(54) Title: RP105 AGONISTS AND ANTAGONISTS

(57) Abstract

Methods of treating or preventing allergic diseases with RP105 antagonists are disclosed. Methods of identifying RP105 antagonists are disclosed.
FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

<table>
<thead>
<tr>
<th>Code</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL</td>
<td>Albania</td>
</tr>
<tr>
<td>AM</td>
<td>Armenia</td>
</tr>
<tr>
<td>AT</td>
<td>Austria</td>
</tr>
<tr>
<td>AU</td>
<td>Australia</td>
</tr>
<tr>
<td>AZ</td>
<td>Azerbaijan</td>
</tr>
<tr>
<td>BA</td>
<td>Bosnia and Herzegovina</td>
</tr>
<tr>
<td>BB</td>
<td>Barbados</td>
</tr>
<tr>
<td>BE</td>
<td>Belgium</td>
</tr>
<tr>
<td>BF</td>
<td>Burkina Faso</td>
</tr>
<tr>
<td>BG</td>
<td>Bulgaria</td>
</tr>
<tr>
<td>BJ</td>
<td>Benin</td>
</tr>
<tr>
<td>BR</td>
<td>Brazil</td>
</tr>
<tr>
<td>BY</td>
<td>Belarus</td>
</tr>
<tr>
<td>CA</td>
<td>Canada</td>
</tr>
<tr>
<td>CF</td>
<td>Central African Republic</td>
</tr>
<tr>
<td>CG</td>
<td>Congo</td>
</tr>
<tr>
<td>CH</td>
<td>Switzerland</td>
</tr>
<tr>
<td>CI</td>
<td>Côte d’Ivoire</td>
</tr>
<tr>
<td>CM</td>
<td>Cameroon</td>
</tr>
<tr>
<td>CN</td>
<td>China</td>
</tr>
<tr>
<td>CU</td>
<td>Cuba</td>
</tr>
<tr>
<td>CZ</td>
<td>Czech Republic</td>
</tr>
<tr>
<td>DE</td>
<td>Germany</td>
</tr>
<tr>
<td>DK</td>
<td>Denmark</td>
</tr>
<tr>
<td>EE</td>
<td>Estonia</td>
</tr>
<tr>
<td>ES</td>
<td>Spain</td>
</tr>
<tr>
<td>FI</td>
<td>Finland</td>
</tr>
<tr>
<td>FR</td>
<td>France</td>
</tr>
<tr>
<td>GA</td>
<td>Gabon</td>
</tr>
<tr>
<td>GB</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>GE</td>
<td>Georgia</td>
</tr>
<tr>
<td>GH</td>
<td>Ghana</td>
</tr>
<tr>
<td>GN</td>
<td>Guinea</td>
</tr>
<tr>
<td>GR</td>
<td>Greece</td>
</tr>
<tr>
<td>HU</td>
<td>Hungary</td>
</tr>
<tr>
<td>IE</td>
<td>Ireland</td>
</tr>
<tr>
<td>IL</td>
<td>Israel</td>
</tr>
<tr>
<td>IS</td>
<td>Iceland</td>
</tr>
<tr>
<td>IT</td>
<td>Italy</td>
</tr>
<tr>
<td>JP</td>
<td>Japan</td>
</tr>
<tr>
<td>KE</td>
<td>Kenya</td>
</tr>
<tr>
<td>KG</td>
<td>Kyrgyzstan</td>
</tr>
<tr>
<td>KP</td>
<td>Democratic People’s Republic of Korea</td>
</tr>
<tr>
<td>KR</td>
<td>Republic of Korea</td>
</tr>
<tr>
<td>KZ</td>
<td>Kazakhstan</td>
</tr>
<tr>
<td>LC</td>
<td>Saint Lucia</td>
</tr>
<tr>
<td>LI</td>
<td>Liechtenstein</td>
</tr>
<tr>
<td>LK</td>
<td>Sri Lanka</td>
</tr>
<tr>
<td>LR</td>
<td>Liberia</td>
</tr>
<tr>
<td>LS</td>
<td>Lesotho</td>
</tr>
<tr>
<td>LT</td>
<td>Lithuania</td>
</tr>
<tr>
<td>LU</td>
<td>Luxembourg</td>
</tr>
<tr>
<td>LV</td>
<td>Latvia</td>
</tr>
<tr>
<td>MC</td>
<td>Monaco</td>
</tr>
<tr>
<td>MD</td>
<td>Republic of Moldova</td>
</tr>
<tr>
<td>MG</td>
<td>Madagascar</td>
</tr>
<tr>
<td>MK</td>
<td>The former Yugoslavia</td>
</tr>
<tr>
<td>ML</td>
<td>Mali</td>
</tr>
<tr>
<td>MN</td>
<td>Mongolia</td>
</tr>
<tr>
<td>MR</td>
<td>Mauritania</td>
</tr>
<tr>
<td>MW</td>
<td>Malawi</td>
</tr>
<tr>
<td>MX</td>
<td>Mexico</td>
</tr>
<tr>
<td>NE</td>
<td>Niger</td>
</tr>
<tr>
<td>NL</td>
<td>Netherlands</td>
</tr>
<tr>
<td>NO</td>
<td>Norway</td>
</tr>
<tr>
<td>NZ</td>
<td>New Zealand</td>
</tr>
<tr>
<td>PL</td>
<td>Poland</td>
</tr>
<tr>
<td>PT</td>
<td>Portugal</td>
</tr>
<tr>
<td>RO</td>
<td>Romania</td>
</tr>
<tr>
<td>RU</td>
<td>Russian Federation</td>
</tr>
<tr>
<td>SD</td>
<td>Sudan</td>
</tr>
<tr>
<td>SE</td>
<td>Sweden</td>
</tr>
<tr>
<td>SG</td>
<td>Singapore</td>
</tr>
<tr>
<td>SI</td>
<td>Slovenia</td>
</tr>
<tr>
<td>SK</td>
<td>Slovakia</td>
</tr>
<tr>
<td>SN</td>
<td>Senegal</td>
</tr>
<tr>
<td>SZ</td>
<td>Swaziland</td>
</tr>
<tr>
<td>TD</td>
<td>Chad</td>
</tr>
<tr>
<td>TG</td>
<td>Togo</td>
</tr>
<tr>
<td>TJ</td>
<td>Tujikistan</td>
</tr>
<tr>
<td>TM</td>
<td>Turkmenistan</td>
</tr>
<tr>
<td>TR</td>
<td>Turkey</td>
</tr>
<tr>
<td>TT</td>
<td>Trinidad and Tobago</td>
</tr>
<tr>
<td>UA</td>
<td>Ukraine</td>
</tr>
<tr>
<td>UD</td>
<td>Uganda</td>
</tr>
<tr>
<td>US</td>
<td>United States of America</td>
</tr>
<tr>
<td>US</td>
<td>United States of America</td>
</tr>
<tr>
<td>VN</td>
<td>Viet Nam</td>
</tr>
<tr>
<td>YU</td>
<td>Yugoslavia</td>
</tr>
<tr>
<td>ZW</td>
<td>Zimbabwe</td>
</tr>
</tbody>
</table>
RP105 AGONISTS AND ANTAGONISTS

