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(54) **Title:** HUMAN MONOCLONAL ANTIBODIES AND PROCESSES AND MATERIALS FOR MAKING SUCH ANTIBODIES

(57) **Abstract**

An anti-allergic agent is disclosed which comprises a human monoclonal antibody which binds to an airborne allergen that provokes an allergic IgE response, an allergen-binding fragment of such human monoclonal antibody or an immune complex of such human monoclonal antibody or allergen-binding fragment with said allergen. Pharmaceutical compositions and methods employing the anti-allergic agent, cell lines for making the human monoclonal antibodies and purified and isolated DNA for making the human monoclonal antibodies or allergen-binding fragments thereof are also disclosed. Also disclosed is a process for making multiple clones from an immortalized B-cell population, each of which clones secretes a human monoclonal antibody that binds to the desired antigen.

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HUMAN MONOCLONAL ANTIBODIES AND PROCESSES AND MATERIALS FOR MAKING SUCH ANTIBODIES

FIELD OF THE INVENTION

The present invention relates to anti-allergic agents which bind to airborne allergens, which agents are selected from human monoclonal antibodies (HuMAbs) or allergen-binding fragments or immune complexes of the human monoclonal antibodies or fragments with said allergen. The invention also relates to processes, cells and nucleic acids (*e.g.*, cDNA, recombinant vectors, etc.) for making such anti-allergic agents. In addition, the invention relates to a process for making a human monoclonal antibody to a desired antigen.

BACKGROUND OF THE INVENTION

Many airborne allergens cause degranulation of IgE-sensitized basophils/mast cells that results in allergic symptoms (such as rhinitis, conjunctivitis, atopic dermatitis and pollen-induced asthma) in sensitized persons. These allergens include pollens of trees and grasses (*e.g.*, ragweed), house dust mites, cat and dog dander, etc.

For example, proteins isolated from aqueous extracts of birchpollen are responsible for most of the allergic symptoms occurring in middle, eastern and northern Europe, as well as in certain areas of North America during the early and middle parts of the spring season. Twenty-two percent of allergic patients in Central Europe suffer from birch pollinosis.

Many airborne allergens such as the two major allergens from birchpollen have been characterized at the molecular level. For example, Bet vI is a 17 kD glycoprotein, which has been localized on ultra-thin sections of pollen grain in the cytoplasmic matrix (Grote, M., *J. Histochem. Cytochem.*, *39*: 1395-401 (1991). Ninety-seven per cent of patients with birchpollen allergy have IgE antibodies that recognize Bet vI (Valenta et al., *J. Allergy Clin. Immunol.*, *88*: 889-94 (1991c).

The gene coding for Bet vI has been cloned and found to be very homologous to the pea disease resistant response gene (Breiteneder et al., *EMBO J.*, *8*: 1935-8 (1989). The N-terminus of the protein is not subjected to post-translational processing.

Bet vII, the second major birch allergen, is recognized by the IgE antibodies of 9% of patients allergic to birch (Valenta, 1991c, *supra*). Bet vII relates to profilin, a ubiquitous component of the cytoskeleton which constitutes a family of functional plant pan-allergens, that is present in trees, grasses and weeds. Approximately 20% of all patients allergic to pollen also respond to the profilins (Valenta et al., *Science*, *253*: 557-60 (1991b). Both recombinant Bet vI and Bet vII allergens expressed in *E. coli* are recognized by IgE from birchpollen allergic patients (Valenta, 1991c, *supra*).

The molecular characterization of Bet vI and of Bet vII helps to explain the common observation that patients allergic to birchpollen are often sensitive to other related tree pollens. A high degree of homology at the nucleic acid level among the major allergens from birch (Bet vI), alder (Aln gI), hazel (Cor aI), and hornbeam (Car bI) at the nucleic acid level has been reported (Valenta et al., *J. Allergy Clin. Immunol.*, *87*: 677-82 (1991a). Partial N-terminal amino acid sequence analysis of Bet vI, Aln gI, Cor aI and Car bI (40-45 amino acid residues) demonstrated high sequence identity-homology and putative common epitopes (Ipsen, H. and O. Hanssen, "The NH_2 Terminal Amino-Acid Sequence of The Major Allergen of Birch (*Betulaverrucosa*), Bet vI, And Alder (*Alnus glutinosa*) Aln gI Pollens," 16th Norkiske Allergological Congress, Tromso, 1987. Accordingly, immunotherapy with birchpollen extract has been found effective in reducing IgE response as well as skin and nasal sensitivity in patients allergic to birch-, hazel- or hornbeam-pollen (Wihl et al., *Allergy*, *43*: 363-9 (1988); and Ipsen et al., *Allergy*, *43*: 370-7 (1988).

Up to 70% of patients allergic to birchpollen also demonstrate an intolerance to fruits and/or vegetables. This syndrome has collectively been called an allergy to BPRF (birchpollen-related food stuff) (Eriksson et al., *Allergy*, *37*: 437-43 (1982). True cross-reactivity of IgE antibodies from apple-sensitive patients with Bet vI on western blot analysis, and binding of BIP I, a mouse monoclonal antibody specific for Bet vI, to a 17 kD antigen from apple extract have been demonstrated (Ebner et al., *J. Allergy Clin. Immunol.*, *88*: 588-94 (1991).

The offending allergens of the major airborne allergy caused by house dust have been identified as proteins from three *Dermatophagoides* species: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and *Dermatophagoides microceras*. The most important allergens from these species are referred to as Group I (respectively Der pI, Der fI, and Der mI) and Group II (Der pII, Der fII, and Der mII). Complementary DNAs coding for those

proteins have been cloned, and recombinant allergens have been expressed in various systems (for a review, see Baldo, B. A., *Curr. Opin. Immunol.*, *3*: 841-50 (1991). It has been shown that human IgE antibodies react with both the Group I determinants that are common for the three mite species and the species-specific determinants (Chua et al., *J. Exp. Med.*, *167*: 175-82 (1988).

At present, treatment of those allergies is mainly directed to alleviation of symptoms, based on the usage of antihistamines and/or steroids. Classical immunotherapy, *e.g.*, desensitization, despite its limited efficacy and its potential toxicity, will probably continue to be used. However, an efficient and effective treatment of the underlying allergic mechanisms would be highly desirable.

Specific modulation of the immune response to allergens could take advantage of the use of allergen-specific monoclonal antibodies. Classically, this would be done with non-human monoclonal antibodies (MAbs) such as murine antibodies. In fact, rodent MAbs to some of the allergens discussed above have been prepared, *e.g.*, MAbs against Bet vI (Jarolim et al., *Int. Arch. Allergy Appl. Immunol.*, *90*: 54-60 (1989). However, such non-human MAbs may elicit an antigenic response if used to treat allergic reactions in humans.

It would therefore be more desirable to have human monoclonal antibodies (HuMAbs) which bind to these allergens and/or block the allergen-induced degranulation of IgE-sensitized basophils/mast cells. Single cross-reacting HuMAbs could even be used to treat allergy caused by distinct allergens which share common epitopes, as described above for tree pollens.

In the past, producing clones of immortalized human B-cells that secrete HuMAbs of a predetermined specificity has been difficult. The ability to obtain even one such clone after substantial effort may be considered unusual. It would thus be desirable to provide a process to produce a multiplicity of antibody-secreting human B-cell clones of a given specificity, from which multiplicity of cells a selection can be made to obtain one antibody or a set of HuMAbs with the desired characteristics. These clones and cDNA encoding the human monoclonal antibodies produced by such clones could be used to produce a wide variety of HuMAbs to specific antigens.

SUMMARY OF THE INVENTION

The present invention fills the above-mentioned needs by providing methods and materials for the treatment of allergies. In particular, a first aspect

of the present invention involves an anti-allergic agent comprising human monoclonal antibody (HuMAb) which binds to an airborne allergen that provokes an allergic IgE response, an allergen-binding fragment of such HuMAb, or an immune complex of such HuMAb or allergen-binding fragment with said allergen. The anti-allergic agent is a HuMAb of any human isotype (*i.e.*, IgG, IgA, IgD, IgE, or IgM). However, it is preferably an IgG, IgA or IgD antibody; and more preferably, it is of the IgG class. The anti-allergic agent of the invention may be employed in pharmaceutical compositions in combination with a pharmaceutically acceptable carrier and in methods of treating allergic reactions.

In one embodiment, the anti-allergic agent is a HuMAb which binds to birch antigen or house-dust-mite antigen. Preferably, it is a HuMAb which binds to the Bet vI or Bet vII antigens of birchpollen or to the Der pI or Der pII antigens of house-dust mites.

In another embodiment, the anti-allergic agent comprises a HuMAb which inhibits the allergen-induced degranulation of IgE-sensitized basophils/mast cells, an allergen-binding fragment of such HuMAb, or an immune complex of such HuMAb or allergen-binding fragment with said allergen.

The antibody or fragment of the invention preferably comprises at least one CDR (complementarity determining region) of an amino acid sequence defined by amino acid residues 1-127 of SEQ ID NO. 2, 1-114 of SEQ ID NO. 4, 1-127 of SEQ ID NO. 6, 1-107 of SEQ ID NO. 8, 1-119 of SEQ ID NO. 10, 1-108 of SEQ ID NO. 12, 1-118 of SEQ ID NO. 14, 1-113 of SEQ ID NO. 16, 1-123 of SEQ ID NO. 18, and/or 1-111 of SEQ ID NO. 20; or one or more somatic variant of such sequence. (All SEQ IDs are given in the SEQUENCE LISTING immediately before the Claims.)

In one embodiment, the antibody or fragment comprises a V_H segment having an amino acid sequence defined by amino acid residues 1-127 of SEQ ID NO. 2, 1-127 of SEQ ID NO. 6, 1-119 of SEQ ID NO. 10. 1-118 of SEQ ID NO. 14, or 1-123 of SEQ ID NO. 18, or by a CDR somatic variant of one of said sequences, and/or a V_L segment having an amino acid sequence defined by amino acid residues 1-114 of SEQ ID NO. 4, 1-107 of SEQ ID NO. 8, 1-108 of SEQ ID NO. 12, 1-113 of SEQ ID NO. 16, or 1-111 of SEQ ID NO. 20, or by a CDR somatic variant of one of said sequences.

The antibody of the invention preferably comprises a V_H segment having an amino acid sequence defined by amino acid residues 1-127 of SEQ ID

NO. 2, 1-127 of SEQ ID NO. 6, 1-119 of SEQ ID NO. 10, 1-118 of SEQ ID NO. 14, or 1-123 of SEQ ID NO. 18, in combination with a V_L segment having an amino acid sequence defined by amino acid residues 1-114 of SEQ ID NO. 4, 1-107 of SEQ ID NO. 8, 1-108 of SEQ ID NO. 12, 1-113 of SEQ ID NO. 16, or 1-111 of SEQ ID NO. 20, respectively. In one embodiment, the antibody comprises a V_H segment having the amino acid sequence defined by amino acid residues 1-127 of SEQ ID NO. 2, 1-127 of SEQ ID NO. 6, 1-119 of SEQ ID NO. 10, 1-118 of SEQ ID NO. 14, or 1-123 of SEQ ID NO. 18, and/or a V_L segment having the amino acid residues 11-114 of SEQ ID NO. 4, 1-107 of SEQ ID NO. 8, 1-108 of SEQ ID NO. 12, 1-113 of SEQ ID NO. 16, or 1-111 of SEQ ID NO. 20, or which comprises a CDR somatic variant of one or both of said amino acid sequences. More preferably, the antibody comprises a V_H segment having the amino acid sequence defined by amino acid residues 1-127 of SEQ ID NO. 2, 1-127 of SEQ ID NO. 6, 1-119 of SEQ ID NO. 10, 1-118 of SEQ ID NO. 14, or 1-123 of SEQ ID NO. 18, in combination with a V_L segment having the amino acid residues 1-114 of SEQ ID NO. 4, 1-107 of SEQ ID NO. 8, 1-108 of SEQ ID NO. 12, 1-113 of SEQ ID NO. 16, or 1-111 of SEQ ID NO. 20, respectively.

The antibody fragment of the invention preferably comprises a V_H segment having an amino acid sequence defined by amino acid residues 1-127 of SEQ ID NO. 2, 1-127 of SEQ ID NO. 6, 1-119 of SEQ ID NO. 10, 1-118 of SEQ ID NO. 14, or 1-123 of SEQ ID NO. 18, and/or a V_L segment having an amino acid sequence defined by amino acid residues 1-114 of SEQ ID NO. 4, 1-107 of SEQ ID NO. 8, 1-108 of SEQ ID NO. 12, 1-113 of SEQ ID NO. 16, or 1-111 of SEQ ID NO. 20.

In another embodiment, the anti-allergic agent comprises a HuMAb which inhibits the allergen-induced degranulation of IgE-sensitized basophils/mast cells, an allergen-binding fragment of such HuMAb, or an immune complex of such HuMAb or allergen-binding fragment with said allergen.

The invention also provides a human B-cell line established by CD40 cross-linking and Epstein-Barr virus (EBV) transformation, which established cell line produces a HuMAb which binds to an airborne allergen that provokes an allergic IgE response and/or which inhibits the allergen-induced degranulation of IgE-sensitized basophils/mast cells. Preferably, a HuMAb-producing clone is isolated.

The invention further provides purified and isolated DNA which encodes the heavy and/or light chains of a HuMAb or allergen-binding fragment according to the invention. Preferably, the isolated nucleic acid comprises:

a nucleotide sequence defined by base numbers 58-438 of SEQ ID NO. 1, 58-438 of SEQ ID NO. 5, 58-414 of SEQ ID NO. 9, 58-411 of SEQ ID NO. 13, or 58-426 of SEQ ID NO. 17, or by a CDR-encoding somatic variant of one of said sequences, and/or

a nucleotide sequence defined by base numbers 61-402 of SEQ ID NO. 3, 61-381 of SEQ ID NO. 7, 61-384 of SEQ ID NO. 11, 61-399 of SEQ ID NO. 15, or 67-399 of SEQ ID NO. 19, or by a CDR-encoding somatic variant of one of said sequences; or

a functional equivalent of one or both of said nucleotide sequences.

More preferably, the isolated nucleic acid comprises a nucleotide sequence selected from a sequence defined by base numbers 58-438 of SEQ ID NO. 1, 58-438 of SEQ ID NO. 5, 58-414 of SEQ ID NO. 9, 58-411 of SEQ ID NO. 13, or 58-426 of SEQ ID NO. 17, and/or a nucleotide sequence selected from a sequence defined by base numbers 61-402 of SEQ ID NO. 3, 61-381 of SEQ ID NO. 7, 61-384 of SEQ ID NO. 11, 61-399 of SEQ ID NO. 17, or 67-399 of SEQ ID NO. 19.

A second major aspect of the invention is not limited to airborne allergens and more broadly relates to making HuMAbs to any desired antigen. In particular, a process is provided for making a HuMAb to a desired antigen comprising the steps of:

establishing an immortalized human B-cell population from a patient having antibodies that bind to the desired antigen, said immortalization comprising infecting the B-cells with Epstein-Barr virus and crosslinking the CD40 of such B-cells;

culturing said immortalized B-cells;

isolating multiple clones from such immortalized B-cells, each of which clones secretes a human monoclonal antibody that binds to the desired antigen;

and using one or more of such clones to produce one or more human monoclonal antibody or an antigen-binding fragment of such human monoclonal antibody.

Preferably, nucleic acids derived from the clone which encodes said HuMAb or an antigen-binding fragment thereof are used to produce the desired HuMAb or antigen-binding fragment. The clone may also be hybridized with a

myeloma or a heteromyeloma, *e.g.*, either a human myeloma or a hybrid human/mouse heteromyeloma), cell to produce a hybridoma that proliferates in culture and produces the desired HuMAb.

DETAILED DESCRIPTION OF THE INVENTION

The invention may employ a B-cell population including resting B-cells which retain their surface-bound immunoglobulin and/or activated B-cells which secrete HuMAbs. If desired, the B-cell population may be sorted to select for activated B-cells or for resting B-cells, *e.g.*, as described below and in WO 91/09115.

A starting human B-cell population for use in accordance with the present invention, *e.g.*, in providing an anti-allergic agent, can be identified using means conventional in the art. A small amount of blood can be taken from patients and tested for Ig against the desired antigen, *e.g.*, an airborne allergen by ELISA, radioimmunoprecipitation assay, western blotting, etc. Patients who react positively in such a test or in a conventional skin test and/or a RAST® or a CAPS® test (available from Pharmacia, Sweden) with an antigen, *e.g.*, an airborne allergen of interest, are sources of B-cells that can be used to immortalize and isolate a clone producing the desired HuMAb as described further below. Alternatively, B-cells from a patient who tests negatively in such tests, but nevertheless has serum that tests positive for an antibody to the antigen, *e.g.*, by ELISA, radioimmunoprecipitation assay, western blotting, etc., may also be employed as a starting B-cell population. A larger sample can then be taken from each patient identified by the above procedures.

