu	Lys	Tyr	Leu	Tyr	Pro	Asp	Ile	Pro	Lys	Asp	Lys	Ala	Phe	Gly	660	665	670

-continued

Lys His Tyr Ser Ser Gln Pro Cys Glu Val Ser Arg Pro Thr Glu Arg
 675 680 685
 Gly Asp Lys Gly Tyr Val Pro Ser Val Phe Ile Pro Ile Ser Thr Ile
 690 695 700
 Arg Ser Asp Ser Thr Glu Pro His Ser Pro Ser Asp Leu Leu Pro Met
 705 710 715 720
 Ser Pro Ser Val Tyr Ala Val Leu Arg Glu Asn Leu Ser Pro Thr Thr
 725 730 735
 Ile Glu Thr Ala Met Lys Ser Pro Tyr Ser Ala Glu
 740 745

<210> SEQ ID NO 5
 <211> LENGTH: 704
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Unknown: STAT4-beta
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<400> SEQUENCE: 5

Met Ser Gln Trp Asn Gln Val Gln Gln Leu Glu Ile Lys Phe Leu Glu
 1 5 10 15
 Gln Val Asp Gln Phe Tyr Asp Asp Asn Phe Pro Met Glu Ile Arg His
 20 25 30
 Leu Leu Ala Gln Trp Ile Glu Asn Gln Asp Trp Glu Ala Ala Ser Asn
 35 40 45
 Asn Glu Thr Met Ala Thr Ile Leu Leu Gln Asn Leu Leu Ile Gln Leu
 50 55 60
 Asp Glu Gln Leu Gly Arg Val Ser Lys Glu Lys Asn Leu Leu Leu Ile
 65 70 75 80
 His Asn Leu Lys Arg Ile Arg Lys Val Leu Gln Gly Lys Phe His Gly
 85 90 95
 Asn Pro Met His Val Ala Val Val Ile Ser Asn Cys Leu Arg Glu Glu
 100 105 110
 Arg Arg Ile Leu Ala Ala Ala Asn Met Pro Val Gln Gly Pro Leu Glu
 115 120 125
 Lys Ser Leu Gln Ser Ser Ser Val Ser Glu Arg Gln Arg Asn Val Glu
 130 135 140
 His Lys Val Ala Ala Ile Lys Asn Ser Val Gln Met Thr Glu Gln Asp
 145 150 155 160
 Thr Lys Tyr Leu Glu Asp Leu Gln Asp Glu Phe Asp Tyr Arg Tyr Lys
 165 170 175
 Thr Ile Gln Thr Met Asp Gln Ser Asp Lys Asn Ser Ala Met Val Asn
 180 185 190
 Gln Glu Val Leu Thr Leu Gln Glu Met Leu Asn Ser Leu Asp Phe Lys
 195 200 205
 Arg Lys Glu Ala Leu Ser Lys Met Thr Gln Ile Ile His Glu Thr Asp
 210 215 220
 Leu Leu Met Asn Thr Met Leu Ile Glu Glu Leu Gln Asp Trp Lys Arg
 225 230 235 240
 Arg Gln Gln Ile Ala Cys Ile Gly Gly Pro Leu His Asn Gly Leu Asp
 245 250 255
 Gln Leu Gln Asn Cys Phe Thr Leu Leu Ala Glu Ser Leu Phe Gln Leu
 260 265 270

