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**WO 01/29069 A1**

(54) Title: N-FORMYL PEPTIDE RECEPTOR COMPLEX WITH A G-PROTEIN KINASE SIGNAL PATHWAY MODIFICATION AGENT

(57) Abstract: A method of inhibiting a pro-inflammatory response of a human peripheral blood mononuclear cell or polymorphonuclear cell, or fixed tissue cell is described. The cell is contacted with a pro-inflammatory agent to stimulate a pro-inflammatory response. Then, the cell is contacted with a G protein kinase signal pathway modification agent, thereby inhibiting inflammatory response signal transduction pathways mediated by G protein. A receptor complex is described wherein a G protein kinase signal pathway modification agent binds to a cell surface receptor of a human peripheral blood mononuclear cell or polymorphonuclear cell that has been stimulated by a pro-inflammatory agent.

N-FORMYL PEPTIDE RECEPTOR COMPLEX WITH  
G-PROTEIN KINASE SIGNAL PATHWAY MODIFICATION AGENT

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FIELD OF THE INVENTION

10 This invention relates to N-formyl peptide receptors, which are found on the surfaces of peripheral blood cells, and particularly to complexes of such receptors with agents that alter or disrupt the G-protein signal pathways, particularly certain N-formyl peptides, and to methods for altering signal transduction due to co-stimulation by pro-inflammatory agents.

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BACKGROUND OF THE INVENTION

The human body has evolved to develop defense mechanisms to bacterial infections by using bacterially-generated N-formylmethionyl peptides as  
20 chemoattractants for phagocytes, in particular, neutrophils and monocytes. Of the N-formyl peptides, f-Met-Leu-Phe (FMLP) was identified as the most potent in its ability to recruit phagocytes and to stimulate release of lysosomal enzymes by neutrophils (Showell et al., *J. Exp. Med.* 143:1154-1169, 1976). Synthetic tetrapeptides, particularly f-Met-Ile-Phe-Leu and f-Met-Leu-Phe-Ile,  
25 have also subsequently been shown to evoke neutrophil responses (Rot et al., *Proc. Natl. Acad. Scie. USA* 84:7967-7971, 1987). The potency of these peptides were initially ascribed to: (1) a formyl group at the N-terminus, (2) a methionine side chain, and (3) leucine and phenylalanine side chains.

30 Cloning of the N-formyl peptide receptor (FRP) cDNA followed by the delineation of the primary structure of the FPR protein provided a major breakthrough in understanding the mechanism of action of N-formyl peptides (Boulay et al., *Biochemistry* 29: 11123-11133, 1990; Boulay et al., *Biochem.*

*Biophys. Res. Commun.* 168: 1103-1109, 1990). FPR was initially found on neutrophils and monocytes but have subsequently been shown to be expressed in the human brain, hepatocytes, dendritic cells, and astrocytes (Lacy et al., *J. Neuroimmunol.* 61: 71-78, 1995; Sozzani et al., *J. Immunol.* 155: 3292-3295, 1995). Two other FPR genes were later isolated, FPR2 (also known as FPRL1 and FPRH1) (Bao et al., *Genomics* 13: 437-440, 1992; Murphy et al., *J. Biol. Chem.* 267: 7637-7643, 1992; Ye et al., *Biochem. Biophys. Res. Commun.* 184: 582-589, 1992) and FPRL2 (Bao et al., *supra*). FPRL1 consists of 351 amino acids and shares 70% identity with FPR but was found to have a 400-fold lower affinity to FMLP ( $K_d=1\text{nM}$ ) than the original FPR. FPRL2 shares 56% identity with FPR and 83% identity with FPRL1. However, unlike FPRL1, which is expressed in both monocytes and neutrophils, FPRL2 was found to be expressed in monocytes but not in neutrophils (Durstin et al., *Biochem. Biophys. Res. Commun.* 201: 174-179, 1994).

The FPR contains seven hydrophobic domains spanning the plasma membrane, connected by hydrophilic sequences exposed to either the extracellular space or the intracellular space (Murphy, *Annu. Rev. Immunol.* 12: 593-633, 1994). The first and third intracellular loops are relatively small, consisting of 5 and 16 amino acids, respectively. The carboxy terminal is exposed to the intracellular space, while the N-terminal is exposed to the extracellular space. The intracellular sequences further contain a G-protein-coupling domain (a domain essential for function of the receptor as discussed below) and a potential phosphorylation domain.

The FMLP binding domain appears to be located in the first and third extracellular domains of FPR (Quehenberger et al., *J. Biol. Chem.* 268: 18167-18175, 1993). In addition, there have been suggestions of interconvertible states of FPR, alternating between high-affinity binding and low-affinity binding states for FMLP binding (Kermode et al., *Biochem. J.* 276: 715-723, 1991). The G-protein is thought to regulate such interconvertible states. FPR alone, without association with the G-protein, represents a low-affinity state, whereas FPR bound to the G-protein exhibits a high affinity binding state. The model

further dissects the G-protein-bound FPR into three different states that account for the different potencies observed by different peptides. State I represents the low affinity binding state in which FPR is not associated with the G-protein but can be converted to the high affinity state of State II upon binding to the G-protein. State III is a high affinity intermediate step in which the receptor binds the ligand, while releasing the  $G_\alpha$  subunit of the G-protein. State IV is a transient, low affinity state, in which the remaining G-protein subunits ( $G_\beta$  and  $G_\gamma$ ) disassociate from the receptor. The disassociation of the G-protein subunit  $G_\alpha$  leads to the effector functions of the G-protein, causing cellular activation. A similar model was proposed by Sklar et al. (*J. Biol. Chem.* 264: 8483-8486, 1989).

According to the above model, potency of a peptide is determined by the residence time at State III (Kermode et al., *supra*). The authors propose that most potent formyl peptides such as fMet-Leu-Phe-Phe and fMet-Leu-Phe-NHBzl temporarily stabilize and maintain State III, which triggers an immediate degranulation response but the duration at State III allows for a sustained signal for chemotactic responses, allowing maximal migration. Less potent formyl peptides mediate a rapid conversion from the high affinity State III to the low affinity State IV. Although the initial degranulation response at State III is unaffected, the chemotactic signaling ability is minimized and thus cellular migration is limited. Therefore, in general, a highly potent ligand can be said to effect chemotactic responses by binding to the high affinity state of FPR, while a less potent ligand effects the degranulation response by binding to the low affinity state of FPR.

Agonist activity and antagonistic activity can also be defined using this model. An agonist stabilizes the activated receptor State III and the degree of stabilization is reflected in its potency. An antagonist, on the other hand, binds to the receptor but destabilizes the activated State III and inactivates the receptor. Such a model is not limited to FPR.

Furthermore, desensitization is said to occur when a ligand binds to the receptor and the same or different receptor becomes refractory to subsequent stimulation by the same or different ligand. This desensitized state can form after receptor occupancy in the normal course of cell activation, resulting in destabilization of State III, or prior to receptor occupancy, in which State III is never achieved. When the refractory state or receptor inactivation is induced by one stimulant and affects multiple nonliganded receptors, this situation is called heterologous desensitization.

The most well-studied N-formyl peptide is f-Met-Leu-Phe (FMLP or fMLP). However, more potent peptides have been characterized with receptors on rabbit neutrophils *in vitro*, in particular, fMet-Leu-Phe-Phe, fMet-Leu-Phe-NHBzl (fMet-Leu-Phe benzylamide), and fNle-Leu-Phe-Tyr (N-formyl-L-norleucyl-Leu-Phe-Tyr) (Kermode et al., *supra*). These show both maximal migration (on the order of 20-35  $\mu\text{m}$ ) and degranulation (on the order of  $\text{ED}_{50}$  of  $10^{-10}$  to  $10^{-11}$ ). More recent studies suggest that nonformylated peptides may also bind to FPR and can act as potent activators of neutrophil function. For example, Met-Met-Trp-Leu-Leu is a potent pentapeptide and is comparable in activity to FMLP (Chen et al., *J. Biol. Chem.* 270: 23398-23401, 1995). Conversion of the pentapeptide to an N-formylated form boosted its potency 100-500 fold, demonstrating that N-formylation still plays a significant role in the potency of a peptide, although bioactivity does not appear to be strictly determined by N-formylation.

Other modifications to peptides have shown that some peptides can be converted to potent agonists for FPR (Derian et al., *Biochemistry* 35: 1265-1269, 1996; Higgins et al., *J. Med. Chem.* 39: 1013-1017, 1996). Such modifications include urea substitution of the amino terminal group and carbamate modifications. Furthermore, alteration of amino acid composition of the MLF peptide has been shown to also convert agonists to antagonists of FPR, as determined by superoxide anion release and neutrophil adhesion to vascular endothelium.

Chemotaxis stimulated by N-formyl peptides via FPR in neutrophils has been well- studied. Even prior to stimulation by N-formyl peptides, neutrophils transiently bind to P-selectin expressed on endothelial cells. Activation of neutrophils mediated by N-formyl peptides generated at the site of inflammation lead to neutrophil accumulation at this site. N-formyl peptides upregulate L-selectin on neutrophils and direct rolling of neutrophils along the endothelium, followed by upregulation of integrins on the surface of neutrophils. Integrins mediate cell-cell and cell-extracellular matrix interactions and bind to laminin, fibronectin, vitronectin, as well as to ICAM (intracellular cell adhesion molecule) and VCAM (vascular cell adhesion molecule) found on the endothelium. Upon binding of the integrins to ICAM and VCAM, a signal is transduced to the interior of the neutrophil through interactions with the cytoskeleton. Neutrophils then shed L-selectin and begin to spread along the endothelium. Upregulation of E-selectin and ICAM-1 on the surface of endothelial cells then mediate the migration of neutrophils across the endothelium (Luscinskas et al., *J. Immunol.* 146: 1617-1625, 1991). Upon crossing the endothelial barrier, neutrophils migrate toward the site of inflammation by sensing a concentration gradient of the N-formyl peptide. Upon reaching their destination, which contains a high concentration of the peptide, neutrophils unleash their anti-microbial actions.

Stimulated neutrophils rapidly activate respiratory burst oxidase, which catalyzes the generation of the superoxide radical  $O_2^-$ . The superoxide radical reacts with other molecules to produce hydrogen peroxides and hypochlorous acid, both of which are highly reactive agents and are therefore effective in interfering with microbial functions. Degranulation is also an effective means for destroying foreign microbes. However, degranulation can also damage host tissue. Phagocytosis is another mechanism by which neutrophils eliminate foreign microbes. Many of these functions are stimulated via the G-protein, using phospholipases as second messengers, three of which have been characterized.

The phospholipase C, PLC<sub>β2</sub>, generates two second messengers, 1,4,5-ionsitol triphosphate (IP<sub>3</sub>) and diacylglycerol (DG). The βγ subunits of the G-protein generated during activation of the FPR activate PLC<sub>β2</sub>. IP<sub>3</sub> binds to certain calcium channels to stimulate the release of calcium from intracellular storage, resulting in an increase in the cytosolic concentration of calcium that is observed during stimulation by chemoattractants. DG, in concert with released calcium, activates protein kinase C (PKC). G-protein activated PLC kinase has recently been reported in the literature (Beaven, et al, *J.of Immunology* 160:5136-5144, 1998) as a major pathway for mast cell degranulation in rat peritoneal cells in vitro, associated with Ca<sup>2+</sup> increases.

Phospholipase A<sub>2</sub> (PLA<sub>2</sub>) generates arachidonic acid from the phospholipids of the inner face of the plasma membrane. Arachidonic acid provides the precursors for the inflammatory mediators such as leukotrienes and prostaglandins. PLA<sub>2</sub> is activated upon phosphorylation by the mitogen-activated protein (MAP) kinase.

The third phospholipase is phospholipase D (PLD), which generates phosphatidic acid and choline from phosphatidylcholine. Phosphatidic acid may be involved in activation of respiratory burst oxidase in addition to playing a role in the production of DG, which activates PKC. However, activation of PLD requires calcium, and FMLP cannot stimulate PLD in calcium-depleted cells (Kessels et al., *J. Biol. Chem.* 266: 23152-23156, 1991). In addition, it appears that the G-protein Arf and G-protein Rho regulate PLD activity (Brown et al., *Cell* 75: 1137-1144, 1993; Cockcroft et al., *Science* 263: 523-526, 1994; Singer et al., *J. Biol. Chem.* 270: 14944-14950, 1995).

Protein phosphorylation plays a central role in signal transduction initiated by FMLP. Three major protein kinases are involved in the phosphorylation of proteins as a result of FMLP stimulation.

As discussed above, PKC is activated by DG, which is generated by PLC. PKC act to phosphorylate serine and threonine residues. PKC consists of six

different isoforms, three of which are sensitive to intracellular calcium ( $\alpha$ ,  $\beta$ , and  $\gamma$  forms) and three that are not ( $\delta$ ,  $\epsilon$ , and  $\zeta$  forms). Neutrophils contain the  $\alpha$ ,  $\beta$ , and  $\zeta$  forms but not the  $\gamma$  form. The calcium-dependent and DG-dependent PKC (PKC- $\beta$ ) responds to FMLP and phorbol ester stimulation by translocating from the cytosol to the membrane. It then phosphorylates a number of cytosolic proteins, such as those involved in the respiratory burst oxidase system. FMLP can also activate the calcium-independent, DG-dependent and phosphatidyl serine-dependent PKC form but their function is unclear.