FIELD OF INVENTION

An object of this invention is to provide a method for treating or preventing allergic disease, including asthma and atopic dermatitis, B cell neoplasms, including chronic lymphocyte leukemia, hairy cell leukemia, prolymphocytic leukemia myelomas, autoimmune diseases such as systemic lupus Erythematous (SLE), rheumatoid arthritis(RA), Multiple Sclerosis (MS), acquired hemolytic anemia and diabetes, among others, in a mammal using an antagonist to RP105 receptor.

BACKGROUND OF THE INVENTION

Mature B cells comprise 10 to 15 percent of human peripheral blood lymphocytes, 50 percent of splenic lymphocytes, and approximately 10 percent of bone marrow lymphocytes. Mature B cells are derived from bone marrow precursor cells that arise continuously throughout life. B cells express on their surface intramembrane immunoglobulin (Ig) molecules that function as B cell antigen receptors in a complex of Ig-associated α and β signaling molecules with intracellular signaling events. B cells also express surface receptors for the Fc region of IgG molecules as well as receptors for activated complement components. The primary function of B cells is to produce antibodies.

B cells undergo several selection steps during differentiation from immature cells to mature B cells or antibody-secreting cells. Only B cells which have undergone the selection process join the mature B cell pool and differentiate into antibody secreting cells upon stimulation. These selections allow for maintenance of a stable B cell pool without the production of autoreactive B cells which react to create an autoimmune response and efficient antibody production with adequate specificity and affinity. von Boehmer, H. Cell 1994 76:210; Liu et al. Immunol. Today 1992 13:17; Liu et al. Nature (Lond.) 1989 342:929. The selection steps involve apoptosis, survival or proliferation of B cells. A study in double transgenic mice revealed that apoptosis is important in the negative selection that destroys B cells bearing aberrant specificity against self-antigens. Hartley et al. Cell 1993 72:325. The autoantigen itself or the autoantigen in combination with other signals triggered apoptosis in these hazardous cells. In contrast, positive selection requires a condition where only cells receiving a signal are able to survive and grow. Otherwise, cells undergo apoptosis. In the germinal center, only cells bearing a surface immunoglobulin with sufficient affinity against an antigen are allowed to survive and grow.
Liu et al. Immunol. Today 1992 13:17. In this situation, stimulatory signals such as the antigen itself or the CD40 ligand are shown to protect cells from apoptosis and induce survival or proliferation. Liu et al. Immunol. Today 1992 13:17; Liu et al. Nature (Lond.) 1989 342:929. Thus, apoptosis plays an essential role in the selection process. A signal that is capable of inducing or blocking apoptosis has been implicated as a pivotal system for selecting B cells.

Lymphocytes such as B cells are one of the most sensitive cells to immediate radiation induced damage. Anderson, R.E., and Warner, N.L. Adv. Immunol. 1976 24:215. Quiescent lymphocytes have been found to be more sensitive than actively cycling cells. The damaged cells are believed to undergo apoptosis. This process, which does not involve cell division, is sometimes referred to as interphase death. It has been hypothesized that lymphocyte signaling systems which are important in facilitating the B cell selection process are also related to cell death by irradiation.

Miyake et al. J. Exp. Med. 1994 180:1217-1224 disclose a monoclonal antibody (mAb), RP14, that protects murine B cells from apoptosis induced by irradiation or dexamethasone. A molecule recognized by this antibody was found to be expressed on the murine B cells. This murine B cell surface molecule, referred to as RP105, has been further characterized. Miyake et al. J. Immunol. 1995 154:3333-3340. RP105 is a murine B cell antigen. It is monomeric, with a size of approximately 105 kDa. It is expressed on mature B cells, but not on either immature or pre-B cells. This antigen is believed to transmit a signal into murine B cells that results in protection from radiation or dexamethasone induced apoptosis.

The N-terminal amino acid sequence of the murine RP105 molecule has been determined. A cDNA clone was also isolated with a probe corresponding to the obtained amino acid sequence. DNA sequencing revealed that an encoded murine polypeptide is a type 1 transmembrane protein consisting of 641 amino acids in a mature form. Northern hybridization with the clone detected a transcript of approximately 3 kb. This transcript was found in mouse spleen, but not thymus, kidney, muscle, heart, brain or liver. Transfection of the clone into a pro-B cell line resulted in the expression of RP105.