Suitable sources of B-cells from a selected patient include peripheral blood, tonsils, adenoid tissue, spleen (if removed for a medical necessity), mucosa-infiltrating cells, cells from biological fluids (*e.g.*, bronchioalveolar lavage or nasal lavage fluid), or any other source of B-cells from the body. Typically, peripheral blood is employed as the B-cell source.

The blood is first treated to separate the peripheral blood lymphocytes (PBLs) from the red blood cells and platelets by means conventional in the art. For example, the peripheral blood may be diluted with an appropriate isotonic medium, *e.g.*, RPMI 1640 medium (cat. 041-01870 M. Gibco, USA). The diluted blood is loaded onto a suitable separation medium such as FICOLL® (available from Pharmacia, Sweden). After centrifugation, the PBLs may be aspirated

from the interface between the serum and the FICOLL. The purified PBLs may be frozen in liquid nitrogen for later use. The plasma is then analyzed by conventional techniques such as ELISA, radioimmunoprecipitation assay, western blotting, etc., to confirm the presence of significant amounts of the desired antibody (*e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA, IgD, IgM and/or IgE antibody) against the antigen of interest, *e.g.*, an airborne allergen such as the 17 kD allergen of the pollen extract Bet vI.

The purified PBLs may be used directly or may be further enriched and/or sorted as discussed below. For example, T-cells may be removed by rosetting with sheep erythrocytes treated with 2-aminoethylisothiouronium bromide. Further selection for an antigen-specific B-cell subpopulation can be carried out by a variety of techniques including panning, immunoadsorbent affinity chromatography, fluorescent-activated cell sorting (FACS), etc. These techniques are described for example by Casali et al., *Science*, *234*: 476-479 (1986); in U.S. patent No. 4,325,706; and by Mage, Hubbard et al., Parks et al., and Haegert, in *Meth. Enzymol.*, *108*: 118-124, 139-147, 197-241 and 386-392 (1984), respectively. The PBLs may also be treated with magnetic beads whose surface is coated with a material to selectively sort the desired B-cells. Such beads may be coated, *e.g.*, with anti-Ig isotype for the desired Ig to be separated, anti-surface antigen to select for non-naive B-cells, or a purified antigen or allergen.

The resulting enriched and/or sorted B-cell population is then subjected to the B-cell immortalization process described in WO 91/09115. Briefly, the B-cells are transformed with EBV and their CD40 molecules are crosslinked. Reference is made to WO 91/09115 for the variations that may be employed in this activation/immortalization process.

The treated B-cell population may be washed by an appropriate isotonic medium (*e.g.*, with RPMI 1640), pelleted and then resuspended in medium. The cells are then transformed with EBV by the addition of a suitable EBV strain, preferably a strain such as the one released by the B95.8 cell line available from the ATCC (ATCC CRL 1612). The amount of EBV used may vary depending on the strain of the virus and the number of B-cells to be transformed. For example, with a sample containing $14x10^6$ non-sorted PBLs, 200 µl of a concentrated EBV (strain B95.8) suspension is typically used. Incubation with the virus is typically carried out for about 1 to 24 hours, preferably for about 2 hours, at 37°C; but other conditions may be employed, if desired.

The EBV-infected cells are preferably washed and resuspended in an appropriate medium such as LINOLEA® 15% Fetal Calf Serum (FCS) [Yssel et al., *J. Immunol. Methods*, *72*: 219-24 (1984)] or DMEM-F12 (cat. 041-01331M, Life Technologies, Paisley, Scotland) 15% Horse Serum. The concentration of the PBLs in the suspension may vary depending, for example, on whether a sorting step for antigen-specific B-cells was performed as described above. Lower concentrations can be employed when PBLs have been enriched in the desired B-cells. Typical concentrations for non-selected PBLs are from about $1x10^3$ to about $5x10^4$ cells/ml. If the B-cell population is first sorted as discussed above, the concentration may be decreased depending upon the efficiency of the sorting, *e.g.*, up to about $1x10^2$ cells/ml.

An agent capable of crosslinking CD40 antigen is added to the suspended cells. The crosslinking agent may include T-cells, other transfected cells expressing CD40 ligand or membranes therefrom. Other suitable agents are described in WO 91/09115. Preferably, the agent is an immobilized monoclonal antibody specific for the CD40 antigen, *e.g.*, immobilized on irradiated fibroblasts expressing the human or murine Fc-gamma receptor (ATCC CRL 10680).

The monoclonal antibody to CD40 can be any which binds to the CD40 marker on the B-cells of the suspension and also to the Fc-gamma receptor of the L-cells. Preferably, the monoclonal antibody is selected from MAb 89 and G28-5. These antibodies are described by Valle et al., *Eur. J. Immunol.*, *19*: 1463-1467 (1989), and by Ledbetter et al., *J. Immunol.*, *138*: 788-794 (1987), respectively. The hybridoma corresponding to MAb 89 has been deposited with the European Collection of Animal Cell Cultures, PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire, SP4 0JG, U.K. under accession No. 89091401.

Typically, the PBLs, the CD40 antibody and the L-cells are simply mixed together in appropriate amounts. The CD40 antibody may be present in a concentration of from about 0.01 µg/ml to about 50 µg/ml, preferably, from about 0.1 µg/ml to about 5 µg/ml, more preferably about 0.5 µg/ml.

The treated cell suspension is divided among an appropriate number of wells of a tissue microplate to provide a suitable cell concentration for amplification and screening. If enriched suspensions are employed as the result of an antigen-selective screening as discussed above, fewer cells per well may be used.

Typically, the initial culture phase takes 10-20 days in the case of non-selected PBLs and 5 days or even less in the case of an antigen-specific enriched B-cell population, which would allow an earlier detection of specific antibodies. During this phase, fresh medium is added as necessary. The duration of this initial culture phase is adjusted to allow detection of the antigen-specific B-cells, while preventing them from being overgrown by non-specific B-cells. A sample of supernatant from each well is screened by an appropriate assay for the desired HuMAb positive characteristics, *e.g.*, by ELISA, western blotting, radioimmunoprecipitation assay, etc.

In a preferred screening method, supernatants are contacted with a labeled protein (*e.g.*, radiolabeled with ^{125}I) and polyclonal or monoclonal anti-human Ig coupled to a substrate or insoluble support. The anti-human Ig can be a mixture of isotypes (*i.e.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA, IgD, IgE and/or IgM) or an individual isotype (*e.g.*, IgG_4). Alternatively, if one is screening for an IgG HuMAb, the supernatants can be contacted with a labeled protein and protein G coupled to a substrate or insoluble support. The presence of the desired HuMAb is determined by detecting labeled protein in the immunoprecipitated product. The immunoprecipitation screens (with anti-human Ig and with protein G) may be employed serially.]

Cell lines which test positive for the desired HuMAb characteristics are subcloned (3-10 cells/well) and cloned (0.5-1.0 cells/well) by techniques well-known in the art, *e.g.*, by culturing in limiting dilution conditions for 7-20 days in additional medium as needed, and sorted, *e.g.*, by FACS sorting or by micromanipulation. Supernatants of the clones are screened by the procedures described above.

Positive clones are expanded in a larger volume and amplified by conventional incubation.

HuMAbs can be purified from the supernatant of the amplified clones by conventional immunoglobulin purification methodology. For example, the HuMAb may be precipitated with solid ammonium sulfate, reconstituted in sterile water, and dialyzed extensively against a buffer such as phosphate-buffered saline (PBS). The dialysate may then be applied to an immunoaffinity column, *e.g.*, a column having anti-human Ig or Protein G covalently coupled to Sepharose. After washing, the desired HuMAb may be eluted from the column by any appropriate eluent, *e.g.*, acidic buffer, chaotrophic agents, etc. [for example, see Current Protocols in Immunology. edited by John E. Coligan et al., John Wiley and Sons, New York].

By the term "human monoclonal antibody" as used herein, we mean to include HuMAbs that are isolated from human B-cells as discussed above (*e.g.*, whether the antibody is prepared by culturing the immortalized human B-cells or recombinantly from human B-cell cDNAs encoding such a HuMAb) and HuMAbs that are made by recombining the variable portions of a HuMAb of the present invention of one isotype (*e.g.*, an IgG_4) with the constant region of a human antibody of another isotype (*e.g.*, a human IgG_1, IgG_2, IgG_3, IgG_4, IgA, IgD, IgM or IgE). Recombinant methods for making these HuMAbs are described below.

By the terms "antigen-binding fragment" or "allergen-binding fragment", we mean an antibody fragment such as an Fab, $(Fab)_2$, Fv, single-chain binding protein, or any other binding polypeptide which contains one or more complementarity-determining regions (CDRs) of the variable region of a light or heavy chain of a HuMAb of the present invention. These fragments can be prepared by well-known methods. For example, fragments can be made from the full-length HuMAb protein, *e.g.*, by papain or pepsin cleavage, or by chemical oxidation, followed by separation of the resulting fragments. Alternatively, recombinant DNA technology may be used. For example, cDNA encoding the variable regions of both heavy and light chains may be engineered to produce the Fv portion of the HuMAb of the invention. See, for example, the methodology of U.S. patent No. 4,642,334 which may be employed.

By the terms "fragment" or "subsequence" of a HuMAb of the present invention, we mean an antibody fragment such as an Fab, $F(ab')_2$, Fv, single-chain binding protein, or any other binding polypeptide which contains one or more CDRs of the variable region of a light or heavy chain of a HuMAb of the present invention (*e.g.*, an Fab, Fv, CDR, etc. of a HuMAb in accordance with the present invention either alone or linked to any desired molecule which can alter its biological activity, *e.g.*, a receptor or ligand, an enzyme, a toxin, a carrier, etc.). These fragments can be prepared by well-known methods. For example, fragments can be made from the full-length HuMAb protein, *e.g.*, by cleavage with papain or pepsin, or by chemical oxidation, followed by separation of the resulting fragments. Alternatively, recombinant DNA technology may be used. For example, cDNAs encoding the variable regions of the heavy and the light chains may be engineered to produce the Fv portion of the HuMAb of the invention. See, for example, the methodology of U.S. patent No. 4,642,334.

By the terms "CDR somatic variant" and "CDR-encoding somatic variant" as used herein we mean a nucleic acid or amino acid sequence corresponding to any one of SEQ ID NOS. 1 through 20 or a subsequence of such a sequence containing at least one CDR or CDR-encoding region thereof, but having at least one replacement mutation, addition and/or deletion in one or more of the CDRs or CDR-encoding regions of the sequence or subsequence, such that a human monoclonal antibody including said at least one replacement mutation, addition and/or deletion has a binding affinity for the antigen of 10^8 M^{-1} or greater, preferably 10^9 M^{-1} or greater, preferably 10^{10} M^{-1} or greater.

By the term "affinity" as used herein, we mean the measure of the binding strength between an antigenic determinant and an antigen binding site of a human monoclonal antibody of the invention or a fragment thereof as measured by the affinity constant (Ka), *e.g.*, determined by the method described below.

By the term "V_H segment" as used herein, we mean the variable region of the heavy chain of a human monoclonal antibody of the invention.

By the term "V_L segment" as used herein, we mean the variable region of the light chain of a human monoclonal antibody of the invention.

By the term "Fv fragment" as used herein, we mean an antigen-binding fragment of an antibody that contains the variable regions of the heavy (V_H) and light (V_L) chains. Those V_H and V_L regions can be linked to form a single-chain Fv (scFv).

By the term "Fab fragment" as used herein, we mean the antigen-binding fragment resulting from the digestion with papain of a human monoclonal antibody of the invention.

By the term "$F(ab')_2$ fragment" as used herein, we mean the antigen-binding fragment resulting from the digestion with pepsin of a human monoclonal antibody of the invention.

By the term "functional equivalent" as used herein, we mean a nucleic acid sequence that encodes the same amino acid sequence as the identified nucleic acid sequence.

By the term "activated B cell" and "activation" as used herein we mean a human B cell that has been CD40-crosslinked and EBV-transformed and expresses and secretes human antibodies.

By the term "immune complex" as used herein, we mean a complex of an airborne allergen and its corresponding HuMAb or HuMAbs or allergen-binding fragment or fragments in accordance with the present invention. These immune complexes may be prepared by conventional techniques. For example, a

predetermined weight ratio such as a one to five molar ratio of purified or recombinant allergen (or a fragment thereof) and of HuMAb(s) binding to it may be incubated for 2 hours at 37°C, and stored for later use at -80°C.

The B-cell clones of the invention may be used in conventional DNA recombinant methods to produce the HuMAbs of the invention or fragments thereof. For example, RNA from the B-cell clones may be isolated according to the single-step method described by Chomczynski et al., *Anal. Biochem.*, *162*: 156-9 (1987). Briefly, about 10^7 cells are lysed in guanidinium thiocyanate denaturing solution. After acidification of the mixture with 2M sodium acetate, pH 4, RNA is extracted with phenol and chloroform/isoamyl alcohol. RNA is then precipitated with isopropanol, and the RNA pellet is redissolved in denaturing solution, reprecipitated with isopropanol, and washed with 75% ethanol.

cDNA is obtained by reverse transcription, *e.g.*, using the Superscript Reverse Transcriptase Kit (cat. 20898 BRL, Gaithersburg, MD, USA), and oligo $dT_{12\text{-}18}$ primers (Cat. 27.7858-01, Pharmacia, Uppsala, Sweden). The cDNA is then used as template in the polymerase chain reaction (PCR). The primers may be designed to include restriction sites, to allow for the directional cloning of the PCR products. For the heavy chain, primers specific for the leader sequence of all the different human V_H families are used individually in conjunction with primers located at the 3'-end of the constant region corresponding to the isotype previously determined by isotyping the HuMAb by ELISA or other appropriate method (*e.g.*, radioimmunoprecipitation assay, etc.). The light chain is amplified with individual combinations of primers corresponding to the 3'-end of the kappa or lambda chain in conjunction with a series of primers annealing to the leader sequence of the V kappa or the V lambda genes. Thus, full-length heavy and light chains starting at the initiation codon in the leader sequence and ending at the stop codon may be generated.

After appropriate restriction cleavage, both full-length heavy and light chains can then be cloned in any appropriate expression vector designed to be compatible with the restricted PCR products. Appropriate vectors include for example baculovirus vectors and plasmids compatible with CHO cells or other host cells. Examples of suitable vectors and hosts are described in the review "Engineered antibody molecules" in *Immunol. Reviews*, *130* (1992). Heavy and light chains can be cloned individually in distinct vectors, or in tandem in one vector. The recombinant plasmids or viral vectors may be cloned in bacteria, and a few clones may be sequenced on both strands to check for the

absence of alteration of the insert. One clone each for the heavy and the light chain, or one clone containing both chains may then be selected for expression in the appropriate host cells. Depending on the vector used, it will be introduced in appropriate prokaryotic or eukaryotic cells either by transfection or infection. After cloning the cells expressing the recombinant HuMAb, supernatant fluid from the cultured cells is collected, and the HuMAb therein can be purified, *e.g.* by immunoaffinity, HPLC or any other appropriate methods.

The full-length PCR product for the heavy chain can be modified for example to replace the original heavy chain constant region by another one, so substituting, *e.g.*, a human IgG_4 isotype to a human IgG_1, IgG_2, IgG_3, IgA, IgD, IgM or IgE isotype. This can be accomplished through a second PCR using as 5'-primer the V_H leader specific primer, and as 3'-primer a primer annealing to the 3'-end of the V_H region (*e.g.*, IgG_4) and including a tail corresponding to the 5'-end of the desired constant region (*e.g.*, IgG_1, IgG_2, etc.) cloned in an appropriate plasmid. After amplification, the heavy chain coding DNA generated may be digested with appropriate enzymes and ligated into the new expression vector (containing the sequence of the desired heavy chain, *e.g.*, IgG_1). This will allow the production of a recombinant HuMAb with the variable region of the originally isolated HuMAb (*e.g.*, the variable region from an IgG_4 HuMAb) and the constant region of a different human isotype (*e.g.*, the constant region of a human IgG_1, IgG_2, etc.). This type of recombined HuMAb will have the characteristic binding of the IgG_4 HuMAb, but will be able to display the effector functions normally associated with the human IgG_1, IgG_2, etc. isotype.

Hybridomas may also be made with the B-cells of the invention by techniques conventional in the art. For example, the B-cells of the invention may be fused with an appropriate myeloma cell or with a heterohybridoma cell to increase or stabilize the immunoglobulin secretion. See for example Kudo et al., *J. Immunol. Methods*, *145*: 119-125 (1991); and Zanella et al., *J. Immunol. Methods*, *156*: 205-215 (1992).

The anti-allergic agent of the present invention may be used therapeutically to treat existing symptoms associated with the antigen of interest, *e.g.*, an allergic reaction, or prophylactically to prevent or inhibit the occurrence of such symptoms. The anti-allergic agent may be used alone or in combination with at least one other anti-allergic agent to form a cocktail. For example, such a cocktail may include two or more of the HuMAbs of the invention, each of which binds to one or more epitopes on an antigen of interest, or to different molecular components of an allergenic extract. When

such a cocktail is employed, the proportions of the various HuMAbs may vary, depending for example upon their binding characteristics and/or ability to inhibit mast cell/basophil degranulation.