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Arg	Arg	Gln	Leu	Glu	Lys	Leu	Glu	Glu	Gln	Ser	Thr	Lys	Met	Thr	Tyr	275	280	285
Glu	Gly	Asp	Pro	Ile	Pro	Met	Gln	Arg	Thr	His	Met	Leu	Glu	Arg	Val	290	295	300
Thr	Phe	Leu	Ile	Tyr	Asn	Leu	Phe	Lys	Asn	Ser	Phe	Val	Val	Glu	Arg	305	310	315
Gln	Pro	Cys	Met	Pro	Thr	His	Pro	Gln	Arg	Pro	Leu	Val	Leu	Lys	Thr	325	330	335
Leu	Ile	Gln	Phe	Thr	Val	Lys	Leu	Arg	Leu	Leu	Ile	Lys	Leu	Pro	Glu	340	345	350
Leu	Asn	Tyr	Gln	Val	Lys	Val	Lys	Ala	Ser	Ile	Asp	Lys	Asn	Val	Ser	355	360	365
Thr	Leu	Ser	Asn	Arg	Arg	Phe	Val	Leu	Cys	Gly	Thr	Asn	Val	Lys	Ala	370	375	380
Met	Ser	Ile	Glu	Glu	Ser	Ser	Asn	Gly	Ser	Leu	Ser	Val	Glu	Phe	Arg	385	390	395
His	Leu	Gln	Pro	Lys	Glu	Met	Lys	Ser	Ser	Ala	Gly	Gly	Lys	Gly	Asn	405	410	415
Glu	Gly	Cys	His	Met	Val	Thr	Glu	Glu	Leu	His	Ser	Ile	Thr	Phe	Glu	420	425	430
Thr	Gln	Ile	Cys	Leu	Tyr	Gly	Leu	Thr	Ile	Asp	Leu	Glu	Thr	Ser	Ser	435	440	445
Leu	Pro	Val	Val	Met	Ile	Ser	Asn	Val	Ser	Gln	Leu	Pro	Asn	Ala	Trp	450	455	460
Ala	Ser	Ile	Ile	Trp	Tyr	Asn	Val	Ser	Thr	Asn	Asp	Ser	Gln	Asn	Leu	465	470	475
Val	Phe	Phe	Asn	Asn	Pro	Pro	Pro	Ala	Thr	Leu	Ser	Gln	Leu	Leu	Glu	485	490	495
Val	Met	Ser	Trp	Gln	Phe	Ser	Ser	Tyr	Val	Gly	Arg	Gly	Leu	Asn	Ser	500	505	510
Asp	Gln	Leu	His	Met	Leu	Ala	Glu	Lys	Leu	Thr	Val	Gln	Ser	Ser	Tyr	515	520	525
Ser	Asp	Gly	His	Leu	Thr	Trp	Ala	Lys	Phe	Cys	Lys	Glu	His	Leu	Pro	530	535	540
Gly	Lys	Ser	Phe	Thr	Phe	Trp	Thr	Trp	Leu	Glu	Ala	Ile	Leu	Asp	Leu	545	550	555
Ile	Lys	Lys	His	Ile	Leu	Pro	Leu	Trp	Ile	Asp	Gly	Tyr	Val	Met	Gly	565	570	575
Phe	Val	Ser	Lys	Glu	Lys	Glu	Arg	Leu	Leu	Leu	Lys	Asp	Lys	Met	Pro	580	585	590
Gly	Thr	Phe	Leu	Leu	Arg	Phe	Ser	Glu	Ser	His	Leu	Gly	Gly	Ile	Thr	595	600	605
Phe	Thr	Trp	Val	Asp	His	Ser	Glu	Ser	Gly	Glu	Val	Arg	Phe	His	Ser	610	615	620
Val	Glu	Pro	Tyr	Asn	Lys	Gly	Arg	Leu	Ser	Ala	Leu	Pro	Phe	Ala	Asp	625	630	635
Ile	Leu	Arg	Asp	Tyr	Lys	Val	Ile	Met	Ala	Glu	Asn	Ile	Pro	Glu	Asn	645	650	655
Pro	Leu	Lys	Tyr	Leu	Tyr	Pro	Asp	Ile	Pro	Lys	Asp	Lys	Ala	Phe	Gly	660	665	670

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Lys His Tyr Ser Ser Gln Pro Cys Glu Val Ser Arg Pro Thr Glu Arg
675 680 685

Gly Asp Lys Gly Tyr Val Pro Ser Val Phe Ile Pro Ile Ser Thr Met
690 695 700

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tatcctgaca ttcccaaaga c 21

<210> SEQ ID NO 7
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<400> SEQUENCE: 7

ctctcaacac cgcatacaca c 21

<210> SEQ ID NO 8
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 8

gacttactat gtcaggaact c 21

<210> SEQ ID NO 9
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<212> TYPE: PRT
<213> ORGANISM: Mus sp.

