

Description**Field of the Invention**

5 The invention relates generally to monoclonal antibodies and their associated hybridomas, and more particularly to monoclonal antibodies specific for human interleukin-4.

BACKGROUND

10 Human interleukin-4 (IL-4) was first cloned and characterized by Yokota et al., *Proc. Natl. Acad. Sci.*, Vol. 83, pgs. 5894-5898 (1986). IL-4 is a highly pleiotropic lymphokine which affects many different components of the immune system. It has T cell growth factor (TCGF) activity, and B cell growth factor activity. It is capable of potentiating the TCGF activity of interleukin-2 (IL-2) and the colony-forming activity of granulocyte-macrophage colony-stimulating factor (GM-CSF). It induces the preferential production of
 15 IgG₁ and IgE, and induces the expression of human leukocyte class II DR antigens. These activities suggest several possible therapeutic uses, e.g. as a potentiating agent for IL-2 anticancer therapy, as a potentiating agent for GM-CSF-stimulated bone marrow regeneration, or as an agent to treat bare lymphocyte syndrome: Touraine, *Lancet*, Pgs. 319-321 (February 7, 1981); Touraine and Betuel, *Human Immunology*, Vol. 2, pgs. 147-153 (1981); and Sullivan et al., *J. Clin. Invest.*, Vol. 76, pgs. 75-79 (1985).

20 International Patent Application No. WO 87/02990 describes and claims mammalian proteins and muteins thereof designated interleukin-4s. Products specifically described include native human and murine IL-4s, muteins thereof.

The IgE-inducing activity of IL-4 could have important consequences for persons suffering from allergic diseases. The availability of IL-4 antagonists could provide an alternative to the use of glucocorticoid
 25 steroids, which have many deleterious side effects, especially with prolonged usage: e.g. Goodman and Gillman, *The Pharmacological Basis of Therapeutics*, 6th Ed. (MacMillan Publishing Company, New York, 1980). In particular, strongly blocking monoclonal antibodies specific for human IL-4 provide a means for constructing antagonists by generating anti-idiotypic antibodies, e.g. U.S. patent 4,731,237, or by mimotope screening, e.g. Geysen et al., *J. Immunol. Meth.*, Vol. 102, pgs. 259-274 (1987); or PCT patent applications
 30 WO 86/00991 and WO 86/06487.

An important aspect of any therapy involving drugs is the ability to predict and/or monitor concentration levels in the patient's blood or other body fluids. Monoclonal antibodies are widely used for this purpose: e.g. Springer, ed., *Hybridoma Technology in the Biosciences and Medicine* (Plenum Press, N.Y., 1985); and U.S. Patents 4,562,003, 4,486,530, and 4,255,329.

35 In the production of genetically engineered proteins such as IL-4, separation of the expressed protein from the transformed host cells and/or their culture supernatants is a major problem. Frequently separation procedures involve one or more passes of crude material through immunoadsorbent columns. Monoclonal antibodies specific for the protein to be purified are crucial elements of such columns. Such monoclonal antibodies can also be used to measure the degree of purification achieved by a particular protocol, e.g. by
 40 "Western" blot analysis, Burnette, *Anal. Biochem.*, Vol. 112, pgs. 195-203 (1981).

From the foregoing it is evident that the availability of monoclonal and/or polyclonal antibodies specific for IL-4 could facilitate its medical and veterinary applications by improving current methods of purification, and by providing means for monitoring concentrations of IL-4 in body fluids, such as blood, urine, or the like.

SUMMARY OF THE INVENTION

45 The invention provides compounds and compositions useful for the detection, purification, measurement and/or inhibition of human IL-4. The compounds and compositions are derived from hybridomas IC1.11B4.6 and MP4.25D2.11 producing monoclonal antibodies specific for human IL-4. The compounds and compositions of the invention include the hybridomas themselves, monoclonal antibodies produced by the hybridomas, heavy chain and light chain variable region polypeptides and other fragments of such antibodies such as half-molecules comprising a light chain joined to a heavy chain by natural disulfide bonds, Fab fragments, F(ab)₂ fragments, Fv fragments, and the like. The invention also includes methods of
 50 using the above compounds and compositions to detect, purify, and measure the concentration of human IL-4, and kits for practising such methods.

