(54) NUCLEIC ACID ENCODING EOSINOPHIL EOTAXIN RECEPTOR

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(57) ABSTRACT

The eosinophil eotaxin receptor has been isolated, cloned and sequenced. This receptor is a human β-chemokine receptor and has been designated “CC CKR3”. The eosinophil eotaxin receptor may be used to screen and identify compounds that bind to the eosinophil eotaxin receptor. Such compounds would be useful in the treatment and prevention of atopic conditions including allergic rhinitis, dermatitis, conjunctivitis, and particularly bronchial asthma.
NUCLEIC ACID ENCODING EOSINOPHIL EOTAXIN RECEPTOR

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. § 119(e) from provisional application Case Number 19634PV, filed Apr. 26, 1996 and from provisional application Case Number 19697PV, filed Apr. 26, 1996 as U.S. Ser. No. 60/016,158.

FIELD OF THE INVENTION

This invention relates to an eosinophil eotaxin receptor ("CC CKR3"), in particular, the human eosinophil eotaxin receptor and nucleic acids encoding this receptor. This invention further relates to assays which may be used to screen and identify compounds that bind to the eosinophil eotaxin receptor. Such compounds would be useful in the treatment and prevention of atopic conditions including allergic rhinitis, dermatitis, conjunctivitis, and particularly bronchial asthma.

BACKGROUND OF THE INVENTION

Eosinophils play prominent roles in a variety of atopic conditions including allergic rhinitis, dermatitis, conjunctivitis, and particularly bronchial asthma (for a reviews see e.g. Gleich, G. J., et al., Eosinophils. J. I. Gallin, I. M. Goldstein, R. Snyderman, Eds., Inflammation: Basic Principles and Clinical Correlates (Raven Press, Ltd., New York, 1992) and Seminario, M. C., et al. (1994) Current Opinion in Immunology 6, 860-864). A pivotal event in the process is the accumulation of eosinophils at the involved sites. While a number of the classical chemoattractants, including CSa, LTB4, and PAF, are known to attract eosinophils (Gleich, G. J., et al., Eosinophils. J. I. Gallin, I. M. Goldstein, R. Snyderman, Eds., Inflammation: Basic Principles and Clinical Correlates (Raven Press, Ltd., New York, 1992)), these mediators are promiscuous, acting on a variety of leukocytes including neutrophils, and are unlikely to be responsible for the selective accumulation of eosinophils. In contrast, the chemokines a family of 8-10 kDa proteins are more restricted in the leukocyte subtypes they target and are potential candidates for the recruitment of eosinophils in atopic diseases and asthma (Baggiolini, M., Dewald, B. and Moser, B. (1994) Advances in Immunology 55, 97-179). Although there is a mounting body of evidence that eosinophils are recruited to sites of allergic inflammation by a number of β-chemokines, particularly eotaxin and RANTES, the receptor which mediates these actions has not been identified.


While elucidation of the actions of β-chemokines on eosinophils has contributed greatly to the understanding of eosinophil biology, information regarding the cell surface receptors which mediate these effects remain sparse. Furthermore, there are no reports describing binding studies of any of the β-chemokines to primary eosinophils. The known β-chemokine receptors are members of the G protein-coupled receptor superfamily. Two of these receptors, CC CKR1 (12, 13) and CC CKR2 (MCP-1R) (Charo, I. F., et al. (1994) Proceedings of the National Academy of Sciences 91, 2752-2756; Myers, S. J., et al. (1995) Journal of Biological Chemistry 270, 5786-5792; Franci, C., et al. (1995) Journal of Immunology 154, 6511-6517) found on monocytes, have been extensively studied and their selectivity for the different chemokines defined. However, neither of these receptors has the necessary ligand selectivity or the appropriate expression patterns required to mediate the effects of the β-chemokines on eosinophils. For example, CC CKR1 binds
RANTES with high affinity, but binds eotaxin poorly, and while the effects of eotaxin on CC CKR2 have not been studied this receptor has no avidity for RANTES (Myers, S. J., et al. (1995) Journal of Biological Chemistry 270, 5786-5792).

[0007] A review of the role of chemokines in allergic inflammation is provided by Kita, H., et al., J. Exp. Med. 183, 2421-2426 (June 1996). In particular, this review discusses the role which the receptor CKR-3 plays in the process of allergic inflammation. The cloning, expression and characterization of the human eosinophil eotaxin receptor has been reported by Daugherty, B., J. Exp. Med. 183, 2349-2354 (May 1996). This publication discloses the cloning and functional expression of the chemokine receptor CC CKR3, as well as its characterization.

[0008] The cloning and expression of a human eosinophil receptor was allegedly achieved by Combadiere, C., et al., J. Biological Chem. 270 (27), 16491-16494 (Jul. 14, 1995). However, in a subsequent retraction (J. Biological Chem. 270, 30235 (1995)) they confirmed that the receptor which was actually cloned and expressed was not CC CKR3, but was another CC chemokine receptor CC CRK5. This receptor was subsequently characterized by Kita, M., et al., J. Biological Chem. 271 (13), 7725-7730 (Mar. 29, 1996).

[0009] A human eotaxin receptor has been reported by Ponath, P. D., et al., J. Exp. Med. 183, 2437-2448 (June 1996) and Gerard, C. J., et al., PCT Publication No. WO 96/22771 (Jul. 25, 1996). However, the sequence disclosed in this publication possesses an error in the assignment of threonine rather than serine at position # 276 of the receptor. In addition, functionality of the receptor was not fully demonstrated.

[0010] A retrovirus designated human immunodeficiency virus (HIV-1) is the etiological agent of the complex disease that includes progressive destruction of the immune system (acquired immune deficiency syndrome; AIDS) and degeneration of the central and peripheral nervous system. This virus was previously known as LAV, HTLV-III, or ARV. Entry of HIV-1 into a target cell requires cell-surface CD4 and additional host cell cofactors. Fusin has been identified as a cofactor required for infection with virus adapted for growth in transformed T-cells, however, fusin does not promote entry of macrophage-tropic viruses which are believed to be the key pathogenic strains of HIV in vivo. It has recently been recognized that for efficient entry into target cells, human immunodeficiency viruses require the chemokine receptors CCR-5 and CXCR-4, as well as the primary receptor CD4 (Levy, N. Engl. J. Med., 335(20), 1528-1530 (Nov. 14 1996). The principal cofactor for entry mediated by the envelope glycoproteins of primary macrophage-tropic strains of HIV-1 is CCR5, a receptor for the β-chemokines RANTES, MIP-1α and MIP-1β (Deng, et al., Nature, 381, 661-666 (1996)). HIV attaches to the CD4 molecule on cells through a region of its envelope protein, gp120. It is believed that the CD-4 binding site on the gp120 of HIV interacts with the CD4 molecule on the cell surface, and undergoes conformational changes which allow it to bind to another cell-surface receptor, such as CCR5 and/or CXCR-4. This brings the viral envelope closer to the cell surface and allows interaction between gp41 on the viral envelope and a fusion domain on the cell surface, fusion with the cell membrane, and entry of the viral core into the cell. It has been shown that β-chemokine ligands prevent HIV-1 from fusing with the cell (Dragic, et al., Nature, 381, 667-673 (1996)). It has further been demonstrated that a complex of gp120 and soluble CD4 interacts specifically with CCR-5 and inhibits the binding of the natural CCR-5 ligands MIP-1α and MIP-1β (Wu, et al., Nature, 384, 179-183 (1996)); Ito, et al., Nature, 384, 184-187 (1996)).