<400> SEQUENCE: 9

Met Glu Val Gly Trp Tyr Arg Ser Pro Phe Ser Arg Val Val His Leu
1 5 10 15

Tyr Arg Asn Gly Lys
20

<210> SEQ ID NO 10
<211> LENGTH: 171
<212> TYPE: DNA
<213> ORGANISM: Unknown
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<223> OTHER INFORMATION: Description of Unknown: STAT4-alpha
sequence
<220> FEATURE:
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<222> LOCATION: (1)..(168)

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tat gtt cct tct gtt ttt atc ccc atc tca aca atc cga agt gat tca 48
Tyr Val Pro Ser Val Phe Ile Pro Ile Ser Thr Ile Arg Ser Asp Ser
1 5 10 15

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aca gag cca cat tct cca tca gac ctt ctt ccc atg tct cca agt gtg      96
Thr Glu Pro His Ser Pro Ser Asp Leu Leu Pro Met Ser Pro Ser Val
                20                      25                      30

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tat gcg gtg ttg aga gaa aac ctg agt ccc aca aca att gaa act gca      144
Tyr Ala Val Leu Arg Glu Asn Leu Ser Pro Thr Thr Ile Glu Thr Ala
                35                      40                      45

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atg aag tct cct tat tct gct gaa tga      171
Met Lys Ser Pro Tyr Ser Ala Glu
                50                      55

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<210> SEQ ID NO 11
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<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Description of Unknown: STAT4-alpha
sequence

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<400> SEQUENCE: 11

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Tyr Val Pro Ser Val Phe Ile Pro Ile Ser Thr Ile Arg Ser Asp Ser
1                5                10                15
Thr Glu Pro His Ser Pro Ser Asp Leu Leu Pro Met Ser Pro Ser Val
                20                      25                      30
Tyr Ala Val Leu Arg Glu Asn Leu Ser Pro Thr Thr Ile Glu Thr Ala
                35                      40                      45
Met Lys Ser Pro Tyr Ser Ala Glu
                50                      55

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<210> SEQ ID NO 12
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Tyr Val Pro Ser Val Phe Ile Pro Ile Ser Thr Met
1                5                10