A computer search showed similarity of murine RP105 to a number of molecules including CD14, decorin and biglycan, which are human proteoglycans in extracellular tissue; the Drosophila toll, tartan, connectin, chaoptin and slit proteins (which are responsible for dorsal/ventral polarity, epidermal/subepidermal structure, target recognition of a subset of motor neurons, photoreceptor morphogenesis and pathway finding by commissural axons during embryogenesis); and the α-subunit of platelet glycoprotein Ib
(involved in platelet adhesion to vascular endothelial cells). Miyake et al. J. Immunol. 1995 154:3333-3340. The property shared by murine RP105 and these molecules is tandem repeats of a leucine-rich motif (LRM). These repeated motifs are observed in members of the leucine-rich repeat protein family and have been implicated in protein-protein interactions such as cell adhesion or receptor-ligand binding. The murine RP105 molecule has 22 tandem repeats of a leucine-rich motif, as well as amino and carboxyl flanking regions that are characteristically conserved among members of this family. Thus, murine RP105 is believed to be a member of the leucine-rich repeat family and the first one that is expressed specifically on mature B cells.

A human gene encoding a novel human B cell surface molecule has now been found.

SUMMARY OF THE INVENTION

In one aspect, the invention provides an anti-RP105 antibody.

In another aspect, the invention provides a composition comprising the anti-RP105 antibody and a pharmaceutically acceptable carrier, as well as a method for treating or preventing allergic disease, including asthma and atopic dermatitis, B cell neoplasms, including chronic lymphocyte leukemia, hairy cell leukemia, prolymphocytic leukemia, myelomas, autoimmune diseases such as systemic lupus Erythamatosus (SLE), rheumatoid arthritis (RA), Multiple Sclerosis (MS), acquired hemolytic anemia or diabetes; comprising administering to a patient in need of such treatment or prevention a therapeutically effective amount of anti-RP105 antibody or its composition.

BRIEF DESCRIPTION OF FIGURES

Figure 1. (A) Effect of RP105 antiserum on PBL proliferation and (B) levels of soluble CD23 in culture supernatants.

DETAILED DESCRIPTION OF THE INVENTION

Definition

In general, the following words or phrases have the indicated definition when used in the description, examples, and claims:

"RP105", "RP105 polypeptide", or "RP105 receptor" is defined as a polypeptide which has at least 80% identity to a polypeptide of SEQ ID NO: 2. The definitions includes any fragment of RP105 that can be used to raise antibodies against an RP105 epitope.
Thus, the definition covers any fragment of RP105 capable of functioning as an immunogen by itself or capable of functioning as an antigen in a conjugate created by the recombinant or in vitro fusion of the RP105 fragment and an immunogen. In one embodiment, for example, RP105 also includes a polypeptide sequence defined as SEQ ID NO:4 disclosed in US patent no. 5,707,829 issued January 13, 1998, which is incorporated by reference in its entirety.


Preferred parameters for polypeptide sequence comparison include the following: 1) Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)
Gap Penalty: 12
Gap Length Penalty: 4
A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for peptide comparisons (along with no penalty for end gaps).

Preferred parameters for polynucleotide comparison include the following:
Comparison matrix: matches = +10, mismatch = 0
Gap Penalty: 50
Gap Length Penalty: 3
Available as: The "gap" program from Genetics Computer Group, Madison WI. These are the default parameters for nucleic acid comparisons.

By way of example, a polynucleotide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:2, that is be 100% identical, or it may include up to a certain integer number of nucleotide alterations as compared to the reference sequence. Such alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5’ or 3’ terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:2 by the numerical percent of the respective percent identity(divided by 100) and subtracting that product from said total number of nucleotides in SEQ ID NO:2, or:

\[ n_n \leq x_n \cdot (y) \]

wherein \( n_n \) is the number of nucleotide alterations, \( x_n \) is the total number of nucleotides in SEQ ID NO:2, and \( y \) is, for instance, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, etc., and wherein any non-integer product of \( x_n \) and \( y \) is rounded down to the nearest integer prior to subtracting it from \( x_n \). Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:1 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

Similarly, a polypeptide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:1, that is be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the % identity is less than 100%. Such alterations are selected from the group
consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino acids in SEQ ID NO:1 by the numerical percent of the respective percent identity (divided by 100) and then subtracting that product from said total number of amino acids in SEQ ID NO:1, or:

\[ n_a = x_a - (x_a \cdot y), \]

wherein \( n_a \) is the number of amino acid alterations, \( x_a \) is the total number of amino acids in SEQ ID NO:1, and \( y \) is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and wherein any non-integer product of \( x_a \) and \( y \) is rounded down to the nearest integer prior to subtracting it from \( x_a \).

Anti-RP105 antibody is defined as any antibody that binds to an RP105 or fragment thereof. The definition includes antibodies of all immunoglobulin types, such as IgG, IgA, IgM, IgD and IgE, and fragments thereof, such as Fab, F(ab')2, and Fv, and includes antibodies and antibody fragments of all origins, such as polyclonal antibodies, monoclonal antibodies, humanized antibodies and human antibodies produced in transgenic animals or transgenic animal cell culture.

The terms "blocking RP105 antibody", "antagonist antibody to RP105", and "antibody capable of blocking RP105" are defined such that the ability of the RP105 to bind to a ligand is impaired or eliminated.

"Highly stringent conditions" include overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA; followed by washing the filters in 0.1x SSC at about 65°C.

An RP105 for use in anti-RP105 antibody preparation can be isolated from natural sources or synthesized by in vitro or recombinant techniques. What follows is a description of each of these three methods of making RP105.

In a preferred embodiment of the invention, the RP105 or antigenic fragment thereof is produced by recombinant techniques. In one embodiment, the recombinant RP105 or fragment thereof can be used as immunogen without an adjuvant. In a further
embodiment, the immunogen is a fusion protein that contains the amino acid sequence of a suitable adjuvant fused to RP105. In yet another embodiment, the recombinant RP105 is covalently fused in vitro to an adjuvant or is administered concomitantly with an adjuvant. In a particularly preferred embodiment of the invention, recombinant cells expressing the RP105 as a surface protein anchored to the cell membrane are used to immunize the desired animal species. Recombinant cells expressing RP105 as cell surface protein will generate the desired immunogenic response in the animal without the use of an adjuvant.

The following is a general discussion of methods for the design and construction of recombinant RP105 expression systems.