The MAP kinase (MAPK) reportedly is activated by the  $\beta\gamma$  subunits of the G-proteins by the activities of Ras and Raf. Recent literature suggests the involvement of high-intensity Ras signaling in inducing apoptosis (Bar-Sagi, et al, *J. Mol. Cell Biol.* 19(9):5892-901, 1999) as well as in promoting endothelial cell adherence (Finkel, et al, .....). Raf is now posited with a central role in growth signals, including cell survival, growth and differentiation (Rapp, et al, .....). This kinase pathway is also stimulated by C5a and IL-8 (Buhl et al., *J. Biol. Chem.* 270: 19828-19832, 1995; Knall et al., *J. Biol. Chem.* 271: 2832-2838, 1996). MAP kinase induces tyrosine phosphorylation of several regulatory proteins, such as the extracellular signal-regulated kinase (ERK)-1. Recent literature suggests that MAPK pathways are responsible for cytokine production; however, the activation of both TH-1 and TH-2 cytokines, as well as other pro-inflammatory molecules, such as C5a, IL-8 and FMLP, is dependent upon the trimeric G-protein signal transduction.

Phosphatidylinositol 3-kinase (PI3K) is responsible for the formation of PI triphosphate (PIP<sub>3</sub>) that is observed upon stimulation by FMLP. Elevated PIP<sub>3</sub> levels apparently contribute to the activation of the respiratory burst oxidase system and to actin polymerization in neutrophils, which is considered important in regulating cytoskeletal changes and cell migration. Recent literature (Rankin, et al, *J. Exp. Med.* 188(9):1621-32, 1998) has reported that elevated PI3 kinase levels also can promote degranulation of eosinophils, based upon G-protein signaling based activation of IL-5. Further, Sagi-Eisenberg, et

al, *Eur.J.Immunol*, 1998.28: 3468-3478 suggest that G-protein signaling, using the intermediate pathways of PKC and PI3 kinases, may activate the FC $\epsilon$ R receptor by IgE for the release of histamines and other pro-inflammatory cytokines involved in allergic airway hypersensitivity. Uckun, et al, *J.of Biolog. Chemistry*, Vol.274, No.38, Sep., 1999, pp.27028-27038 reports G-protein signaling in the JAK3 kinase pathway, through IgE/FC $\epsilon$ R1 cross-linking, as leading to mast cell degranulation. Beaven, et al, *J. of Immunology*, 1998, 160: 5136-5144 report that G-protein signaling, through the activation of PKC and resulting uptake in Ca<sup>2+</sup>, also leads to secretion and degranulation of mast cells. Thus, G-protein may be essential for the down-stream activation of the FC $\epsilon$ R1 upon IgE antigen challenge, and the corresponding ability to interfere with G-protein signaling, can be an important basis for down-stream inhibition of the activation of the FC $\epsilon$ R receptor.

Several antagonists of rabbit neutrophil FPR have been identified but features that make certain molecules antagonists are still unclear. Certain butoxycarbonyl analogs of FMLP have been shown to competitively inhibit chemotactic peptide-induced cell activation, of which BocPLPLP was found to be the most potent (Schiffman et al., *FEBS Lett.* 117: 1, 1980; Freer et al., *Biochemistry* 19: 2404, 1980; Kanaho et al., *J. Leukocyte Biol.* 47: 420, 1990). Cyclosporin H, a cyclic undecapeptide and an analogue of cyclosporin A, has also been shown to possess antagonist activity to the formyl peptide receptor (Wenzel-Seifert et al., *J. Immunol.* 150: 4591-4599, 1993). A naturally occurring formyl peptide receptor antagonist has also been identified, a retrovirus-derived hexapeptide (Oostendorp et al., *J. Immunol.* 149: 1010, 1992). However, none of these antagonists bind to the formyl peptide receptors with high affinity and are therefore not of practical use as investigative tools or as anti-inflammatory drugs.

Interestingly, inhibition of mast cell degranulation by N-formyl-methionyl-leucyl-phenylalanine was reported in *Inflammation*, Vol. 5, No. 1, pp. 13-16 (1981). There, it was reported that two structurally different chemotactic peptides, i.e., pepstatin and N-formyl-methionyl-leucyl-phenylalanine, inhibit

the increase in vascular permeability in rat skin produced by intradermal injection of 40/80, anti-rat IgE serum, or macromolecular anionic permeability factor isolated from calf lung. It was reported that these peptides appear to act directly on the mast cells. The mechanism of such inhibition is unclear because FPR has not been reported to be expressed on mast cells.

Clearly, there are many unanswered questions regarding the function and mechanism of action of FPR. Furthermore, the finding that FPR is expressed in nonleukocytic cells, such as in the brain and dendritic cells, suggests that FPR may perform novel functions in other cell types that have yet to be elucidated. Thus, additional agents for inhibiting pro-inflammatory response of cells are needed as well as methods for studying the pro-inflammatory response of cells.

#### SUMMARY OF THE INVENTION

It has now been discovered that treatment of human peripheral blood mononuclear cells or polymorphonuclear cells, or fixed tissue cells with a G-protein kinase signal pathway modification agent after stimulation of such cells with a pro-inflammatory agent inhibits the conventional pro-inflammatory response, particularly down-stream pro-inflammatory responses induced by pro-inflammatory agents such as, for example, C5a, FMLP, IL-4, IL-6, IL-8, IL-10, IL-13 and TNF $\alpha$  or by the FC $\epsilon$  receptor. The alteration of the cellular production of G-protein inhibits inflammatory response signal transduction pathways mediated by G protein. Particularly useful G-protein kinase signal pathway modification agents are N-formyl-methionyl-leucyl ("f-Met-Leu") peptides having the formula f-Met-Leu X where X is selected from the group consisting of Tyr, Tyr-Phe, Phe-Phe and Phe-Tyr, most preferably f-Met-Leu-Phe-Phe.

The human peripheral blood mononuclear or polymorphonuclear cells or fixed tissue cells can be lymphocytes and granulocytes, preferably, eosinophils, basophils, and activated T-cells, and mast cells.

The G-protein kinase signal pathway modification agent forms a complex with a cell surface receptor, preferably the formyl peptide receptor ("FPR"), that is present on these cells that, in the presence of pro-inflammatory agents, inhibits phosphorylation of the G-protein subunit  $\gamma$  in preferred embodiments of the present invention. Further, by inhibiting or blocking the down-stream phosphorylation of the G-protein, various pathways dependent upon the trimeric G-protein components block or down-regulate the response of the cell to the pro-inflammatory agents.

In a preferred embodiment of the invention, at the level of the FC $\epsilon$  receptor (FC $\epsilon$ R), this G-protein kinase signal pathway modification agent, in the presence of IgE, inhibits the activation of the FC $\epsilon$ R by IgE.

In accord with the present invention, the pro-inflammatory agents used to stimulate the human peripheral blood mononuclear and polymorphonuclear cells, or fixed tissue cells preferably are cytokines, chemotaxins, or mitogens. Especially preferred pro-inflammatory agents are N-formyl peptides such as FMLP, activated complement fragment (C5a), leukotriene B4 (LTB4), platelet activating factor (PAF), and cytokines such as TNF $\alpha$ , IL-4, IL-6, IL-8, IL-10 and IL-13, plus antigen cross-linked IgE, IgG or IgA.

In another embodiment of the present invention, a method for inhibiting a pro-inflammatory response of a human peripheral blood mononuclear or polymorphonuclear cell is provided, the method comprising contacting the cell with a G- protein kinase signal pathway modification agent, and binding of the agent to a receptor on the cell. The pro-inflammatory mediating cell can be a lymphocyte or a granulocyte. Preferably, the cell is first stimulated by a pro-inflammatory agent such as set forth above.

In a preferred embodiment of the invention, the ligand-bound receptor is a formyl peptide receptor. The down-stream blocking of G-protein activation of the FC $\epsilon$ R receptor provides a similar therapeutic benefit.

The present invention further provides a method for identifying a G protein kinase signal pathway modification agent, the method comprising the steps of:

- 5 a) contacting a pro-inflammatory mediating cell selected from the group consisting of activated T-lymphocyte, mast cell, eosinophil and basophil with a known pro-inflammatory mediator selected from the group consisting of a cytokine, chemotaxin, and mitogen and eliciting a pro-inflammatory response;
- 10 b) adding to step a) a candidate G- protein signal pathway modification agent; and
- c) detecting a decrease in amount of a G protein kinase or failure of the G protein kinase to disassociate in (i) a pro-inflammatory cell simultaneously contacted with said candidate agent and said pro-inflammatory mediator compared to (ii) a pro-inflammatory cell contacted with said pro-
- 15 inflammatory mediator alone.

A preferred G protein kinase is the G protein  $\gamma$  kinase.

20 In another embodiment of the present invention, a receptor complexed with a G-protein kinase signal pathway modification agent is provided on the surface of a pro-inflammatory mediating cell selected from the group consisting of activated T-lymphocyte, mast cell, eosinophil and basophil by contacting the pro-inflammatory mediating cell with the G-protein kinase signal pathway modification agent, thereby inhibiting a pro-inflammatory response.

25

In a further embodiment of the invention, a method for identifying a G protein kinase signal pathway modification agent comprises the steps of:

- 30 contacting a pro-inflammatory mediating cell selected from the group consisting of activated T-lymphocyte, mast cell, eosinophil and basophil with a candidate G- protein signal pathway modification agent; and
- determining the distribution of protein kinases produced by said cell, wherein compared to cells not contacted by the candidate G- protein signal pathway modification agent there is no change in the amount of PLC $\gamma$ ,

Pp60 Src, and ERK-1, there is an increase in the amount of PI3 (102 Kd) and PI3 (83 Kd), and there is a decrease in the amount of Raf, Ras, G-protein  $\alpha$  and G-protein  $\beta$  kinases.

5           Thus, the present invention also provides a new receptor complex of a human peripheral blood mononuclear cell or a polymorphonuclear cell comprising a cell surface receptor and a G- protein signal pathway modification agent wherein, compared to cells not exhibiting said receptor complex, the distribution of protein kinases in the cells is such that there is no change in the  
10 amount of PLC $\gamma$ , Pp60 Src, and ERK-1, there is an increase in the amount of PI3 (102 Kd) and PI3 (83 Kd), and there is a decrease in the amount of Raf, Ras, G-protein  $\alpha$  and G-protein  $\beta$  kinases. Preferably, the cell surface receptor is a FPR receptor. Secondary blocking of G-protein activation of the FC $\epsilon$ R receptor is also preferred.

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          Stimulation by IL-4 of a human peripheral blood mononuclear cell or a polymorphonuclear cell having a cell surface receptor complexed with a G-protein signal pathway modification agent provides a distribution of protein kinases produced by said cell wherein, compared to stimulated cells not  
20 exhibiting said receptor complex, there is an increase in the amount of PLC $\gamma$ , PI3 (102 Kd) and PI3 (83 Kd), and a decrease in the amount of Ras and G-protein  $\gamma$  kinases.

          Stimulation by IL-6 of a human peripheral blood mononuclear cell or a  
25 polymorphonuclear cell having a cell surface receptor complexed with a G-protein signal pathway modification agent provides a distribution of protein kinases produced by said cell wherein, compared to stimulated cells not exhibiting said receptor complex, there is an increase in the amount of PLC $\gamma$ , PI3 (102 Kd), Raf, Pp60 Src, ERK-1 and G-protein  $\alpha$ , and a decrease in the  
30 amount of PI3 (83 Kd), G-protein  $\beta$  and G-protein  $\gamma$  kinases.

          Stimulation by IL-10 of a human peripheral blood mononuclear cell or a polymorphonuclear cell having a cell surface receptor complexed with a G-

protein signal pathway modification agent provides a distribution of protein kinases produced by said cell wherein, compared to stimulated cells not exhibiting said receptor complex, there is an increase in the amount of PI3 (102 Kd), ERK-1 and G-protein  $\gamma$ , and a decrease in the amount of PLC $\gamma$ , PI3 (83 Kd), Ras and kinases.

Stimulation by the pro-inflammatory agent, IL-13, of a human peripheral blood mononuclear cell or a polymorphonuclear cell having a cell surface receptor complexed with a G- protein signal pathway modification agent provides a distribution of protein kinases produced by said cell wherein, compared to stimulated cells not exhibiting said receptor complex, there is an increase in the amount of PLC $\gamma$ , PI3 (102 Kd), PI3 (83 Kd), ERK-1 and Raf, and a decrease in the amount of Ras, Pp60 Src, G-protein  $\alpha$ , G-protein  $\beta$  and G-protein  $\gamma$  kinases.

Stimulation by the pro-inflammatory agent, C5a, of a human peripheral blood mononuclear cell or a polymorphonuclear cell having a cell surface receptor complexed with a G- protein signal pathway modification agent provides a distribution of protein kinases produced by said cell wherein, compared to stimulated cells not exhibiting said receptor complex, there is an increase in the amount of Pp60 Src and Raf, and a decrease in the amount of PLC $\gamma$ , PI3 (102 Kd), G-protein  $\alpha$ , G-protein  $\beta$  and G-protein  $\gamma$  kinases.

Stimulation by the pro-inflammatory agent, TNF $\alpha$ , of a human peripheral blood mononuclear cell or a polymorphonuclear cell having a cell surface receptor complexed with a G- protein signal pathway modification agent provides a distribution of protein kinases produced by said cell wherein, compared to stimulated cells not exhibiting said receptor complex, there is an increase in the amount of Raf, Ras, and Pp60 Src, and a decrease in the amount of PLC $\gamma$ , PI3 (83 Kd) G-protein  $\alpha$ , G-protein  $\beta$  and G-protein  $\gamma$  kinases.

## BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is a graph showing binding of FITC-labeled HK-X (f-Met-Leu-Phe-Phe) to human peripheral blood nucleated cells.

5           FIG. 2A- FIG. 2C are dot plots of FITC-labeled HK-X binding to activated lymphocytes. FIG. 2A shows lymphocytes stimulated with 6  $\mu$ g Concanavalin A (ConA) at 24 hours after placed in culture in addition with 100nM FITC-labeled HK-X; FIG. 2B shows lymphocytes stimulated with 6  $\mu$ g ConA at 120 hours after placed in culture but with no FITC-labeled HK-X; and FIG. 2C shows  
10 lymphocytes stimulated with 6  $\mu$ g ConA at 120 hours after placed in culture in addition with 100nM FITC-labeled HK-X.

          FIG. 3A- FIG. 3B are histograms of the DNA content of lymphocytes from FIG. 2 A-C. FIG. 3A is a histogram of cells from FIG. 2A and FIG. 3B is a  
15 histogram of cells from FIG. 2B and FIG. 2C.

          FIG. 4 is an autoradiograph of  $^{35}$ S-methionyl labeled proteins recovered from whole human neutrophil cell lysates and various resins.

20           FIG. 5A- FIG. 5B show the presence of phosphorylated proteins in mononuclear cells treated with vehicle (0.3% DMSO) as detected by monoclonal antibodies to phosphotyrosine. FIG. 5A is a densitometry of SDS-PAGE of normal cells and FIG. 5B is a photograph of the SDS-PAGE gel of normal cells treated with vehicle (DMSO) only.

25           FIG. 6A- FIG. 6B show the presence of phosphorylated proteins in mononuclear cells treated with HK-X as detected by monoclonal antibodies to phosphotyrosine. FIG. 6A is a densitometry of SDS-PAGE of HK-X treated cells and FIG. 6B is a photograph of the SDS-PAGE of normal cells treated with HK-  
30 X only.

          FIG. 7A- FIG. 7B show the presence of phosphorylated proteins in mononuclear cells treated with IL-8 as detected by monoclonal antibodies to

phosphotyrosine. FIG. 7A is a densitometry of SDS-PAGE of IL-8 treated cells and FIG. 7B is a photograph of the SDS-PAGE of normal cells treated with IL-8 only.

5           FIG. 8A- FIG. 8B show the presence of phosphorylated proteins in mononuclear cells treated with HK-X and IL-8 as detected by monoclonal antibodies to phosphotyrosine. FIG. 8A is a densitometry of SDS-PAGE of HK-X and IL-8 treated cells and FIG. 8B is a photograph of the SDS-PAGE of normal cells treated with HK-X and IL-8.

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          FIG. 9A- FIG. 9B show the presence of phosphorylated proteins in mononuclear cells freshly collected from peripheral blood as detected by monoclonal antibodies to phosphotyrosine. FIG. 9A is a densitometry of SDS-PAGE of freshly collected cells and FIG. 9B is a photograph of the SDS-PAGE gel of freshly collected cells.

15

#### DETAILED DESCRIPTION OF THE INVENTION

          In accord with the present invention, G-protein kinase signal pathway  
20       modification agents have been found to inactivate certain pro-inflammatory responses of human peripheral blood cells that have been stimulated by pro-inflammatory agents or molecules.

          Preferred G-protein kinase signal pathway modification agents, in accord  
25       with the present invention, can bind to receptors found on pro-inflammatory mediating cells such as lymphocytes, particularly activated T-cells, granulocytes such as eosinophils, basophils, and fixed tissue cells such as mast cells.

30       Upon binding of the G protein kinase signal pathway modification agent to its receptor, pro-inflammatory responses are inhibited. In accord with the preferred embodiments of the present invention, the G-protein subunits  $\alpha$ ,  $\beta$ , and  $\gamma$  are modified and also phosphorylation of these subunits is inhibited.

Pro-inflammatory responses that can be inhibited by the agent-receptor complex are secretion, degranulation and migration of the receptor-bearing cell, as well as synthesis and secretion of other pro-inflammatory molecules.

5            Preferably, the G protein kinase signal pathway modification agents of the present invention can inactivate the receptor previously stimulated by a pro-inflammatory molecule but have no effect on non-stimulated cells. Examples of pro-inflammatory agents useful for stimulating the cells are IL-8, N-formyl peptides, activated complement fragment (C5a), leukotriene B4 (LTB4)  
10            and platelet activating factor (PAF). Preferably, the agent-receptor complex of the present invention also can desensitize the receptor from further stimulation by the same pro-inflammatory agent or by a different pro-inflammatory agent.

              The observed effect of the G protein kinase signal pathway modification agent-receptor complex on the receptor-bearing cell can be used to identify G  
15            protein kinase signal pathway modification agents. Screening for such agents can be carried out by contacting a pro-inflammatory mediating cell with a known pro-inflammatory mediator to elicit a pro-inflammatory response, followed by the addition of a candidate agent and by detecting a decrease in the  
20            amount of a G protein kinase in a pro-inflammatory cell contacted simultaneously with the candidate agent and the pro-inflammatory molecule compared to that amount in a pro-inflammatory mediating cell contacted with the pro-inflammatory molecule alone.

25            A particularly useful G protein kinase signal pathway modification agent is f-Met-Leu-Phe-Phe. Other useful agents can be identified by routine testing using one of the above procedures.

              As used herein, pro-inflammatory responses include secretion or  
30            degranulation of pro-inflammatory mediating cells and release of leukotrienes, histamines, and other cytokines. Such responses also include infiltration of eosinophils, basophils and mast cells into inflammatory tissues as a result of chemotactic adhesion, migration and aggregation of lymphocytes, eosinophils,

basophils, mast cells, and neutrophils. Vascular permeability at the site of inflammation and increased production of IgE, IgG and IgA, and their respective FC receptors, also can be associated with pro-inflammatory responses.

5           Inhibition of pro-inflammatory responses can thus include decrease of degranulation and release of leukotrienes, histamines and other cytokines by pro-inflammatory mediating cells, or complete cessation in preferred  
embodiments, following peptide-receptor binding according to the present  
invention. Infiltration and migration of pro-inflammatory mediating cells can  
10 also be greatly reduced, or completely inhibited. Vascular permeability at the site of inflammation and IgE levels also can be reduced.

In order that the invention described herein may be more fully  
understood, the following examples are set forth. It should be understood that  
15 these examples are for illustrative purposes only and are not to be construed as limiting this invention in any manner.

#### EXAMPLE 1: Binding of Labeled HK-X to Peripheral Blood Nucleated Cells

20           The Kd and Bmax (saturation binding) of HK-X ("f-Met-Leu-Phe-Phe") on peripheral blood nucleated cells were determined.  $2 \times 10^5$  cells in 100  $\mu$ l of solution from various fractions (monocytes, lymphocytes, granulocytes)  
prepared by density centrifugation was previously washed in 1% BSA-PBS  
solution containing 0.1% sodium azide. 100  $\mu$ l of the following molar  
25 concentrations of FITC-labeled HK-X (in 1% BSA-PBS solution) was added to each set of tubes according to Table 1:

TABLE 1

Tube #	FITC-labeled HK-X Molar Concentration
1	$9.06 \times 10^{-12}$
2	$2.71 \times 10^{-11}$
3	$5.43 \times 10^{-11}$
4	$7.25 \times 10^{-11}$
5	$9.06 \times 10^{-11}$
6	$2.71 \times 10^{-10}$
7	$5.43 \times 10^{-10}$
8	$7.25 \times 10^{-10}$
9	$9.06 \times 10^{-10}$

The tubes were mixed and vortexed for 30 seconds. The tubes were then held at 4-8°C for 30 minutes. 100 µl of Cal-Lyse was then added, incubated for 5 minutes, or 500 µl of 1% formaldehyde was added. If Cal-Lyse was added, 1 ml of water was added and incubated for a further 5 min. Cells were then analyzed on a flow cytometer (Coulter Epics Elite). Binding results are shown in FIG. 1. Bmax was determined to be 47.66 and Kd= $1.674 \times 10^{-10}$ M. Thus, these results show that HK-X binds to peripheral blood nucleated cells such as monocytes, lymphocytes, and granulocytes.

It was also determined by similar methodology that H-KX binds to activated T-cells, mast cells, eosinophils, and basophils, as well as the known binding to neutrophils.

#### EXAMPLE 2: HK-X Binds to Activated Receptors

Peripheral blood lymphocytes were stimulated with the mitogen Concanavalin A (ConA) at 24 hours or at 120 hours after being placed in culture. The cells were then either exposed to the 100nM FITC-labeled HK-X or were not exposed. Cells were also stained with DAPI for cell cycle determination. Cells were then analyzed by flow cytometry.

FIGs. 2A-2C show the relationship between activated lymphocytes by ConA and the appearance of binding sites for FITC-labeled HK-X. The four quadrants reveal the following characteristics:

5 the upper left quadrants denote cells with greater than 1n content of DNA and increased levels of FITC HK-X binding above background levels [ established by using the profile in FIG. 1;

the upper right quadrants denote cells that contain greater than 1n DNA content and have FITC-ligand binding greater than background;

10 the lower right quadrants contain cells that have 1n DNA content but have bound FITC-ligand above background levels; and

the lower left quadrants contain cells with 1n DNA content and background levels of FITC-ligand.

15 As is apparent from examination of FIGs. 2B and 2C, exposure to the plant lectin or mitogen stimulates cells to enter into the cell cycle and express binding sites to the FITC-labeled HK-X. The longer culture period of 120 hours allowed a greater portion of the cells to enter the cell cycle (compared to FIG. 2A). The most accurate determination of background levels of endogenous  
20 fluorescence was obtained by setting the thresholds (quadrants) using cells cultured with ConA but not stained with the FITC-labeled HK-X (see FIG. 2B).

The statistical analysis confirms the qualitative observations furnished by examination of the dot plots. Approximately 25% of the cells in FIG. 2C  
25 contained greater than 1n DNA content and were above the background levels of FITC-ligand binding. This is in marked contrast to the numbers of cells in the upper right quadrant in FIGs. 2A and 2B, respectively.

30 A more formal examination of the distribution of cells in various phases of the cell cycle was obtained by using a software package "MultiCycle" by Phoenix Software, Phoenix, AZ. This software deconvolutes the DNA histogram and statistically analyses the components for degree of fit and significance.

FIG. 3A shows the same samples contained in Fig. 2A and FIG. 3B shows those contained in Figs. 2B and 2C. As is apparent from examination of the percentage of cells which are diploid (>1n DNA content) the fractions increased from 2.2% in FIG. 2A to 29% in FIGs. 2B and 2C.

5

Thus, it appears that stimulation of human peripheral blood lymphocytes with mitogen initiates duplication of their DNA and, simultaneously with this process, expression of binding sites for the FITC-labeled HK-X. Only a fraction of the cells with DNA content of 1n, or in G<sub>0</sub>/G<sub>1</sub> phase of the cell cycle, show increased expression of binding sites for the ligand. By contrast, all cells with diploid DNA content (>1n) contain binding sites for the ligand.

10

### EXAMPLE 3: Identification and Characterization of HK-X Receptors

15

Receptors that bind HK-X and other N-formyl peptides were isolated and characterized from murine peritoneal mast cells, human polymorphonuclear and mononuclear cell populations. Further, G-protein activation of the FC $\epsilon$ R receptor as a down-stream consequence of increases in PKC, PI3 and mobilization of Ca<sup>2+</sup>, make the FC $\epsilon$  receptor a down-stream effector receptor of G-protein interference.

20

Cells that bind HK-X include mast cells, basophils and eosinophils as evidenced by changed biological reactivity after exposure to HK-X and by binding of a fluorescent or radioactive labelled HK-X. Receptors for HK-X have not been identified. In contrast, neutrophils express formyl peptide receptors on their surface. However, blood lymphocytes and monocytes appear to bind less HK-X than neutrophils.

25

30

## DETAILED MATERIALS AND METHODS:

1. ISOLATION OF CELLS --- Rat peritoneal mast cells were isolated by infusion of 35 ml of Tyrode's Solution into the peritoneal cavity of anesthetized rats. The rats were then sacrificed by injection of an overdose of anesthetic. The peritoneal cells were harvested, placed into 15 ml centrifuge tubes. The cells were pelleted by centrifugation at 250-x g for 10 min at room temperature.

Human peripheral blood mononuclear and polymorphonuclear cells were isolated from peripheral blood obtained from normal donors. The blood was collected in heparin. The various cell types were isolated by centrifugation over Ficoll-Hypaque at 500-x g for 60 min at room temperature. Each fraction was harvested, pooled separately and washed 1x in RPMI 1640 with antibiotics.

2. METABOLIC LABELING OF CELLS WITH <sup>35</sup>S-METHIONINE --- Cells were adjusted to 1 x 10<sup>7</sup> per ml of methionine-less RPMI 1640 media containing 10 μCi of <sup>35</sup>S-methionine and held overnight at 37 °C in the presence of 5 % CO<sub>2</sub>.

3. HARVESTING OF CELLS AND PREPARATION OF CRUDE MEMBRANES --- Cells were washed 3X in PBS and subsequently lysed by sonication in HEPES buffer, pH 7.2 containing 0.3% NP40 and proteinase inhibitor cocktail. The resulting cellular preparation was centrifuged at 600 x g for 10 min and the supernatant collected for further analyses.