The HuMAbs or fragments of the invention may be administered as an immune complex with the sensitizing antigen, *e.g.*, a complex of an anti-Bet vI or anti-Der pI HuMAb with the Bet vI or Der pI antigen. These immune complexes can be prepared in accordance with the procedures described by Jacquemin et al., *Lancet, 335*: 1468-9 (1990); and Machiels et al., *J. Clin. Invest., 85*: 1024-35 (1990); and EP 287361.

The HuMAbs, fragments and/or immune complexes are preferably administered in the form of a pharmaceutical composition containing a therapeutically or prophylactically effective amount of at least one such HuMAb, fragment or immune complex in combination with a pharmaceutically acceptable carrier. Any appropriate carrier may be employed, *i.e.*, a compatible, non-toxic material suitable for delivery of the HuMAb or immune complex in the desired dosage form, *e.g.*, oral, parenteral (subcutaneous, intramuscular or intravenous), or topical dosage forms. Suitable carriers include sterile water, sterile buffered water, sterile saline, etc. Special pharmaceutical compositions to insure a sustained release of the HuMAb, of the fragment and/or of the immune-complex may also be employed.

The concentration of the HuMAb, fragment or immune complex of the invention in the pharmaceutical compositions may vary, *e.g.*, from about 0.1 µg/ml to about 1 mg/ml, preferably from about 1 µg/ml to about 100 µg/ml. The concentration used will depend upon the number of HuMAbs, fragments and/or immune complexes employed in the composition and the dosage form selected, and the dose will be adjusted in a conventional manner by the skilled artisan to levels appropriate to achieve the desired result *in vivo*.

As mentioned above, the anti-allergic agent of the invention can be used prophylactically or therapeutically. Thus, the agent may be administered before the onset of symptoms of the allergic reaction or after the symptoms have appeared. When used prophylactically for seasonal allergy, the anti-allergic agent of the invention may be administered before the start of the allergic season for the particular allergen of interest, preferably from about 4 months to about 2 weeks before the expected start of the allergen's season. When used therapeutically, the anti-allergic agent is administered on an appropriate schedule to relieve the allergic symptoms. Typically the anti-allergic agent should be administrated so as to protect the patient continuously during the

allergenic season. This may be achieved either by daily topical (*e.g.*, intranasal) administration, by parenteral injection every 2 to 3 weeks, or by a few injections of a slow releasing formula mentioned above. The anti-allergic agent of the invention will be administered in a dose effective to alleviate the allergic effect. Amounts effective for this purpose will depend upon many factors, *e.g.*, the sensitivity of the patient or the load of allergen to which the patient has been or will be exposed. In case of allergy to perennial allergens such as house-dust mites, a primary prevention could be attempted by the administration of the anti-allergic agent before the onset of the symptoms in a population known to be at risk for the development of florid hypersensitivity, *e.g.*, children with high serum IgE levels who have allergic parents.

The anti-allergic agent or a HuMAb or fragment thereof produced in accordance with the processes of the invention may be administered in dosages of from about 0.001 µg/kg to about 1 mg/kg, *e.g.*, about 0.01 µg/kg to about 1 µg/kg, preferably, from about 0.01 µg/kg to about 0.1 µg/kg. The proper dosage of an agent of the invention for a particular situation will be determined by using common practices in the art. Generally, treatment may be initiated with smaller dosages that are less than the optimum dose of the agent. Thereafter, the dosage may be increased by small amounts until the optimum effect under the circumstances is reached. The amount and frequency of administration of the agents of the invention will be regulated according to the judgment of the attending clinician considering such factors as age, condition and size of the patient as well as severity of the symptom being treated.

The HuMAbs and fragments of the invention may also be used for diagnostic purposes in the same manner as other antibodies and fragments thereof are currently used in the art. For example, the HuMAbs and fragments of the invention can be used in assays for the antigen, *e.g.*, airborne allergens, to which they bind. The HuMAbs and fragments may be used labeled (*e.g.*, with a radioisotope, fluorescent group, enzyme or other appropriate ligand) or unlabeled as may be conventional in the art for the particular assay of interest (*e.g.*, a sandwich assay with a second labeled antibody). The HuMAbs and fragments may be used in agglutination assays, enzyme immunoassays, etc. They could for example be used to calibrate a dosage of antigen-specific IgG in the serum or in any other biological fluid. Thus, the labeled or unlabeled forms of the HuMAbs and fragments of the invention may be employed as elements of kits for purposes of performing the desired assay. If the presence in the serum from patients of an idiotype expressed by one HuMAb is found to correlate with

clinical evolution of the disease, such a HuMAb could be used to generate an anti-idiotypic murine MAb. Detection of this idiotype in the sera of patients could be used for its prognostic and/or diagnostic value.

The invention disclosed herein is illustrated by the following examples, which should not be construed to limit the scope of the disclosure. The selected vectors and hosts, the concentration of reagents, the temperatures, and the values of other variables are only to exemplify the application of the present invention and are not to be considered limitations thereof. Alternative methods within the scope of the invention will be apparent to those skilled in the art.

Unless otherwise indicated, percentages for solids in solid mixtures, liquids in liquids, and solids in liquids are expressed on a weight/weight, volume/volume and weight/volume basis, respectively.

EXAMPLES

General Methods and Reagents

Protein G (from Group C *Streptococcus sp.*) coupled to Sepharose 4B®, and anti-human Ig polyvalent immunoserum (IgG fraction) coupled to agarose were obtained from Sigma Chemical Co. (St. Louis, MO).

Tissue culture media, FCS, L-glutamine, HEPES buffer and PBS were from GIBCO (Paisley, UK). Bovine serum albumin (BSA) was from Sigma Chemical Co. and gentamycin from Schering-Plough (Levallois-Perret, France).

The transformant EBV strain B 95.8 was produced by culturing transformed marmoset leukocytes (ATCC, CRL 1612) essentially as described by Miller and Lipman [*Proc. Natl. Acad. Sci. USA*, *70*: 190 (1973)].

GENERATION OF HUMAN CLONES SECRETING ANTIBODIES WHICH SELECTIVELY BIND TO BIRCHPOLLEN ALLERGENS

Standard Immunoprecipitation Protocol with Protein G

In order to identify the presence of human antibodies (IgG) against the antigen of interest in biological samples (sera or plasma) or culture supernatants, an immunoprecipitation assay was carried out using radio-labeled antigen and protein G-Sepharose as precipitating reagent. This assay allowed the identification of the four sub-classes of human IgG (IgG_1, IgG_2, IgG_3 and IgG_4). Typically, 50 µl of sera/plasma from patients or 50 µl of culture supernatants (both used at appropriate dilution in PBS, 1% BSA) were

incubated for 45 minutes at room temperature with 50 µl (50 pM) of labeled antigen (diluted in PBS 1% BSA) in a well of a 96-well filtration microplate MultiScreen-HA® (Millipore Co., Bedford, MA) whose bottom was composed of a nitrocellulose membrane (HATF 0.45 µm). Each sample was tested in duplicate. Then, 50 µl of a dilution of protein G coupled to Sepharose 4B® (Sigma Chemical Co.) (15 ml beads diluted to 50 ml in PBS 1% BSA) were added to each well and incubated for 45 minutes at room temperature. The wells were then washed three times with PBS using a vacuum manifold (Millipore Co.), and the dried membranes were collected into appropriate vials using a special collector system (Millipore Co.). The radioactivity corresponding to the complexes labeled antigen/HuMAb was counted in a Wizard gamma-counter (Wallac Oy, Turku, Finland). Positive and negative controls were performed for each plate respectively using the serum of a patient allergic to Bet vI and a HuMAb of unrelated specificity. Specificity of the labeled antigen precipitation obtained with the tested samples was further confirmed through its inhibition by preincubation of these samples with a 100-fold excess of unlabeled antigen.

Derived Immunoprecipitation Protocols

The above standard immunoprecipitation assay has been modified in order to identify human antibodies of isotypes other than IgG or to better identify the IgG subclass and light chain of such antibodies contained in patient sera or culture supernatants. The principle of the different assays was the same as in the standard protocol, but the precipitating reagent, *e.g.* protein G-Sepharose, was changed. The following reagents were used: agarose beads coupled with goat polyspecific antibodies to human IgM, IgG and IgA (Sigma Chemical Co.); Affi-Gel 10® gel (Bio-Rad Laboratories, Richmond, CA) coupled, according to the manufacturer's instructions, with specific goat antibodies to human IgA heavy chain, human lambda light chain or human kappa light chain (Sigma Chemical Co.) or coupled with mouse monoclonal antibodies to human IgG_1, human IgG_2, human IgG_3 or human IgG_4 heavy chain (Calbiochem Co., La Jolla, CA).

These protocols may be employed with other antigens of interest by substituting an appropriately labeled antigen.

Selection of patients and immortalization of the human B lymphocytes

Clinical selection of adult patients was first based on allergic symptoms (rhinitis, conjunctivitis, asthma) occurring early in the spring. Involvement of allergy to birchpollen in causing the symptoms was then confirmed by the PRIST® measurement of the total IgE in their sera, and by the presence of IgE antibodies specific for birchpollen as demonstrated by skin test reactivity and measurement by CAPS® (PHARMACIA, SWEDEN). Ten allergic patients were selected according to these criteria.

Blood (50 ml) from each selected patient was collected in a tube containing EDTA as anticoagulant, then diluted 1:1 with RPMI 1640 medium. This diluted blood was loaded onto Ficoll/Hypaque (Pharmacia, Uppsala Sweden) and centrifuged according to manufacturer's instructions (30 minutes, 600 g.). Peripheral blood mononuclear cells (PBMNC) were harvested at the interface. The upper phase was recovered, aliquoted, and then frozen at -20°C. This plasma was diluted 1:1 in RPMI 1640 and used later for ELISA and Western blot screening. The cells were washed four times in PBS. Purified PBMNC were frozen at a concentration of $1x10^6$ PBLs/ml and kept in liquid nitrogen. The PBMNC from one patient (hereinafter 'Patient A') were selected for transformation on the basis of the presence of large amounts of IgG against birchpollen proteins, and of both IgG and IgE antibodies against the 17 kD allergen of the pollen extract (Bet vI).

$14x10^6$ PBMNC from Patient A were thawed and washed once in RPMI 1640. Viability, as estimated by Blue Trypan exclusion, was above 90%. The cells were pelleted, resuspended in 1 ml of RPMI 1640. 200 µl of concentrated EBV suspension (strain B95.8, produced by culturing transformed marmosets leukocytes, essentially as described by Miller and Lipman [*Proc. Natl. Acad. Sci. USA*, *70*: 190 (1973)]) were added, and this mixture was incubated for 2 hours at 37°C, 5% CO_2 in humidified incubator. The cells were then washed twice in RPMI 1640 complete medium and the pellet was resuspended at $5x10^4$ cells/ml in Linolea complete medium which consisted of Yssel's modified Iscove's medium [Yssel *et al.*, *J. Immunol. Methods 72*: 219 (1984)] supplemented with 15% heat-inactivated FCS, 2 mM L-glutamine, 50 µg/ml gentamycin. Irradiated L cells (7,000 rads) stably expressing the Fc-gamma receptor II for human IgG (CDw32) were added at a final concentration of $5x10^4$ cells/ml, together with the murine monoclonal anti-human CD40 antibody MAb 89 used at a final concentration of 0.5 µg/ml. Aliquots (100 µl each) of this

mixture were then distributed in each well of Microwells 96U round-bottom plates (Nunc ref. 1-63320, Denmark), and the plates were incubated at 37°C, 5% CO_2 humidified atmosphere. After 5 days of incubation, 125 µl of fresh Linolea complete medium containing 0.5 µg/ml anti-CD40 MAb 89 were added to each well.

At day 12, the supernatants were first screened by indirect ELISA for the presence of anti-birchpollen antibodies. 100 µl was collected from each well and replaced by the same volume of fresh Linolea complete medium. At day 19, the same screening was repeated on every well to confirm the first assay, and to identify lines which become lately positive.

A total of 51 positive lines from Patient A were thus identified, all of which were transferred in 2 ml into 24 wells multidish (Nunc, ref. 1-43982, Denmark) for amplification. After expansion, $5x10^6$ cells from each line were frozen and kept in liquid nitrogen. Twenty-five lines from Patient A were kept in culture for further expansion, characterization of antigenic specificity, and cloning. One cell line (G5F2) was found by Western blotting to secrete antibody binding to Bet vI. This line as well as four other ones (G17E7, G27E9, G11A9, G28C10) were found to immunoprecipitate purified radiolabeled Bet vI (see below). These five cell lines were cloned by limiting dilution in Microtest III Tissue Culture Plates (Falcon, New Jersey, USA) flat-bottom microwells. One clone was derived from each of those five lines, *i.e.*, clones G5F2A4, G17E7A1, G27E9B1, G11A9F3 and G28C10C10. Each clone was maintained in culture for more than 4 months and showed stable proliferation and secretion.

Confirmation of the monoclonality by ELISA and by PCR

Supernatant fluids from each clone were tested in ELISA specific for human IgM, IgG, or IgA, for human IgG_1, IgG_2, IgG_3, or IgG_4 subclasses, and for kappa and lambda light chains. Each clone produced only IgG of a single subclass, and only one class of light chain (see Table 1 below). Furthermore, PCR amplification of cDNA derived from the clones with primers specific for the different families of heavy chain variable region genes and light chain variable region genes established that only one heavy chain and one light chain was productively expressed by each clone (see PCR protocol below, and results in Table 1 below).

TABLE 1

HUMABS SPECIFIC TO BET VI

antibody	G5F2A4	G11A9F3	G17E7A1	G2E9B1	G28C10C10
Indirect ELISA	+	+	+	+	+
Capture ELISA	–	+	+	+	+
Western Blot	+	–	–	–	–
Immunopre-cipitation	+	+	+	+	+
Heavy chain Light chain	IgG_1 kappa	IgG_4 kappa	IgG_4 lambda	IgG_2 kappa	IgG_4 kappa
Affinity (K_a)	(1.4 – 7.9) x 10^{-9}M	(1.3 – 9.8) x 10^{-9}M	(1.6 – 1.8) x 10^{-10}M	4x 10^{-9} – 2.5 x 10^{-10}M	(1.5 – 3) x 10^{-9}M

N.D.: NOT DETERMINED

Nature and characterization of the pollen extracts

All the screening work with the birchpollen was carried out using natural antigen, to ensure the clinical relevance of the allergens studied (*i.e.*, to ensure that the allergens had native conformation and glycosylation). We used a pollen extract from *Betula pendula* commercialized for the bronchial challenge of patients (BIRCH POLLEN INHALATION, Smith Kline Beecham Pharmaceuticals, London, U.K.). To extend the study of the specificity of our HuMAbs, we also extracted the proteins from the pollen of *Betula alba* (purchased from Sigma ref. p-6770 St. Louis, MI). Aqueous extraction was followed by precipitation with $(NH_4)_2SO_4$ essentially as described by Vallier *et al.*, *Clin. Exp. Allergy*, *22*: 774 (1992).

The total protein concentrations of the birchpollen extracts were estimated by the absorption at 280 nm of the solution, and the resulting estimate was then confirmed using the Bio-Rad DC Protein Assay kit (Bio-Rad, Richmond, CA). The concentration of protein of the *Betula pendula* extract was found to be 100 µg/ml. To make an estimation of the proportion of Bet vI in the extract, we ran a 12% acrylamide SDS-PAGE (sodium dodecylsulfate polyacrylamide gel electrophoresis) under reducing conditions. Several dilutions of the pollen extract and known amounts of low-molecular-weight protein standards were loaded on a gel. After completion of the run, the gel was silver-stained, and a visual estimation of the concentration of Bet vI was made by the comparison with the molecular weight standards. It was estimated that the Bet vI concentration was about 5 µg/ml in the pollen extract, and that it represented about 10 to 20% of the proteins in the extract.

Indirect ELISA for the detection of anti-birchpollen HuMAbs

Several dilutions of the birchpollen extract were tested, and then the extract was diluted 1:200 in coating buffer (Carbonate Buffer, pH 9.6, Na_2CO_3, 15 mM, $NaHCO_3$, 35 mM). This diluted solution was coated onto the wells of 96-microwell ELISA plates (Immunoplate Maxisorp F96 certified, NUNC, Denmark) overnight at room temperature. The plates were then washed manually once with washing buffer: Phosphate Buffer Saline, Tween 20 (cat. T21-0309 Technicon Diagnostics, USA) 0.05%. Sera were diluted 1 to 100 in dilution buffer, TBS-B-T buffer: Tris 20 mM, NaCl 150 mM, BSA, cat A-9647 Sigma St Louis MI, USA) 1%, Tween 20 0.05%, and 100 µl of diluted sera were added to each of the wells. Supernatant fluids from the lines and clones were used undiluted. Sera or supernatants were incubated for 2 hours at 37°C, then washed once as described above. 100 µl aliquots of rabbit anti-human IgG coupled to alkaline phosphatase (cat D336 Dakopatts, Denmark) were added to each well at a 1:2000 dilution in TBS-B-T buffer. The antibodies were incubated for 1 hour at 37°C. Wells were then washed three times with washing buffer. 100 µl of alkaline phosphatase substrate (disodium 4-nitrophenyl-phosphate, PNPP, 1 mg/ml of 10% diethanolamine solution, pH 9.8) was then added to each well, and the colorimetric reaction was read at 405/490 nm. Background was determined as the optical density (O.D.) of control wells without human antibodies.