Compositions of the invention may also be useful as agonists or antagonists of human IL-4. In particular, compositions comprising fragments of antagonistic anti-IL-4 antibodies, e.g., monoclonal anti-

bodies of MP4.25D2.11, are useful therapeutic agents for treating atopic diseases.

Compositions of the invention also include messenger RNA (mRNA) extracted from hybridomas IC1.11B4.6 and MP4.25D2.11. Such mRNAs are useful in cloning and expressing fragments of the IC1.11B4.6 and MP4.25D2.11 antibodies in bacteria, yeast, or other hosts.

Antibodies comprise an assembly of polypeptide chains linked together by disulfide bridges. Two principal polypeptide chains, referred to as the light chain and the heavy chain, make up all major structural classes (isotypes) of antibody. Both heavy chains and light chains are further divided into subregions referred to as variable regions and constant regions. Heavy chains comprise a single variable region and three different constant regions, and light chains comprise a single variable region (different from that of the heavy chain) and a single constant region (different from those of the heavy chain). The variable regions of the heavy chain and light chain are responsible of the antibody's binding specificity.

As used herein, the term "heavy chain variable region" means a polypeptide (1) which is from 110 to 125 amino acids in length, and (2) whose amino acid sequence corresponds to that of a heavy chain of a monoclonal antibody of the invention, starting from the heavy chain's N-terminal amino acid. Likewise, the term "light chain variable region" means a polypeptide (1) which is from 95 to 115 amino acids in length, and (2) whose amino acid sequence corresponds to that of a light chain of a monoclonal antibody of the invention, starting from the light chain's N-terminal amino acid.

The terms Fab, Fc, F(ab)₂, and Fv are employed with their standard immunological meanings: e.g. Klein, *Immunology* (John Wiley, New York, 1982), or Parham, Chapter 14, in Weir, ed. *Immunochemistry*, 4th Ed. (Blackwell Scientific Publishers, Oxford, 1986).

As used herein the term "monoclonal antibody" refers to homogenous populations of immunoglobulins which are capable of specifically binding to human IL-4. It is understood that human IL-4 may have one or more antigenic determinants comprising (1) peptide antigenic determinants which consist of single peptide chains within human IL-4, (2) conformational antigenic determinants which consist of more than one spatially contiguous peptide chains whose respective amino acid sequences are located disjointly along the human IL-4 polypeptide sequence; and (3) post-translational antigenic determinants which consist, either in whole or part, of molecular structures covalently attached to human IL-4 after translation, such as carbohydrate groups, or the like. The antibodies of the invention may be directed against one or more of these determinants.

As used herein the term "binding composition" means a composition comprising two polypeptide chains (1) which, when operationally associated, assume a conformation having high binding affinity for human IL-4, and (2) which are derived from a hybridoma producing monoclonal antibodies specific for human IL-4. The term "operationally associated" is meant to indicate that the two polypeptide chains can be positioned relative to one another for binding by a variety of means, including association in a native antibody fragment, such as Fab or Fv, or by way of genetically engineered cysteine-containing peptide linkers at the carboxyl termini. Normally, the two polypeptide chains correspond to the light chain variable region and heavy chain variable region of a monoclonal antibody specific for human IL-4.

Brief Description of the Figures

Figure 1 is a diagrammatic representation of an expression vector suitable for expressing unglycosylated human IL-4 in a bacterial host;

Figure 2 illustrates the 215 nm absorption profile in the final purification step of human IL-4; and Figure 3 (parts A and B) shows neutralization of ¹²⁵I-CHO HuIL-4 binding to Daudi cells.

DETAILED DESCRIPTION OF THE INVENTION

Hybridomas of the invention are produced by well-known techniques. Usually, the process involves the fusion of an immortalizing cell line with a B-lymphocyte that produces the desired antibody. Alternatively, non-fusion techniques for generating immortal antibody-producing cell lines are possible, and come within the purview of the present invention, e.g. virally induced transformation: Casali et al., "Human Monoclonals from Antigen-Specific Selection of B Lymphocytes and Transformation by EBV", *Science*, Vol. 234, pgs. 476-479 (1986). Immortalizing cell-lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine, and human origin. Most frequently, rat or mouse myeloma cell-lines are employed as a matter of convenience and availability.