4. SEPHAROSE and SEPAROSE HK-X CHROMATOGRAPHIC SEPARATION OF VARIOUS CELLULAR PROTEINS --- The cellular preparation was passed through a column of Sepharose unsubstituted resin or to Sepharose HK-X resin and divided into two portions, A and B.

Aliquot A: was passed over HK-X substituted Sepharose column. Columns washed and then eluted with buffer containing HK-X (5 mg/ml), and then with 0.1 M glycine, pH 2.5.

Aliquot B was bound to a HK-X substituted column in the presence of soluble HK-X and the proteins eluted. Each fraction was concentrated and lyophilized.

The approach undertaken in this step involved binding of HK-X to a Sepharose resin to make a HK-X substituted resin. Prior to exposure to HK-X substituted resin, the labeled cellular protein mixture was passed over a resin not substituted with HK-X to remove any protein species reacting with the native resin. Thus, when the cellular proteins including the receptor proteins were passed through the HK-X substituted resin under proper ionic environments, the receptor proteins (for the HK-X receptor) among the other proteins bound tightly with the HK-X. The resin was washed with a gentle agent, such as phosphate buffer at neutral pH, to remove any low affinity binding proteins. Subsequently, the resin was exposed to an excess amount of free HK-X to competitively elute receptor proteins bound to the resin. The radioactive proteins released at each of these steps was concentrated and analyzed on a 12% SDS-PAGE system as detailed in the following step.

5. 12% SDS-PAGE --- 25 uL of the radioactive cellular preparation containing 250 cpm to 2000 cpm radioactivity was applied to each lane of the gel. The gel was run at 90 V at 30 mA until good resolution of colored standards was obtained. The standards were phosphorlyase b (MW = 94,000); bovine serum albumin (MW= 68,000); ovalbumin (MW= 43,000); carbonic anhydrase (MW= 30,000); and soybean trypsin inhibitor (MW= 21,000).

6. ESTIMATION OF MOLECULAR WEIGHT OF RESOLVED RADIOACTIVE PROTEINS --- The relative mobilities were calculated for the standards and for each distinct molecular weight species visualized on the gel. A plot of log molecular weight of the standards was plotted against relative mobility for each standard. The data were entered in PRISM software and the molecular weights of the unknown proteins were predicted from a Standard Curve Program. The results were compared to FPR receptor proteins published in the literature (Goetzl et al., *Biochemistry* 20:5717-5722, 1981).

FIG. 4 shows the result of a representative experiment. All proteins present in the cell lysate are shown in Lane A. In Lane B, the unbound material from the Sepharose column without HK-X substitution shows a pattern of protein band distribution similar to the entire cell lysate. Lane C contains the pre-elution material. Lane D is a blank lane. In Lane E, three protein bands are visible when the column was eluted in the presence of 1 mg of HK-X (competitor). The molecular weights are estimated to be ~94,000, ~68,000 and ~40,000 Daltons, respectively. This experimental condition established the specificity of the binding.

Comparison of the relative abundance of the bands eluted in this experiment to that reported by Goetzl et al. revealed interesting differences (see Table 2). Examination of the normalized proportions of receptors reveals a substantial difference in the distribution of the three species recovered from FMLP column (Goetzl) and from HK-X column (this Example). The 68 Kd species was the dominant species recovered by Goetzl et al. from the FMLP affinity column whereas the 40 Kd species was the predominant species recovered from HK-X affinity column.

TABLE 2

Differences in the Reported Recovery of Formyl Receptors from FMLP-Sepharose  
from that Recovered from HK-X-Sepharose

Molecular Weight	# of total residues containing methionine	Proportion of total receptor population by FMLP	Proportion of total receptor population by HK-X	Normalized proportions of receptor proteins	
				FMLP	HK-X
94,000	14	15	26	1.07	1.8
68,000	11	80	28	7.27	2.54
40,000	4	5	41	1.25	10.25

5

It is clear from the radioautograph in FIG. 4 that there are three molecular species eluted from rat peritoneal mast cells by the addition of 1 mg of HK-X. The calculated molecular weights of these proteins agree with the molecular sizes reported by Goetzl et al. The molecular weights of proteins recovered after competition with HK-X or acid from rat peritoneal mast cells and neutrophils were the same as those reported by Goetzl for human neutrophils.

10

Although the total mean number of residues of methionine in the 40 Kd molecule is 5.1, it has a more intense radiographic image than either the 68,000 and 94,000 species which contain 11 and 14 residues per molecule, respectively. In this Example, the relative abundance of each of the protein species recovered was determined by biosynthetic radiolabeling with <sup>35</sup>S-methionine. The relative proportions recovered from affinity chromatography were determined by integration of the areas under the curves from the autoradiographs. Goetzl measured the relative abundance of each protein species by determination of protein by chemical methods. Therefore, to more accurately compare the distribution of the proteins recovered in the two studies, the distribution values were normalized by dividing the relative abundance of each species by its number of methionine residues. This ratio

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corrects for potential variations in the values due to differences in methodologies. The corrected ratios revealed a vastly different binding and/or recovery profile between the values reported by Goetzl and the values obtained in this Example. One possibility is that the detergent disassociated receptor  
5 complex binds to FMLP-substituted Sepharose in a selective manner different from that of HK-X substituted Sepharose.

In summary, in this Example, HK-X was used to selectively recover the binding proteins in a cell lysate recovered after protein labeling with <sup>35</sup>S-methionine in vitro. Three molecular weight species were isolated which agree  
10 with previously reports on the size of peptides associated with the FMLP receptor complex.

It also has been demonstrated by the techniques used in this Example that HK-X binds to receptors on human polymorphonuclear and mononuclear  
15 cells, particularly activated T-cells, mast cells, eosinophils, and basophils.

#### EXAMPLE 4: Signal Transduction and Mechanism of Action

Leukocytes respond to a large number of chemoattractants and other proinflammatory mediators. Some mediators cause chemotaxis, activation of enzyme systems and release of pathologically significant mediators. The typical N-formyl peptides (the archetypal one -- FMLP), activated complement fragment  
20 (C5a), leukotriene B4 (LTB4), platelet activating factor (PAF), and some chemotactic cytokines (such as IL-8) are well-recognized chemotactic and pro-inflammatory agents. These agents bind to G-protein-coupled receptors (GPCRs) with subsequent generation of multiple signal transduction mediated by protein kinase systems. The cascades resulting for the initial events are  
25 complex and interrelated, yet are responsible for the entire behavior of all nucleated cells. Programmed cell death (apoptosis), generation of immune responses, removal of self-recognizing T cells, and control of synthesis of  
30

extracellular matrices are just a few examples of the action of signal transduction pathways.

5 Protein kinases were identified by their capacity to transfer a phosphate group from a phosphate donor onto an acceptor amino acid located within a protein. Usually the  $\gamma$  phosphate of ATP is the donor. The three major acceptor amino residues within proteins are tyrosine, serine and threonine. As of 1999, over 115 protein kinases have been identified and described in the literature.

10 The behavior of cells in response to stimulation with FMLP is well described in the literature (Prosnitz et al., *Pharmacol. Ther.* 74: 73-102, 1997). FMLP binding to phagocytes stimulates phosphorylation, which correlates with cellular functions. FMLP and other chemoattractants stimulate phosphatidylinositol 3-kinase (PI3K) which in turn activates protein kinase  
15 (PKC). In neutrophils, FMLP binding initiates phosphorylation of an extracellular regulated kinase, (ERK-1) which belongs to a general family of kinases termed mitogen-activated protein kinases (MAP kinases). Some of the members of the MAP kinase family are: Raf-1 and Ras.

20 Members of the protein kinase families usually differ in molecular weight to such an extent that they can be resolved one from another by SDS-PAGE technology. Further, phosphotyrosine proteins can be detected from the entire mass of intracellular proteins by monoclonal antibodies which recognize only the phosphotyrosine epitope (Ross et al., *Nature (London)* 294: 654, 1981;  
25 Frackleton et al., *Mol. Cell Biol.* 3: 1343, 1983).

30 Changes in protein kinases mediated by the addition of HK-X to human peripheral blood mononuclear and polymorphonuclear cells were analyzed in order to elucidate the mechanism of action of the HK-X. HK-X was added alone and with the addition of FMLP or IL-8, which are known chemotactic and pro-inflammatory agents.

## DETAILED MATERIALS AND METHODS:

1. ISOLATION OF CELLS --- Human peripheral blood mononuclear and polymorphonuclear cells were isolated from peripheral blood obtained from normal donors. The blood was collected in heparin. The various cell types were isolated by centrifugation over Ficoll-Hypaque at 500-x 9 for 60 min at room temperature. Each fraction was harvested, pooled separately and washed 1x in RPMI 1640 with antibiotics.

2. CULTURE AND TREATMENT OF CELLS ---  $10^7$  cells per ml of media were held at 37°C for 30 min prior to the addition of stimulants in order to allow cells to reach a steady state within the phosphorylated protein pools. Then the following stimulants were added to each ml of cells:

- A. 100 uL of vehicle (0.3% DMSO solution in media) .
- B. 100 uL of HK-X contained 20 ug HK-X .
- C. 100 uL of FMLP contained 0.1 ug FMLP .
- D. 100 uL of IL-8 contained 0.1 ug IL-8 (recombinant human IL-8).
- E. 100 uL of HK-X contained 20 ug HK-X plus 100 uL of FMLP contained 0.1 ug FMLP.
- F. 100 uL of HK-X contained 20 ug HK-X plus 100 uL of IL-8 contained 0.1 ug IL-8.
- G. cell culture media without any stimulants.

Cells were incubated for an additional 30 min at 37°C in 5% CO<sub>2</sub>.

3. HARVESTING OF CELLS AND SDS-PAGE ANALYSIS --- Cells were pelleted at 250 x g for 5 min at room temperature. The supernatant was removed and 25 uL of 2X SDS-PAGE starting buffer added. The pellets were boiled for 15 min and centrifuges at 10,000 x g for 5 min. Small samples were removed for gel electrophoresis on 12% acrylamide gels.

4. IMMUNOBLOT DETECTION OF PHOSPHOPROTEINS --- The proteins were transferred onto nylon membrane at 13 V for 30 min and subsequently blocked with 1% BSA for 12 hr. The antibody conjugated with

HRP in 0.3% BSA was added for 60 min. Membranes were washed, fixed and photographed.

5           5.       DATA ANALYSIS ---- The photographs were taken to a professional laboratory and a negative copy was made of each gel using very high contrast and low grain film. The subsequent photographs were scanned at 600 dpi and densitometric analysis performed using Image Pro Plus software, SPSS. Molecular weights were calculated for each band.

10           In order to standardize the amount of cellular protein applied to each lane of the SDS-PAGE, the same number of cells were used for each treatment and approximately the same volume of sample applied to each lane. In FIGs. 5 through 9, the chemiluminescence patterns of phosphoproteins detected by monoclonal anti-phosphotyrosine antibody are shown. A negative of the  
15           chemiluminescence images was made by a professional photographer in order to obtain the highest degree of resolution of the bands and their corresponding intensity of chemiluminescence. For example, in FIG. 5A, the protein kinases present in cells exposed to vehicle for 30 minutes revealed 9 distinct protein species as shown by peaks in a densitometry analysis of the gel. For the sake of  
20           clarity, peaks in the densitometry analysis are marked by arrows and the corresponding molecular species in the gel are also marked by the same arrows. (The origin of the gel is on the right side of the gel where the larger molecular weight species are located).

25           In FIG. 6, the protein kinase responses of cells to HK-X are depicted. An apparent increase in a molecular weight species of approximately 83 Kd was detected which was not observed in other treatments. Striking differences were seen after the simultaneous treatment of cells with 20 ug HK-X and 0.1 ug IL-8 and 20 ug HK-X and 0.1 ug FMLP (data not shown). The smaller molecular  
30           weight species appeared greatly reduced (left most portion of gel). This reduction is reflected quantitatively by the area of the peaks. This area in the densitometric tracing is shown by the horizontal double-headed arrow. The protein kinase content and distribution of fresh mononuclear cells is shown in

FIG. 9. The distribution of kinases of these cells is strikingly similar to that of vehicle treated cells (FIG. 5) and HK-X treated cells (FIG. 6). The kinase response patterns to FMLP stimulation alone and to HK-X plus FMLP stimulation is not shown. However, the patterns of each of these were substantially identical to that observed with IL-8 alone and HK-X plus IL-8, respectively.

The pattern and distribution of protein kinases for peripheral blood polymorphonuclear cells was essentially the same as that for the mononuclear cells. The primary difference between the two cell types was that mononuclear cells were more metabolically active than the polymorphonuclear cells.

Using the densitometric analytical approach, the area under the peaks for each molecular weight species was calculated. Thus, the quantitative assessment of each kinase as a percent of the total kinase content was calculated. In addition the molecular weight of known kinases was compared to that calculated from the relative migration (Rf) calculation in this experiment. Thus, the kinases in this study could be identified.

Table 3 shows the quantitative change in the distribution of protein kinases from human peripheral blood cells after exposure to HK-X.