All DNA sequences, including the DNA sequence of SEQ ID NO:2, that encode a polypeptide within the amino acid sequence of SEQ ID NO:1 are suitable for use in the recombinant production of RP105. Additional DNA sequences suitable for use herein include a polynucleotide which has at least 80% identity to a polynucleotide which encode polypeptide of SEQ ID NO: 1. Further, sequences suitable for use also include any polypeptide-encoding DNA sequence that hybridizes under highly stringent conditions to the complement of the DNA sequence of SEQ ID NO: 2, or to the complement of any other DNA sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 1. In one embodiment, candidate hybridizing DNA sequences can be obtained by designing DNA sequences that encode variants of the amino acid sequence of SEQ ID NO: 1. Such variants include, for example, deletions from, or insertions and substitutions of, residues within the amino acid sequence of SEQ ID NO: 1. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct is encoded by a DNA sequence that hybridizes under highly stringent conditions to the complement of the DNA sequence of SEQ ID NO: 2, or to the complement of any other DNA sequence that encodes a polypeptide having the amino acid sequence of SEQ ID NO:1.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for above-described polynucleotides. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., Basic Methods in Molecular Biology (1986) and Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). Preferred such methods include, for instance, calcium phosphate transfection, DEAE-dextran mediated transfection, transfection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.
Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, E. coli, Streptomyces and Bacillus subtilis cells; fungal cells, such as yeast cells and Aspergillus cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used, for instance, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector which is able to maintain, propagate or express a polynucleotide to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook et al., MOLECULAR CLONING, A LABORATORY MANUAL (supra). Appropriate secretion signals may be incorporated into the desired polypeptide to allow secretion of the translated protein into the lumen of the endoplasmic reticulum, the periplasmic space or the extracellular environment. These signals may be endogenous to the polypeptide or they may be heterologous signals.

If RP105 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide. If produced intracellularly, the cells must first be lysed before the polypeptide is recovered. Polypeptides of the present invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

In addition to the recombinant production of RP105 described above, RP105 can also be produced by in vitro chemical synthesis. In one embodiment, the desired RP105 is constructed by the solid phase synthesis method described by Merrifield, Science, 232; 342-347 (1986). In this method, a growing polypeptide chain is covalently anchored, usually by
its C-terminus, to an insoluble solid support such as beads of polystyrene resin, and the appropriately blocked amino acids and reagents are added in the proper sequence. This permits the quantitative recovery of the desired peptide product by simply filtering and washing the beads. It will be appreciated that any method of peptide synthesis now or hereafter developed can be used to synthesize RP105 for use in the present invention.

*Anti-RP105 Antibody Preparation*

Polyclonal antibodies to the RP105 generally are raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the RP105 and an adjuvant. Immunization with recombinant cells expressing the RP105 (e.g. mouse or CHO cells expressing RP105) may be satisfactory, or it may be useful to separate the RP105 and conjugate it or a fragment containing the amino acid sequence of the desired RP105 antigenic site to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl2, or R1N=C=NR, where R and R1 are different alkyl groups.

Animals ordinarily are immunized against the cells or immunogenic conjugates of RP105 with monophosphoryl lipid. A (MPL)/trehalose dicorynomycolate (TDM)(Ribi Immunochem, Research, Inc., Hamilton, Mont.) and injecting the solution intradermally at multiple sites. Two weeks later the animals are boosted with the original amount of conjugate in MPL/TDM. 7 to 14 days later animals are bled and the serum is assayed for anti-RP105 titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same RP105, but conjugated to a different protein and/or through a different cross-linking agent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are used to enhance the immune response.

Monoclonal antibodies are prepared by recovering spleen cells from immunized animals and immortalizing the cells in conventional fashion, e.g., by fusion with myeloma cells or by Epstein-Barr (EB)-virus transformation and screening for clones expressing the desired antibody. The hybridoma technique described originally by Koehler and Milstein, Eur. J. Immunol., 6: 511 (1976) and also described by Hammerling et al., In: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981) has been widely
applied to produce hybrid cell lines that secrete high levels of monoclonal antibodies against many specific antigens.

The hybrid cell lines can be maintained in vitro in cell culture media. The cell lines producing the antibodies can be selected and/or maintained in a medium containing hypoxanthine-aminopterin thymidine (HAT). In fact, once the hybridoma cell line is established, it can be maintained on a variety of nutritionally adequate media. Moreover, the hybrid cell lines can be stored and preserved in any number of conventional ways, including freezing and storage under liquid nitrogen. Frozen cell lines can be revived and cultured indefinitely with resumed synthesis and secretion of monoclonal antibody.

The secreted antibody is recovered from tissue culture supernatant or ascites fluid by conventional methods such as immune precipitation, ion-exchange chromatography, affinity chromatography such as protein A/protein G column chromatography, or the like. The antibodies described herein are also recovered from hybridoma cell cultures by conventional methods such as precipitation with 50% ammonium sulfate. The purified antibodies can then be sterile filtered.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins.

The monoclonal antibodies herein include hybrid and recombinant antibodies produced by splicing a variable (including hypervariable) domain of an anti-RP105 antibody with a constant domain (e.g. "humanized" antibodies), or a light chain with a heavy chain, or a chain from one species with a chain from another species, or fusions with heterologous proteins, regardless of species of origin or immunoglobulin class or subclass designation, as well as antibody fragments (e.g., Fab, F(ab')2, and Fv), so long as they exhibit the desired biological activity. (See, e.g., Cabilly, et al., U.S. Pat. No. 4,816,567; Mage & Lamoyi, in Monoclonal Antibody Production Techniques and Applications, pp. 79-97 (Marcel Dekker, Inc., New York, 1987).)
Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method described by Kohler and Milstein, supra, or may be made by recombinant DNA methods (Cabilly, et al., supra).

"Humanized" forms of non-human (e.g. murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab′, F(ab′)2 or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore humanized antibody may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin.

DNA encoding the monoclonal antibodies of the invention is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells. Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences. Morrison, et al., Proc. Nat. Acad. Sci. 81, 6851 (1984), or by covalently joining to the immunoglobulin coding sequence all or
part of the coding sequence for a non-immunoglobulin polypeptide. In that manner, "chimeric" or "hybrid" antibodies are prepared that have the binding specificity of an anti RP105 monoclonal antibody herein.

Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody of this invention, or they are substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody comprising one antigen-combining bivalent antibody comprising one antigen-combining site having specificity for an RP105 and another antigen-combining site having specificity for a different antigen.

Chimeric or hybrid antibodies also may be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., Nature 321: 522-525 (1986); Riechmann et al., Nature 332, 323-327 (1988); Verhoeyen et al., Science 239, 1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (Cabilly, supra), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

It is important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three dimensional models of the parental and humanized sequences. Three dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probably three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of
these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequence so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

Alternatively, it is now possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy chain joining region (J) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant animals will result in the production of human antibodies upon antigen challenge, and the antibodies can be harvested from the animal's blood or other body fluid. See, e.g., Jakobovits et al., *Proc. Natl. Acad. Sci. USA* 90, 2551-255 (1993); Jakobovits et al., *Nature* 362, 255-258 (1993). In addition, the cells expressing the desired antibody can be isolated from the animal host and used to produce the antibody in cell culture, and the antibody can be harvested from the cell culture by standard methods.

*Therapeutic Compositions and Administration of Anti-RP105 Antibodies*

Therapeutic formulation of anti-RP105 antibodies are prepared for storage by mixing antibody having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (*Remington's Pharmaceutical Sciences*, supra), in the form of lyophilized cake or aqueous solutions. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids, antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins, hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol, salt-forming couterions such as sodium; and/or nonionic surfactants such as Tween, Pluronics or polyethylene glycol (PEG).
The anti-RP105 antibody to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution. The anti-RP105 antibody ordinarily will be stored in lyophilized form or in solution.

Therapeutic anti-RP105 antibody compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper piercable by a hypodermic injection needle.

The route of anti-RP105 antibody administration is in accord with known methods, e.g., injection or infusion by intravenous, intraperitoneal, intercerebral, intramuscular, intraocular, intrarterial, intracerebrospinal, or intralesional routes, or by sustained release systems as noted below. Preferably the antibody is given systemically.


Anti-RP105 antibody can also be administered by inhalation. Commercially available nebulizers for liquid formulations, including jet nebulizers and ultrasonic nebulizers are useful for administration. Liquid formulations can be directly nebulized and lyophilized powder can be nebulized after reconstitution. Alternatively, anti-RP105 antibody can be aerosolized using a fluorocarbon formulation and a metered dose inhaler, or inhaled as a lyophilized and milled powder.

An "effective amount" of anti-RP105 antibody to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, the type of anti-RP105 antibody employed, and the condition of the patient. Accordingly, it will be
necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. Typically, the clinician will administer the anti-RP105 antibody until a dosage is reached that achieves the desired effect. The progress of this therapy is easily monitored by conventional assays.

In the treatment and prevention of allergic disease, including asthma and atopic dermatitis, B cell neoplasms, including chronic lymphocyte leukemia, hairy cell leukemia, prolymphocytic leukemia myelomas, autoimmune diseases such as systemic lupus Erythmatosus (SLE), rheumatoid arthritis (RA), Multiple Sclerosis (MS), acquired hemolytic anemia and diabetes in a mammal using an antibody to RP105 receptor by an anti-RP105 antibody, the antibody composition will be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the antibody, the particular type of antibody, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The "therapeutically effective amount" of antibody to be administered will be governed by such considerations, and is the minimum amount necessary to prevent, ameliorate, or treat allergic disease, including asthma and atopic dermatitis, B cell neoplasms, including chronic lymphocyte leukemia, hairy cell leukemia, prolymphocytic leukemia myelomas, autoimmune diseases such as systemic lupus Erythmatosus (SLE), rheumatoid arthritis (RA), Multiple Sclerosis (MS), acquired hemolytic anemia and diabetes in a mammal using an antibody to RP105 receptor. Such amount is preferably below the amount that is toxic to the host.

As a general proposition, the initial pharmaceutically effective amount of the antibody administered parenterally will be in the range of about 0.1 to 50 mg/kg of patient body weight per day, with the typical initial range of antibody used being 0.3 to 20 mg/kg/day, more preferably 0.3 to 15 mg/kg/day. The desired dosage can be delivered by a single bolus, by multiple bolus administration, or by continuous infusion administration of antibody, depending on the pattern of pharmacokinetic decay that the practitioner wishes to achieve.

As noted above, however, these suggested amounts of antibody are subject to a great deal of therapeutic discretion. The key factor in selecting an appropriate dose and scheduling is the result obtained, as indicated above.

The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the allergic disease, including asthma and atopic
dermatitis, B cell neoplasms, including chronic lymphocyte leukemia, hairy cell leukemia, prolymphocytic leukemia, myelomas, autoimmune diseases such as systemic lupus Erythematosus (SLE), rheumatoid arthritis (RA), Multiple Sclerosis (MS), acquired hemolytic anemia and diabetes in a mammal using an antibody to RP105 receptor in question. The effective amount of such other agents depends on the amount of anti-RP105 antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as used herebefore or about from 1 to 99% of the heretofore employed dosages.

The following examples are offered by way of illustration and not by way of limitation.

Examples

Effect of RP105 antiserum on peripheral blood lymphocyte (PBL) proliferation.

Antiserum to human RP105 was generated in mice by genetic immunization using a full length RP105 expression vector. This antiserum was used to characterize the function of RP105 in B lymphocyte responses. More specifically, peripheral blood lymphocytes were purified by differential density gradient purification. Diluted whole blood was layered onto Ficoll gradients (Pharmacia LKB Biotech, Piscataway, NJ) and centrifuged at 400xg for 30 minutes at 22°C. Cells were recovered from the interface of the gradient and washed twice in RPMI 1640 medium (Life technologies Inc., Gaithersburg, MA). PBLs were adjusted to 2x10^6 cells/ml in RPMI 1640 medium supplemented with 10% Foetal Bovine Serum (HyClone Laboratories, Logan, UT), 25mM Hepes buffer, 2mM L-Glutamine and 50μg/ml Gentomycin (Life Technologies Inc., Gaithersburg, MA). 100ul of cell suspension was added to wells of a 96 well flat bottomed microtitre plate (Falcon Labware, Franklin Lakes, NJ) and 50ul of diluted mouse antiserum to human RP105 or control mouse antiserum were added to the cells. A further 50ul of medium were added to the wells and cells were incubated at 37 oC in 5% CO2 for 66 hours. After this time 100ul of cell culture supernatant was removed from wells and stored at -70oC, and 1μCi 3H Thymidine was added to wells for a further 6 hours. Cells were harvested and proliferation was monitored by β-scintillation counting.