Techniques for obtaining the appropriate lymphocytes from mammals injected with the target antigen are well known. Generally, peripheral blood lymphocytes (PBLs) are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. A host

mammal is injected with repeated dosages of the purified antigen, and the mammal is permitted to generate the desired antibody-producing cells before these are harvested for fusion with the immortalizing cell-line. Techniques for fusion are also well known in the art, and in general involve mixing the cells with a fusing agent, such as polyethylene glycol. Hybridomas are selected by standard procedures, such as HAT selection. From among these hybridomas, those secreting the desired antibody are selected by assaying their culture medium by standard immunoassays, such as Western blotting, ELISA, RIA, or the like. Antibodies are recovered from the medium using standard protein-purification techniques, e.g. Tijssen, *Practice and Theory of Enzyme Immunoassays* (Elsevier, Amsterdam, 1985). Many references are available for guidance in applying any of the above techniques: e.g. Kohler et al., *Hybridoma Techniques* (Cold Spring Harbor Laboratory, New York, 1980); Tijssen, *Practice and Theory of Enzyme Immunoassays* - (Elsevier, Amsterdam, 1985); Campbell, *Monoclonal Antibody Technology* (Elsevier, Amsterdam, 1984); Hurrell, *Monoclonal Hybridoma Antibodies: Techniques and Applications* (CRC Press, Boca Raton, FL, 1982); and the like.

The use and generation of fragments of antibodies is also well known; e.g. Fab fragments: Tijssen, *Practice and Theory of Enzyme Immunoassays* (Elsevier, Amsterdam, 1985); and Fv fragments: Hochman et al. *Biochemistry*, Vol. 12, pgs. 1130-1135 (1973), Sharon et al., *Biochemistry*, Vol. 15, pgs 1591-1594 (1976) and Ehrlich et al., U.S. Patent 4,355,023; and antibody half molecules: Auditore-Hargreaves, U.S. Patent 4,470,925. Moreover, such compounds and compositions of the invention can be used to construct bi-specific antibodies by known techniques; e.g., by further fusions of hybridomas (i.e. to form so-called quadromas): Reading, U.S. Patent 4,474,493; or by chemical reassociation of half molecules: Brennan et al., *Science*, Vol. 229, pgs. 81-83 (1985).

Hybridomas and monoclonal antibodies of the invention are produced against either glycosylated or unglycosylated versions of recombinantly-produced mature human IL-4. Generally, unglycosylated versions of human IL-4 are produced in *E. coli*, and glycosylated versions are produced in mammalian cell hosts, e.g. CVI or COS monkey cells, mouse L cells, or the like. Recombinantly-produced mature human IL-4 is produced by introducing an expression vector into a host cell using standard protocols; e.g. Maniatis et al., *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982); Okayama and Berg, *Mol. Cell. Biol.*, Vol. 2, pgs. 161-170 (1982) and Vol. 3, pgs. 280-289 (1983); Hamer, *Genetic Engineering*, Vol. 2, pgs. 83-100 (1980) and U.S. Patent 4,599,308; Kaufman et al., *Mol. Cell. Biol.*, Vol. 2, pgs. 1304-1319 (1982); or the like.

Construction of bacterial or mammalian expression vectors is well known in the art, once the nucleotide sequence encoding a desired protein is known or otherwise available; e.g. DeBoer in U.S. Patent 4,511,433 discloses promoters for use in bacterial expression vectors; Goeddel et al., in U.S. Patent 4,601,980, and Riggs, in U.S. Patent 4,431,739, disclose the production of mammalian proteins by *E. coli* expression systems; and Riggs (cited above), Ferretti et al., *Proc. Natl. Acad. Sci.*, Vol. 83, pgs. 599-603 (1986), Sproat et al., *Nucleic Acids Research*, Vol. 13, pgs. 2959-2977 (1985), and Mullenbach et al., *J. Biol. Chem.*, Vol. 261, pgs. 719-722 (1986), disclose how to construct synthetic genes for expression in bacteria. Accordingly, these references are incorporated by reference. The amino acid sequence of mature human IL-4 is disclosed by Yokota et al. (cited above), and the cDNA encoding human IL-4 carried by the pcD vector described by Yokota et al. (cited above) is deposited with the American Type Culture Collection (ATCC), Rockville, MD, under accession number 67029. Many bacterial expression vectors and hosts are available commercially or through the ATCC. Preferably, human IL-4 for immunizing host animals is isolated from culture supernatants of COS, CVI, or mouse L cells which have been transiently transfected by the above-mentioned pcD vector.