[0011] Humans who are homozygous for mutant CCR-5 receptors which do not serve as co-receptors for HIV-1 in vitro appear to be unusually resistant to HIV-1 infection and are not immunocompromised by the presence of this genetic variant (Nature, 382, 722-725 (1996)). Absence of CCR-5 appears to confer protection from HIV-1 infection (Nature, 382, 668-669 (1996)). Other chemokine receptors may be used by some strains of HIV-1 or may be favored by non-sexual routes of transmission. Although most HIV-1 isolates studied to date utilize CCR-5 or fusin, some can use both as well as the related CCR-2B and CCR-3 as co-receptors (Nature Medicine, 2(11), 1240-1243 (1996)). The determination that chemokine receptors are critical co-receptors for the entry of HIV into cells was pronounced a “1996 Breakthrough of the Year” by Science Magazine (Science, 274, 1987-1991 (Dec. 20, 1996)).

[0012] The use of orally-active agents which modulate the action of the eosinophil eotaxin receptor would be a significant advance in the treatment and prevention of atopic conditions including allergic rhinitis, dermatitis, conjunctivitis, and particularly bronchial asthma. Further, agents which could block the eosinophil eotaxin receptor in humans who possess normal chemokine receptors should prevent infection in healthy individuals and slow or halt viral progression in infected patients.

[0013] It would also be desirable to know the molecular structure of the eosinophil eosinophil receptor in order to analyze this new receptor family and understand its normal physiological role. This could lead to a better understanding of the in vivo processes which occur upon ligand-receptor binding. Further, it would be desirable to use cloned-eosinophil eotaxin receptor as essential components of an assay system which can identify new agents for the treatment and prevention of atopic conditions.

SUMMARY OF THE INVENTION

[0014] The present invention relates to a novel receptor which is the eosinophil eotaxin receptor. This receptor is a human β-chemokine receptor and has been designated “CC CRK3”. One aspect of the present invention is directed to the human eosinophil eotaxin receptor, free from receptor-associated proteins. A further aspect of this invention is the human eosinophil eotaxin receptor which is isolated or purified.

[0015] Another aspect of this invention are eosinophil eotaxin receptors which are encoded by substantially the same nucleic acid sequences, but which have undergone changes in splicing or other RNA processing-derived modifications or mutagenesis induced changes, so that the expressed protein has a homologous, but different amino acid sequence from the native forms. These variant forms may have different and/or additional functions in human and animal physiology or in vitro in cell based assays.

[0016] The present invention further provides the eosinophil eotaxin receptor, CC CRK3, which is a 13-chemokine
receptor and which was cloned from primary eosinophils, and expressed in AML14.3D10 cells. This receptor binds the potent eosinophil attractants, eotaxin, RANTES and MCP-3 with high affinity. In addition, eotaxin and RANTES, and to a lesser extent MCP-3, induce Ca\(^{2+}\)-fluxes in cells expressing CC CRK3. Correlation with the binding properties of primary eosinophils provide conclusive evidence that CC CRK3 is the primary endogenous receptor which mediates the effects of β-chemokines on eosinophils.

[0017] The present invention further relates to assays which employ a novel receptor which is the eosinophil eotaxin receptor. This receptor is a human β-chemokine receptor and has been designated “CC CRK3”. One aspect of the present invention is directed to assays employing the human eosinophil eotaxin receptor, free from receptor-associated proteins. A further aspect of this invention is directed to assays which employ the human eosinophil eotaxin receptor which is isolated or purified. In addition, the present invention provides assays in which the eosinophil eotaxin receptor is expressed in an AML14.3D10 cell line.

DETAILED DESCRIPTION OF THE INVENTION

[0018] The present invention is directed to an eosinophil eotaxin receptor “CC CRK3” which is a G protein-coupled receptor and has been cloned from human eosinophils and which when stably expressed in AML14.3D10 cells binds eotaxin, RANTES and MCP-3 with high affinity. Competition binding studies against 125I-human eotaxin gives Kd values of 0.1, 2.7, and 3.1 nM, respectively for the three β-chemokines. CC CRK3 also binds MCP-1 with lower affinity, but does not bind MIP-1α or MIP-1β. Eotaxin, RANTES, and to a lesser extent MCP-3, but not the other chemokines activate CC CRK3 as determined by the ability to stimulate a Ca\(^{2+}\)-flux in clones expressing the receptor. Competition binding studies on primary eosinophils give binding affinities for the different chemokines which are indistinguishable from those measured with CC CRK3. Since CC CRK3 is prominently expressed in eosinophils it is concluded that CC CRK3 is the eosinophil eotaxin receptor. Eosinophils also express a much lower level of a second chemokine receptor, CC CRK1, which appears to be responsible for the effects of MIP-1α.

[0019] The eosinophil eotaxin receptor is a protein containing various functional domains, including one or more domains which anchor the receptor in the cell membrane, and at least one ligand binding domain. As with many receptor proteins, it is possible to modify many of the amino acids, particularly those which are not found in the ligand binding domain, and still retain at least a percentage of the biological activity of the original receptor. In accordance with this invention, it is suggested that certain portions of the eosinophil eotaxin receptor are not essential for its activation by β-chemokines. Thus this invention specifically includes modified functionally equivalent eosinophil eotaxin receptors which have deleted, truncated, or mutated portions. This invention also specifically includes modified functionally equivalent eosinophil eotaxin receptors which contain modified and/or deletions in other domains, which are not accompanied by a loss of functional activity.

[0020] Additionally, it is possible to modify other functional domains such as those that interact with second messenger effector systems, by altering binding specificity and/or selectivity. Such functionally equivalent mutant receptors are also within the scope of this invention.

[0021] A further aspect of this invention are nucleic acids which encode an eosinophil eotaxin receptor or a functional equivalent from human or other species. These nucleic acids may be free from associated nucleic acids, or they may be isolated or purified. For most cloning purposes, cDNA is a preferred nucleic acid, but this invention specifically includes other forms of DNA as well as RNAs which encode an eosinophil eotaxin receptor or a functional equivalent.

[0022] Yet another aspect of this invention relates to vectors which comprise nucleic acids encoding an eosinophil eotaxin receptor or a functional equivalent. These vectors may be comprised of DNA or RNA; for most cloning purposes DNA vectors are preferred. Typical vectors include plasmids, modified viruses, bacteriophage and cosmids, yeast artificial chromosomes and other forms of episomal or integrated DNA that can encode an eosinophil eotaxin receptor. It is well within the skill of the ordinary artisan to determine an appropriate vector for a particular gene transfer or other use.