As shown in Figure 1, PBLs cultured in the presence of RP105 antiserum significantly increased PBL proliferation above responses induced by control mouse antiserum at an equivalent dilution. CD40 mAb and interleukin-4, potent stimulators of PBL proliferation, also induced strong proliferative responses. In one donor, RP105 antiserum induced similar levels of proliferation to CD40 and IL-4 activation whereas in a
second donor a weaker response was observed relative to stimulation with CD40 mAb and IL-4. This data indicates that RP105 receptor activation results in the proliferation of PBLs and that in some donors this is equivalent to CD40 and IL-4 stimulation.

In the same experiment, culture supernatants from PBLs were assayed for soluble CD23 (sCD23). sCD23 was detected in cell culture supernatants from PBLs stimulated with CD40 and IL-4, however no significant increases in sCD23 could be detected in cells treated with RP105 antisera or control antiserum. This indicates that RP105 does not induce the release of sCD23 from B cells indicating that stimulation via CD40/IL-4 and RP105 receptors differ in their downstream signaling.

Activation of B lymphocytes results in increased B cell proliferation, expression of co-stimulatory molecules, production of cytokines, differentiation, production of immunoglobulins and resistance to apoptosis. RP105 receptor stimulation with specific antiserum resulted in B cell activation with increased proliferation. This indicates that inhibition of RP105 signalling using blocking RP105 antibodies will inhibit B cell activation and thereby decrease antibody production and the capacity of B cells to present antigen to T cells. This would be expected to have important beneficial effects in immune disorders where chronic activation of B cells results in the production of antibodies to allergenic proteins (asthma, allergic dermatitis, atopy) autoantibodies to self antigens (SLE, MS, RA) and diseases where B cells have been implicated in activation of T cells (RA, SLE, MS, diabetes). In addition, the capacity of RP105 to stimulate B cell proliferation and protection from apoptosis may be a mechanism of survival for chronically activated B cells as well as B lymphocyte neoplasms such as chronic lymphocyte leukemia, hairy cell leukemia, prolymphocytic leukemia and myelomas. Antagonist antibodies to RP105 would have utility in preventing B cell activation in these diseases leading to deletion of autoreactive or chronically activated B cells, and removal of survival signals for B cell lymphomas through blockade of the RP105 receptor. Thus in summary the invention provides a method for treating or preventing allergic disease, including asthma and atopic dermatitis, B cell neoplasms, including chronic lymphocyte leukemia, hairy cell leukemia, prolymphocytic leukemia, myelomas, autoimmune diseases such as systemic lupus Erythematosus (SLE), rheumatoid arthritis (RA), Multiple Sclerosis (MS), aquired hemolytic anemia or diabetes; comprising administering to a patient in need of such treatment or prevention a therapeutically effective amount of blocking RP105 antibody or its composition.

Stimulatory RP105 antibodies that induce receptor signaling may also be used to screen for inhibitors (antagonists), which include both small molecular weight compounds.
and even blocking RP105 antibodies, of the RP105 signaling pathway. The antagonist thus
discovered will have utility in treating immune disorders where chronic activation of B cells
results in the production of antibodies to allergenic proteins (asthma, allergic dermatitis,
atopy) autoantibodies to self antigens (SLE, MS, RA) and diseases where B cells have been
implicated in activation of T cells (RA, SLE, MS, diabetes). More specifically the present
invention relates to a method for identifying an antagonist of a RP105 which comprises:

contacting a cell expressing on the surface thereof a RP105 polypeptide, said
RP105 polypeptide being associated with a second component capable of providing a
detectable signal in response to a stimulatory RP105 antibody with a candidate compound
to be screened; and

determining whether the compound inhibits RP105 polypeptide by measuring the
level of said detectable signal generated from the interaction of the stimulatory RP105
antibody with RP105 polypeptide. In one embodiment detectable signal response is B cell
proliferation. Preferred stimulatory antibody is monoclonal antibody.

In another embodiment, a method for identifying an antagonist of a RP105
polypeptide comprises:

determining the inhibition of binding of a stimulatory RP105 antibody to cells
which have the RP105 polypeptide on the surface thereof, or to cell membranes containing
the polypeptide, in the presence of a candidate compound under conditions to permit
binding to the polypeptide, and determining the amount of stimulatory RP105 antibody
bound to the polypeptide, such that a compound capable of causing reduction of binding of
stimulatory RP105 antibody is an antagonist.

Thus the present invention also relates to an antagonist obtainable by the above
methods, and a method of treating immune disorders where chronic activation of B cells
results in the production of antibodies to allergenic proteins (asthma, allergic dermatitis,
atopy) autoantibodies to self antigens (SLE, MS, RA) and diseases where B cells have been
implicated in activation of T cells (RA, SLE, MS, diabetes) by administering to a patient
suffering from such ailments by administering an effective amount of such antagonist.

Thus the present invention provides a method for treating or preventing allergic
disease, including asthma and atopic dermatitis, B cell neoplasms, including chronic
lymphocyte leukemia, hairy cell leukemia, prolymphocytic leukemia, myelomas,
autoimmune diseases such as systemic lupus Erythematous (SLE), rheumatoid
arthritis(RA), Multiple Sclerosis (MS), acquired hemolytic anemia or diabetes; comprising
administering to a patient in need of such treatment or prevention a therapeutically effective
amount of antagonist of RP105.
In addition, agonist RP105 mAbs similar to polyclonal antibodies demonstrated in Figure 1 would be expected to have utility in B cell immunodeficiencies resulting from bone marrow transplant, rheumatic illnesses or due to treatment for leukemia, and for example in primary immunodeficiency syndromes, Wolf-Hirschhorn syndrome, Nijmegen breakage syndrome and X-linked agammaglobulinemia.