Antibodies and antibody fragments characteristic of hybridomas of the invention, particularly IC1.11B4.6, can also be produced by recombinant means by extracting messenger RNA, constructing a cDNA library, and selecting clones which encode segments of the antibody molecule: e.g. Wall et al., *Nucleic Acids Research*, Vol. 5, pgs. 3113-3128 (1978); Zalsut et al., *Nucleic Acids Research*, Vol. 8, pgs. 3591-3601 (1980); Cabilly et al., *Proc. Natl. Acad. Sci.*, Vol. 81, pgs. 3273-3277 (1984); Boss et al., *Nucleic Acids Research*, Vol. 12, pgs. 3791-3806 (1984); Amster et al., *Nucleic Acids Research*, Vol. 8, pgs. 2055-2065 (1980); and Moore et al., U.S. Patent 4,642,234. In particular, such techniques can be used to produce interspecific monoclonal antibodies, wherein the binding region of one species is combined with a non-binding region of the antibody of another species; e.g. Liu et al., *Proc. Natl. Acad. Sci.*, Vol. 84, pgs. 3439-3443 (1987).

Uses of monoclonal antibodies for purification and measurement are well known, e.g. affinity chromatography: *Affinity Chromatography: Principles and Methods* (Pharmacia, Orebro, Sweden, 1979); Secher et al., *Nature*, Vol. 285, pgs. 446-450 (1980), and U.S. Patent 4,423,147; and European patent application 0190711 (13 August 1986); and immunoassay techniques: Tijssen (cited above); U.S. Patent 4,486,530; and Burnette

(cited above). Affinity chromatography can be used to purify human IL-4 by extracting it from a sample, such as a culture supernatant of cells transformed or transfected with a human IL-4 expression vector. Such a purification process is referred to herein as an immunopurification process. Typically, it involves covalently attaching a monoclonal antibody specific for human IL-4 to a solid-phase support (referred to herein as an "immunoabsorbent") which is placed in a column or chamber through which the sample is passed. Human IL-4 from the sample preferentially binds to the binding sites of the attached monoclonal antibodies, while the rest of the material from the sample is washed from the column or chamber. The human IL-4 is then eluted from the immunoabsorbent by standard techniques, e.g. low pH, high salt concentration, or the like.

"Two site" or "sandwich" immunoassays are the preferred immunoassays of the invention, e.g. as disclosed in U.S. Patent 4,486,530. Accordingly, this patent is incorporated by reference. Such assays entail the use of two different sets of anti-IL-4 antibodies, at least one of which consists of a monoclonal antibody of the invention, such as that produced by IC1.11B4.6. Antibodies from one of the two sets are attached to the surface of a solid-phase support. The attached antibodies are then exposed to a sample suspected of containing human IL-4. The IL-4 molecules bind to the attached antibodies. Next, the second set of antibodies is applied to the bound IL-4, and these bind to one or more antigenic determinants distinct from that (or those) to which the first set of antibodies is bound. The IL-4 is then detected by an indirect or direct signal-generating means associated with the second set of antibodies. For example, the antibodies can be directly conjugated to a signal-generating moiety, such as an enzyme, rare earth chelator, or an organic dye. Or they can be indirectly linked to one or more signal-generating moieties through additional antibodies, or high affinity complexes, such as the avidin-biotin complexes. Quantitative measurements of IL-4 concentration are made by comparing the signal generated by the sample with signals generated by IL-4 standards containing known concentrations of human IL-4.