[0023] A further aspect of this invention are host cells which are transformed with a gene which encodes an eosinophil eotaxin receptor or a functional equivalent. The host cell may or may not naturally express an eosinophil eotaxin receptor on the cell membrane. Preferably, once transformed, the host cells are able to express the eosinophil eotaxin receptor or a functional equivalent on the cell membrane. Depending on the host cell, it may be desirable to adapt the DNA so that particular codons are used in order to optimize expression. Such adaptations are known in the art, and these nucleic acids are also included within the scope of this invention.

[0024] The receptors of this invention were cloned from RNA isolated from eosinophils. Degenerate PCR was used with primers designed from both CCCRKR1 and CCCRKR2, and clones screened by expression in the AML14.3D10 cell line. The cloning was made difficult by several factors. First, prior to this invention there was very little information available about the biochemical characteristics and intracellular signalling/effector pathways used by these receptors. Making screening procedures uncertain. Second, this receptor could not be expressed and/or functionally coupled in the cell lines normally used for cloning receptors such as COS, CHO, HEK293. After repeated failures using standard lines, an obscure eosinophilic-like cell line, AML14.3D10, was tried and found to be suitable for expression of the receptors described in this invention.

[0025] The present invention further relates to assays which employ a novel receptor which is the eosinophil eotaxin receptor. This receptor is a human β-chemokine receptor and has been designated “CC CRK3”. One aspect of the present invention is directed to assays employing the human eosinophil eotaxin receptor, free from receptor-associated proteins. A further aspect of this invention is directed to assays which employ the human eosinophil eotaxin receptor which is isolated or purified. In addition, the present invention provides assays in which the eosinophil eotaxin receptor is expressed in an AML14.3D10 cell line.

[0026] A particular embodiment of this invention is directed to an assay to determine the presence of a com-
compound which binds to the eosinophil eotaxin receptor. Thus, this invention also comprises a method to determine the presence of a compound which binds to an eosinophil eotaxin receptor comprising:

(a) introducing a nucleic acid which encodes an eosinophil eotaxin receptor into a cell under conditions so that eosinophil eotaxin receptor is expressed;

(b) introducing a detector molecule or a nucleic acid encoding a detector molecule into the cell, wherein the detector molecule is directly or indirectly responsive to a eosinophil eotaxin-ligand binding event;

(c) contacting the cell with a compound suspected of binding to the eosinophil eotaxin receptor; and

(d) determining whether the compound binds to the eosinophil eotaxin receptor by monitoring the detector molecule.

In a preferred embodiment of the present invention, the eosinophil eotaxin receptor is expressed in AML14.3D10 cells.

In another preferred embodiment of the present invention, the binding of the compound suspected of binding to the eosinophil eotaxin receptor is compared to the binding of the influence of eotaxin, RANTES and MCP-3.

A further embodiment of this invention is directed to an assay to determine the presence of a compound which antagonizes the binding of a known ligand to the eosinophil eotaxin receptor. Thus, this invention further comprises a method to determine the presence of a compound which antagonizes the eosinophil eotaxin receptor comprising:

(a) introducing a nucleic acid which encodes the eosinophil eotaxin receptor into a cell under conditions so that eosinophil eotaxin receptor is expressed;

(b) introducing a detector molecule or a nucleic acid encoding a detector molecule into the cell, wherein the detector molecule is directly or indirectly responsive to an eosinophil eotaxin-ligand antagonism event;

(c) contacting the cell with a compound suspected of antagonizing the eosinophil eotaxin receptor;

(d) contacting the cell with a compound which is a known ligand of the eosinophil eotaxin receptor; and

(e) determining whether the compound antagonizes the action of the known ligand to the eosinophil eotaxin receptor by monitoring the detector molecule.

In a preferred embodiment of the present invention, the eosinophil eotaxin receptor is expressed in AML14.3D10 cells.

In another preferred embodiment of the present invention, the known ligand of the eosinophil eotaxin receptor is eotaxin, RANTES and MCP-3.

One aspect of this invention is the development of a sensitive, robust, reliable and high-throughput screening assay which may be used to detect ligands which bind to the eosinophil eotaxin receptor, in particular, antagonists of the action of chemokines on eosinophils.

In particular, a typical protocol of such an assay is as follows. Assay buffer (50 mM HEPES, pH 7.2 w/0.5% BSA, 5 mM MgCl₂, 1 mM CaCl₂, 100 μM PMFS and 10 μg/ml phosphoramidon, leupeptin, aprotinin and chymostatin), test compound (or equivalent volume of solvent), 20 pM 125I-human eotaxin (2000 Ci/mmol), 25 ng unlabeled human eotaxin (non-specific binding wells only), and AML14.3D10 cells expressing eotaxin receptor cells, or eosinophils, are added sequentially in 96-well, round-bottom polystyrene plates to a final volume of 250 μl. Assay plates are then mixed and incubated for 60 minutes at 31°C. After incubation, assay plates are harvested onto Packard 96-well GF/C Unifilter plates treated with 0.33% polyethyl- yleneimine (PEI) using Packard Filtermate 196 cell harvester. Wells and filters are washed with 200 μl, 50 mM HEPES, pH 7.2 with 0.5M NaCl and 0.02% NaN₃. After filtration, GF/C plates are dried and sealed. 25 μl Packard Microscint-O scintillant are then added to each well and counted for 2 minutes on Packard Topcount (liquid 125I setting).

Ligands detected using assays described herein may be used in the treatment and prevention of conditions which would be benefited by the modification of the activity of the eosinophil eotaxin receptor, such as in the treatment and prevention of atopic conditions including allergic rhinitis, dermatitis, conjunctivitis, and particularly bronchial asthma.

A further aspect of this invention is directed to novel ligands which are identified using the subject assays.

The eosinophil eotaxin receptor and fragments are immunogenic. Thus, another aspect of this invention is antibodies and antibody fragments which can bind to eosinophil eotaxin receptor or an eosinophil eotaxin receptor fragment. These antibodies may be monoclonal antibodies and produced using either hybridoma technology or recombinant methods. They may be used as part of assay systems or to deduce the function of an eosinophil eotaxin receptor present in a cell.

A further aspect of this invention are antisense oligonucleotides which can bind to eosinophil eotaxin receptor nucleotides and modulate receptor function or expression.

A further aspect of this invention is a method of increasing the amount of eosinophil eotaxin receptor in a cell comprising, introducing into the cell a nucleic acid encoding an eosinophil eotaxin receptor, and allowing expression of the eosinophil eotaxin receptor.

As used throughout the specification and claims, the following definitions shall apply:

Ligand—any molecule which binds to an eosinophil eotaxin receptor of this invention. These ligands can have either agonist, partial agonist, partial antagonist or antagonist activity.

Free from receptor-associated proteins—the receptor protein is not in a mixture or solution with other membrane receptor proteins.