TABLE 3

	Protein Kinase	Molecular Weight	% Change with HK-X
25	PLC $\gamma$	150 Kd	NC
	PI3	102 Kd	+57
	PI3	83 Kd	+55
	Raf	65-68 Kd	-42
	Pp60 Src	62 Kd	NC
30	G-protein $\alpha$	56 Kd	-49
	ERK-1	44-49 Kd	NC
	G-protein $\beta$	33-35 Kd	-25
	Ras	21 Kd	-32
35	G-protein $\gamma$	9 Kd	NC

Table 4 shows the quantitative amount of G protein  $\gamma$  kinase for various experiments, which confirmed and extended the previous qualitative data. Although it was attempted to use the same number of cells were used for each treatment and approximately the same amount of intracellular protein recovered was applied to each lane, the total amount of kinases differed as seen by the areas observed.

TABLE 4

Summary of Protein Kinase from Peripheral Blood Cells After Exposure to HK-X and IL-8 and FMLP

Protein Kinase	MW (kD)	Vehicle	HK-X Alone	FMLP Alone	IL-8 Alone	HK-X + FMLP	HK-X + IL-8	Fresh Normal Cells
G-protein $\gamma$	9	10	11.4	10.1	14.8	2.7	3.4	11.9
Total areas		164,413	246,023	189,169	170,985	98,097	128,937	176,442

Table 5 illustrates the results of another experiment that shows the change in the distribution of protein kinases from human peripheral blood cells after exposure to HK-X compared to costimulatory exposure to (1) HK-X and (2) FMLP or IL-8.

Tables 6 and 7 illustrate the results of a further experiment that shows the change in the distribution of protein kinases from human peripheral blood cells after exposure to HK-X compared to costimulatory exposure to (1) HK-X and (2) Ca<sup>2+</sup>, TNF $\alpha$ , IL-4, IL-6, IL-10 or IL-13.

**Table 5.**  
**Summary of Tyrosine Protein Kinases Identification and Distribution in Peripheral Blood Cells**

-----PERCENT DISTRIBUTION -----

<b>Protein Kinase</b>	<b>Molecular Weight</b>	<b>Vehicle</b>	<b>HK-X Alone</b>	<b>HK-X + fMLP</b>	<b>HK-X + IL-8</b>	<b>Fresh Normal Cells</b>
PI 3 Kinase	102 kd	5	4	5.1	4	3
PI 3 Kinase	83 Kd	5.2	7.7	11	10.4	4.9
Raf	65-68 Kd	7	7.8	9.5	8.9	5
Ras	21 Kd	18	12.7	5.7	11.3	18.9
Pp60 Src	62 Kd	6.3	5	6.6	8.8	8.7
ERK-1	44-49 Kd	6.6	6.9	4.8	8.6	8.5
G-Protein α	56 Kd	6.9	6.3	7.8	8.2	12.3
G-Protein β	33-35 Kd	6.6	7	4.4	5.3	9.3
G-Protein γ	9 Kd	10	11.4	2.7	3.4	11.9

Table 6.

Summary of Tyrosine Protein Kinases Identification and Distribution in Peripheral Blood Cells

-----PERCENT DISTRIBUTION -----

<u>Protein Kinase</u>	<u>Molecular Weight</u>	<u>C5α Plus</u> <u>HK-X</u>	<u>TNFα Plus</u> <u>HK-X</u>	<u>IL-4 Plus</u> <u>HK-X</u>	<u>HK-X</u>
PLCγ	150 Kd	2.2	2.4	7.7	4.2
PI 3 Kinase	102 kd	2.7	3.1	6.0	3
PI 3 Kinase	83 Kd	6.9	5.5	7.7	6.8
Raf	65-68 Kd	6.4	5.7	3.1	3
Ras	21 Kd	16.5	20.9	9.9	16.4
Pp60 Src	62 Kd	5.8	7.4	4.5	5.1
ERK-1	44-49 Kd	6.5	5.9	6.4	6.0
G-Protein α	56 Kd	5.5	6.3	7.9	7.5
G-Protein β	33-35 Kd	6.2	6.2	8.0	8.2
G-Protein γ	9 Kd	8.0	7.4	5.8	9.4

Table 7.

Summary of Tyrosine Protein Kinases Identification and Distribution in Peripheral Blood Cells

-----PERCENT DISTRIBUTION -----

<b>Protein Kinase</b>	<b>Molecular Weight</b>	<b>IL-6 Plus HK-X</b>	<b>IL-10 Plus HK-x</b>	<b>IL-13 Plus HK-X</b>	<b>Vehicle</b>
PLC $\gamma$	150 Kd	5.0	3.8	11.2	5.8
PI 3 Kinase	102 kd	3.5	3.4	13.3	4.0
PI 3 Kinase	83 Kd	4.3	5.7	7.2	7.6
Raf	65-68 Kd	6.7	2.6	5.7	3.6
Ras	21 Kd	17.6	15.8	9.8	13.3
Pp60 Src	62 Kd	6.7	4.7	4.4	4.8
ERK-1	44-49 Kd	8.9	8.8	6.9	5.7
G-Protein $\alpha$	56 Kd	8.7	8.1	4.9	7.9
G-Protein $\beta$	33-35 Kd	6.6	8.5	5.2	7.2
G-Protein $\gamma$	9 Kd	4.4	11.1	3.4	6.7

A number of important events transpire during stimulation of cells bearing receptors for formyl peptides. These include:

- 5 A. Upon binding FMLP or other analogues, the FPR interacts with the G-protein pool that is common to other chemoattractant receptors such as the LTB<sub>4</sub> and C5a receptors (Jacobs et al., *J. Leukoc. Biol.* 57: 679-686, 1995; McLeish et al., *Mol. Pharm.* 36: 384-390).
- 10 B. Following stimulation with FMLP or other chemoattractants, cells become refractory to further or subsequent responsiveness to the same or other chemoattractants. When the refractory state or receptor inactivation is induced by one stimulant and affects multiple unliganded receptors, this situation is heterologous desensitization. It has been speculated that this form of desensitization may result from the phosphorylation of the unliganded receptors by downstream-activated kinases, as with the FC $\epsilon$ R receptor.
- 15 C. IL-8, C5a, and FMLP desensitized each other's receptors. These studies were extended to include PAF and LTB<sub>4</sub> receptors. The mechanisms by which this process may be initiated may involve receptor phosphorylation by PKC. However, the FPR does not contain intracellular domains capable of being phosphorylated by PKC, but another kinase appears to be responsible.
- 20 D. Desensitization of downstream FPR responses occurs in the absence of FPR phosphorylation.

HK-X treatment alone did not specifically down-regulate kinases. G-protein  $\gamma$  kinase was selectively and significantly decreased after simultaneous stimulation of mononuclear cells with HK-X and IL-8 or with HK-X and FMLP. These observations suggest the following mechanism of action of HK-X, which is described below.

30 HK-X and potential pro-inflammatory molecules, such as IL-8 and FMLP can be present together. There is a down-regulation of one of the trimeric G-protein complexes. Specifically, G-protein  $\gamma$  phosphorylation was decreased under the steady state conditions. Therefore, HK-X in the presence of IL-8 or FMLP appears to initiate a receptor desensitization that either directly or indirectly inhibits phosphorylation of G-protein  $\gamma$  subunit.

There are a number of corollaries, which can be drawn from the data presented here and in previous reports. They are:

- 5 1. HK-X demonstrates specificity for cells capable of responding to pro-inflammatory stimuli mediated by specific pro-inflammatory receptors.
2. If HK-X is maintained in the system during inflammatory events, HK-X should maintain desensitization of the potentially responding cells.
3. Using an in vitro culture system as described in this report, other cell  
10 targets and other pro-inflammatory mediators can be screened for their sensitivity to the action of HK-X. Thus, a simple assay can be used to rapidly screen for potential therapeutic efficacy and to establish therapeutic predictability.

15 In part, HK-X appears to mediate its therapeutic efficacy through desensitization of pro-inflammatory mediator receptors. Downstream from this event, the inactivation of the inflammatory cell is mediated and maintained by either directly or indirectly through inhibition of phosphorylation of G-protein  $\gamma$  subunit.

20 The present invention has been described in detail including the preferred embodiments thereof. However, it will be appreciated that those skilled in the art may make modifications and improvements within the spirit and scope of the invention as set forth in the claims.

25

What is claimed is:

1. A method of inhibiting a pro-inflammatory response of a human peripheral blood mononuclear cell or polymorphonuclear cell, or a fixed tissue cell when contacted with a pro-inflammatory agent, said method comprising contacting said cell with a G protein kinase signal pathway modification agent, thereby inhibiting inflammatory response signal transduction pathways mediated by G protein.
2. The method of claim 1, wherein the human peripheral blood mononuclear cell is selected from lymphocytes and monocytes or a polymorphonuclear cell selected from granulocytes.
3. The method of claim 1, wherein the human peripheral blood mononuclear cell or polymorphonuclear cell is selected from eosinophils, basophils, and activated T-cells.
4. The method of claim 1, wherein the fixed tissue cell is selected from mast cells, dendritic cells, astrocytes or macrophages.
5. The method of claim 1, wherein the pro-inflammatory agent is selected from cytokines, chemotaxins, and mitogens.
6. The method of claim 1, wherein the pro-inflammatory agent is selected from fMLP, activated complement fragment, leukotriene B<sub>4</sub>, platelet activating factor, IL-4, IL-6, IL-8, IL-10, IL-13 and TNF $\alpha$ .
7. The method of claim 1, wherein the G protein kinase signal pathway modification agent is a f-Met-Leu peptide.
8. The method of claim 1, wherein the G protein kinase signal pathway modification agent is f-Met-Leu-Phe-Phe.

9. A method of inhibiting a pro-inflammatory response of a human peripheral blood mononuclear cell or polymorphonuclear cell, or a fixed tissue cell when contacted with a pro-inflammatory agent, said method comprising forming a complex of a cell surface receptor with a G protein kinase signal pathway modification agent, thereby inhibiting a pro-inflammatory response.

10. The method of claim 9, wherein the cell surface receptor is a formyl peptide receptor.

11. The method of claim 9, wherein the pro-inflammatory response includes inhibiting a down-stream receptor, wherein the downstream receptor is a FC receptor that is a receptor for an agent selected from an immunoglobulin, a cytokine selected from the group consisting of IL-4, IL-6, IL-8, IL-10, IL-13 and TNF $\alpha$ , or a chemokine selected from the group consisting of C5a, FMLP, PAF, LTB<sub>4</sub>.

12. The method of claim 9, wherein the human peripheral blood mononuclear cell is selected from lymphocytes and monocytes or a polymorphonuclear cell selected from eosinophils and basophils.

13. The method of claim 9, wherein the human peripheral blood mononuclear cell or polymorphonuclear cell is selected from, eosinophils, basophils, and activated T-cells.

14. The method of claim 9, wherein the fixed tissue cell is selected from mast cells, dendritic cells, astrocytes and macrophages.

15. The method of claim 9, wherein the pro-inflammatory agent is selected from cytokines, chemokines, chemotaxins, and mitogens.

16. The method of claim 9, wherein the pro-inflammatory agent is selected from fMLP, activated complement fragment, leukotriene B<sub>4</sub>, and platelet activating factor, or IL-4, IL-6, IL-8, IL-10, IL-13 and TNF $\alpha$ .

17. The method of claim 9, wherein the G protein kinase signal pathway modification agent is a f-Met-Leu peptide.

18. The method of claim 9, wherein the G protein kinase signal pathway modification agent is f-Met-Leu-Phe-Phe.

19. A receptor complex comprising a G protein kinase signal pathway modification agent and a cell surface receptor of a human peripheral blood mononuclear cell or polymorphonuclear cell, or a fixed tissue cell that has been stimulated by a pro-inflammatory agent.

20. The receptor complex of claim 19, wherein the human peripheral blood cell is a mononuclear cell selected from lymphocytes and monocytes or a polymorphonuclear cell selected from eosinophils and basophils.

21. The receptor complex of claim 19, wherein the fixed tissue cell is selected from mast cells, dendritic cells, astrocytes and macrophages.

22. The receptor complex of claim 19, wherein the G protein kinase signal pathway modification agent is a f-Met-Leu peptide.

23. The receptor complex of claim 19, wherein the G protein kinase signal pathway modification agent is f-Met-Leu-Phe-Phe.

24. The receptor complex of claim 19, wherein the pro-inflammatory agent is selected from cytokines, chemokines, chemotaxins, and mitogens.

25. The receptor complex of claim 19, wherein the pro-inflammatory agent is selected from fMLP, activated complement fragment, leukotriene B<sub>4</sub>, and platelet activating factor or IL-4, IL-6, IL-8, IL-10, IL-13 and TNF $\alpha$ .

26. A method for identifying a G protein kinase signal pathway modification agent, the method comprising the steps of:

- a) contacting a pro-inflammatory mediating cell selected from the group consisting of activated T-lymphocyte, mast cell, eosinophil and basophil with a known pro-inflammatory mediator selected from the group consisting of a cytokine, chemokine, chemotaxin, and mitogen and eliciting a pro-inflammatory response;
- b) adding to step a) a candidate G protein kinase signal pathway modification agent; and
- c) detecting a decrease or increase in amount of a regulating G protein kinase in (i) a pro-inflammatory mediating cell simultaneously contacted with said candidate antagonist and said pro-inflammatory mediator compared to (ii) a pro-inflammatory mediating cell contacted with said pro-inflammatory mediator alone.

27. The method of claim 26, wherein the pro-inflammatory mediator is selected from fMLP, activated complement fragment, leukotriene B<sub>4</sub>, and platelet activating factor, or IL-4, IL-6, IL-8, IL-10, IL-13 and TNF $\alpha$ .