Capture ELISA for the detection of anti-birchpollen HuMAbs

A panel of murine monoclonal antibodies against natural Bet vI were generated after immunization of BALB/c mice with the total birchpollen extract. Among the antibodies which were able to bind the 17 kD protein from the birchpollen extract as shown by immunoprecipitation (see below), some, like MAb 202E8, have been found to present Bet vI to the HuMAbs in a capture ELISA. Such murine anti-Bet vI antibodies purified from ascites by ion-exchange chromatography on DEAE column are diluted at 2 µg/ml in carbonate buffer (see above) and coated onto the wells of 96-microwell ELISA plates overnight at room temperature. Plates are then washed manually once with washing buffer. 100 µl of birchpollen extract diluted 1/100 in TBS-B-T buffer is added to each well, except in control wells, where only TBS-B-T buffer is added. After 2 hour of incubation at 37°C, the plates are washed once. 100 µl of supernatants of human clones cultures are added to each wells, and are incubated for 2 hours at 37°C. After washing, 100 µl aliquots of rabbit anti-human IgG coupled to alkaline phosphatase (cat D336 Dakopatts, Denmark) are added to each well at a 1:2000 dilution in TBS-B-T buffer. The antibodies are incubated for 1 hour at 37°C. After 3 washes, 100 µl of alkaline phosphatase substrate (PNPP) is then added to each well, and the colorimetric reaction is read at 405/490 nm. Background is determined as the optical density (O.D.) of control wells without addition of birchpollen extract.

ELISA for the detection of cross-reactivity of the anti-birchpollen HuMAbs with other pollens

Pollen extracts of Alder, Oak, Hazel, Willow, Plane, Beech, Em, and extracts from potato, carrot, apple and celery, commercialized for skin test of patients (BIRCH POLLEN INHALATION, Smith Kline Beecham Pharmaceuticals, London, U.K.) were diluted 1:200 in carbonate buffer (see above) and coated onto the wells of 96-microwell ELISA plates (Immunoplate Maxisorp F96 certified, NUNC, Denmark) overnight at room temperature. The plates were then washed manually once with washing buffer. 100 µl of purified HuMAbs were added and incubated for 2 hours at 37°C, then washed once as described above. 100 µl aliquots of rabbit anti-human IgG coupled to alkaline phosphatase (cat D336 Dakopatts, Denmark) were added to each well at a 1:2000 dilution in TBS-B-T buffer. The antibodies were incubated for 1 hour at 37°C. Wells were then washed three times with washing buffer. 100 µl of alkaline phosphatase substrate (disodium 4-nitrophenyl-phosphate, 1 mg/ml of

10% diethanolamine solution, pH 9.8) was then added to each well, and the colorimetric reaction was read at 405/490 nm. Background was determined as the optical density (O.D.) of control wells without human antibodies. Results are shown in Table 2 below: the O.D. specific for each allergen was expressed as the percentage of the O.D. measured with Bet vI. It is seen that the HuMAbs cross-react with the pollen of *Betula alba*, as well as the pollen of Alder. All the other allergens gave an O.D. below 20% of the O.D. observed with Bet vI.

TABLE 2

CROSS-REACTIVITY OF ANTI-BET VI HUMABS WITH ALLERGENS FROM DIFFERENT SOURCES

	G5F2A4	G11A9F3	G17E7A1	G27E9B1	G28C10C10
Bet VI	>50%	>50%	>50%	>50%	>50%
Bet AI	>50%	>50%	>50%	>50%	>50%
Alder	>50%	>50%	>50%	>50%	<50%
Oak	<50%	<50%	<50%	<50%	<50%
Hazel	<50%	<50%	<50%	<50%	<50%

The O.D. observed with the different allergens is expressed as a percent of the O.D. measured with Bet VI.

Western Blot assay

15% SDS-acrylamide gels (14x12x0.75 cm) were used with O'Farrell/Laemmli Tris-Glycine-SDS buffer (BufferEZE formula 2, cat. 8327603, Kodak, Rochester, NY, USA). Natural birchpollen proteins were treated with 2-mercaptoethanol, loaded at 2 µg/cm, and subjected to electrophoresis.

Proteins separated by SDS-PAGE were transferred to 0.45 micron nitrocellulose membrane (Hybond-C super, Amersham, UK) using a Tris 25 mM, glycine 192 mM, methanol 20% buffer. Transfer was performed with a BIOLYON cell (cat 50111, Biolyon, Dardilly, France) for 40 minutes at 250 mA. After transfer, unbound membrane sites were blocked by immersion with rocking in Tris 10 mM, NaCl 500 mM, Tween 20 0.05%, BSA 3%, pH 7.4, for 1 hour at room temperature.

Nitrocellulose strips (2 mm in width) were incubated with atopic sera (diluted 1:20 in Tris 10 mM, NaCl 500 mM, Tween 20 0.05%, pH 7.4, BSA 1%) or with undiluted culture supernatants overnight at 4°C and were then washed twice with Tris 10 mM, NaCl 500 mM, Tween 20 0.05% (TNT) for 30 minutes. One of the following procedures was then used for detection:

(1) *chemiluminescence*: For the detection of IgE, the strips were incubated with the murine monoclonal anti-human IgE I27 (dilution 1:5000 in TNT) for 1 hour at room temperature with rocking. After washing, goat anti-mouse Ig peroxidase conjugate (TAGO ref. 6540 diluted 1:500 in TNT) was added for 1 hour at room temperature. After washing, detection was performed with the ECL Western blotting detection reagents (cat 2106 Amersham, UK) according to the manufacturer's instructions. The strips were then loaded into a Kodak X O-matic cassette together with a photographic film (X O-mat AR, Kodak).

(2) *colorimetric reaction*: Nitrocellulose strips were incubated for 1 hour at room temperature with peroxidase-labeled sheep Fab anti-human IgG (cat. 1087-690 Boehringer Mannheim GmbH, W.-Germany) diluted 1:2000 Tris 10 mM, NaCl 500 mM, Tween 20 0.05%, pH 7.4, BSA 1% . After washing as above, color development was performed with 3,3'-diaminobenzidine tetrahydrochloride (cat. D-5905 Sigma, St. Louis MI USA) 0.1%, $NiCl_2$ 0.003%, H_2O_2 0.03%.

Immunoprecipitation of purified Bet vI

Bet vI is purified by immunoaffinity column: a murine MAb against Bet vI 202B8, was prepared by conventional techniques and covalently coupled to an AFFIGEL HZ® hydrazide gel column (cat 153-6048, Biorad, Richmond, CA, USA). Purified Bet vI was then iodinated with the Iodo Beads kit (cat. 28665X, Pierce, Rockford, USA). After iodination, the labeled material was subjected to SDS-PAGE in a 15% gel, which is dried after completion of the run. An X O-Mat film is exposed to the dried gel to check for the purity of the iodinated agent, and to control the efficacy of the labeling.

Typically, 50 µl of sera/plasma from patients or 50 µl of culture supernatants (both used at appropriate dilution in PBS, 1% BSA) were incubated for 45 minutes at room temperature with 20,000 cpm of ^{125}I-Bet vI (diluted in PBS 1% BSA) in a well of a special 96-well filtration microplate MultiScreen-HA (Millipore Co., Bedford, MA) whose bottom was composed of a nitrocellulose

membrane (HATF 0.45 µm). Each sample was tested in duplicate. Then, 50 µl of a dilution of protein G coupled to sepharose 4B (Sigma Chemical Co.) (15 ml beads diluted to 50 ml in PBS 1% BSA) were added to each well and incubated for 45 minutes at room temperature. The wells were then washed three times with PBS using a vacuum manifold (Millipore Co.) and the dried membranes were collected into appropriate vials using a special collector system (Millipore Co.). The radioactivity corresponding to the complexes ^{125}I-Bet vI – IgG anti-Bet vI was counted in a Wizard gamma-counter (Wallac Oy, Turku, Finland). The following Table 3 shows the results of immunoprecipitation of 100,000 CPM of ^{125}I-labeled birchpollen extract by the HuMAbs alone or in the presence of a 100-fold excess of unlabeled purified Bet vI. AAI, an anti-Der pI purified HuMAb (see below) was introduced as a negative control.

Table 3

IMMUNOPRECIPITATION OF ^{125}I BIRCHPOLLEN EXTRACT HUMABS ANTI-BPE

HuMAb	CPM Immuno-precipitated	HuMAb	CPM Immuno-precipitated
G5F2A4	6,030	G5F2A4 + Bet vI	294
G11A9F3	8,788	G11A9F3 + Bet vI	332
G17E7A1	7,444	G17E7A1 + Bet vI	363
G27E9B1	2,547	G27E9B1 + Bet vI	377
G28C10C10	10,361	G28C10C10 + Bet vI	288
AA 1	326	AA 1 + Bet vI	202

Specific IgE inhibition (CAPS inhibition)

CAPS® (Pharmacia, Sweden) bearing the birchpollen are incubated at 37°C for 2 hours with different dilutions of the monoclonal antibody in the buffer provided by the manufacturer. The CAPS® are then used with the sera of patients which have previously been shown to contain IgE antibodies against Bet vI only, as judged from a Western Blot assay as described above. The CAPS® are further processed according to the manufacturer's instructions with an automatic CAPS® machine (Pharmacia, Sweden). Specific IgE inhibition is

measured by comparing the radioactivity bound to the CAPS with and without pre-incubation with the HuMAbs.

Measurement of affinity of anti-Bet vI HuMAbs

To determine the binding affinity of the human monoclonal antibodies to Bet vI, the dissociation constant of HuMAbs-Bet vI complexes was measured. Purified HuMAbs were incubated with increasing concentrations of natural ^{125}I-Bet vI (0.15-2.5 pM) in a final volume of 250 µl PBS-Tween 1%. Each tested condition was performed in duplicate. Nonspecific binding controls were performed in duplicate by addition of a volume of birchpollen extract containing 250 pM of unlabeled Bet vI. After 4 hours' incubation at 4°C, 200 µl of each sample were distributed in one well of 96-well special filtration plates (MultiScreen-HA, 0.45 µm) containing 50 µl of protein G coupled to sepharose (Sigma Chemical cat. P-3296, St Louis, MI). After 1 hour's incubation at 4°C, plates were washed four times with PBS, and dried membranes were collected from each well. Radioactivity corresponding to the complexes ^{125}I-Bet vI/ HuMAb/protein G-beads retained on the membranes was counted using a Wizard gamma-counter (Wallac). Specific binding of ^{125}I-Bet vI was calculated, then plotted versus free ^{125}I-Bet vI concentrations and subjected to Scatchard analysis using a Ligand software.

The values of the equilibrium dissociation constants (K_d) obtained for the human monoclonal antibodies are presented in Table 1 above.

Sequencing of the Variable Region Genes of the Anti-Bet vI HuMAbs

RNA from the B-cell clones in Table 1 above have been isolated according to the single-step method described by Chomczynski et al., *Anal. Biochem.*, *162*: 156-9 (1987). Briefly, about 10^7 cells were lysed in guanidinium thiocyanate denaturing solution. After acidification of the mixture with 2M sodium acetate, pH 4, RNA was extracted with phenol and chloroform/isoamyl alcohol (24:1). RNA was then precipitated with isopropanol, and the RNA pellet was redissolved in denaturing solution. reprecipitated with isopropanol, and washed with 75% ethanol.

cDNA was obtained by reverse transcription, using the Superscript Reverse Transcriptase Kit (cat. 20898 BRL, Gaithersburg. MD, USA), with oligo

dT_{12-18} primers (Cat. 27.7858-01, Pharmacia, Uppsala, Sweden). The cDNA was then used as template in a polymerase chain reaction (PCR). PCR amplifications were performed with Taq polymerase (Perkin Elmer, Norwalk, Connecticut) using the reaction buffer, Taq buffer, provided by the manufacturers: 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, pH 8.3 and 0.0% (w/v) gelatin. All PCR reaction mixtures contained 200 ng of each primer, and 2.5 of Taq Polymerase. Amplifications were performed in a Trio-Thermoblock Thermal Cycler (Biometra, GmbH) and consisted of 35 cycles: 1 minute's denaturation at 94°C, 2 minutes' primer annealing at 60°C, and 3 minutes' extension at 72°C. After the last cycle, the reaction mixtures were incubated for 10 minutes at 72°C to insure complete extension of all products. The primers were designed to include restriction sites, to allow for the directional cloning of the PCR products. For the heavy chain, primers (listed in SEQ ID NOS. 21, 22, 23, 24, 25 and 26) specific for the leader sequence of the six different human V_H families were used individually in conjunction with a primer (listed in SEQ ID NO. 27) located at the 3'-end of the gamma constant region corresponding to the sub-class previously determined by isotyping the HuMAb by ELISA (IgG_4). The light chain was amplified with individual combinations of primers corresponding to the 3'-end of the kappa or lambda chain (listed in SEQ ID NOS. 28 and 29, respectively) in conjunction with a series of primers (listed in SEQ ID NOS. 30, 31, 32, 33, 34 and 35) annealing to the leader sequence of the different V kappa gene families or with a series of primers (listed in SEQ ID NOS. 36, 37, 38, 39 and 40) annealing to the leader sequence of the different V lambda gene families. Thus, full-length heavy and light chains starting at the initiation codon in the leader sequence and ending at the stop codon have been generated. Two independent PCRs were performed for both the heavy and the light chains.

Those PCR products were loaded on agarose gels, and purified with GELase (Epicentre, cat. G21223, WI) according to the manufacturer's instructions. Purified PCR products from heavy and light chains were used as templates for sequencing reactions with leader PCR primers and with primer hybridizing at the 5'-end of the gamma and of the kappa or lambda constant region gene respectively. The sequencing reaction was performed on a 373 DNA Sequencer with TaqDyeDeoxy Terminator Cycle Sequencing Kit (both from Applied Biosystems Inc., Foster City, CA). Direct sequencing of both strands of the products of the two independent PCRs were therefore obtained, and the sequences were compared. No difference was found between the

sequences of the two PCRs from the same cells. Sequences were compared with the Gene Bank release 77 (July 1993) using the DNAstar (WI. USA) software. No variable-region germline sequence showing 100% homology with any heavy or light chain of the anti-allergen HuMAb was found. Some of the V_H and V_L genes showed a level of homology with germline sequences higher than 94%. Those were most probably derived from the identified germline sequence, or from a closely related one with differences resulting from somatic mutation. Indeed, the ratio of replacement *vs.* silent mutation in regard to their distribution in the CDR *vs.* the FR (framework) regions suggests that they have been selected by the antigen. This correlates well with the high affinity of the HuMAbs for BET vI. The DNA and amino acid sequences of one functional heavy and light chain variable region for each clone are shown in SEQ ID NOs. 1-20 as indicated in Table 4 below:

Table 4

SEQ ID NO.	CLONE	CHAIN TYPE
1 & 2	G17E7A1	VH2-D-JH5-Cγ1 (heavy chain)
3 & 4	G17E7A1	VL-JL 1 (λ light chain)
5 & 6	G27E9B1	VH2-DJH1-Cγ1 (heavy chain)
7 & 8	G27E9B1	VKL-JK1-Cκ (κ light chain)
9 & 10	G5F2A4	VH3-D-JH4-Cγ1 (heavy chain)
11 & 12	G5F2A4	VK-JK5-Cκ (κ light chain)
13 & 14	G11A9F3	VH3-JH6-Cγ1 (heavy chain)
15 & 16	G11A9F3	VK-JK1-Cκ (κ light chain)
17 & 18	G28C10C10	VH5-JH5-Cγ1 (heavy chain)
19 & 20	G28C10C10	VKL-JK1-Cκ (κ light chain)

The sequences of the V_H genes and encoded amino acids listed in SEQ ID NO. 1-2, 5-6, 9-10, 13-14 and 17-18 and the sequences of the V_L genes and encoded amino acids listed in SEQ ID NO. 3-4, 7-8, 11-12, 15-16 and 19-20 are further characterized below. Framework (FR) and CDRs are identified as below, in agreement with the system of Kabat et al. (Kabat, E.A., T.T. Wu, H.M. Perry,

G17E7A1 - V_H Segment
SEQ ID NO. 1 & 2

amino acid residue nos.	region
-19 to -1	Signal peptide
+1 to +30	FR 1
+31 to +37	CDR1
+38 to +51	FR 2
+52 to +67	CDR2
+68 to 99	FR 3
+100 to +112	D
+113 to +127	JH5