Free from associated nucleic acids—the nucleic acid is not covalently linked to DNA which it is naturally covalently linked in the organism’s chromosome.

Isolated receptor—the protein is not in a mixture or solution with any other proteins.
Isolated nucleic acid—the nucleic acid is not in a mixture or solution with any other nucleic acid. Functional equivalent—a receptor which does not have the exact same amino acid sequence of a naturally occurring eosinophil chemokine receptor, due to alternative splicing, deletions, mutations, or additions, but retains at least 1%, preferably 10%, and more preferably 25% of the biological activity of the naturally occurring receptor. Such derivatives will have a significant homology with the natural eosinophil chemokine receptor and can be detected by reduced stringency hybridization with a DNA sequence obtained from an eosinophil chemokine receptor. The nucleic acid encoding a functional equivalent has at least about 50% homology at the nucleotide level to a naturally occurring receptor nucleic acid.

Purified receptor—the receptor is at least about 95% pure. Purified nucleic acid—the nucleic acid is at least about 95% pure.

Single-letter abbreviations for amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

Orphan Cloning of an Eosinophil Chemokine Receptor

RT/PCR conducted using oligonucleotide primers developed from the amino acid residues clustered within transmembrane helices II (TMII) and VII (TMVII) of the β-chemokine receptors, CC CKR1 (Neote, K., et al. (1993) Cell 72, 415-425) and MCP-1R (Charo, I. F., et al. (1994) Proceedings of the National Academy of Sciences 91, 2752-2756) on total RNA isolated from eosinophils yielded DNA fragments of ~700 bases, a size consistent with that expected for a G protein coupled receptor. Analysis of several TMII to TMVII clones provided a novel sequence which was 76% homologous with human CC CKR1 at the nucleic acid level. Completion of the cloning of the 3' and 5' ends gave a sequence for a protein of 355 residues in length, 63% identical to CC CKR1, and 51% identical to CC CKR2B, its closest homologues.

The amino acid sequence of the human eosinophil chemokine receptor CC CKR3 is depicted below (SEQ ID NO:1):

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Met Thr Thr Ser Leu Asp Thr Val Gly Thr Phe Gly
Thr Thr Ser Tyr Thr Asp Thr Val Gly Leu Leu Cys
Glu Lys Ala Asp Thr Arg Ala Met Ala Gin Phe
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Val Pro Pro Leu Tyr Ser Leu Val Phe Thr Val Gly
Leu Leu Gly Asn Val Val Val Val Val Met Ile Leu Ile
Lys Tyr Arg Arg Leu Arg Ile Met Thr Asn Ile Tyr
Leu Leu Lys Leu Ala Lys Ser Asp Leu Leu Phe Leu
Val Thr Pro Phe Trp Ile His Tyr Val Arg Gly
His Asn Trp Val Phe Gly His Gly Met Cys Lys Leu
Leu Ser Gly Phe Tyr His Thr Gly Val Leu Tyr Ser Glu
Ile Phe Phe Ile Ile Leu Leu Thr Ile Asp Arg Tyr
Leu Ala Ile Val His Ala Val Phe Ala Leu Arg Ala
Arg Thr Val Thr Phe Gly Val Ile Thr Ser Ile Val
Thr Trp Gly Leu Ala Val Ala Leu Leu Leu Pro Glu
Phe Ile Phe Tyr Glu Thr Glu Leu Phe Gly Glu
Thr Leu Cys Ser Ala Leu Tyr Pro Glu Asp Thr Val
Tyr Ser Trp Arg His Phe His Thr Leu Arg Met Thr
Ile Phe Cys Leu Val Leu Leu Val Leu Met Val Ala
Ile Cys Tyr Thr Glu Ile Ile Lys Thr Thr Leu Arg
Cys Pro Ser Lys Lys Lys Tyr Lys Ala Ile Arg Leu
Ile Phe Val Ile Met Ala Val Phe Ile Phe Trp
Thr Pro Tyr Asn Val Ala Ile Leu Leu Ser Ser Tyr
Gln Ser Ile Leu Phe Gly Asn Asp Cys Gly Arg Ser
Ile Cys Tyr Thr Glu Ile Ile Lys Thr Thr Leu Arg
Ile Pro Ser Lys Lys Lys Tyr Lys Ala Ile Arg Leu
Ile Phe Val Ile Met Ala Val Phe Ile Phe Trp
Thr Pro Tyr Asn Val Ala Ile Leu Leu Ser Ser Tyr
Gln Ser Ile Leu Phe Gly Asn Asp Cys Gly Arg Ser
Lys His Leu Asp Leu Val Met Leu Val Thr Glu Val
Ile Ala Tyr Ser His Cys Met Asn Pro Val Ile
Tyra Phe Val Val Gly Arg Phe Arg Lys Tyr Leu
Arg His Phe His Arg His Arg Leu Met His Leu
Gly Arg Tyr Ile Phe Phe Phe Leu Pro Ser Glu Lys Leu
Glu Arg Thr Ser Ser Val Ser Pro Ser Thr Ala Glu
Pro Glu Leu Ser Ile Val Phe
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The sequence for the cDNA encoding the human eosinophil chemokine receptor CC CKR3 beginning with nucleotide 3587 and ending with nucleotide 4651 is depicted below (SEQ ID NO:2):

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ATGA CAACCTC A
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3601 AGATACAGTT GAGACCTTTG TGCTCTGTGA AAAAGCTGAT CCGCTGTACT CCCTGGGTT GGTGATGATC CTCATAAAAT ACCTGCTCAA CCTGGCCATT

3651 ATGCTGCTGA AAAAGCTGAT ACACAGAAGCAT TGATGGCCCA GTTGGCGCC

3701 CGGCTGCTC GCTGCTGGCC CTCCTGGGGA ACGGGCGAT

3751 GGTATGATC CTCTGAAAAT ACACAGAAGCT CCGATTATG ACCAAACAT

3801 ACCPCTGCA CCTGCGCCATT TGGAGGCTGC TCTCCCTGCT ACOCCTTCCA
or a degenerate variation thereof.

[0060] The 5' genomic DNA flanking sequence encoding the human eosinophil cotoxin receptor further comprises the region beginning with nucleotide 1 and ending with nucleotide 3586 as depicted below (SEQ ID NO:3):

    1  GGAATCCCTAC CTTCCCATAT AGAGCTAGGG GGCATGGAGC GCTCTCTGCCT
    51  AAGATGGGCA CCCCACAGGA AGTTCTCCCT GTGGGCAACT TCCCTACAGT
   101  ATGGATGACG CAGATGGGTG GATTGGAGAG TGGGACAGGG AGATGAGGGAA
   151  TACTTTTAGG GCCGAGGCT CAGCAGGACT GAGGATGAGG TGATGGAATG
   201  GTCGACCTCC GTGAGACGCT TGGTGAGGAG GATGAGGAGG GATGAGGAGG
   251  CATAGGCTAAG GCCGCTGACT CGTGGAGAGG GACAGGACAG GTAGGAGAGG
   301  GCCGTCAGTT CGTCTTCCCT TCCCTGAGCG CGAAGCGAGC TCTGAGGAGG
   351  ACACGAGTTC TCTCCCTGA CCAGAGACCA CGAGAGACCA CAGAGAGAC
   401  CTAAGTTCAC TTAATGATAC GATGCTCACT CTGGAGCTGT GGGGCTTAAG
   451  AGAAGTGGGC AGAGCTAGGG GCCGAGGCT AGAGCTAGGG GCCGAGGCT
   501  AGAAGTGGGC AGAGCTAGGG GCCGAGGCT AGAGCTAGGG GCCGAGGCT

Continued...
or a degenerate variation thereof.