28. A cell surface receptor complex comprising a G-protein kinase signal pathway modification agent binding with a receptor on the surface of a pro-inflammatory mediating cell selected from the group consisting of activated T-lymphocyte, mast cell, eosinophil and basophil, whereby inhibition of a pro-inflammatory cell response to a pro-inflammatory agent is provided.

29. A method for identifying a G protein kinase signal pathway modification agent, said method comprising the steps of:

- contacting a pro-inflammatory mediating cell selected from the group consisting of activated T-lymphocyte, mast cell, eosinophil and basophil with a candidate trimeric G- protein signal pathway modification agent; and
- determining the distribution of protein kinases produced by said cell, wherein compared to cells not contacted by the candidate trimeric G-protein signal pathway modification agent there is no change in the amount of

PLC $\gamma$ , Pp60 Src, and ERK-1, there is an increase in the amount of PI3 (102 Kd) and PI3 (83 Kd), and there is a decrease in the amount of Raf, Ras, G-protein  $\alpha$  and G-protein  $\beta$  kinases.

30. A cell surface receptor complex comprising:  
a cell surface receptor of a human peripheral blood mononuclear cell or a polymorphonuclear cell and  
a G- protein signal pathway modification agent,  
wherein, compared to cells not exhibiting said cell surface receptor complex, the distribution of protein kinases in the cells is such that there is no change in the amount of PLC $\gamma$ , Pp60 Src, and ERK-1, there is an increase in the amount of PI3 (113 Kd) and PI3 (83 Kd), and there is a decrease in the amount of Raf, Ras, G-protein  $\alpha$  and G-protein  $\beta$  kinases.

31. The cell surface receptor complex of claim 30, wherein the cell surface receptor is a FPR receptor.