G17E7A1 - V_L Segment
SEQ ID NO. 3 & 4

amino acid residue nos.	region
-20 to -1	Signal peptide
+1 to +22	FR 1
+24 to +36	CDR 1
+37 to +51	FR 2
+52 to +58	CDR 2
+59 to +90	FR 3
+91 to +96	CDR 3
+97 to +102	N
+103 to +114	Jλ1

amino acid residue nos.	region
-19 to -1	Signal peptide
+1 to +30	FR 1
+31 to +37	CDR1
+38 to +51	FR 2
+52 to +67	CDR2
+68 to 99	FR 3
+100 to +110	D
+111 to +127	JH1

amino acid residue nos.	region
-20 to -1	Signal peptide
+1 to +23	FR 1
+24 to +34	CDR 1
+35 to +49	FR 2
+50 to +56	CDR 2
+57 to +88	FR 3
+89 to +94	CDR 3
+95 to +107	Jκ1

amino acid residue nos.	region
-19 to -1	Signal peptide
+1 to +30	FR 1
+31 to +35	CDR1
+36 to +49	FR 2
+50 to +66	CDR2
+67 to 98	FR 3
+99 to +103	D
+104 to +119	JH4

amino acid residue nos.	region
-20 to -1	Signal peptide
+1 to +23	FR 1
+24 to +34	CDR 1
+35 to +49	FR 2
+50 to +56	CDR 2
+57 to +88	FR 3
+89 to +97	CDR 3
+98 to +108	Jκ5

amino acid residue nos.	region
-19 to -1	Signal peptide
+1 to +30	FR 1
+31 to +35	CDR1
+36 to +49	FR 2
+50 to +66	CDR2
+67 to +98	FR 3
+99	N
+100 to +108	D4
+109 to +110	N
+111 to +118	JH6

amino acid residue nos.	region
-20 to -1	Signal peptide
+1 to +23	FR 1
+24 to +39	CDR 1
+40 to +54	FR 2
+55 to +61	CDR 2
+62 to +93	FR 3
+94 to +97	CDR 3
+98 to +101	N
+102 to +113	Jκ1

amino acid residue nos.	region	amino acid residue nos.	region
-19 to -1	Signal peptide	-20 to -1	Signal peptide
+1 to +29	FR 1	+1 to +23	FR 1
+30 to +35	CDR1	+24 to +34	CDR 1
+36 to +49	FR 2	+35 to +49	FR 2
+50 to +66	CDR2	+50 to +56	CDR 2
+67 to 98	FR 3	+57 to +88	FR 3
+99 to +107	D	+89 to +94	CDR 3
+108 to +123	JH5	+95 to +98	N
		+99 to +111	Jκ1

[In order to enable the alignment of V region genes as they are sequenced, Kabat has assigned numbers to the amino acid residues and their corresponding codons. These numbers are not included in the Sequence Listings herewith, nor in the Tables immediately above, although they are classically used to accommodate the variations in size of the different V genes.]

Expression of Recombinant Anti-Allergen HuMAbs in Baculovirus

To isolate RNA from the B cell clones, the guanidinium thiocyanate single-step method described by Chomczynski et al., *supra*, can be used. cDNA is obtained by reverse transcription of the RNA using a Superscript Reverse Transcriptase Kit with oligo dT_{12-18} primers (Cat. 27.7858-01, Pharmacia, Uppsala, Sweden). The cDNA is then used as a template in the PCR performed with Taq polymerase. The primers are designed to include restriction sites such as *Eco*RI and *Not*I, to allow for the directional cloning of the PCR products into the baculovirus vector pVL1393. For the heavy chain, primers (listed in SEQ ID NO. 21, 22, 23, 24, 25 and 26) specific for the appropriate leader sequence of the human V_H family were used in conjunction with a primer (listed in SEQ ID NO. 27) located at the 3'-end of the gamma constant region corresponding to the sub-class previously determined by isotyping the HuMAb by ELISA. The light chain is amplified with primer (listed

in SEQ ID NO. 28 and 29) corresponding to the 3'-end of the appropriate kappa or lamda light-chain constant region in conjunction with a primer (listed in SEQ ID NO. 30, 31, 32, 33, 34 and 35) annealing to the leader sequence of the different V lamda gene families, as appropriate. Thus, full-length heavy-chain cDNAs and light-chain cDNAs can be generated. Two independent PCRs are performed for both the heavy and the light chains.

After appropriate restriction cleavage of these PCR products, both full-length heavy chains and full-length light chains are cloned in baculovirus vector restricted with the same enzymes. Heavy and light chains are cloned individually in distinct pVL1393 baculovirus vectors (Invitrogen Co., San Diego, CA). The recombinant vectors are then used to transform competent DH5α *E. coli* bacteria (Gibco BRL, Gaithersburg, MD), and a number of single colonies (*e.g.*, ten) are selected. 100 ml cultures of each bacterial clone are obtained and vector DNA is purified with Qiagen plasmid-Kit (Diagen, GmbH). Both strands of the complete insert from double-stranded DNA vector are sequenced with (1) two primers flanking the insert – the first (listed in SEQ ID NO. 41) annealing 5' in the promoter region of the polyhedrin gene and the second (listed in SEQ ID NO. 42) annealing 3' in the polyhedrin gene itself; and (2) a series of forward and backward primers distributed about 400 bp apart along the heavy and the light chain sequences, *i.e.*, the forward primers for the heavy chain are listed in SEQ ID NOS. 43 and 44, the backward primers for the heavy chain are listed in SEQ ID NOS. 45, 46 and 47, and the backward primer for the light chain is listed in SEQ ID NO. 48. Double-stranded DNA sequencing can be done on a 373 DNA Sequencer with TaqDyeDeoxy Terminator Cycle Sequencing Kit (both from Applied Biosystems Inc., Foster City, CA).
A recombinant baculovirus vector clone can be selected for both heavy and light chains, which should show a perfect match with the variable region sequences obtained from the PCR products, and with the published sequences of the constant regions of the heavy gamma and kappa light chains respectively. Recombinant baculovirus vectors are cotransfected with wild type baculovirus DNA in Sf9 insect cells, using the transfection module (Invitrogen Co, San Diego, CA). Recombinant baculoviruses recovered from the cell culture supernatant of these transfected cells are then cloned in Sf9 cells by limiting dilution and screened by hybridization with the labeled inserts. After two runs of cloning, followed by production, recombinant baculoviruses containing the heavy chain cDNAs or the light chain cDNAs are titrated, and used to infect insect cells at a Multiplicity of Infection (MOI) of 5. After 5 days of

culture, production of human heavy or light chain can be confirmed by ELISA and/or *in vivo* labeling. One baculovirus clone expressing the heavy chain and one expressing the light chain can be used to co-infect Sf9 cells, both at a MOI of 5. After 5 days of infection, the presence in the supernatant of an antibody binding specifically to Bet vI can be confirmed by immunoprecipitation.

Brief details of the primers referred to in this section are given in the following Table 5:

TABLE 5: SEQUENCE LISTINGS AND PRIMERS

SEQ. ID. NO.	NATURE OF PRIMER	SEQ. ID. NO.	NATURE OF PRIMER
21	Leader VH1	22	Leader VH2
23	Leader VH3	24	Leader VH4
25	Leader VH5	26	Leader VH6
27	Constant gamma 3'-end	28	Constant kappa 3'-end
29	Constant lambda 3'-end	30	Leader VK1
31	Leader VK2	32	Leader VK3
33	Leader VK4	34	Leader VK5
35	Leader VK6	36	Leader VLAMBDA1
37	Leader VLAMBDA2	38	Leader VLAMBDA3
39	Leader VLAMBDA4	40	Leader VLAMBDA5
41	5' of the promoter of the polyhydedrin gene	42	3' in the polyhedrin gene itself
43	first forward primer for the gamma heavy chain	44	second forward primer for the gamma heavy chain
45	first backward primer for the gamma heavy chain	46	second backward primer for the gamma heavy chain
47	third backward primer for the gamma heavy chain	48	backward primer for the kappa light chain

Repertoire Cloning

As demonstrated above, by dilution cloning of an immortalized and/or activated B cell population in accordance with the present invention, a series of amplified B cell subpopulations can be provided for screening for antibodies that bind to the desired antigen, *e.g.*, by the standard and derived immunoprecipitation protocols described above. Thus, by using the immortalization, amplification and screening techniques of the present

invention, it is possible to produce and identify an immortalized and/or secreting B cell subpopulation that consists of from about 5 to about 50 different, amplified B cell clones, at least one of which expresses a HuMAb against the desired antigen, *e.g.* Bet vI, Bet vII, Der pI, Der pII, etc.

For example, as discussed above, we produced amplified, immortalized and/or secreting B cell subpopulations from 5 patients expressing HuMAbs against Bet vI, Der pI, or Der pII. By the time of the first screening, each of these subpopulations contained from about 5 to about 50 different amplified B cell clones in a total number of about $5x10^5$ to about $50x10^5$ B cells.

Thus, the percentage of B cells producing HuMAbs against the antigen of interest in a B cell subpopulation of the present invention is greatly enhanced in comparison to other techniques which start with naturally occurring B cell populations. This amplified subpopulation can make it possible to uncover HuMAbs to allergen even when it is not possible to isolate a single clone as we accomplished with the anti-Bet vI clones discussed above.

For example, in an amplified, immortalized and/or secreting B cell subpopulation in accordance with the present invention, including 40 different B cell clones, the number of possible V_H/V_L combinations from a cDNA library encoding the V_H segments and V_L segments from these B cells is 1600 (40 x 40). This very low number makes it far easier to isolate the specific combination of V_H and V_L segments responsible for the one (or more) amplified HuMAb clone in the subpopulation which binds to the desired antigen. Thus, applying standard techniques such as repertoire cloning and phage display to the amplified, immortalized and/or secreting B cell subpopulation of the invention enables the identification and isolation of a series of HuMAbs against the desired antigen by recombinant techniques.

For example, a cDNA library encoding the mRNA repertoire of V_H and/or V_L segments of all the HuMAbs expressed in such a subpopulation (*e.g.*, a subpopulation screened as containing a clone expressing a HuMAb to Bet vII) can be prepared by reverse transcriptase PCR amplification of the mRNA from the subpopulation using appropriate primers. These repertoire cloning techniques are now standard in the art: see, for example, Marks et al., *J. Mol. Biol.*, *222*: 581-597 (1991); Huse et al., *Science*, *246*: 1275-1281 (1989); WO 90/14430; WO 92/15678; WO 91/16427; and WO 92/01047. The DNAs encoding the V_H and V_L segments may be assembled into appropriate vectors for direct cloning and expression in a host, *e.g.*, by the methods described by Hoogenboom et al., *Nucleic Acids Research*, *19*: 4133-4137 (1991). The

expressed V_H and V_L segments or Fab (*e.g.*, for an anti-Bet vII HuMAb) may then be screened for binding to the desired antigen by the standard and derived immunoprecipitation protocols described above using labeled antigen, *e.g.* ^{125}I-Bet vII. The DNA encoding the V_H or V_L segments from the identified clones can then be sequenced and operatively linked to DNA segments encoding the constant regions for the desired HuMAb isotype heavy or light chains, *e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA, etc. heavy chains or κ or λ light chains, to create a complete HuMAb or a fragment thereof (*e.g.*, Fab, $F(ab')_2$, Fv etc.) against the desired antigen, *e.g.*, Bet vII.

Alternatively, the cDNA repertoire encoding the V_H and/or V_L segments can be included in a vector appropriate to display the V_H and/or V_L segments on the surface of a suitable host. See, for example, Marks et al., *J. Mol. Biol.*, *222*: 581-597 (1991) and Hoogenboom et al., *supra*, who disclose methodologies for displaying Fv, single-chain Fv (scFv) or Fab fragments of such a cDNA library on the surface of bacteriophage. The host cells (*e.g.*, phage) that display the scFv on their surface and bind to the desired antigen can then be identified by ELISA or any other suitable assay. The DNA encoding the V_H and/or V_L segments from binding host cells can be separated and reassembled into appropriate vectors for direct cloning and expression in a host, *e.g.*, by the method described by the Marks et al. The DNA sequences can then be assembled into a full-length HuMAb or fragment thereof by the methodologies described above.

An amplified B-cell subpopulation of the present invention as described above may be employed with single-cell and multiple-cell PCR techniques to obtain the DNA sequences encoding the variable regions of the HuMAbs produced by these cells. For example, a CD40-crosslinked B-cell population of the invention (which may also be EBV-transformed) may be diluted to provide (on average) either a small number of B-cells (*e.g.*, 10 B-cells per well) or a single B-cell or less per well. The methodologies disclosed, for example, by Larrick et al., *Biotechnology*, *7*: 934 (1989), Embleton et al., *Nucleic Acids Research*, *10*: 3831-3837 (1992), Liu et al., *Proc. Natl. Acad. Sci.*, *89*: 7610-7614 (1992) and Lew et al. *Immunology*, *75*: 3-9 (1992), may be employed to obtain accurate and complete heavy and light chain variable region (V_H and V_L) genes from these cells. These DNA sequences may be included in an appropriate recombinant system to express, for example, an Fv or ScFv, and the fact that they represent a HuMAb to the desired antigen may then be confirmed by the immunoprecipitation assays described above for

binding to the desired antigen. Previous isotyping of the active HuMAbs in the B-cell starting population or subpopulation may then be used to construct the full-length HuMAb.

It is most likely that the pairs of heavy and light chains identified under the above conditions will be those of the identified antibody, since the screening procedures described above are selective for high-affinity antibody which can only be obtained with a given combination of heavy and light chain. Furthermore, the identification of the heavy and light chain isotypes in the supernatants of the oligoclonal cell lines will also be of great help in determining whether the selected pair does indeed correspond to the initially identified clone.

Inhibition of basophil degranulation

The protocols for the degranulation of human basophils and for the measurement of histamine released were essentially as described by Weyer et al., "Seasonal increase of spontaneous histamine release in washed leukocytes from rhinitis patients sensitive to grass pollen", *Clin. Exp. Immunol. 79(3)* (March 1990), 385-391.

A first series of degranulations was performed to select appropriate patients and to determine the concentration of allergen to be used in the assays. Briefly, 30 ml of peripheral blood were drawn from each of six patients into a syringe containing 120 µl of heparin. 6 ml of glucose-dextran was added and most of the red cells and platelets were allowed to sediment for 45 minutes at room temperature. Peripheral blood leukocytes, including basophils, were recovered in the plasma and glucose-dextran solution: the cellular concentration ranged from (1 to 2)x10^6/ml. 50 µl of differing concentrations (ranging from 10^{-10} to 6x10^{-6} g/ml) of the immuno-affinity purified natural Bet vI were incubated with 500 µl of leukocytes for 40 minutes at 37°C. Incubation was stopped by the addition of 50 µl EDTA, and the supernatant was recovered for analysis by fluorometry. The dosage of total histamine after cell lysis with 0.4N perchloric acid represents 100% of histamine release, whereas the dosage of spontaneous histamine release, after incubation with the HuMAbs mixture in the absence of allergen, represents 0% of histamine release.

The histamine release curve was established for each patient as a function of the allergen concentration, and the concentration of Bet vI giving 50% of the maximum histamine release was selected for the inhibition test: this concentration varied from 10^{-9} to 10^{-11} g/ml, according to the patient. Three

patients (patient 1: A.R.; patient 2: S.A.; patient 3: R.V.) were selected for their low level of spontaneous histamine release and for their good response to Bet vI.

For the inhibition assays, 50 µl of natural Bet vI at either 10^{-10} or $5x10^{-11}$ g/ml were incubated for 90 minutes at 37°C with 50 µl of a mixture of the five HuMAbs, each of which was at a concentration of 20 µg/ml in the mixture, or with 50 µl of TrisACM (0% histamine release) or with 50 µl of G26F6B2, an IgG HuMAb of unrelated specificity (as negative control, at a concentration of 100 µg/ml). 400 µl of the leukocyte suspension were then challenged with the allergen, protected or not as described above. Every dosage was done in triplicate. The following Table 6 shows that the mixture of the five HuMAbs was able to inhibit strongly the Bet vI-induced degranulation of basophils from the three allergic patients studied.

TABLE 6

INHIBITION OF BET VI-INDUCED DEGRANULATION OF BASOPHILS BY ANTI-BET VI HUMABS

	Patient 1 (A.R.)		Patient 2. (S.A.)		Patient 3 (R.V.)	
Concentration of Bet vI (g/ml)	10^{-10}	$5x10^{-11}$	10^{-10}	$5x10^{-11}$	10^{-9}	10^{-10}
No HuMAbs	65	44	26	40	45	18
With HuMAbs	33	9.5	0	18	24	10

Skin test inhibition in monkeys

Passive sensitization of the skin of monkeys is performed by intradermal injection of 1 ml of the serum from an allergic patient in one forearm. 24 hours later, 10 ml of Blue Evans dye is administered to the animal just before challenge by prick skin test with serial dilutions of the birchpollen (Bet vI) extract in the area of pre-sensitized skin. The specific reactions are evaluated by comparison of the size of the flare and weal reaction elicited with the same series of skin prick tests in the control non-sensitized forearm.