[0061] The sequence for the cDNA encoding human eosinophil eotaxin receptor further comprises the terminator region beginning with nucleotide 4652 and ending with nucleotide 5099 as depicted below (SEQ ID NO:4):

4652 TAGCTGAG TSCAGAATA TSSCTAAAGA GGAAGGACCA AGGAGATGAA GCAAACACAT TCCAGTGCAA TAGCAGTAGA GCAGCGTACT AAAATGAGTT
4701 AAGCCTTCC ACACTCACCT CTAAAACAGT CACTGAAGCT CTTGAAGACA CTGAAATATA TGCATGTACC CTAAGGTCAT TACCACAGGC CATCATCAAC CCTAAAAAGC AGAGCTTTGC ACCTACATTT TAATGCACCT GAATGTTAGA
4751 CCTTCAAACT CACACACCAG CAGGGGCTGG TTCTCTCTCT TAGTTACTAT

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or a degenerate variation thereof.

[0062] As will be appreciated by one skilled in the art, there is a substantial amount of redundancy in the set of codons which translate specific amino acids. Accordingly, this invention also includes alternative base sequences wherein a codon (or codons) are replaced with another codon such that the amino acid sequence translated by the DNA sequences remains unchanged. For purposes of this specification, a sequence bearing one or more such replaced codons will be defined as a degenerate variation. Also included are mutations (exchanges of individual amino acids) which one skilled in the art would expect to have no effect on functionality, such as valine for leucine, arginine for lysine, and asparagine for glutamine.


Expression of the Human CC CKR3 in AML14.3D10 Cells

[0064] Once a full length cDNA encoding CC CKR3 was isolated and cloned into the expression vector pBJ/NEO the resulting plasmid designated pBJ/NEO/CCCCKR3, was transfected into the AML14.3D10 line.

[0065] The CC CKR3 transfected AML14.3D10 cell line has been placed on restricted deposit with American Type Culture Collection in Rockville, Md. as ATCC No. CRL-12079, on Apr. 5, 1996.

[0066] Stable clones were selected for neomycin resistance, and a number were chosen for further analysis. To demonstrate expression of receptor protein, a western blot was performed using antisera generated against a peptide derived from the predicted C-terminus of CC CKR3. Immuno-reactive bands migrating at approximately 45-55 kd are present in primary eosinophils and the 3.16 clone, indicating that CC CKR3 is indeed expressed in these cells. There was no immuno- reactive bands present in neutrophils indicating that the antisera was indeed identifying an eosinophil-specific protein. A nonspecific pattern of immuno-reactivity was detected in untransfected AML14.3D10 cells, and furthermore, this pattern was identical in clone 3.49 indicating that this neomycin-resistant clone is a non-expressor of CC CKR3. Of the 27 neomycin resistant clones studied, 19 failed to express CC CKR3. The other 8 did express the receptor as judged both by Western analysis, and by the ability of eotaxin and RANTES to induce Ca" influxes. The non-expressing clones were used as negative controls in subsequent experiments.

Binding to CC CKR3 on Intact AML14/CCCKR3.16 Cells

[0067] Because preliminary experiments with three different CC CKR3 expressing clones indicated that they bound 125I-eotaxin, competition studies using this labeled ligand were performed to characterize the binding properties of the receptor. As shown in Table 1, unlabeled human eotaxin competed with an Kd of 0.1 nM. Results with murine eotaxin were essentially identical. Scatchard analysis demonstrated that eotaxin binds with a single affinity and that the different clones expressed 2-4x10^5 receptors/cell. The ability to bind eotaxin is due to CC CKR3 since neither immunoreactive negative clones, such as 3.49, nor untransfected cells displayed any specific binding. Clearly, CC CKR3 is a high affinity receptor for eotaxin. Cross-competition studies with the two other β-chemokines known to be eosinophil chemoattractants, RANTES and MCP-3, demonstrated that they too have considerable affinity for CC CKR3, with Kd’s of about 3 nM (See Table 1). In contrast, MIP-1α competed with much lower affinity (Kd=60 nM), and MIP-1β failed to compete at all (See Table 1). Similarly, the α-chemokine IL-8 did not inhibit eotaxin binding.

[0068] Competition studies were also carried out against 125I-MCP-3. Again, human and murine eotaxin competed strongly with Kd’s of 0.2 and 0.3 nM (Table 1). RANTES and MCP-3 also demonstrated high affinity with Kd’s of 0.5 and 0.7 nM, values about 4-fold lower than observed against eotaxin. As in the studies with eotaxin, MCP-1 competed weakly (Kd=16 nM), and MIP-1α, and MIP-1β failed to compete at all. Thus despite some small quantitative differ-
nces the overall ligand selectivity of the receptor is the same whether measured by competition against eotaxin or MCP-3, and the order of potency, eotaxin > MCP-3 = RANTES >> MCP-1, is identical.

CC CKR3 is Functionally Coupled in AML14.3D10 Cells

In order to determine whether human CC CKR3 was functionally coupled when expressed in the AML14.3D10 line, intracellular Ca\(^{2+}\) levels were measured in response to various 3-chemokines. Both 100 nM eotaxin and RANTES induced Ca\(^{2+}\)-fluxes in cells expressing the receptor. Surprisingly, 1 µM of MCP-3 was required to induce a response, and that response was smaller than those observed for eotaxin or RANTES. No response at all was generated by addition of MIP-1α, MIP-1β, MCP-1 or IL-8 at concentrations as high as 1 µM. The responses to eotaxin, RANTES, and MCP-3 are due to the specific expression of CC CKR3 since none of these mediators induced fluxes in untransfected cells or in clone 3.49. While the preliminary functional characterization by Combadiere et al. differs greatly from the present invention, they were not able to demonstrate any specific binding to cells putatively expressing the receptor, and such functional data have now been retracted (Combadiere, C., et al. (1995) *Journal of Biological Chemistry* 270, 30235).