Agonists and antagonists of the present invention may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of an agonist or an antagonist, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject’s condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 μg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

All references cited in this specification are expressly incorporated by reference.
We claim:

1. A method for treating or preventing allergic disease, including asthma and atopic dermatitis, B cell neoplasms, including chronic lymphocyte leukemia, hairy cell leukemia, prolymphocytic leukemia, myelomas, autoimmune diseases such as systemic lupus Erythamatosus (SLE), rheumatoid arthritis (RA), Multiple Sclerosis (MS), acquired hemolytic anemia or diabetes; comprising administering to a patient in need of such treatment or prevention a therapeutically effective amount of antagonist for RP105.

2. An antagonist to which is blocking RP105 antibody.

3. The antibody of claim 2 which is monoclonal antibody.

4. The antibody of claim 2 which is polyclonal antibody.

5. The antibody of claim 2 which is humanized antibody.

6. A method for identifying an antagonist of a RP105 which comprises:
   contacting a cell expressing on the surface thereof a RP105 polypeptide, said RP105 polypeptide being associated with a second component capable of providing a detectable signal in response to a stimulatory RP105 antibody with a candidate compound to be screened; and
   determining whether the compound inhibits RP105 polypeptide by measuring the level of said detectable signal generated from the interaction of the stimulatory RP105 antibody with RP105 polypeptide.

7. The method of claim 7 in which said detectable signal response is B cell proliferation.

8. The method of claim 7 in which said stimulatory RP105 antibody is monoclonal antibody.
(A) Effect of RP105 antiserum on PBL proliferation and (B) levels of soluble CD23 in culture supernatants.

**FIG. IA**

![Graph showing the effect of RP105 antiserum on PBL proliferation for Donor A.](image)

**FIG. IB**

![Graph showing the levels of soluble CD23 for Donor B.](image)

**FIG. IC**

![Graph showing the effect of RP105 antiserum on PBL proliferation for Donor A.](image)

**FIG. ID**

![Graph showing the levels of soluble CD23 for Donor B.](image)
### SEQUENCE LISTING