The invention includes kits of reagents for use in immunoassays, particularly sandwich immunoassays. Such kits include (1) a solid-phase support, (2) a first antibody which is monoclonal and is capable of binding to a first antigenic determinant of human IL-4, (3) a second antibody selected from the group consisting of a monoclonal antibody capable of binding to a second antigenic determinant of human IL-4 and a polyclonal antibody specific for human IL-4 (referred to herein as a "polyclonal antibody composition"), and (4) a signal-generation means associated with one of the three antibodies. Depending on the particular embodiment, kits may include a selection of two of the three anti-IL-4 antibody types, either a monoclonal antibody specific for a first antigenic determinant and a monoclonal antibody specific for a second antigenic determinant, or a monoclonal antibody specific for a first or second antigenic determinant and a polyclonal antibody composition. The antibodies may be in solution or in lyophilized form. One of the sets of antibodies may be available for application to the surface of the solid support when the kit is used, or may come pre-attached to the solid support. The signal-generating means may come pre-associated with one of the two antibody types, or may require combination with one or more components, e.g. buffers, antibody-enzyme conjugates, enzyme substrates, or the like, prior to use. Many types of signal-generating means are available and can make up one or more components of a kit. Various signal-generating means are disclosed by Tijssen (cited above). Kits of the invention may also include additional reagents, e.g. blocking reagents for reducing nonspecific binding to the solid-phase surface, washing reagents, enzyme substrates, and the like. The solid-phase surface may be in the form of microtiter plates, microspheres, or the like, composed of polyvinyl chloride, polystyrene, or the like materials suitable for immobilizing proteins. Such materials having solid-phase surfaces are referred to herein as "support means". Preferably, an enzyme which catalyzes the formation of a fluorescent or colored product is a component of the signal-generating means. More preferably, the enzyme is selected from the group consisting of peroxidase, alkaline phosphatase, and beta-galactosidase. Substrates and reaction conditions for these enzymes are well known in the art; e.g. Tijssen (cited above).

Compositions of the invention can also be used as components of pharmaceutical compositions directed against IL-4-related diseases. Pharmaceutical compositions that comprise monoclonal antibodies (or binding fragments thereof) having agonistic effects can be used to enhance IL-4 activity. Or compositions that comprise monoclonal antibodies (or binding fragments thereof) having blocking or antagonistic effects can be used to suppress IL-4 activity. Such compositions contain a therapeutic amount of at least one of the monoclonal antibodies of the invention, or binding fragments thereof, in a pharmaceutically effective carrier. A pharmaceutical carrier can be any compatible, non-toxic substance suitable for delivering the compositions of the invention to a patient. Sterile water, alcohol, fats, waxes, and inert solids may be included in a carrier. Pharmaceutically acceptable adjuvants (buffering agents, dispersing agents) may also be incorporated into the pharmaceutical composition. Generally, compositions useful for parenteral administration of such drugs are well known; e.g. Remington's Pharmaceutical Science, 15th Ed. (Mack Publishing

Company, Easton, PA, 1980). Alternatively, compositions of the invention may be introduced into a patient's body by implantable drug delivery systems; e.g. Urquhart et al., Ann. Rev. Pharmacol. Toxicol., Vol. 24, pgs. 199-236 (1984).

6 EXAMPLES

The following examples serve to illustrate the present invention. Selection of vectors and hosts as well as the concentration of reagents, temperatures, and the values of other variables are only to exemplify application of the present invention and are not to be considered limitations thereof.

10 Example I. Production of Glycosylated Human IL-4 by Transfection of COS 7 Monkey Cells with pcD-human-IL-4

The expression vector pcD-human-IL-4 and host cell COS 7 are available from the American Type
 15 Culture Collection under accession numbers 67029 and CRL 1651, respectively. The pcD-human-IL-4 clone was amplified, the plasmid DNA was purified, and then a standard transfection protocol was used to transfect COS 7: About 1×10^6 COS 7 cells are seeded onto 100 mm. tissue-culture plates containing Dulbecco's Modified Eagle's medium (DME), 10% fetal calf serum, and 4 mM L-glutamine. About 24 hours after seeding, the medium is aspirated from the plates and the cells are washed twice with serum-free
 20 buffered (50 mM Tris) DMF. To each plate is added 4 ml serum-free buffered DME (with 4 mM L-glutamine), 80 microliters DEAE-dextran, and 5 micrograms of pcD-human-IL-4 DNA. The cells are incubated in this mixture for 4 hours at 30°C, after which the mixture is aspirated off and the cells are washed once with serum-free buffered DME. After washing, 5 ml of DME with 4 mM L-glutamine, 100 µM chloroquine, and 2% fetal calf serum is added to each plate, and the cells are incubated for 3 hours, and
 25 then twice washed with serum-free buffered DME. Next, 5 ml DME with 4 mM L-glutamine and 4% fetal calf serum is added and the cells are incubated at 37°C for 24 hours. Afterwards the cells are washed 1-3 times with DME or PBS, 5 ml serum-free DME (with 4mM L-glutamine) is added, and the cells are incubated at 37°C until culture supernatants are harvested 5 days later.