Inhibition of passive cutaneous anaphylaxis is determined by the effect of injection of any of the anti-Bet vI HuMAbs, either alone or in different combination, at different times and dosages. The HuMAb is administrated

either locally, in the sensitized area, just before the skin test, or systemically, *e.g.* by i.v. or i.m. injection. The HuMAb (or HuMAbs) can also be administrated as immune complexes prepared *in vitro* at different ratios with the challenging allergen in a series of skin tests. Comparison of the flare and weal reaction (*e.g.*, by measuring its surface area) elicited by the allergen alone with the reaction elicited by the same dose of allergen in combination with one or several HuMAbs can be used to establish the skin-test-inhibition capacity of the HuMAbs.

Generation of Anti-Der PI and Anti-Der PII HuMAbs

It has long been recognized that the mites of the genus *Dermatophagoides* are the most prevalent allergens sensitizing more than 10% of the population, and being responsible for allergic diseases such as asthma, rhinitis and atopic dermatitis. More recently, *Euroglyphus maynei* has also been recognized as a frequent source house dust allergens.

Studies on *Dermatophagoides* mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and *Dermatophagoides microceras*) have demonstrated that at least seven groups of mite allergens are of clinical importance, out of which three are bound by IgE from more than 70% of allergic patients.

One of the major mite allergens, Der pI, has been purified and cloned. It shows large sequence homology with cystein proteases. This 27 kD glycoprotein, which is associated with fecal particles, has been shown to react with the anti-mite IgE antibodies in up to 80% of allergic sera. Human IgE reacts with both the group I determinants that are common for the three mite species and the species-specific determinants.

Der pII, a member of the group II allergen, is a very stable molecule of 14 kD, which has been cloned and sequenced. Evidence for Der pII being lysozyme are accumulating. This allergen is recognized by the IgE of more than 80% of allergic patients, and strong cross-reactivity with the other members of the group II allergen has been demonstrated.

Group III allergen consist of proteins of 30 kD, which are recognized by the IgE of 70 to 100% of allergic patients. Sequence analysis revealed that they are trypsins.

GENERATION OF HUMAN CLONES SECRETING ANTIBODIES WHICH SELECTIVELY BIND TO *DERMATOPHAGOIDES PTERONYSSINUS* (DPT)

We have used essentially the same strategy as described above to generate HuMAbs against Der pI and Der pII. *Dermatophagoides pteronnysinus* extract and natural Der pI and Der pII, purified by immunoaffinity and kindly provided by Dr. Jean-Marie Saint-Remy, Clinical Allergy Unit, ICP, Brussels, Belgium (Saint-Remy et al., *Allergy, 43* (1988), 338-347), were used for the screening and the charaterization of the HuMAbs. All the materials and conditions used for immunoprecipitation and ELISA assays were exactly as described above for Bet vI, except where mentioned. Anti-DPT immunoglobulin fractions depleted for IgM were purified from the blood of allergic and non-allergic patients as previously described (Saint-Remy et al., *Allergy, 43* (1988), 338-347).

Selection of patients and immortalization of the human B lymphocytes

Among ten allergic patients, two allergic children have been selected for their sensitivity to house-dust mites, as judged by clinical and laboratory data: positive history of asthma, positive skin test with house-dust mite extract, and presence of high level of total IgE (>1000IU/ml) and specific anti-DPT IgE in the serum as detected by RAST. Both children had been undergoing classical desensitization with house-dust mite extract for several months when blood was drawn. $16x10^6$ PBLs from a first allergic child (Ro3), and $3.5x10^6$ PBLs from a second allergic child (Ro12) were immortalized with EBV, while being activated through their CD40 molecules. After 10 days of culture, the lines were screened for their secretion of IgG antibody that was able either to immunoprecipitate immunopurified and radioiodinated natural Der pI (see protocol above) or to bind to immunopurified natural Der pII in an indirect ELISA assay. The indirect ELISA assay is exactly the same as the one described for Bet vI, except that 1 µg/ml of either Der pI or Der pII diluted in coating buffer was used to coat the plates instead of the birchpollen extract. Lines which scored positive (signal.> twice the background) in either of the two tests were subsequently cloned. Clonality was checked by the criteria mentioned for anti-Bet vI HuMAbs: (1) detection of single IgG subclass and single light chain in the supernatant of each line by ELISA; (2) amplification with only one of the six leader variable-region gene primers corresponding to the six different variable-regions family, and with only either kappa or lambda light-chain primers. Two clones were

obtained which secrete IgG that is able to immunoprecipitate Der pI: HuMAb AA1 is an IgG_4-kappa, whereas AK5 is an IgG_1-kappa. Another clone, AK6, produces IgG which immunoprecipitates Der pII. Table 7 summarises the characteristics of the three HuMAbs, and Table 8 illustrates the result of immunoprecipitation of ^{125}-I labeled Der pI by the HumAbs alone, or in the presence of a 100-fold excess of cold (*i.e.*, unlabeled) Der pI.

TABLE 7

HUMABS ANTI-DER PI AND ANTI-DER PI

Antibody	AA7A3	AK5	AK6A3
ELISA	+	+	+
Western Blot	-	-	+
Immunoprecipitation	Der pI	Der pI	Der pII
Heavy Chain Light Chain	IgG_4 kappa	IgG_1 kappa	IgG_1 lambda
Affinity (KA)	(2.7 – 5.4) x 10^{10} M	(2.5 – 5) x 10^{10} M	N.D.

N.D. = Not Determined

TABLE 8

IMMUNOPRECIPITATION OF PURIFIED ^{125}I-DER PI BY ANTI-DER PI HUMABS

HuMAb	CPM Immunoprecipitated
Control	552
AA7A3	1890
AA7A3 + cold Der PI	480
AK5	3550
AK5 + cold Der PI	470

Inhibition of the binding of the HuMAbs to DPT by human, immunoaffinity-purified ,DPT-specific, polyclonal non-IgM immunoglobulins

10 µg/ml of *Dermatophagoides pteronnysinus* extract was diluted in coating buffer to coat the ELISA plates. A mixture of anti-DPT immunoglobulin fractions depleted for IgM at 30 µg/ml with either biotinylated AA7A3 or AK5 HuMAbs at a concentration of 1µg/ml was then added to the coated plates. The HuMAbs were detected with 2-phenylenediamine (cat P5412 Sigma) labeled with avidin-peroxydase (cat. A3151 Sigma Chemical Co., St. Louis, MO) as substrate. Results represent the percentage of inhibition of the binding of the HuMAbs to DPT by human DPT-specific immunoglobulins: 0% inhibition corresponds to the O.D. obtained with the HuMAbs in the absence of DPT-specific polyclonal immunoglobulins, whereas 100% inhibition is given by the O.D. obtained when the HuMabs were incubated in the wells in the presence of 25 µg/ml of non-biotinylated HuMab as competitor. Results from the analysis of the immunoglobulin fractions of 20 allergic patients (10 suffering from asthma, and 10 from atopic dermatitis) and of 10 non-allergic patients are presented in Table 9 below. It appears that, whereas the binding of AA7A3 to DPT is inhibited by the immunoglobulin fractions of most of the patients, none of the non-allergic subjects had DPT-specific immunoglobulins able to compete with AA7A3. This suggest that AA7A3 binds to an epitope frequently recognised by the allergic patient, but not by non-allergic persons. AK5 binds to an epitope recognised less frequently by both allergic and non-allergic persons.

Inhibition of the binding of human DPT-specific IgE to DPT by the HuMAbs

Again, 10 µg/ml of *Dermatophagoides pteronnysinus* extract was diluted in the coating buffer to coat the ELISA plates. A mixture of sera diluted 1:4 in dilution buffer with either AA7A3 or AK5 HuMAbs at a concentration of 25 µg/ml was then added to the coated plates. Detection of human IgE was performed with a human IgE-specific rabbit antiserum coupled to peroxydase (cat. A139, Tago, Inc., Burlingame, CA USA). Results represent the percentage of inhibition of human IgE binding to DPT by the HuMAbs: 0% inhibition corresponds to the O.D. obtained with the diluted sera in the absence of HuMAbs. whereas 100% inhibition is given by the O.D. obtained when the sera were incubated in the presence of 20 µg/ml of DPT extract as soluble competitor. As shown in Table 10, both AA7A3 and AK5 are able to inhibit significantly the binding of human IgE from allergic patients to DPT.

TABLE 9

INHIBITION OF THE BINDING OF THE ANTI-DER PI HUMABS TO THE DPT EXTRACT BY HUMAN NON-IGM IMMUNOGLOBULINS SPECIFIC FOR THE DPT

patient	AA7A3	AK5
Atopic Dermatitis		
Co.	37	0
De.	12	0
Jo.	33	0
Gru.	47	0
Ca.	12	0
Des	2	14
Pe.	5	3
Ge.	20	0
Ro.	27	12
Wa.	42	15
mean	**24**	**4**
Allergic Asthma		
Al.	44	0
Ma.	40	0
Wn.	0	22
Db.	38	15
Fe.	52	19
Te.	46	0
Vi.	46	13
We.	32	20
Co.	32	6
Da.	29	0
mean	**36**	**10**
Non-allergic		
NL.1	0	0
NL.2	0	0
NL.4	0	N.D.
NL.5	0	13
NL.6	0	3
NL.7	0	N.D.
NL.8	0	N.D.
NL.9	0	0
NL.10	0	N.D.
NL.11	0	6
mean	**0**	**4**

N.D. = Non Determined

TABLE 10
INHIBITION OF THE BINDING OF HUMAN IGE TO THE DPT EXTRACT BY THE ANTI-DER PI HUMABS

patient	AA7A3	AK5
Atopic Dermatitis		
Co.	12	31
De.	12	20
Jo.	53	10
Gr.	N.D.	N.D.
Ca.	3	9
Des.	12	47
Cas..	25	0
De.	19	12
Gre.	59	10
Wi.	0	3
mean	**22**	**16**
Allergic Asthma		
Al.	25	26
Ma.	10	32
Wn.	44	29
La.	4	0
Ro.	0	33
Pi.	68	98
Ve.	100	99
Bru.	57	44
Mo.	23	17
Thi.	43	46
mean	**37**	**42**

N.D. = Not Determined

Whereas the present invention has been described in conjunction with the specific embodiments set forth above, many alternatives, modifications and variations thereof will be apparent to those of ordinary skill in the art. All such alternatives. modifications and variations are intended to fall within the spirit and scope of the present invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: [All countries except US]

(A) NAME: SCHERING CORPORATION

(B) STREET: 2000 GALLOPING HILL ROAD

(C) CITY: KENILWORTH

(D) STATE: NEW JERSEY

(E) COUNTRY: UNITED STATES OF AMERICA

(F) POSTAL CODE: 07033

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(A) NAME: SERGE J. E. LEBECQUE

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(F) POSTAL CODE: 69380

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(D) STATE:

(E) COUNTRY: FRANCE

(F) POSTAL CODE: 69130

(ii) TITLE OF INVENTION: HUMAN MONOCLONAL ANTIBODIES AGAINST HUMAN PROTEINS AND METHODS OF MAKING AND USING SUCH ANTIBODIES

(iii) NUMBER OF SEQUENCES: 48

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Schering-Plough Corporation

(B) STREET: One Giralda Farms

(C) CITY: Madison

(D) STATE: New Jersey

(E) COUNTRY: USA

(F) ZIP: 07940

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: Apple Macintosh

(C) OPERATING SYSTEM: Macintosh 6.0.8

(D) SOFTWARE: Microsoft Word 5.1a

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

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(A) APPLICATION NUMBER: 93400944.0

(B) FILING DATE: 09-APR-1993

(C) COUNTRY: EUROPE (FR)

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(B) REGISTRATION NUMBER: 31,895 (USA)

(C) REFERENCE/DOCKET NUMBER: SF0368K

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 201-822-7398

(B) TELEFAX: 201-822-7039

(C) TELEX: 219165

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 438 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
ATG GAC ATA CTT TGT ACC ACG TCC TGG TTG CTG ACC ATC CCT TCA TGG   48
Met Asp Ile Leu Cys Thr Thr Ser Trp Leu Leu Thr Ile Pro Ser Trp
               -15                 -10                 -5

GTC TTG TCC CAG ATC ACC TTG ATG GAG TCG GGT CCT TCG CTG GTG AAA   96
Val Leu Ser Gln Ile Thr Leu Met Glu Ser Gly Pro Ser Leu Val Lys
            +1                  5                  10

CCC ACT CAG ACC CTC ACG CTG ACC TGC ACC TTC TCT GGA TTC TCA CTC  144
Pro Thr Gln Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu
     15                  20                  25

AAC ACT CGT GCA GTG GCT GTG GCC TGG ATC CGT CAG CCC CCA GGC AAG  192
Asn Thr Arg Asp Val Ala Val Ser Trp Ile Arg Gln Pro Pro Gly Lys
 30                  35                  40                  45

GCC CTG GAT TGG CTT GCA CTC ATT TTT TGG GAT GAT GAT AAG CGT TAC  240
Ala Leu Asp Trp Leu Ala Leu Ile Phe Trp Asp Asp Asp Lys Arg Tyr
                50                  55                  60

AGC CCA TCT CTG AGG AGC AGG CTC ACC ATC ACC AAG GAC ACC TCC AAA  288
Ser Pro Ser Leu Arg Ser Arg Leu Thr Ile Thr Lys Asp Thr Ser Lys
            65                  70                  75
```

```
AAC CAG GTG GTC CTT ACA ATG ACC AAC ATG GAC CCT GTC GAC ACA GGC  336
Asn Gln Val Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala
        80                  85                  90

ACA TAT TTC TGC GCA CAC GTG CCC ACC GGA TAT TGT GAT GGT CCC AGG  384
Thr Tyr Tyr Cys Ala His Val Pro Thr Gly Tyr Cys Asp Gly Pro Arg
     95                 100                 105

TGC AAC CGG TGG TTC GAC TCC TGG GGC CAG GGA ACC CTG GTC ACC TGC  432
Cys Asn Arg Trp Phe Asp Ser Trp Gly Gln Gly Thr Leu Val Thr Cys
110                 115                 120                 125

TCC TTA                                                          438
Ser Leu
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 146 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Met Asp Ile Leu Cys Thr Thr Ser Trp Leu Leu Thr Ile Pro Ser Trp
               -15                 -10                  -5

Val Leu Ser Gln Ile Thr Leu Met Glu Ser Gly Pro Ser Leu Val Lys
            +1                  5                  10

Pro Thr Gln Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu
     15                 20                 25

Asn Thr Arg Asp Val Ala Val Ser Trp Ile Arg Gln Pro Pro Gly Lys
 30                 35                 40                 45

Ala Leu Asp Trp Leu Ala Leu Ile Phe Trp Asp Asp Asp Lys Arg Tyr
                50                 55                 60

Ser Pro Ser Leu Arg Ser Arg Leu Thr Ile Thr Lys Asp Thr Ser Lys
            65                 70                 75

Asn Gln Val Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala
         80                 85                 90

Thr Tyr Tyr Cys Ala His Val Pro Thr Gly Tyr Cys Asp Gly Pro Arg
     95                 100                105
```

Cys Asn Arg Trp Phe Asp Ser Trp Gly Gln Gly Thr Leu Val Thr Cys
110 115 120 125

Ser Leu

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 402 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
ATG GCC TGG GCT GCT CTG CTG CTC CTC ACT CTC CTC GCT CAC TGC ACA   48
Met Ala Trp Ala Ala Leu Leu Leu Leu Thr Leu Leu Ala His Cys Thr
-20                 -15                 -10                  -5

GGG TCC TGG GCC CAG TCT GTG CTG ACG CAG CCG CCC TCA GTG TCC GGG   96
Gly Ser Trp Ala Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly
                +1                  5                  10

GCC CCA GGG CAG ACG GTC ACC ATC TCC TGC ACT GGG AGC AGC TCC AAC  144
Ala Pro Gly Gln Thr Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn
        15                  20                  25

CTC GGG GCA GGT TAT GAT GTT CAC TGG TAC CGG CAA CTT CCA GAG GCT  192
Leu Ala Gly Tyr Asp Val His Trp Tyr Gln Arg Gln Leu Pro Glu Ala
    30                  35                  40

GCC CCC ACA CTC CTC ATC TAT GGT AAT AGC AAT CGG CCC TCA GGG GTC  240
Ala Pro Thr Leu Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val
45                  50                  55                  60

CCT GAC CGA TTC TCT GGC TCC AAG TCT GGC ACC TCA GCC TCC CTG GCC  288
Pro Asp Arg Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala
                65                  70                  75

ATT ACT GGG CTC CAG GCT GAC GAT GAG GCT GAT TAT TAC TGC CAG TCC  336
Ile Thr Gly Leu Gln Ala Asp Asp Glu Ala Asp Tyr Tyr Cys Gln Ser
            80                  85                  90

TAT GAC AAC AGC CTG GGT GCC TCC CTG TTT GTC TTC GGA ACT GGG ACC  384
Tyr Asp Asn Ser Leu Gly Ala Ser Leu Phe Val Phe Gly Thr Gly Thr
        95                  100                 105
```