Binding Properties of Primary Eosinophils

The selectivity of CC CKR3 for the various β-chemokines mirrors the effectiveness of these ligands as eosinophil chemotactants suggesting that CC CKR3 is the primary mediator of chemokine-induced eosinophil chemotaxis. To provide additional pharmacological evidence, binding studies were conducted on primary eosinophils. When measured by competition against \(^{125}\)I-eotaxin, unlabeled human eotaxin gave a Kd=0.1 nM, a value identical to that obtained on cloned CC CKR3 (see Table 1). Scatchard showed a single binding affinity, and 4x10\(^5\) sites/cell. The number of binding sites varied by less than 2-fold for the 3 donors used in the studies. The affinities for RANTES and MCP-3 were also identical to those measured on CC CKR3, and as with CC CKR3, neither MIP-1α, or MIP-1β, showed any ability to compete with radiolabeled eotaxin (see Table 1). Similarly, the Kd’s obtained by competition against \(^{125}\)I-MCP-3 on eosinophils are effectively indistinguishable to those measured against cloned CC CKR3 (see Table 1). All of the observations and measurements, taken together with the Western blot showing expression of CC CKR3, verify that CC CKR3 is the eosinophil receptor, and appears to be largely responsible for mediating the effects of most β-chemokines on eosinophils.

Stably expressed in the eosinophilic line AML14.3D10, CC CKR3 binds eotaxin, RANTES and MCP-3, with high affinity, with a rank order of potency of eotaxin>RANTES>MCP-3. MCP-1 binds with much lower affinity, while MIP-1α and MIP-1β fail to bind at all. The selectivity of CC CKR3 mirrors most of the binding activity of primary eosinophils. In fact, when measured by competition against \(^{125}\)I-eotaxin, the binding affinities on eosinophils for all of these β-chemokines are indistinguishable from those obtained with cloned CC CKR3. Moreover, CC CKR3 was cloned from eosinophils, and as shown by Western blotting is heavily expressed in these cells. The abilities of the different chemokines to activate CC CKR3 are consistent with the binding data as eotaxin, RANTES, and to a lesser extent MCP-3 all stimulate Ca\(^{2+}\) fluxes in clones which express the receptor, while MCP-1, MIP-1α and MIP-1β do not, even at concentrations as high as 1 µM. Thus, based on its properties, and expression, CC CKR3, is the eosinophil eotaxin receptor.

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[0072] Competition binding experiments were carried out against the indicated iodinated ligand as follows and as described herein. Equilibrium binding of β-chemokines to AML14.3D10 cells expressing CC CKR3 and to primary eosinophils was examined with increasing concentrations of unlabeled human eotaxin, murine eotaxin, RANTES, MCP-3, or MCP-1 to compete against fixed concentrations of either \(^{125}\)I-human eotaxin, or \(^{125}\)I-MCP-3. Also the competition with 100 nM concentrations of MIP-1α, and MIP-1β was examined. The experiments were carried out either with AML14.3D10 cells expressing CC CKR3, or with eosinophils. All values are the averages of triplicate determinations. Typically, 4000-6000 cpm of iodinated ligand was bound in the absence of competitor with S/N ratios exceeding 15. Human and murine eotaxin are the human and murine chemokines, respectively. "N.B." means that no competition was observed. All results are the averages of the number of experiments shown in parenthesis.

[0073] Various changes and modifications may be made in the products and processes of the present invention without departing from the spirit and scope thereof. The various embodiments and the examples which have been set forth herein are given for the purpose of illustrating the present invention and shall not be construed as being limitations on the scope or spirit of the instant invention.

**EXAMPLE 1**

mRNA Isolation and cDNA Cloning

[0074] Total RNA was isolated from purified eosinophils with Trizol reagent (BRL) and used in a RT/PCR reaction (Daugherty, B. L., et al. (1991) *Nucleic Acids Research* 19, 2471-2476) using oligonucleotide primers designed from the human CC CKR1 and MCP-1RB cDNA sequences.

**[0075]** 5'-PCR primer (TMII) (SEQ ID NO:5):

5'-'AACCCTGCCCAT(C,T)CTCTG(A,T)GTGC-3'

**[0076]** 3'-RT/PCR primer (TMVII) (SEQ ID NO:6):

5'-'GACC(C,T)CTCTC(C,A)CAAGGAGG-3'.

**[0077]** The resultant PCR product of ~700 bp was subcloned into plasmid pNoTA (Five Prime, Three Prime, Inc) and sequenced using Sequenase (USB). The remaining 5' and 3' sequence encoding CC CRK3 was cloned by rapid amplification of cDNA ends (RACE) using both the 5'-RACE and 3'-RACE kits (Clontech) with the following primer sequences:

**[0078]** (5'-RACE) (SEQ ID NO:7):

5'-'TCTCTCTTACAAGGCTGCTG-3';

**[0079]** (3'-RACE) (SEQ ID NO:8):

5'-'CTCTCTCTCTCTAATCAATCC-3'.

**[0080]** The resultant PCR products (5'-RACE, ~450 bp; 3'-RACE, ~700 bp) were subcloned into pCRII (Invitrogen) and sequenced. Upon identification of the 5'-end of the cDNA containing the initiator ATG codon and the 3'-end containing the termination codon TAG, a new set of PCR primers were designed to reamplify the entire coding region from eosinophil total RNA for expression of CC CRK3. The primer sequences used for RT/PCR were:

**[0081]** 5'-PCR primer (SEQ ID NO:9):

5'-'ATATATAGCTTCTACACATAGGATAATACAG-3';

**[0082]** 3'-RT/PCR primer (SEQ ID NO:10):

5'-'ATATATCTTACACGCGCCGCTTAAACACAACTAGAGGTTCC-3'.

**[0083]** The resultant PCR product of 1105 bp was digested with HindIII and NotI and subcloned into plasmid pBlu/Neo to yield pHJ/NEO/CCCRK3. The plasmid pHJ/Neo was prepared essentially as follows. Plasmid pDS5/Igh/Neo (Daugherty, B. (1991) Nucleic Acids Research 19, 2471-2476) was digested with the restriction enzyme Sall, filled in with E. coli DNA polymerase I Klenow fragment to create a blunt end and subsequently digested with the restriction enzyme NotI. The CMVIE intron A fragment from plasmid p89-I was digested with ClaI, filled in to create a blunt end and subsequently digested with HindIII. These fragments were used in a three-way ligation with a HindIII and NotI fragment of the human C5a receptor cDNA. The C5a receptor fragment was excised with HindIII and NotI and replaced with the eotaxin receptor cDNA of 1105 bp obtained by RT/PCR with oligonucleotides SEQ ID NO:9 and SEQ ID NO: 10 after digestion with HindIII and NotI. Several clones were sequenced and one clone comprising the consensus sequence was chosen for expression of CC CRK3 in heterologous cells.