32. The cell surface receptor complex of claim 30, wherein stimulation by IL-4 of the human peripheral blood mononuclear cell or the polymorphonuclear cell provides a distribution of protein kinases produced by said cell wherein, compared to stimulated cells not exhibiting said receptor complex, there is an increase in the amount of PLC $\gamma$ , PI3 (102 Kd) and PI3 (83 Kd), and a decrease in the amount of Ras and G-protein  $\gamma$  kinases.

33. The cell surface receptor complex of claim 30, wherein stimulation by the pro-inflammatory agent, IL-13, of the human peripheral blood mononuclear cell or the polymorphonuclear cell provides a distribution of protein kinases produced by said cell wherein, compared to stimulated cells not exhibiting said receptor complex, there is an increase in the amount of PLC $\gamma$ , PI3 (113 Kd) and PI3 (83 Kd), and a decrease in the amount of Raf, Ras, Pp60 Src, G-protein  $\alpha$  and G-protein  $\beta$  kinases.

34. The cell surface receptor complex of claim 30, wherein stimulation by the pro-inflammatory agent, C5a, of the human peripheral blood mononuclear cell or the polymorphonuclear cell provides a distribution of protein kinases produced by said cell wherein, compared to stimulated cells not exhibiting said receptor complex, there is an increase in the amount of PI3 (83 Kd) and Raf, and a decrease in the amount of PLC $\gamma$ , PI3 (113 Kd), G-protein  $\alpha$  and G-protein  $\beta$  kinases.

35. The cell surface receptor complex of claim 30, wherein stimulation by the pro-inflammatory agent, TNF $\alpha$ , of the human peripheral blood mononuclear cell or the polymorphonuclear cell provides a distribution of protein kinases produced by said cell wherein, compared to stimulated cells not exhibiting said receptor complex, there is an increase in the amount of Raf, ERK-1, Ras, G-protein  $\alpha$ , G-protein  $\beta$  and G-protein  $\gamma$ , and a decrease in the amount of PLC $\gamma$ , PI3 (113 Kd) and Pp60 Src kinases.

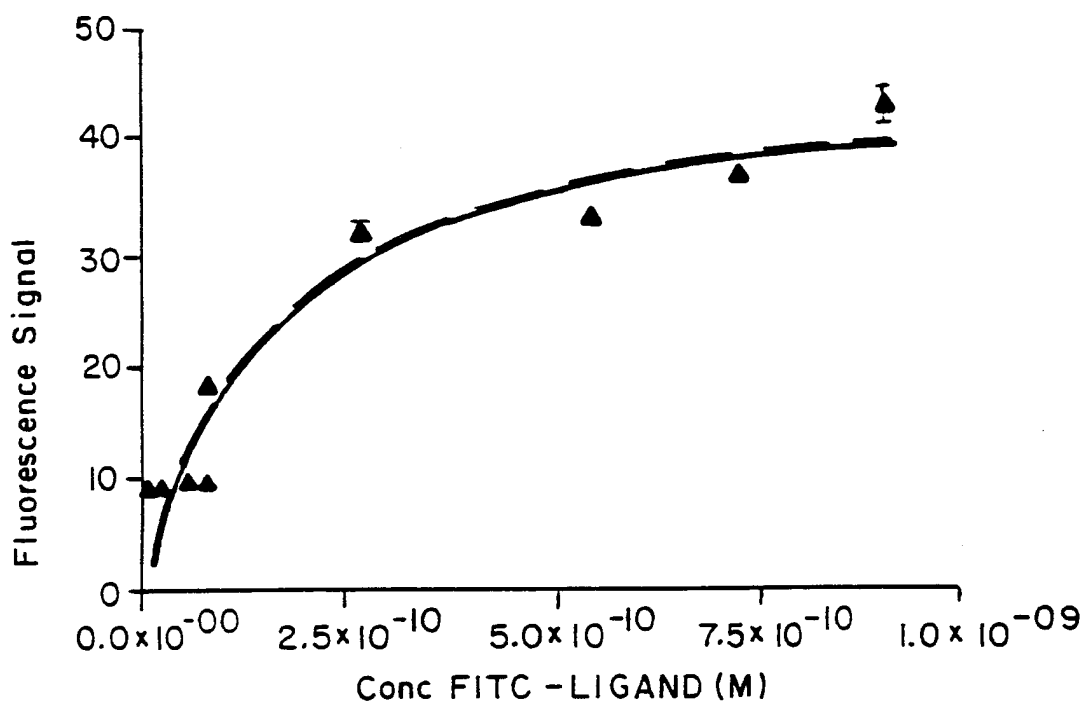


FIG. 1

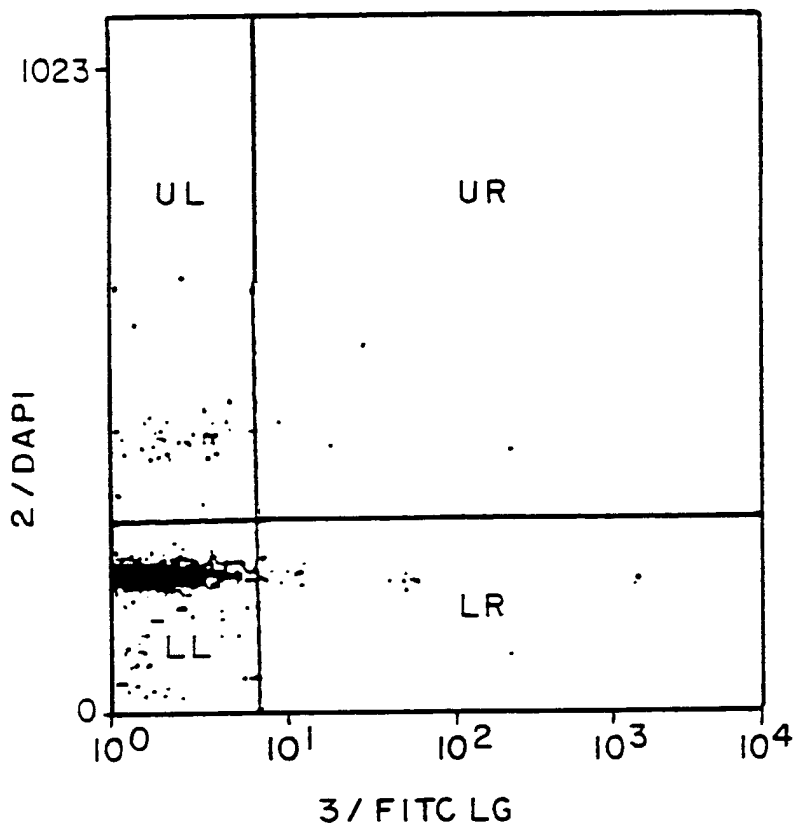
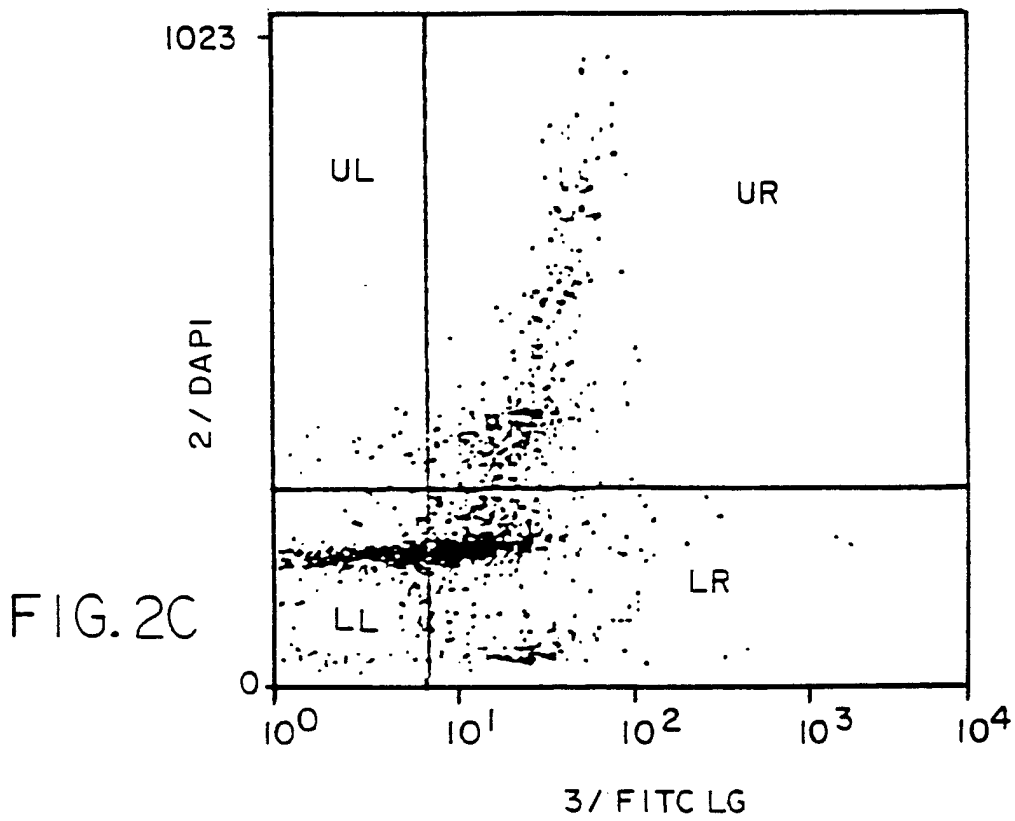
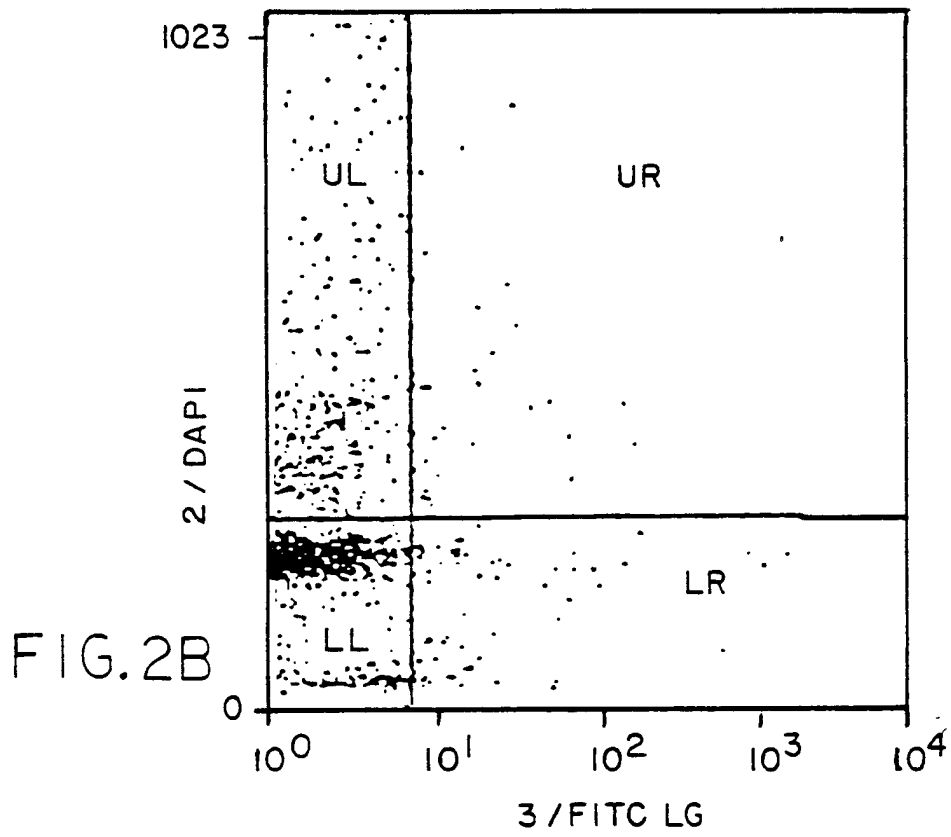
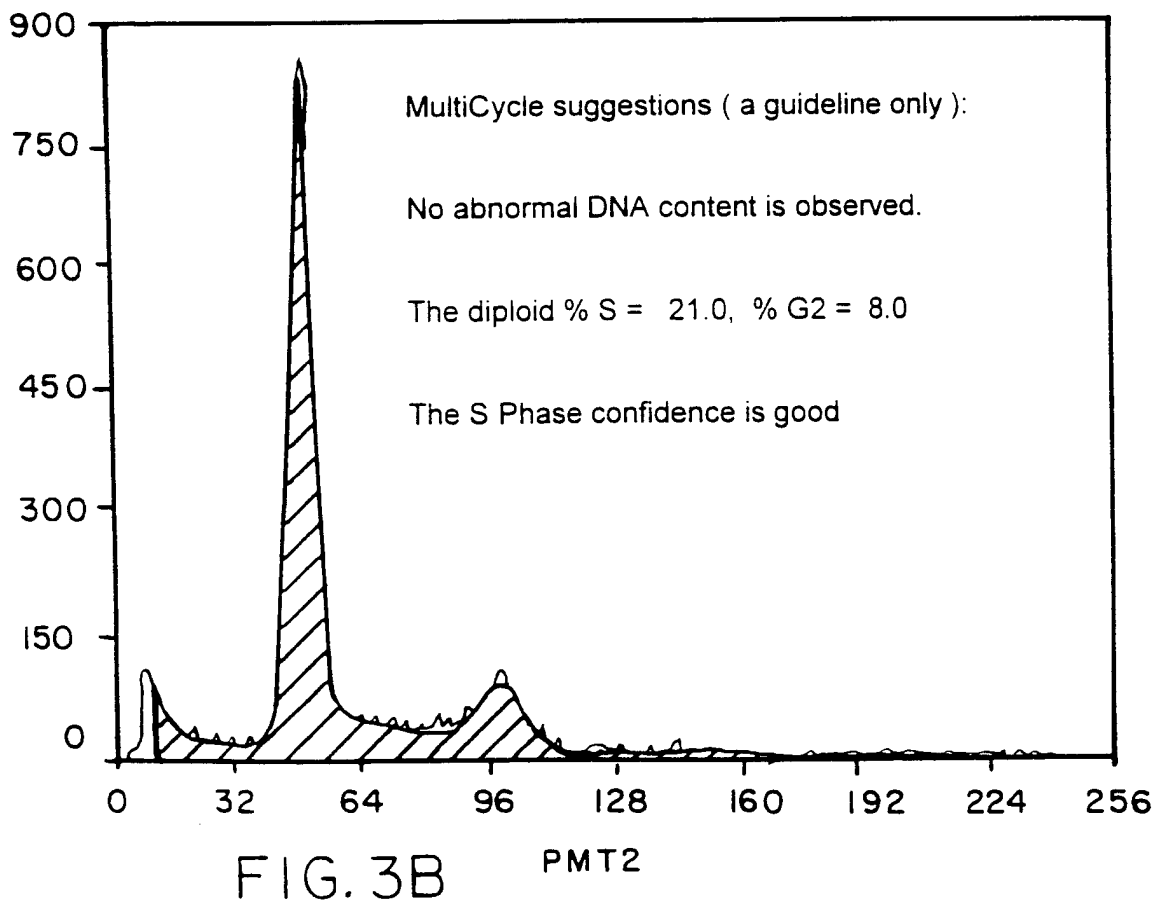
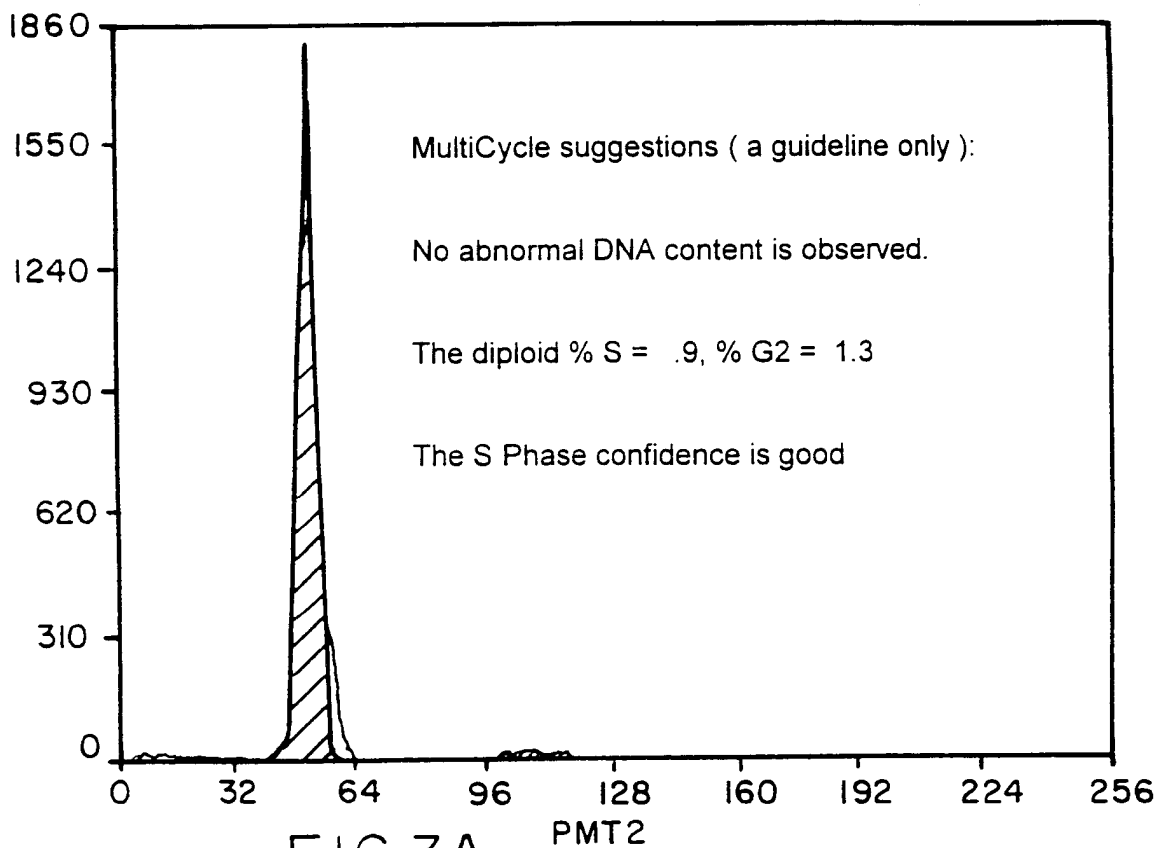