AGG GTC ACC GTC CTA GGT 402
Arg Val Thr Val Leu Gly
110

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 134 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Ala Trp Ala Ala Leu Leu Leu Leu Thr Leu Leu Ala His Cys Thr
-20 -15 -10 -5

Gly Ser Trp Ala Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly
+1 5 10

Ala Pro Gly Gln Thr Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn
15 20 25

Leu Ala Gly Tyr Asp Val His Trp Tyr Gln Arg Gln Leu Pro Glu Ala
30 35 40

Ala Pro Thr Leu Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val
45 50 55 60

Pro Asp Arg Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala
65 70 75

Ile Thr Gly Leu Gln Ala Asp Asp Glu Ala Asp Tyr Tyr Cys Gln Ser
80 85 90

Tyr Asp Asn Ser Leu Gly Ala Ser Leu Phe Val Phe Gly Thr Gly Thr
95 100 105

Arg Val Thr Val Leu Gly
110

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 438 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
ATG GAC ATA CTT TGT ACC ACG CTC CTG CTG CTG ACC ATC CCT TCA TGG   48
Met Asp Ile Leu Cys Thr Thr Leu Leu Leu Leu Thr Ile Pro Ser Trp
               -15                 -10                 -5

GTC TTG TCC CAG ATC ACC TTG AAG GAG TCT GGT CCT ACG CTG GTG AAA   96
Val Leu Ser Gln Ile Thr Leu Lys Glu Ser Gly Pro Thr Leu Val Lys
            +1                              10

CCC ACA CAG ACC CTC ACG CTG ACC TGC ACC TTC TCT GGG TTC TCA CTC  144
Pro Thr Gln Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu
     15                  20                  25

ACC ACT CGT GGA GTG GGT GTG GGC TGG CTC CGT CAG CCC CCC GGA AAG  192
Thr Thr Arg Gly Val Gly Val Ser Trp Leu Arg Gln Pro Pro Gly Lys
 30                  35                  40                  45

GCC CCG GAG TGG CTT GCA GTC ATT TAT TGG GAT GAT GAT AAG CGC TAC  240
Ala Pro Glu Trp Leu Ala Val Ile Tyr Trp Asp Asp Asp Lys Arg Tyr
               50                  55                  60

AGC CCA TCT CTG AGG AGC AGG CTC ACC ATC AGC AAG GAC TCG TCC AAA  288
Ser Pro Ser Leu Arg Ser Arg Leu Thr Ile Ser Lys Asp Ser Ser Lys
            65                  70                  75

AAC CAG GTG GTC CTT ACA TTG ACC AAC ATG GAC CCT GTG GAC ACA GGC  336
Asn Gln Val Val Leu Thr Leu Thr Asn Met Asp Pro Val Asp Thr Ala
         80                  85                  90

ACA TAT TAC TGT GCA CAC GGG CCG TAT TCC ATC TCG TCG GGT GTC GGC  384
Thr Tyr Tyr Cys Ala His Gly Pro Tyr Ser Ile Ser Ser Gly Val Gly
     95                  100                 105

AAA GCA GAA TCC TTT GGC GAC TGG GGC CAG GGC ACC CCG GTC ACC GTC  432
Lys Ala Glu Ser Phe Gly Asp Trp Gly Gln Gly Thr Pro Val Thr Val
110                 115                 120                 125

TCC TCA                                                         438
Ser Ser
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 146 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Met Asp Ile Leu Cys Thr Thr Leu Leu Leu Thr Ile Pro Ser Trp
-15 -10 -5

Val Leu Ser Gln Ile Thr Leu Lys Glu Ser Gly Pro Thr Leu Val Lys
+1 10

Pro Thr Gln Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu
15 20 25

Thr Thr Arg Gly Val Gly Val Ser Trp Leu Arg Gln Pro Pro Gly Lys
30 35 40 45

Ala Pro Glu Trp Leu Ala Val Ile Tyr Trp Asp Asp Asp Lys Arg Tyr
50 55 60

Ser Pro Ser Leu Arg Ser Arg Leu Thr Ile Ser Lys Asp Ser Ser Lys
65 70 75

Asn Gln Val Val Leu Thr Leu Thr Asn Met Asp Pro Val Asp Thr Ala
80 85 90

Thr Tyr Tyr Cys Ala His Gly Pro Tyr Ser Ile Ser Ser Gly Val Gly
95 100 105

Lys Ala Glu Ser Phe Gly Asp Trp Gly Gln Gly Thr Pro Val Thr Val
110 115 120 125

Ser Ser

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 381 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

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ATG AGG CTC CCT GCT CAG CTC CTG GGG CTC CTG CTG TTC TGG CTC CCA   48
Met Arg Leu Pro Ala Gln Leu Leu Gly Leu Leu Leu Phe Trp Leu Pro
-20                 -15                 -10                 -5

GGT GCC AAA TGT GAC ATC CAG ATG ACC CAG TCT CCT TCC ACC CTG TCT   96
Gly Ala Lys Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser
                +1                  5                  10

GCC TCT GTA GGC GAC AGA GTC ACC ATC ACC TGC CGG GCC AGT CAG AGT  144
Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser
         15                  20                  25

ATT AGT AAG TAC TTG GCC TGG TAT CAG CAG AGA CCA GGG AAA GCC CCT  192
Ile Ser Lys Tyr Leu Ala Trp Tyr Gln Gln Arg Pro Gly Lys Ala Pro
     30                  35                  40

AAC CTC CTG ATC TCT CAG ACA TCT ACT TTA CAA ACC GGG GTC CCA TCA  240
Asn Leu Leu Ile Ser Gln Thr Ser Thr Leu Gln Thr Gly Val Pro Ser
 45                  50                  55                  60

CGG TTT AGT GGC AGT GGA TCT GGG ACA GAG TTC ACT CTC ACC ATC AGC  288
Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser
                65                  70                  75

AGC CTG CAG CCT GAT GAC TTT GCA ACC TAT TAC TGC CAA CAG TAT AAG  336
Ser Leu Gln Pro Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Lys
            80                  85                  90

ACT TAT TGG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAT CGA      381
Thr Tyr Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Asn Arg
        95                  100                 105
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 127 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
Met Arg Leu Pro Ala Gln Leu Leu Gly Leu Leu Leu Phe Trp Leu Pro
-20                 -15                 -10                 -5

Gly Ala Lys Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser
                +1                  5                  10
```

Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser
15 20 25

Ile Ser Lys Tyr Leu Ala Trp Tyr Gln Gln Arg Pro Gly Lys Ala Pro
30 35 40

Asn Leu Leu Ile Ser Gln Thr Ser Thr Leu Gln Thr Gly Val Pro Ser
45 50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser
65 70 75

Ser Leu Gln Pro Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Lys
80 85 90

Thr Tyr Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Asn Arg
95 100 105

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 414 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

ATG GAG TTT GGG CTG AGC TGG CTT TTT CTC GTG GCT TTT TTA AAA GGT 48
Met Glu Phe Gly Leu Ser Trp Leu Phe Leu Val Ala Phe Leu Lys Gly
-19 -10

GTC CAG TGT GAG GTG CAG CTG TTG GAG TCT GGG GGA GGC TTG GTA CAG 96
Val Gln Cys Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln
+1 5 10

CCT GGG GGG TCC CTG AGA CTC TCC TGT GTA GGC TCT GGA TTC ACC TTC 144
Pro Gly Gly Ser Leu Arg Leu Ser Cys Val Gly Ser Gly Phe Thr Phe
15 20 25

ACC AAC TAT GCC ATG ACC TGG GTC CGC CAG GCT CCA GGG AAG GGG CTG 192
Thr Asn Tyr Ala Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
30 35 40 45

GAG TGG GTC TCA GCT ATT AGT GGT AGT GGT AAT TAC ACA TAC TAC TCA 240
Glu Trp Val Ser Ala Ile Ser Gly Ser Gly Asn Tyr Thr Tyr Tyr Ser
50 55 60

```
GAC TCC GTG AAG GGC CGG TTC ACC ATC TCC AGA GAC AAT TCC AAG AGC  288
Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Ser
              65                  70                  75

ACG CTA TCT TTG CAT ATG AAC AGC CTC AGA GCT GAG GAC ACG GCC CTT  336
Thr Leu Tyr Leu His Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu
         80                  85                  90

TAT TAC TGT GCG AAA GCG GCA GTC CAC GCG TAC TTC TTT GAC TTC TGG  384
Tyr Tyr Cys Ala Lys Ala Ala Val His Ala Tyr Phe Phe Asp Tyr Trp
     95                  100                 105

GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA                          414
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110                 115
```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 138 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
Met Glu Phe Gly Leu Ser Trp Leu Phe Leu Val Ala Phe Leu Lys Gly
-19                                 -10

Val Gln Cys Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln
             +1                  5                  10

Pro Gly Gly Ser Leu Arg Leu Ser Cys Val Gly Ser Gly Phe Thr Phe
     15                  20                  25

Thr Asn Tyr Ala Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
 30                  35                  40                  45

Glu Trp Val Ser Ala Ile Ser Gly Ser Gly Asn Tyr Thr Tyr Tyr Ser
                    50                  55                  60

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Ser
             65                  70                  75

Thr Leu Tyr Leu His Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu
         80                  85                  90

Tyr Tyr Cys Ala Lys Ala Ala Val His Ala Tyr Phe Phe Asp Tyr Trp
     95                  100                 105
```

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 384 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
ATG AGG CTC CCT GCT CAG CTC CTG GGG CTC CTA CTG CTC TGG GTC CCA   48
Met Arg Leu Pro Ala Gln Leu Leu Gly Leu Leu Leu Trp Val Pro
-20                 -15                 -10                 -5

GGT GCC GGC TGT GAC ATC CAG TTG ACC CAG TCT CCA TCC TCC CTG TCT   96
Gly Ala Gly Cys Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser
                +1                  5                  10

GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC CGG GTG AGT CAG GGC  144
Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Val Ser Gln Gly
        15                  20                  25

ATT AGC AAT TGT TTA AAT TGG TAT CGG CGG AAA CCA GGT AAA GTT AAT  192
Ile Ser Asn Cys Leu Asn Trp Tyr Arg Arg Lys Pro Gly Lys Val Asn
    30                  35                  40

AAG GTC CTC TTC TAT AGT GCA TCC AAT TTC CAA TCT GGA GTC CCA TCT  240
Lys Val Leu Phe Tyr Ser Ala Ser Asn Phe Gln Ser Gly Val Pro Ser
45                  50                  55                  60

CGG TTC AGT GCC AGT GCG TCT GGG ACA GAT CTC ACT CTC AGT GTC AAC  288
Arg Phe Ser Ala Ser Ala Ser Gly Thr Asp Leu Thr Leu Ser Val Asn
                65                  70                  75

ACC CTG CAG CCT GAA GAT GTT GCA ACT TGT TAC TGT CAA CGG ACT GAC  336
Thr Leu Gln Pro Glu Asp Val Ala Thr Cys Tyr Cys Gln Arg Thr Asp
            80                  85                  90

AAT GCC CTT CAT CAC TTC GCC CAA GGG ACA CGA CTT GAG ATT AAA CGA  384
Asn Ala Leu His His Phe Ala Gln Gly Thr Arg Leu Glu Ile Lys Arg
        95                  100                 105
```

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 128 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Met Arg Leu Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp Val Pro
-20 -15 -10 -5

Gly Ala Gly Cys Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser
+1 5 10

Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Val Ser Gln Gly
15 20 25

Ile Ser Asn Cys Leu Asn Trp Tyr Arg Arg Lys Pro Gly Lys Val Asn
30 35 40

Lys Val Leu Phe Tyr Ser Ala Ser Asn Phe Gln Ser Gly Val Pro Ser
45 50 55 60

Arg Phe Ser Ala Ser Ala Ser Gly Thr Asp Leu Thr Leu Ser Val Asn
65 70 75

Thr Leu Gln Pro Glu Asp Val Ala Thr Cys Tyr Cys Gln Arg Thr Asp
80 85 90

Asn Ala Leu His His Phe Ala Gln Gly Thr Arg Leu Glu Ile Lys Arg
95 100 105

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 411 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

```
ATG GAG TTT GGG CTG AGC TGG GTT TTC CTC GTT GCT CTT TTA AGA GGT   48
Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu Leu Arg Gly
-19                                 -10

GTC CAG TGT CAG GTG CAC CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG   96
Val Gln Cys Gln Val His Leu Val Glu Ser Gly Gly Gly Val Val Gln
            +1              5                  10

CCG GGG GGG TCC CTG AGA CTC TCC TGT GCA GCC TCT GGC TTC TAC TTC  144
Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Tyr Phe
     15                 20                  25

AGT AGT TAT CAT GTG TAC TGG GTC CGC CAG GCT CCA GCC AAG GGG CTG  192
Ser Ser Tyr His Val Tyr Trp Val Arg Gln Ala Pro Ala Lys Gly Leu
30                  35                  40                  45

GAG TGG GTG GCA GTT ATT TCA TAT GAT GGC CTC AAG ACA TCC TAT GCA  240
Glu Trp Val Ala Val Ile Ser Tyr Asp Gly Leu Lys Thr Ser Tyr Ala
                50                  55                  60

GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGG GAC AAT TCC AAG AGC  288
Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Ser
            65                  70                  75

ACA CTG TTT CTG CAG ATG GAC AGC CTG AAT ACT GAA GAC ACG GCT GTC  336
Thr Leu Phe Leu Gln Met Asp Ser Leu Asn Thr Glu Asp Thr Ala Val
        80                  85                  90

TAC TAC TGT GCG AGC CCT TAT TGT GGT AAT GCC AGC TGC TAT GCT CCC  384
Tyr Tyr Cys Ala Ser Pro Tyr Cys Gly Asn Ala Ser Cys Tyr Ala Pro
     95                 100                 105

TAC TTC TAC TAC AAT ATT GAC GTC TGG GGC CAA GGG ACC ACG GTC ACC  432
Tyr Phe Tyr Tyr Asn Ile Asp Val Trp Gly Gln Gly Thr Thr Val Thr
110                 115                 110                 115

GTC TCC TCA                                                     411
Val Ser Ser
```

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 137 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu Leu Arg Gly
-19 -10

Val Gln Cys Gln Val His Leu Val Glu Ser Gly Gly Gly Val Val Gln
+1 5 10

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Tyr Phe
15 20 25

Ser Ser Tyr His Val Tyr Trp Val Arg Gln Ala Pro Ala Lys Gly Leu
30 35 40 45

Glu Trp Val Ala Val Ile Ser Tyr Asp Gly Leu Lys Thr Ser Tyr Ala
50 55 60

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Ser
65 70 75

Thr Leu Phe Leu Gln Met Asp Ser Leu Asn Thr Glu Asp Thr Ala Val
80 85 90

Tyr Tyr Cys Ala Ser Pro Tyr Cys Gly Asn Ala Ser Cys Tyr Ala Pro
95 100 105

Tyr Phe Tyr Tyr Asn Ile Asp Val Trp Gly Gln Gly Thr Thr Val Thr
110 115 110 115

Val Ser Ser

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 399 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

ATG AGG CTC CCT GCT CAG CTC CTG GGG CTG CTA GTG CTC TGG GTC CCA 48
Met Arg Leu Pro Ala Gln Leu Leu Gly Leu Leu Val Leu Trp Val Pro
-20 -15 -10 -5

GCA TCC AGT GGG GAA GTT GTG ATG AGT CAG TCT CCA CTC TCC CTG CCC 96
Ala Ser Ser Gly Glu Val Val Met Ser Gln Ser Pro Leu Ser Leu Pro
+1 5 10

```
GTC ACC CTT GGA CAG CCG GCC TCC ATC TCT TGC AGG TCT AAT CGA AGC  144
Val Thr Leu Gly Gln Pro Ala Ser Ile Ser Cys Arg Ser Asn Arg Ser
        15                  20                  25

CTC GTC AGC AGC GAT AGA AAC ACC TAC TTG AAT TGG TTT CAA CAC AGG  192
Leu Val Ser Ser Asp Arg Asn Thr Tyr Leu Asn Trp Phe Gln His Arg
    30                  35                  40

CCA GGC CAA TCT CCA AGG CGC CTA ATT TAT AAG GTT TCT AAC CGG GAC  240
Pro Gly Gln Ser Pro Arg Arg Leu Ile Tyr Lys Val Ser Asn Arg Asp
45                  50                  55                  60

TCT GGG GTC CCA GAC AGA TTC AGC GGC AGT GGG TCA GAC ACT GAT TTT  288
Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Asp Thr Asp Phe
                65                  70                  75

ACA TTG AAA ATC AGC AGG GTG GAG GCT GAG GAT GTT GGG GTT TAC TAC  336
Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
            80                  85                  90

TGC ATG CAA GGT ACA TGG GGG CCT CCG GGG ACG TTC GGC CAA GGG ACC  384
Cys Met Gln Gly Thr Trp Gly Pro Pro Gly Thr Phe Gly Gln Gly Thr
        95                  100                 105