**EXAMPLE 2**

Transfection into AML14.3D10 Human Eosinophilic Cell Line

**[0084]** AML14.3D10 cells (Paul, C. C., et al. (1995) Blood 86, 3737-3744) were cultured in RPMI-1640, 10% FBS, 1 mM sodium pyruvate, 0.5 μM β-mercaptoethanol and 2 mM L-glutamine (complete medium). Cells were harvested at a density of 0.3x10^5/mL, washed once in PBS, resuspended in RPMI at 10^7/mL, and 25 μg of plasmid was added. Electroporation was carried out at 300 V, 960 μF using a Gene Pulser (BioRad). Following electroporation, cells were chilled at 0°C for 10 min and then plated in complete medium at 106/T75 flask and cultured at 37°C, 5% CO2. After 16-24 hr, cells were pelleted and resuspended in complete medium containing 2 mg/mL Genetin (GIBCO). Cells were maintained in selection medium for 8-10 days until individual surviving clusters appeared. Individual colonies were then transferred to 96-well plates and expanded. AML14/CCCRK3 sublines were assayed for the ability to generate a Ca<sup>2+</sup> flux in response to either RANTES or eotaxin. Positive sublines were then probed by western blotting with an antibody raised against the predicted C-terminus of CC CRK3. Cell lines positive in both sets of assays were then characterized for their ability to bind to a variety of CC chemokines, including eotaxin, RANTES, MCP-3, MIP-1α, MIP-1β and MIP-1.

**EXAMPLE 3**

Purification of Eosinophils

**[0085]** Primary eosinophils were isolated from granulophoresis preparations obtained from allergic and asthmatic donors (Bach, M. K., et al. (1996) Journal of Immunological Methods 130, 277-281). The leukocytes were mixed with equal volumes of HBSS and layered over LSM (Organon Teknika) as described (Rollins, T. E., et al. (1998) Journal of Biological Chemistry 263, 520-526). After lysis of erythrocytes with NH<sub>4</sub>Cl, the granulocytes were subsequently treated with anti-CD 16 microbeads followed by MACS separation (Miltenyi Biotech) (Hanel, T. T., et al. (1991) Journal of Immunological Methods 145, 105-110). Typically the eosinophil preparations were >99% pure as determined using the LeukoStat staining kit (Fisher).

**EXAMPLE 4**

Generation of α-CC CRK3 Antisera and Immunoblotting

**[0086]** Polyclonal rabbit antisera was generated to CC CRK3 using the C-terminal decapeptide sequence TAEPEL-SIV. Peptide synthesis, coupling to thyroglobulin and production of antisera was performed (Miller, D. K., et al. (1993) Journal of Biological Chemistry 268, 18062-18069). Whole cells were boiled and sonicated in Laemli sample buffer (Laemmli, U. K. (1970) Nature 227, 680-685), electrophoresed on 4-20% SDS gels (Novex), transferred to
polyvinylidene difluoride membranes (BioRad), and blocked with 5% nonfat dry milk in TBST (20 mM Tris, 200 mM NaCl, 0.1% Tween-20) for 16 hr at 4°C. The membrane was incubated with antisera at 1:1000 in TBST for 1 hr at room temperature, washed, and subsequently incubated with goat anti-rabbit HRP (Zymed) at 1:4000 in TBST for 30 min also at room temperature. After washing, the membrane was treated with ECL western blotting reagents (Amersham) for 1 min, covered in plastic wrap and exposed to film for 2 min.

EXAMPLE 5

Chemokine Binding Assays

Recombinant MCP-3, MCP-1, RANTES, murine and human eotaxin were obtained from Peprotech (Princeton, N.J.), 125I-MCP-3 and 125I-MIP-1α were obtained from New England Nuclear (Boston, Mass.), and 125I-human-eotaxin was obtained from Amersham. Binding of 125I-labeled ligands (typically a total of 2x10⁶ cpm) in the presence of varying concentrations of unlabeled ligands to intact cells (typically 1.5x10⁵, 10⁶, or 10⁷ for experiments with labeled eotaxin, MCP-3, or MIP-1α, respectively) were performed at 32°C. (Van Riper, G., et al. (1993). Journal of Experimental Medicine 177, 851-856).

EXAMPLE 6

Ligand-Induced Ca²⁺ Fluxes

Human CC CKR3 expressing AML14 clones or purified eosinophils were incubated with 1.25 μg/ml Indo-1 (Molecular Probes, Eugene, Ore.) in RPMI 1640, 10 mM HEPES, 5% FBS, for 60 min at 37°C. (Van Riper, G., et al. (1993). Journal of Experimental Medicine 177, 851-856). Loaded cells were washed and incubated at 37°C in buffer containing calcium ions. Calcium fluxes were performed on a FACS analyzer (Becton Dickinson & Co., Mountain View, Calif.) with an excitation wavelength of 365 nm and dual emission wavelengths of 405 and 488 nm.

EXAMPLE 7

CC CKR3 Binding Assay

Assay buffer (50 mM Hepes, pH 7.2 w/0.5% BSA, 5 mM MgCl₂, 1 mM CaCl₂, 100 μM PMSF and 10 μg/ml phosphoramidon, leupeptin, aprotinin and chymostatin), test compound (or equivalent volume of solvent), 20 pM 125I-human eotaxin (2000 Ci/mmol), 25 ng unlabeled human eotaxin (non-specific binding wells only), and AML14.3D10 cells expressing eotaxin receptor cells, or eosinophils, are added sequentially to 96-well, round-bottom, polystyrene plates to a final volume of 250 μl. Assay plates are then mixed and incubated for 60 minutes at 31°C. After incubation, assay plates are harvested onto Packard 96-well GF/C Unifilter plates treated with 0.3% polyethylenimine (PEI) using Packard Filtermate 96 Cell Harvester. Wells and filters are washed with 200 ul. 50 mM Hepes, pH 7.2 with 0.5M NaCl and 0.02% NaN₃. After filtration, GF/C plates are dried and sealed. 25 ul Packard Microscint-O scintillant are then added to each well and counted for 2 minutes on Packard Topcount (liquid 125I setting).

EXAMPLE 8

Phosphoinositide 3-Kinase (PI-3K) Assay

AML14.3D10 expressing eotaxin receptor (CCCKR3) cells are incubated with test compound and stimulated with eotaxin, RANTES, or MCP-3, pelleted and lysed in 1 mL lysis buffer (1% Nonidet P-40, 100 mM NaCl, 20 mM Tris, pH 7.4, 10 mM iodoacetamide, 46 mM b-glycerophosphate, 10 mM NaF, 1 mM PMSF, 1 μg/mL Leupeptin, 1 μg/mL chymostatin, 1 μg/mL antipain, 1 μg/mL pepstatin A, and 1 μM sodium orthovanadate). Lysates are then pre-cleared for 1 hr with uncoupled protein A Affi-Gel beads. Immunoprecipitation is then performed with p 85 polyclonal antiserum (1 μg/mL. rysate; Upstate Biotechnologies, New York, N.Y.), coupled to protein A Affi-Gel beads (Bio-Rad) at 4°C for 2 hr. Immunoprecipitates are washed and subjected to in vitro lipid kinase assays by using a lipid mixture, 100 ul 0.1 mg/ml PtdIns and 0.1 mg/ml phosphatidylycerine dispersed by sonication into solution in 20 mM HEPES, pH 7.0, and 1 mM EDTA. The reaction is initiated by the addition of 100 mM ATP and 20 uCi [γ-³²P]ATP (3000 Ci/mmol) in 20 μl kinase buffer. The reaction is then terminated after 15 min and the phosphoinositide lipids are separated by thin layer chromatography (TLC) and visualized by exposure to iodine vapor autoradiography.