FIG. 2A





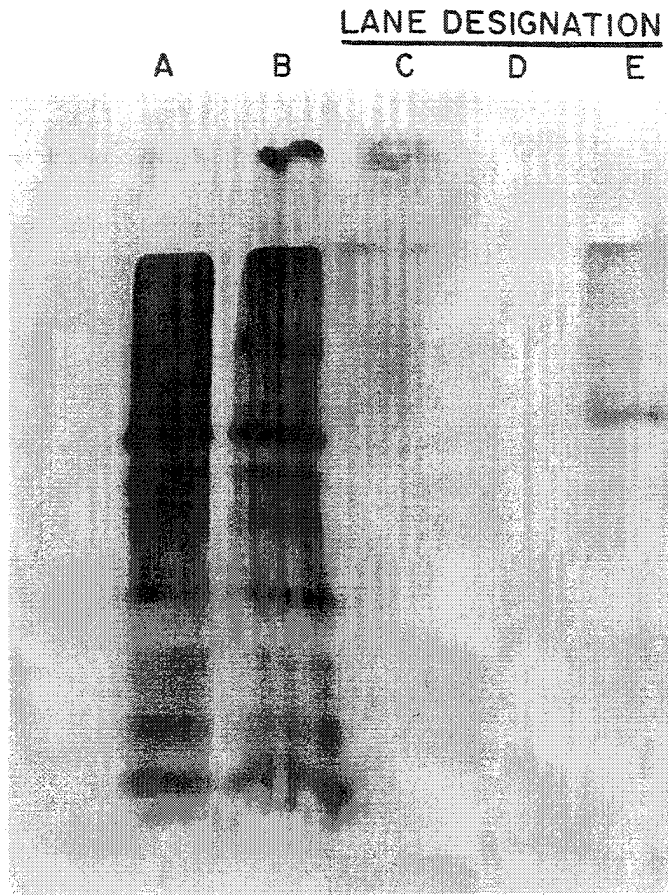


FIG. 4

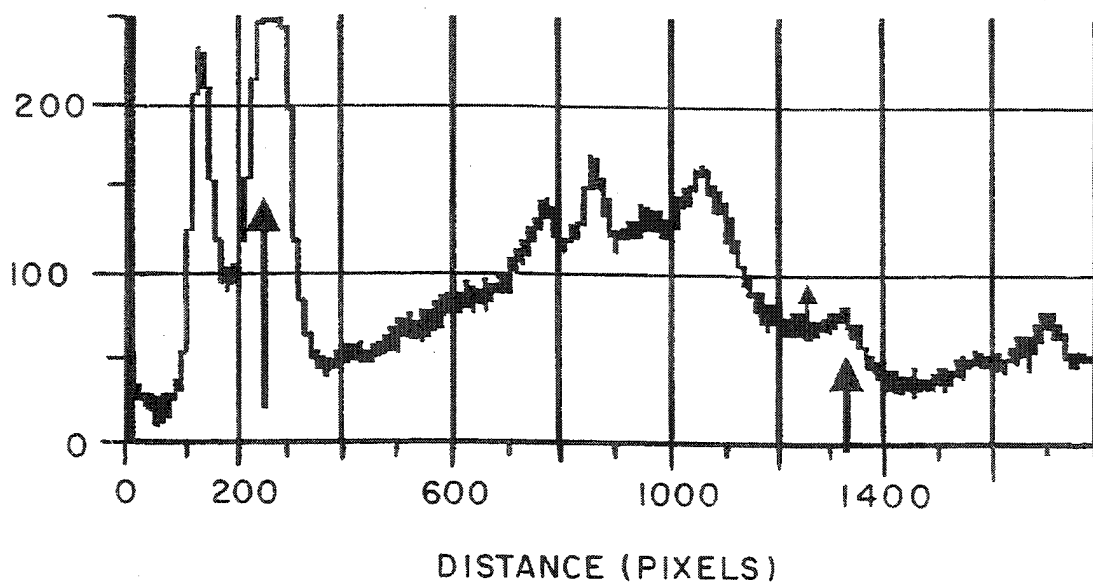


FIG. 5A



FIG. 5B

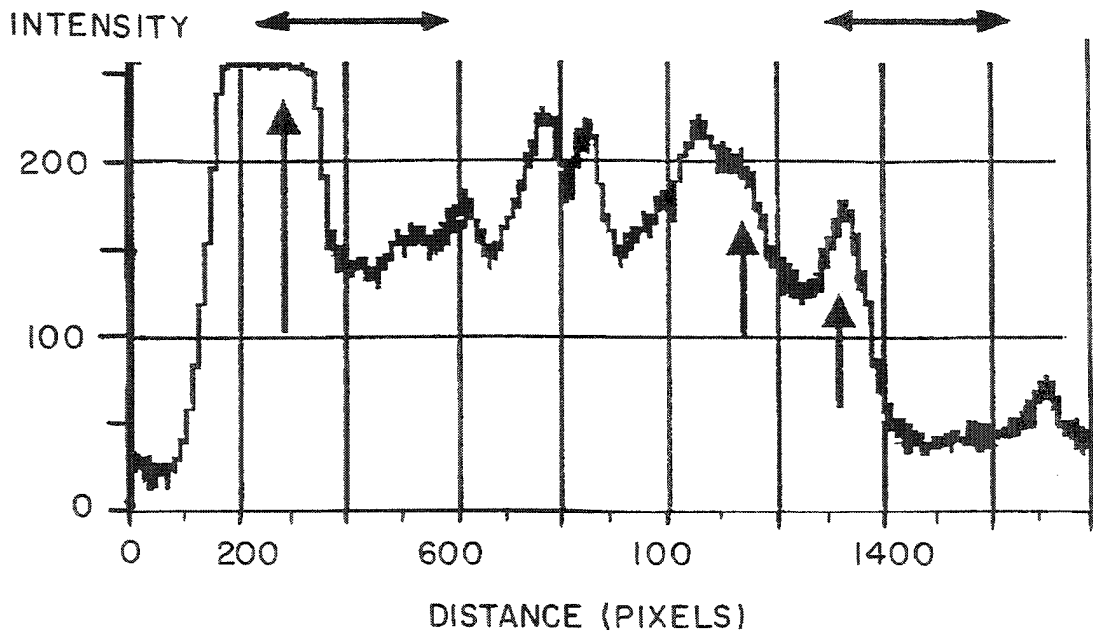


FIG. 6A



FIG 6B

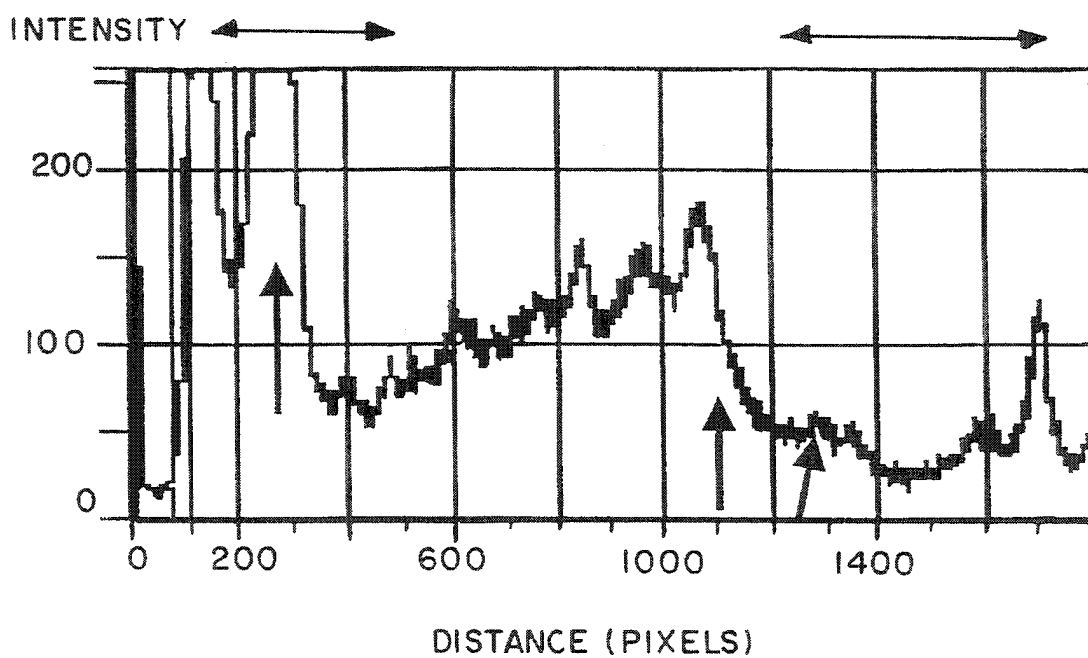


FIG. 7A

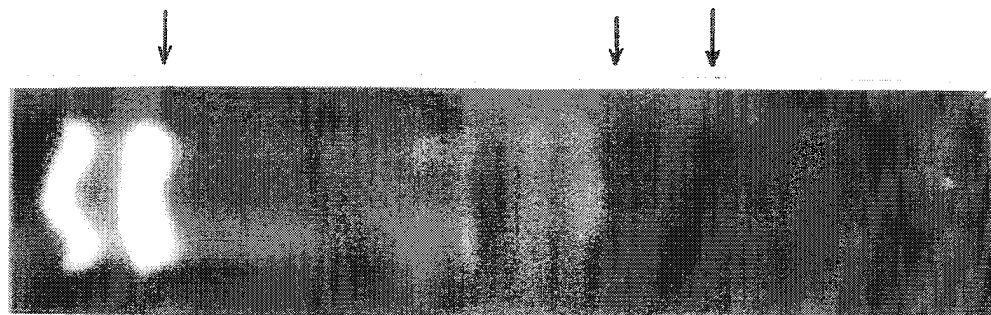


FIG. 7B

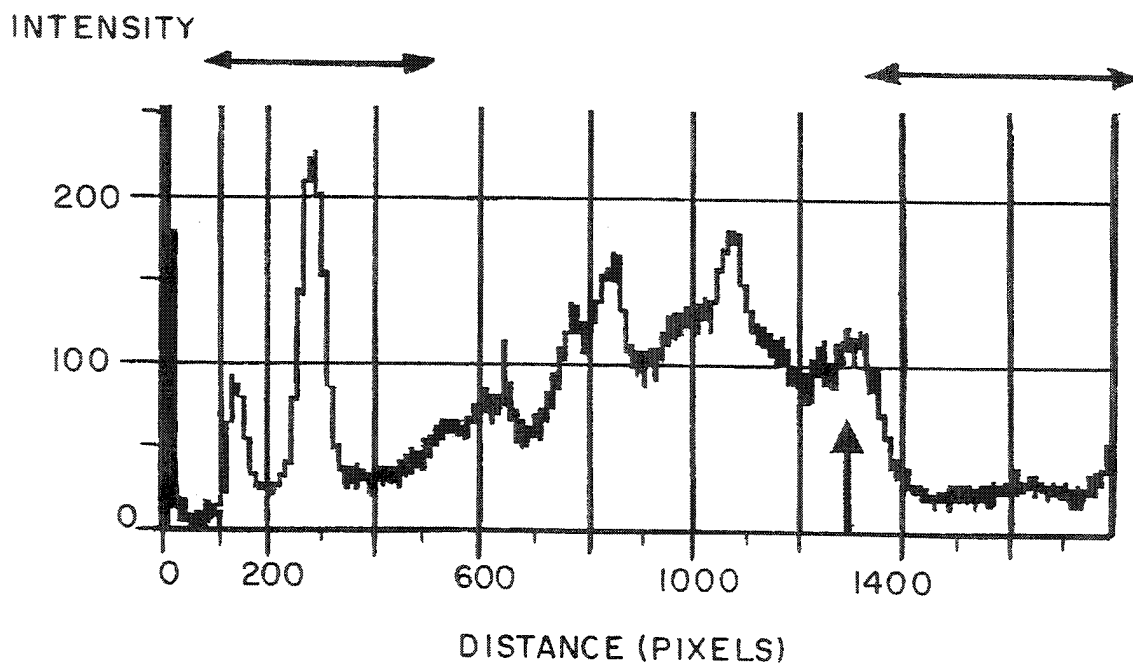


FIG. 8A

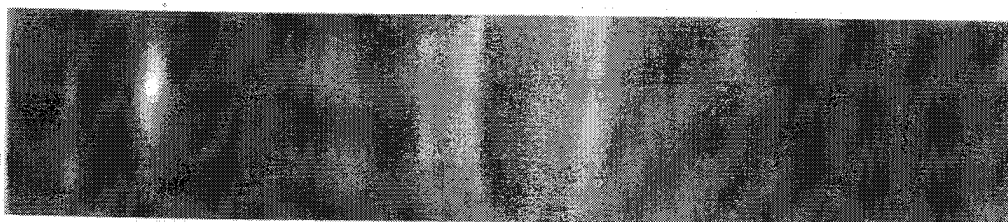


FIG. 8B

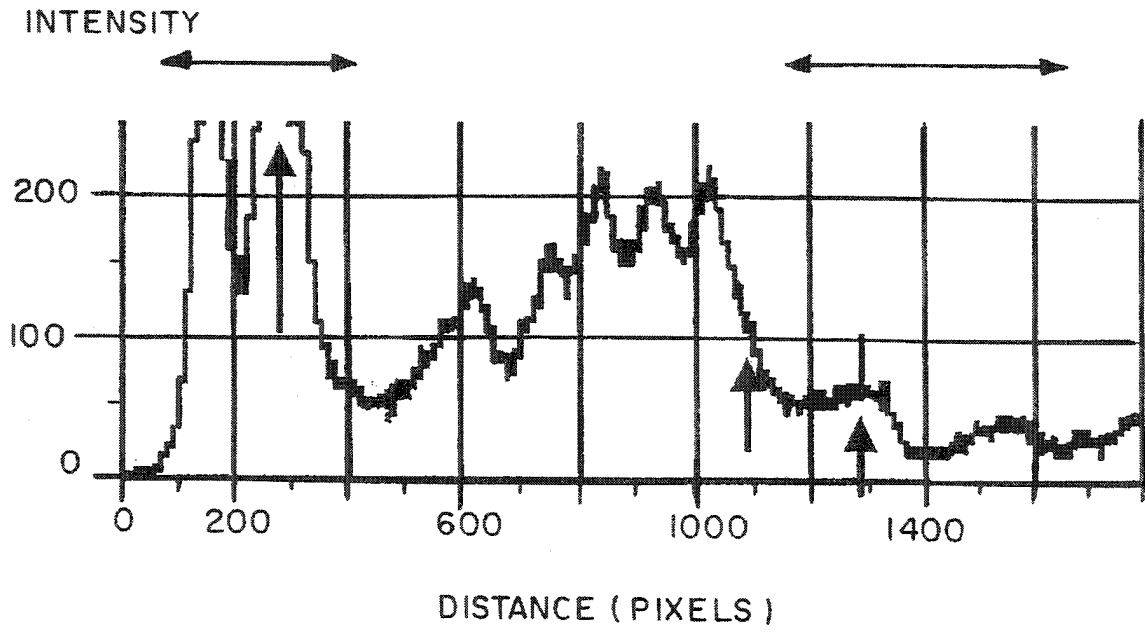


FIG. 9A

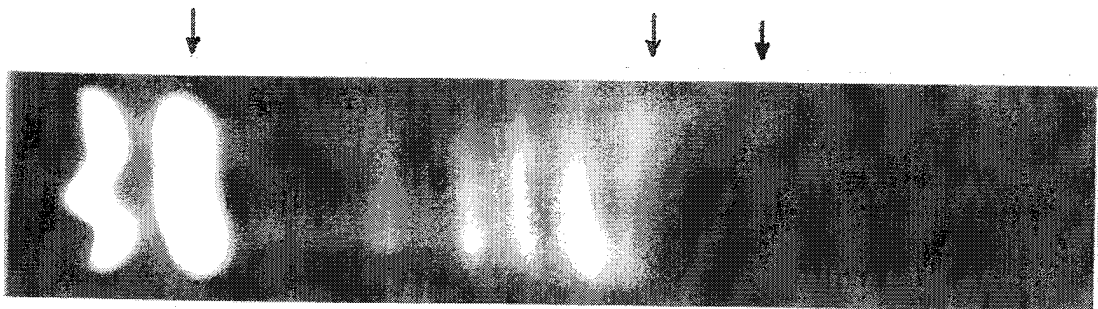


FIG. 9B

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/28183

**A. CLASSIFICATION OF SUBJECT MATTER**  
 IPC(7) : C07K 14/00; G01N 33/53  
 US CL : 435/7.1, 7.8; 530/350, 351  
 According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**  
 Minimum documentation searched (classification system followed by classification symbols)  
 U.S. : 435/7.1, 7.8; 530/350, 351

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
 WEST MEDLINE BIOSIS EMBASE CAPLUS

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BEVILACQUA, M. et al. Nimesulide decreases superoxide production by inhibiting phosphodiesterase type IV. European Journal of Pharmacology. 1994, Vol. 283, No. 3, pages 415-423, see entire document.	1-35
Y	SMITH, R.J. et al. NPC 15669-modulated human polymorphonuclear neutrophil functional responsiveness: effects on receptor-coupled signal transduction. British Journal of Pharmacology. 1995, Vol. 114, No. 8, pages 1694-1702, see entire document.	1-35

Further documents are listed in the continuation of Box C.  See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"G"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 12 FEBRUARY 2001	Date of mailing of the international search report <b>30 MAR 2001</b>
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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-8230	Authorized officer <i>Joseph F. Murphy</i> JOSEPH F. MURPHY Telephone No. (703) 308-0196
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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/28183

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<b>SIMON, J.C. et al. Phorbol myristate acetate-activated keratinocytes stimulate proliferation of resting peripheral blood mononuclear lymphocytes via a MHC-independent, but protein kinase C-and intercellular adhesion molecule 1-dependent, mechanism. Journal of Immunology. 15 January 1991, Vol. 146, No. 2, pages 476-484, see entire document.</b>	1-35
Y	<b>BERETA, J. et al. Inhibition of basil and tumor necrosis factor-enhanced binding of murine tumor cells to murine endothelium by transforming growth factor-<math>\beta^1</math>. Journal of Immunology. 01 May 1992, Vol. 148, No. 9, pages 2932-2940, see entire document.</b>	1-35

专利名称(译)	N-甲酰肽受体复合物与g蛋白激酶信号通路修饰剂		
公开(公告)号	<a href="#">EP1222199A1</a>	公开(公告)日	2002-07-17
申请号	EP2000970817	申请日	2000-10-12
[标]申请(专利权)人(译)	海斯塔泰克有限责任公司		
申请(专利权)人(译)	HISTATEK , LLC		
当前申请(专利权)人(译)	HISTATEK , LLC		
[标]发明人	CLAGETT JAMES A PALMER CRAIG		
发明人	CLAGETT, JAMES, A. PALMER, CRAIG		
IPC分类号	G01N33/50 A61K38/00 A61K38/05 A61K38/07 C07K5/083 C07K5/103 C07K14/705 C07K14/72 C12N5/06 C12Q1/02 G01N33/15 C07K14/00 G01N33/53		
CPC分类号	A61K38/05 A61K38/07 C07K5/081 C07K5/1013 C07K14/723		
优先权	60/159677 1999-10-15 US		
其他公开文献	EP1222199A4		
外部链接	<a href="#">Espacenet</a>		

#### 摘要(译)

描述了抑制人外周血单核细胞或多形核细胞或固定组织细胞的促炎反应的方法。使细胞与促炎剂接触以刺激促炎反应。然后，使细胞与G蛋白激酶信号通路修饰剂接触，从而抑制由G蛋白介导的炎症反应信号转导途径。描述了一种受体复合物，其中G蛋白激酶信号通路修饰剂结合已被促炎剂刺激的人外周血单核细胞或多形核细胞的细胞表面受体。