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<210> SEQ ID NO 13
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<212> TYPE: DNA
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tat gtt cct tct gtt ttt atc ccc atc tca aca atg tgagtaatgt      46
Tyr Val Pro Ser Val Phe Ile Pro Ile Ser Thr Met
1                5                10

```

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tagtcacatg tgaatatatt ttataaaaag ctttcctata ggagatttaa aggtagagca      106

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gagtacacat aactgagaac aaagcattgt aatgtgcaat gtccatttc cttaataaca      166

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taaggctagc cttcagggca cacttaccac aatctattgt gcctaaaatt ataaaattcc      226

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ccttttatat gccatatatg ccacagtaag ttgagtgttc tgatatgaaa tgatgaatta      286

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-continued

gataactcaa tgtcacaaat agatgaagcc ctagaaatga gttcctgaca tagtaagtca	346
cogtgaacta ttattatattt ttaatccttg tccatattga ccttggtatc tctttaagcc	406
gaagtgatc aacagagcca cattctccat cagaccttct tcccatgtct ccaagtgtgt	466
atgcggtgtt gagagaaaac ctgagtccca caacaattga aactgcaatg aagtctcctt	526
attctgctga atga	540

1. A method of predicting the likelihood of successful anti-inflammatory therapy for a patient suffering from an inflammatory disease, the method comprising predicting the likelihood of successful anti-TNF therapy for the patient based on an expression ratio of Stat4 β /Stat4 α in a biological sample from the patient.

2. The method of claim 1, wherein the biological sample is a tissue biopsy or blood.

3. The method of claim 1, wherein the expression ratio is determined by analyzing the expression in isolated peripheral blood mononuclear cells (PBMC).

4. The method of claim 1, wherein the biological sample includes T-cells.

5. The method of claim 1, wherein the inflammatory disease or response is selected from the group consisting of Crohn's disease, ulcerative colitis, inflammatory bowel disease (IBD), rheumatoid arthritis, lupus, psoriasis, and multiple sclerosis (MS).

6. The method of claim 1, wherein the ratio of Stat4 β /Stat4 α ranges from about 2 to 60.0.

7. The method of claim 1, wherein the ratio of Stat4 β /Stat4 α is greater than 10.

8. The method of claim 1, wherein the expression ratio is determined by a technique selected from the group consisting of PCR, quantitative PCR or real-time PCR, semi-quantitative PCR, probe-hybridization, mass spectrometry, and antibody-based quantitation.

9. The method of claim 1 further comprising evaluating clinicopathological data selected from the group consisting of patient age, previous personal and/or familial history of inflammatory diseases, previous personal and/or familial history of response to anti-inflammatory therapy, and presence of one or more single nucleotide polymorphisms (SNPs) associated with the Stat4 isoforms.

10. The method of claim 1, wherein the anti-inflammatory therapy is selected from the group consisting of infliximab, adalimumab, certolizumab pegol, afelimomab, golimumab, etanercept, abatacept, and anakinra.

11. The method of claim 1, wherein the expression ratio of Stat4 β /Stat4 α is applied for clinical detection of disease, disease diagnosis, disease prognosis, or treatment outcome or a combination of thereof relating to an inflammatory disorder.

12. The method of claim 1, wherein predicting the likelihood of successful anti-TNF therapy for the patient based on the Stat4 β /Stat4 α expression ratio includes correlating the ratio of Stat4 β /Stat4 α in the patient to a control sample or a reference value.

13. A method of treating an individual suffering from or suspected of suffering from an inflammatory disease, the method comprising:

(a) determining whether a sample from the individual has a higher Stat4 β /Stat4 α expression ratio as compared to a control or a reference value; and

(b) administering an anti-TNF therapy if the individual has a higher Stat4 β /Stat4 α ratio.

14. The method of claim 13, wherein the individual is suffering from the inflammatory disease selected from the group consisting of Crohn's disease and ulcerative colitis.

15. The method of claim 13, wherein the anti-TNF therapy is selected from the group consisting of infliximab, adalimumab, certolizumab pegol, afelimomab, golimumab, etanercept, abatacept, and anakinra.

16. A method of assessing a patient's risk for developing an inflammatory disease or an inflammatory response, the method comprising:

(a) quantifying the expression level of Stat4 α and Stat4 β isoforms in a biological sample from the patient; and

(b) determining that the patient's risk for the inflammatory disease or the inflammatory response is higher if the patient exhibits a higher Stat4 β :Stat4 α ratio as compared to a control.

17. A method of predicting disease severity in a patient's suspected of suffering from an inflammatory disease, the method comprising:

(a) obtaining the expression level of Stat4 α and Stat4 β isoforms in a biological sample from the patient; and

(b) determining that the disease severity for the patient suffering from the inflammatory disease is higher if the patient exhibits a higher Stat4 β :Stat4 α ratio as compared to a control.

18. A method of preventing or minimizing excessive inflammatory response in an immuno compromised patient, the method comprising:

(a) determining if the patient exhibits higher risk for the inflammatory response based on the patient's Stat4 β :Stat4 α expression level ratio in a biological sample as compared to a control; and

(b) administering an anti-inflammatory therapy to minimize the excessive inflammatory response.

19. The method of claim 18, wherein the excessive inflammatory response is associated with sepsis.

20. The method of claim 18, wherein the immune compromised patient is treated with an immuno suppressive agent.

21. A diagnostic kit to predict the response to anti-inflammatory therapy comprising reagents to specifically quantify the expression levels of Stat4 α and Stat4 β isoforms.

22. The diagnostic kit of claim 21, wherein the reagents are oligonucleotide primers that specifically amplify a portion of Stat4 β and Stat4 α isoforms.

23. The diagnostic kit of claim 21, wherein the reagents are oligonucleotide primers selected from the group consisting of 5'-TAT CCT GAC ATT CCC AAA GAC-3' (SEQ ID NO: 6),

5'-CTC TCA ACA CCG CAT ACA CAC-3' (SEQ ID NO: 7), and 5' GAC TTA CTATGT CAG GAA CTC-3' (SEQ ID NO: 8).

24. The diagnostic kit of claim **21**, wherein the reagents are Stat4 β and Stat4 α -specific antibodies.

25. The diagnostic kit of claim **21**, wherein the reagents are nucleic acid probes that specifically hybridize to at least a portion of Stat4 β and Stat4 α isoforms.

26. The diagnostic kit of claim **25**, wherein the probes bind to a region of Stat4 β comprising SEQ ID NO: 5 under high stringency hybridization conditions.

27. A nucleic acid probe comprising a contiguous region of about 15 nucleotides of SEQ ID NO: 5, wherein the probe is capable of selectively binding to the Stat4 β -specific exon.

28. The nucleic acid probe of claim **27**, wherein the probe comprises a reverse complementary strand capable of selectively binding to SEQ ID NO: 5.

29. The nucleic acid probe of claim **27**, wherein the probe consists essentially of a sequence of about 15-20 nucleotides capable of selectively binding to SEQ ID NO: 5.

30. A method of identifying an agent for modulating an inflammatory response, the method comprising:

(a) contacting a population of cells with a candidate agent; and

(b) identifying the candidate agent as the agent for modulating immunex response if the expression level of Stat4 β isoform is reduced.

31. The method of claim **30**, wherein the candidate agent is a small molecule.

32. The method of claim **30**, wherein the inflammatory response is modulated in a disease selected from the group consisting of Crohn's disease, ulcerative colitis, rheumatoid arthritis, lupus, psoriasis, and multiple sclerosis.

33. The method of claim **30**, wherein the expression level of Stat4 β isoform is reduced without substantially reducing the expression level of Stat4 α isoform.

34. The method of claim **30**, wherein the expression level of Stat4 β isoform is selectively reduced by an agent comprising a siRNA.

* * * * *

专利名称(译)	stat4表达对免疫系统疾病的诊断和预后		
公开(公告)号	US20110200600A1	公开(公告)日	2011-08-18
申请号	US13/063094	申请日	2009-09-09
[标]申请(专利权)人(译)	印第安纳UNIV RES TECH		
申请(专利权)人(译)	印第安纳大学研究与科技股份有限公司		
当前申请(专利权)人(译)	印第安纳大学研究与科技股份有限公司		
[标]发明人	KAPLAN MARK H		
发明人	KAPLAN, MARK H.		
IPC分类号	A61K39/395 C12Q1/68 G01N33/68 G01N33/53 A61K38/02 C07H21/00 C12Q1/02 A61P29/00 A61P1/00 A61P37/06 H01J49/26		
CPC分类号	C12Q1/6883 C12Q2600/158 C12Q2600/156 C12Q2600/112 C12Q2600/136 C12Q2600/106 A61P1/00 A61P29/00		
优先权	61/095684 2008-09-10 US		
外部链接	Espacenet USPTO		

摘要(译)

公开了确定Stat4 α 和Stat4 β 同种型的表达水平的方法和组合物，其用于抗炎治疗的治疗功效，评估个体发展炎性疾病（包括克罗恩病，溃疡性结肠炎，类风湿性关节炎和多发性硬化症）的风险。