EXAMPLE 9

Chemotaxis Assay

AML14.3D10 expressing eotaxin receptor cells are isolated by centrifugation (Van Riper, G., et al. (1994). Journal of Immunology 152, 4055-4061) for 15 min at 150g, washed and resuspended at 10⁶ cells/ml in HBSS (pH 7.4) containing 1 mM CaCl₂ and 1 mM MgCl₂ (chemotaxis buffer). The chemotaxis experiments are then performed in Transwell dishes (6.5 mm, Costar, Cambridge, Mass.). The lower chamber contains 0.6 ml of chemotaxis buffer and is separated from the upper chamber containing 10⁶ cells by a 5-mm pore Nucleopore polycarbonate membrane (Nucleo- pore Corporation, Pleasanton, Calif.). After a 15 min preincubation at 37°C, test compound and eotaxin, RANTES, or MCP-3 are added to the lower chamber to a final concentration of 300 nM. After 2 hrs at 37°C, the upper chamber inserts are removed, and the cells that migrate to the lower chamber are enumerated by a Coulter Counter (Coulter Electronics, Hialeah, Fla.).

EXAMPLE 10

Ligand-Dependent Inositol Phosphate Release Assay

AML14.3D10 expressing eotaxin receptor cells are labeled with [³²P]inositol (10 μCi/ml) for 24 hrs as described (Wu, D., et al. (1993). Science 261, 101-103). Test compound and arious concentrations of eotaxin, MCP-3, or RANTES are then added to the cells for 30 min. The cells are lysed in 10% perchloric acid, neutralized in 2 N KOH and centrifuged. The supernatant is transferred to columns containing 0.5 ml AG1-X8 anion exchange resin, washed with 6 ml borax buffer and eluted with 0.3 ml formic acid (0.1 M). The eluted samples are mixed with scintillation cocktail and counted.
EXAMPLE 11
Acidification Rate Assay

AML14.3D10 expressing eotaxin receptor cells are subject to serum starvation for 16 hrs. The cells are then mixed at a 3:1 (v/v) ratio with low melting temperature agarose. A 10 ul drop of the cell/agarose mixture is pipetted into a sterile Capsule Cup (Molecular Devices) at a cell density of approximately 200,000 cells/cup. The cell/agarose drop forms a gel after about 5 min, and is assembled into the cup between two 3 um porosity polycarbonate membranes with running medium. The assembled capsule cups are placed into the sensor chambers and then placed on the Cytosensor Microphysiometer (Molecular Devices) containing 1 ml of running medium. The chambers are allowed to equilibrate for 1 hr at 37° C. with a flow rate of 100 ul/min. The experiment is initiated with an 8 min exposure of eotaxin, RANTES, MCP-3 and test compound at various concentrations and the acidification rate over baseline will be measured in the medium (McConnell, H. (1992) Science 257, 1906-1912) until the cells return to the unstimulated level.

EXAMPLE 12
Actin Polymerization Assay

AML14.3D10 expressing eotaxin receptor cells are diluted in APA buffer (HBSS; 25 mM Hepes; 0.2% BSA, pH7.2) at a concentration of 4x10⁵/ml. One ul of test compound and eotaxin, RANTES, or MCP-3 added into a 96-well plate and incubated at 37° C. 100 ml of cells are added to the plate and incubated for 20 sec to which 100 ml of APA cocktail (2 mls 8% formaldehyde; 460 uLs 0.33 uM Rhodamine-phalloidin; 1.85 mg 200 ug/ml lysolecithin; 7.25 mls HBSS) is added. Plates are then centrifuged at 2000 RPM for 5 min, cleared and then 100 ul of HBSS is added to all wells which are read in a Fluoroskan II fluorometer.

While the invention has been described and illustrated with reference to certain particular embodiments thereof, those skilled in the art will appreciate that various adaptations, changes, modifications, substitutions, deletions, or additions of procedures and protocols may be made without departing from the spirit and scope of the invention. For example, procedures other than the particular experimental procedures as set forth herein above may be applicable as a consequence of degeneracy and variations in the sequences of the proteins and DNA of the invention indicated above. Likewise, the characterization data observed may vary slightly according to and depending upon the particular assay or characterization method employed, and such expected variations or differences in the results are contemplated in accordance with the objects and practices of the present invention. It is intended, therefore, that the invention be defined by the scope of the claims which follow and that such claims be interpreted as broadly as is reasonable.

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1-35. (canceled)

36. An isolated nucleic acid which encodes a human eosinophil eotaxin receptor, said nucleic acid having the nucleotide sequence as set forth in SEQ ID NO:2.

37. The nucleic acid of claim 36 which further comprises the nucleotide sequence as set forth in SEQ ID NO:3.

38. The nucleic acid of claim 36 which further comprises the nucleotide sequence as set forth in SEQ ID NO:4.

39. A vector comprising a nucleic acid which encodes a human eosinophil eotaxin receptor, said nucleic acid having the nucleotide sequence as set forth in SEQ ID NO:2.

40. The vector according to claim 39 which is selected from the group consisting of: plasmids, modified viruses, yeast artificial chromosomes, bacteriophages, cosmids and transposable elements.

41. An isolated mammalian host cell comprising the vector according to claim 39.

42. The host cell of claim 41 which is from the AML14.3D10 cell line.

43. A method to determine the presence of a compound which binds to a human eosinophil eotaxin receptor comprising:

(a) introducing a nucleic acid which encodes the human eosinophil eotaxin receptor which comprises the nucleotide sequence (SEQ ID NO:2) into a cell under conditions so that eosinophil eotaxin receptor is expressed;

(b) introducing a detector molecule or a nucleic acid encoding a detector molecule into the cell, wherein the detector molecule is directly or indirectly responsive to a eosinophil eotaxin-ligand binding event;

(c) contacting the cell with a compound suspected of binding to the eosinophil eotaxin receptor; and

(d) determining whether the compound binds to the eosinophil eotaxin receptor by monitoring the detector molecule.

44. The method of claim 43 wherein the result of step (d) is compared to that obtained using a known ligand of the eosinophil eotaxin receptor.

45. The method of claim 44 wherein the known ligand of the eosinophil eotaxin receptor is eotaxin.

46. The method of claim 44 wherein the known ligand of the eosinophil eotaxin receptor is RANTES.

47. The method of claim 44 wherein the known ligand of the eosinophil eotaxin receptor is MCP-3.

48. The method of claim 43 wherein the eosinophil eotaxin receptor is expressed in a host cell which does not naturally express the human eosinophil eotaxin receptor

49. The method of claim 48 wherein the host cell is from the AML14.3D10 cell line.

* * * * *