AAG GTG GAA ATC AAA                                             399
Lys Val Glu Ile Lys
    110
```

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 133 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

```
Met Arg Leu Pro Ala Gln Leu Leu Gly Leu Leu Val Leu Trp Val Pro
-20                 -15                 -10                 -5

Ala Ser Ser Gly Glu Val Val Met Ser Gln Ser Pro Leu Ser Leu Pro
                +1                  5                   10

Val Thr Leu Gly Gln Pro Ala Ser Ile Ser Cys Arg Ser Asn Arg Ser
        15                  20                  25

Leu Val Ser Ser Asp Arg Asn Thr Tyr Leu Asn Trp Phe Gln His Arg
    30                  35                  40
```

```
Pro Gly Gln Ser Pro Arg Arg Leu Ile Tyr Lys Val Ser Asn Arg Asp
 45                  50                  55                  60

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Asp Thr Asp Phe
                 65                  70                  75

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
             80                  85                  90

Cys Met Gln Gly Thr Trp Gly Pro Pro Gly Thr Phe Gly Gln Gly Thr
         95                  100                 105

Lys Val Glu Ile Lys
    110
```

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 426 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

```
ATG GGG TCA ACC GCC ATC CTC GCC CTC CTC CTG GCT GTT CTC CAA GGA   48
Met Gly Ser Thr Ala Ile Leu Ala Leu Leu Leu Ala Val Leu Gln Gly
               -15                 -10                 -5

GTC TGT GCC GAG GTG CAG CTG GTG CAG TCT GGA GCA GAG GTG AAA AAG   96
Val Cys Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
            +1                  5                  10

CCG GGG GAG TCT CTG AAA ATC TCC TGT AAG GGT TCC GGA TAT ACC TTT  144
Pro Gly Glu Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Thr Phe
     15                  20                  25

GGC AAC TAC TGG ATC GGC TGG GTG CGC CAG ATG CCC GGG AAA GGC CTG  192
Thr Ser Tyr Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu
 30                  35                  40                  45

GAG TGG ATG GGA TCC ATC TAT CCT CTT GAC TCT GAT ACC AGA TAC AGC  240
Glu Trp Met Gly Ser Ile Tyr Pro Leu Asp Ser Asp Thr Arg Tyr Ser
                 50                  55                  60

CCG TCC TTC GAA GGC CAG GTC ACC ATC TCG GCC GAC AAG TCC ATC AAC  288
Pro Ser Phe Glu Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser
             65                  70                  75
```

ACC GCC TAC CTG CAG TGG AGC AGC CTG AAG GCC TCG GAC ACC GCC ATG 336
Thr Ala Tyr Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met
80 85 90

TAT TAC TGT GCG AGA GTC GAT TCG ATA CTA TAT ACT AGA GTC TAC TAC 384
Tyr Tyr Cys Ala Arg Val Asp Ser Ile Leu Tyr Thr Arg Val Tyr Tyr
95 100 105

TTT GAC CCC TGG GGC CAG GGA ACC CCG GTC AGC GTC TCC TCA 426
Phe Asp Pro Trp Gly Gln Gly Thr Pro Val Ser Val Ser Ser
110 115 120

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 142 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Met Gly Ser Thr Ala Ile Leu Ala Leu Leu Leu Ala Val Leu Gln Gly
-15 -10 -5

Val Cys Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
+1 5 10

Pro Gly Glu Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Thr Phe
15 20 25

Thr Ser Tyr Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu
30 35 40 45

Glu Trp Met Gly Ser Ile Tyr Pro Leu Asp Ser Asp Thr Arg Tyr Ser
50 55 60

Pro Ser Phe Glu Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser
65 70 75

Thr Ala Tyr Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met
80 85 90

Tyr Tyr Cys Ala Arg Val Asp Ser Ile Leu Tyr Thr Arg Val Tyr Tyr
95 100 105

Phe Asp Pro Trp Gly Gln Gly Thr Pro Val Ser Val Ser Ser
110 115 120

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 399 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

```
ATG GAA ACC CCA GCG CAG CTT CTC TTC CTC CTG CTA CTC TGG CTC CCA   48
Met Glu Thr Pro Ala Gln Leu Leu Phe Leu Leu Leu Trp Leu Pro
      -20                 -15                 -10

GTT TCA GAT ACC ACT GGA GAA ATA GTG ATG ACG CAG TCT CCA GCC ACC   96
Val Ser Asp Thr Thr Gly Glu Ile Val Met Thr Gln Ser Pro Ala Thr
   -5                  +1                  5                  10

CTG TCT GTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC AGG GCC AGT  144
Leu Ser Val Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser
               15                  20                  25

CAG AGT ATT AGC AAC AAC TTA GCC TGG TAC CAG CAG AGG CCT GGC CAG  192
Gln Ser Ile Ser Asn Asn Leu Ala Trp Tyr Gln Gln Arg Pro Gly Gln
           30                  35                  40

GCT CCC AGG CTC CTC ATC TAT GGT GCA TCC ACC AGG GCC ACT GGT ATC  240
Ala Pro Arg Leu Leu Ile Tyr Gly Ala Ser Thr Arg Ala Thr Gly Ile
       45                  50                  55

CCA GCC AGG TTC AGT GGA AGT GGG TCT GGG ACA GAG TTC ACT CTC ACC  288
Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr
   60                  65                  70

ATC AGC AGC CTG CAG TCT GAA GAT TGT GCA GTT TAT TAC TGT CAG CAG  336
Ile Ser Ser Leu Gln Ser Glu Asp Cys Ala Val Tyr Tyr Cys Gln Gln
75                  80                  85                  90

TAT GAC AAC TGG CCC CTA TGG GCG TGG GCG TTC GGA CCA GGG ACC ACG  384
Tyr Asp Asn Trp Pro Leu Trp Ala Trp Thr Phe Gly Gln Gly Thr Thr
               95                  100                 105

GTG GAA ATC AAA CGA                                             399
Val Glu Ile Lys Arg
           110
```

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 133 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Met Glu Thr Pro Ala Gln Leu Leu Phe Leu Leu Leu Trp Leu Pro
-20 -15 -10

Val Ser Asp Thr Thr Gly Glu Ile Val Met Thr Gln Ser Pro Ala Thr
-5 +1 5 10

Leu Ser Val Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser
15 20 25

Gln Ser Ile Ser Asn Asn Leu Ala Trp Tyr Gln Gln Arg Pro Gly Gln
30 35 40

Ala Pro Arg Leu Leu Ile Tyr Gly Ala Ser Thr Arg Ala Thr Gly Ile
45 50 55

Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr
60 65 70

Ile Ser Ser Leu Gln Ser Glu Asp Cys Ala Val Tyr Tyr Cys Gln Gln
75 80 85 90

Tyr Asp Asn Trp Pro Leu Trp Ala Trp Thr Phe Gly Gln Gly Thr Thr
95 100 105

Val Glu Ile Lys Arg
110

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CCCGAATTCA TGGACTGGAC CTGGAGG 27

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

CCCGAATTCA TGGACATACT TTGTACCAC 29

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

CCCGAATTCA TGGAGTTTGG GCTGAGC 27

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

CCCGAATTCA TGAAACACCT GTGGTTCTT 29

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

CCCGAATTCA TGGGGTCAAC CGCCATCCT 29

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

CCCGAATTCA TGTCTGTCTC CTTCCTCAT 29

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

GAGAGAGCGG CCGCACTCAT TTACCCGGAG A 31

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

GAGAGAGCGG CCGCTAACAC TCTCCCCTGT TGAA 34

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 38 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

GAGAGAGCGG CCGCCTATGA ACATTCTGTA GGGGCCAC 38

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

CCCGAATTCA TGGACATGAG GGTCCCCGCT CAGCTC 36

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

CCCGAATTCA TGGACACGAG GGCCCCCACT CAG 33

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

CCCGAATTCA TGGTGTTGCA GACCCAGGT 29

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

CCCGAATTCA TGAGGCTCCC TGCTCAGCTC CTG 33

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

CCCGAATTCA TGGAAACCCC AGCGCAGCT 29

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

CCCGAATTCA TGGGGTCCCA GGTTCACCTC 30

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

CCCGAATTCA TGACCTGCTC CCCTCTCCT 29

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

CCCGAATTCA TGGCCTGGAC TCCTCTCTTT CTG 33

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

CCCGAATTCA TGGCCTGGGC TCCACTACT 29

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

CCCGAATTCA TGGCATGGAT CCCTCTCTT 29

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

CCCGAATTCA TGGCCTGGGC TCTGCTGCTC 30

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

ACCTATAAAT ATTCCGGATT ATTCA 25

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

TCTTGCCGGG TCCCAGG 17

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

GGTCTCCAAC AAAGCCCTCC C 21

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

CACAAGCCCA GCAACACCAA GGTGGAC 27

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

GGGGGGAAGA GGAAGACTGA CGGTCC 26

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

GGGTGTACAC CTGTGGTTCT CGGGGCTG 28

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

GCAGGTGTAG GTCTGGGTGC C 21

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

TGGCGGGAAG ATGAAGACAG 20

CLAIMS:

1. An anti-allergic agent comprising human monoclonal antibody which binds to an airborne allergen that provokes an allergic IgE response, an allergen-binding fragment of such human monoclonal antibody, or an immune complex of such human monoclonal antibody or allergen-binding fragment with said allergen.

2. An anti-allergic agent according to claim 1 which is a human monoclonal antibody of the IgG class.

3. An anti-allergic agent according to claim 1 which is a human monoclonal antibody which binds to birch or house dust mite antigen.

4. A human monoclonal antibody according to claim 3, wherein the allergen to which the antibody binds comprises Bet vI, Bet vII, Der pI or Der pII.

5. A human monoclonal antibody or a fragment thereof comprising at least one CDR of an amino acid sequence defined by amino acid residues 1-127 of SEQ ID NO. 2, 1-114 of SEQ ID NO. 4, 1-127 of SEQ ID NO. 6, 1-107 of SEQ ID NO. 8, 1-119 of SEQ ID NO. 10, 1-108 of SEQ ID NO. 12, 1-118 of SEQ ID NO. 14, 1-113 of SEQ ID NO. 16, 1-123 of SEQ ID NO. 18, and/or 1-111 of SEQ ID NO. 20; or one or more somatic variant of such sequence.

6. A human monoclonal antibody or a binding fragment thereof comprising:

a V_H segment having an amino acid sequence defined by amino acid residues 1-127 of SEQ ID NO. 2, 1-127 of SEQ ID NO. 6, 1-119 of SEQ ID NO. 10, 1-118 of SEQ ID NO. 14, or 1-123 of SEQ ID NO. 18, or by a CDR somatic variant of one of said sequences, and/or

a V_L segment having an amino acid sequence defined by amino acid residues 1-114 of SEQ ID NO. 4, 1-107 of SEQ ID NO. 8, 1-108 of SEQ ID NO. 12, 1-113 of SEQ ID NO. 16, or 1-111 of SEQ ID NO. 20, or by a CDR somatic variant of one of said sequences.

7. The antibody according to claim 6 which comprises a V_H segment having an amino acid sequence defined by amino acid residues 1-127 of SEQ ID NO. 2, 1-127 of SEQ ID NO. 6, 1-119 of SEQ ID NO. 10, 1-118 of SEQ ID NO. 14, or 1-123 of SEQ ID NO. 18 and/or comprises a V_L segment having an amino acid sequence defined by amino acid residues 1-114 of SEQ ID NO. 4.

1-107 of SEQ ID NO. 8, 1-108 of SEQ ID NO. 12, 1-113 of SEQ ID NO. 16, or 1-111 of SEQ ID NO. 20, respectively.

8. The antibody according to claim 6 which comprises a V_H segment having the amino acid sequence defined by amino acid residues 1-127 of SEQ ID NO. 2, 1-127 of SEQ ID NO. 6, 1-119 of SEQ ID NO. 10, 1-118 of SEQ ID NO. 14, or 1-123 of SEQ ID NO. 18, and a V_L segment having the amino acid residues 1-114 of SEQ ID NO. 4, 1-107 of SEQ ID NO. 8, 1-108 of SEQ ID NO. 12, 1-113 of SEQ ID NO. 16, or 1-111 of SEQ ID NO. 20, or which comprises a CDR somatic variant of one or both of said amino acid sequences.

9. The antibody according to claim 6 which comprises a V_H segment having the amino acid sequence defined by amino acid residues 1-127 of SEQ ID NO. 2, 1-127 of SEQ ID NO. 6, 1-119 of SEQ ID NO. 10, 1-118 of SEQ ID NO. 14, or 1-123 of SEQ ID NO. 18, and a V_L segment having the amino acid residues 1-114 of SEQ ID NO. 4, 1-107 of SEQ ID NO. 8, 1-108 of SEQ ID NO. 12, 1-113 of SEQ ID NO. 16, or 1-111 of SEQ ID NO. 20, respectively.

10. The antibody according to claim 7, 8 or 9 which is of the IgG_4 isotype.

11. The fragment according to claim 6 which comprises a V_H segment having an amino acid sequence defined by amino acid residues 1-127 of SEQ ID NO. 2, 1-127 of SEQ ID NO. 6, 1-119 of SEQ ID NO. 10, 1-118 of SEQ ID NO. 14, or 1-123 of SEQ ID NO. 18, and/or a V_L segment having an amino acid sequence defined by amino acid residues 1-114 of SEQ ID NO. 4, 1-107 of SEQ ID NO. 8, 1-108 of SEQ ID NO. 12, 1-113 of SEQ ID NO. 16, or 1-111 of SEQ ID NO. 20.

12. The fragment according to claim 11, which comprises a Fv, single chain Fv, Fab or $F(ab')_2$ fragment.

13. An anti-allergic agent comprising human monoclonal antibody which inhibits the allergen-induced degranulation of IgE-sensitized basophils/mast cells, an allergen-binding fragment of such human monoclonal antibody, or an immune complex of such human monoclonal antibody or allergen-binding fragment with said allergen.

14. A human B-cell line established by EBV transformation and CD40 cross linking, which established cell line produces a human monoclonal antibody according to any one of claims 1-10.

15. A human monoclonal antibody-producing clone of an established human B-cell line according to claim 14.

16. Purified and isolated DNA which encodes the heavy and/or light chains of a human monoclonal antibody or allergen-binding fragment according to any one of claims 1-12.

17. An isolated nucleic acid which comprises:

a nucleotide sequence defined by base numbers 58-438 of SEQ ID NO. 1, 58-438 of SEQ ID NO. 5, 58-414 of SEQ ID NO. 9, 58-411 of SEQ ID NO. 13, or 58-426 of SEQ ID NO. 17, or by a CDR-encoding somatic variant of one of said sequences, and/or

a nucleotide sequence defined by base numbers 61-402 of SEQ ID NO. 3, 61-381 of SEQ ID NO. 7, 61-384 of SEQ ID NO. 11, 61-399 of SEQ ID NO. 15, or 67-399 of SEQ ID NO. 19, or by a CDR-encoding somatic variant of one of said sequences; or

a functional equivalent of one or both of said nucleotide sequences.

18. An isolated nucleic acid according to claim 16 comprising a nucleotide sequence selected from a sequence defined by base numbers 58-438 of SEQ ID NO. 1, 58-438 of SEQ ID NO. 5, 58-414 of SEQ ID NO. 9, 58-411 of SEQ ID NO. 13, or 58-426 of SEQ ID NO. 17, and/or a nucleotide sequence selected from a sequence defined by base numbers 61-402 of SEQ ID NO. 3, 61-381 of SEQ ID NO. 7, 61-384 of SEQ ID NO. 11, 61-399 of SEQ ID NO. 17, or 67-399 of SEQ ID NO. 19.

19. A pharmaceutical composition comprising at least one anti-allergic agent according to any one of claims 1-13 and a pharmaceutically acceptable carrier.

20. The use of an anti-allergic agent according to any one of claims 1-13 or a mixture of such agents for the preparation of a pharmaceutical composition to treat allergic reactions.

21. A process for making a human monoclonal antibody to a desired antigen comprising the steps of

establishing an immortalized human B-cell population from a patient having antibodies that bind to the desired antigen, said immortalization comprising infecting the B-cells with Epstein-Barr virus and crosslinking the CD40 of such B-cells;

culturing said immortalized B-cells;

isolating multiple clones from such immortalized B-cells, each of which clones secretes a human monoclonal antibody that binds to the desired antigen;

and using one or more of such clones to produce one or more human monoclonal antibody or an antigen-binding fragment of such human monoclonal antibody.

22. A process according to claim 21, wherein nucleic acid encoding a human monoclonal antibody or an antigen-binding fragment thereof is used to produce the desired human monoclonal antibody or antigen-binding fragment.

23. A process according to claim 21, wherein said clone is hybridized with a myeloma or heteromyeloma cell to produce a hybridoma that proliferates in culture and produces the desired human monoclonal antibody.

24. A human monoclonal antibody produced by a process according to any one of claims 21 to 23.