**<110>** Roshak, Amy K  
Holmes, Stephen D  
Harrop, Jeremy A

**<120>** RP105 AGONISTS AND ANTAGONISTS

**<130>** GH50037

**<140>** Unknown

**<141>**

**<150>** 60/098,030  
**<151>** 1998-08-27

**<160>** 2

**<170>** FastSEQ for Windows Version 3.0

**<210>** 1

**<211>** 650

**<212>** PRT

**<213>** Human

**<400>** 1

<table>
<thead>
<tr>
<th>Met</th>
<th>Ala</th>
<th>Phe</th>
<th>Asp</th>
<th>Val</th>
<th>Ser</th>
<th>Cys</th>
<th>Phe</th>
<th>Phe</th>
<th>Trp</th>
<th>Val</th>
<th>Val</th>
<th>Leu</th>
<th>Phe</th>
<th>Ser</th>
<th>Ala</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>10</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gly</td>
<td>Cys</td>
<td>Lys</td>
<td>Val</td>
<td>Ile</td>
<td>Thr</td>
<td>Ser</td>
<td>Trp</td>
<td>Thr</td>
<td>Ser</td>
<td>Trp</td>
<td>Asp</td>
<td>Gln</td>
<td>Met</td>
<td>Cys</td>
<td>Ile</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>25</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td>Asn</td>
<td>Lys</td>
<td>Thr</td>
<td>Tyr</td>
<td>Asn</td>
<td>Cys</td>
<td>Glu</td>
<td>Asn</td>
<td>Leu</td>
<td>Gly</td>
<td>Leu</td>
<td>Ser</td>
<td>Glu</td>
<td>Ile</td>
<td>Pro</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>40</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asp</td>
<td>Thr</td>
<td>Leu</td>
<td>Pro</td>
<td>Asn</td>
<td>Thr</td>
<td>Trp</td>
<td>Glu</td>
<td>Phe</td>
<td>Leu</td>
<td>Glu</td>
<td>Phe</td>
<td>Ser</td>
<td>Phe</td>
<td>Asn</td>
<td>Phe</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>55</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>Pro</td>
<td>Thr</td>
<td>Ile</td>
<td>His</td>
<td>Asn</td>
<td>Arg</td>
<td>Thr</td>
<td>Phe</td>
<td>Ser</td>
<td>Arg</td>
<td>Leu</td>
<td>Met</td>
<td>Asn</td>
<td>Leu</td>
<td>Thr</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>70</td>
<td>75</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phe</td>
<td>Leu</td>
<td>Asp</td>
<td>Leu</td>
<td>Thr</td>
<td>Arg</td>
<td>Cys</td>
<td>Gln</td>
<td>Ile</td>
<td>Asn</td>
<td>Trp</td>
<td>Ile</td>
<td>His</td>
<td>Glu</td>
<td>Asp</td>
<td>Thr</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>85</td>
<td>90</td>
<td>95</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1/5
Phe Gln Ser His Gln Leu Ser Thr Leu Val Leu Thr Gly Asn Pro 100 105 110
Leu Ile Phe Met Ala Glu Thr Ser Leu Asn Gly Pro Lys Ser Leu Lys 115 120 125
His Leu Phe Leu Ile Gln Thr Gly Ile Ser Asn Leu Glu Phe Ile Pro 130 135 140
Val His Asn Leu Glu Asn Leu Glu Ser Leu Tyr Leu Gly Ser Asn His 145 150 155 160
Ile Ser Ser Ile Lys Phe Pro Lys Asp Phe Pro Ala Arg Asn Leu Lys 165 170 175
Val Leu Asp Phe Gln Asn Asn Ala Ile His Tyr Ile Ser Arg Glu Asp 180 185 190
Met Arg Ser Leu Glu Gln Ala Ile Asn Leu Ser Leu Asn Phe Asn Gly 195 200 205
Asn Asn Val Lys Gly Ile Glu Leu Gly Ala Phe Asp Ser Thr Val Phe 210 215 220
Gln Ser Leu Asn Phe Gly Asp Phe Ile Pro Asn Leu Ser Val Ile Phe Asn 225 230 235 240
Gly Leu Gln Asn Ser Thr Thr Glu Pro Leu Trp Leu Gly Thr Phe Glu 245 250 255
Asp Ile Asp Asp Glu Asp Ile Ser Ser Ala Met Leu Lys Gly Leu Cys 260 265 270
Glu Met Ser Val Glu Ser Leu Asn Leu Gln Glu His Arg Phe Ser Asp 275 280 285
Ile Ser Ser Thr Thr Phe Gln Cys Phe Thr Glu Leu Gln Glu Leu Asp 290 295 300
Leu Thr Ala Thr His Leu Lys Gly Leu Pro Ser Gly Met Lys Gly Leu 305 310 315 320
Asn Leu Leu Lys Leu Val Leu Ser Val Asn His Phe Asp Gln Leu 325 330 335
Cys Gln Ile Ser Ala Ala Asn Phe Pro Ser Leu Thr His Leu Tyr Ile 340 345 350
Arg Gly Asn Val Lys Lys Leu His Leu Gly Val Gly Cys Leu Glu Lys 355 360 365
Leu Gly Asn Leu Gln Thr Leu Asp Leu Ser His Asp Asp Ile Glu Ala 370 375 380
Ser Asp Cys Cys Ser Leu Gln Leu Lys Asn Leu Ser His Leu Gln Thr 385 390 395 400
Leu Asn Leu Ser His Asn Glu Pro Leu Gly Leu Gln Ser Gln Ala Phe 405 410 415
Lys Glu Cys Pro Gln Leu Glu Leu Leu Asp Leu Ala Phe Thr Arg Leu  
420        425        430
His Ile Asn Ala Pro Gln Ser Pro Phe Gln Asn Leu His Phe Leu Gln  
435        440        445
Val Leu Asn Leu Thr Tyr Cys Phe Leu Asp Thr Ser Asn Gln His Leu  
450        455        460
Leu Ala Gly Leu Val Leu Arg His Leu Asn Leu Lys Gly Asn His  
465        470        475        480
Phe Gln Asp Gly Thr Ile Thr Lys Thr Asn Leu Leu Gln Thr Val Gly  
485        490        495
Ser Leu Glu Val Leu Ile Leu Ser Ser Cys Gly Leu Leu Ser Ile Asp  
500        505        510
Gln Glu Ala Phe His Ser Leu Gly Lys Met Ser His Val Asp Leu Ser  
515        520        525
His Asn Ser Leu Thr Cys Asp Ser Ile Asp Ser Leu Ser His Leu Lys  
530        535        540
Gly Ile Tyr Leu Asn Leu Ala Ala Asn Ser Ile Ile Ser Pro  
545        550        555        560
Arg Leu Leu Pro Ile Leu Ser Gln Gln Ser Thr Ile Asn Leu Ser His  
565        570        575
Asn Pro Leu Asp Cys Thr Cys Ser Asn Ile His Phe Leu Thr Trp Tyr  
580        585        590
Lys Glu Asn Leu His Lys Leu Gly Ser Glu Glu Thr Thr Cys Ala  
595        600        605
Asn Pro Pro Ser Leu Arg Gly Val Leu Ser Asp Val Lys Leu Ser  
610        615        620
Cys Gly Ile Thr Ala Ile Gly Ile Phe Phe Leu Ile Val Phe Leu Leu  
625        630        635        640
Leu Leu Ala Ile Leu Leu Phe Phe Cys Ser  
645        650

<210> 2
<211> 2775
<212> DNA
<213> Human

<400> 2

ttagcaagtgc gaccttatcct cactaaaggg aacaaaaagct ggagtcccac cgccggtggcg  
60
gccgctctag gaatagtgga tcccccgggc tcgcaggaatt cgccacacgg gtaaaccgccac  
120
ttaagcaatcc tagcctgtga tgggtttgga cgtcacgtgc tctttttgga tgggtgctgtt  
180

3/5
tgggtctggg ttctcagtaa tgtagccatt tgagaaacctt acttggggac aaagtcetca
2640
tccttatatt aaatgaaaaa agaaaaagaa agcataataa atttaaaaga aaaggtctgag
2700
aaaaaaaaaa aaaaaaact cgaggggggc cggtacccca ttgcacatat atgatctata
2760
aatcgggggg ggaag
2775
**INTERNATIONAL SEARCH REPORT**

IPA63K 39/395, C07K 16/00, 16/18, 16/28, G01N 33/53  
US CL : Please See Extra Sheet.

### A. CLASSIFICATION OF SUBJECT MATTER

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)


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

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG, BIOSIS, CA, EMBASE, MEDLINE, U.S. PATENTS

search terms: rp105

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>US, 5,780,609 A (MARSHALL ET AL.) 14 July 1998, see entire document.</td>
<td>1-8</td>
</tr>
</tbody>
</table>

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

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

Date of the actual completion of the international search: **22 DEC 1999**

Date of mailing of the international search report: **22 DEC 1999**

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231  
Facsimile No. (703) 305-3230

Authorized officer: **PHILLIP GAMBEI**

Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1992)
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :
424/130.1, 133.1, 140.1, 143.1, 144.1, 153.1, 173.1; 435/7.1, 7.2, 7.21; 514/8, 885; 530, 350, 387.1, 387.3, 388.1, 388.2, 388.7, 388.73

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.
424/130.1, 133.1, 140.1, 143.1, 144.1, 153.1, 173.1; 435/7.1, 7.2, 7.21; 514/8, 885; 530, 350, 387.1, 387.3, 388.1, 388.2, 388.7, 388.73