30 Example II. Purification of Glycosylated Human IL-4 from COS 7 Transfection Supernatants

A. Biological Assay for Purification.

T cell growth factor (TCGF) activity was used to assay human IL-4 during purification from the
 35 supernatants produced according to Example I. Several standard assays have been described for TCGF activity: e.g. Devos et al., Nucleic Acids Research, Vol. 11, pgs. 4307-4323 (1983); Thurman et al., J. Biol. Response Modifiers, Vol. 5, pgs. 85-107 (1986); and Robert-Guroff et al., Chapter 9 in Guroff, Ed. Growth and Maturation Factors (John Wiley, New York, 1984). Generally, the TCGF assays are based on the ability
 40 of a factor to promote the proliferation of peripheral T lymphocytes or IL-2-dependent T cell lines; e.g. Gillis et al. J. Immunol., Vol. 120, pg. 2027 (1978). Proliferation can be determined by standard techniques, e.g. tritiated thymidine incorporation, or by colorimetric methods: Mosmann, J. Immunol. Meth., Vol. 65, pgs. 55-63 (1983). The assay for human IL-4 TCGF activity was carried out as follows: Blood from a healthy donor was drawn into a heparinized tube and layered onto Ficoll-Hypaque; e.g., 5 ml of blood per 3 ml Ficoll-Hypaque in a 15 ml centrifuge tube. After centrifugation at 3000 x g for 20 minutes, cells at the interface
 45 were aspirated and diluted in a growth medium consisting of RPMI 1640 containing 10% fetal calf serum, 50 µM 2-mercaptoethanol, 20 µg/ml phytohemagglutinin (PHA), and recombinant human IL-2. After 5-10 days of incubation at 37°C, the PHA-stimulated peripheral blood lymphocytes (PBLs) were washed and used in 2 day colorimetric assays (Mossmann, cited above). Serial two-fold dilutions of an IL-4 standard (supernatants from pcD-human-IL4-transfected COS 7 cells) or the fraction to be tested were performed in
 50 96-well trays utilizing the growth medium described above to yield a final volume of 50 µl/well. 50 µl of the PHA-stimulated PBLs at about $4-8 \times 10^6$ cells/ml were added to each well and the trays were incubated at 37°C for 2 days. Cell growth was then measured according to Mosmann (cited above).

One unit, as used herein, is the amount of factor which in one well (0.1 ml) stimulates 50% maximal proliferation of 2×10^4 PHA-stimulated PBLs over a 48 hour period.

B. Purification

Purification was accomplished by a sequential application of cation exchange chromatography, gel filtration and reverse-phase high pressure liquid chromatography. All operations were performed at 4°C.

The COS-7 cells were removed by centrifugation, and the supernatant was concentrated about 10-fold by ultrafiltration and stored at -80°C until further processed. IL-4 titers were determined by assaying for the ability of the protein to stimulate proliferation of phytohemagglutinin-induced human peripheral blood lymphocytes, i.e. by TCGF activity using the standard assay described above.

Concentrated COS-7 supernatant, having TCGF activity of about 10^4 - 10^6 units/ml and a protein content of about 15-20 mg/ml, is dialyzed against two changes of 50 mM sodium HEPES, pH 7.0 over a 24 hour period (each change being approximately 10-15 times the volume of one concentrate). The dialysate was applied to a column (1 x 2.5 cm) of S-Sepharose (flow rate: 0.2 ml/min) preequilibrated with 50 mM sodium HEPES, pH 7.0. The column was washed with 15 column volumes of equilibrating buffer and then eluted with 20 column volumes of a linear sodium chloride gradient extending from 0 to 0.5 M sodium chloride in 50 mM sodium HEPES, pH 7.0. The elution was terminated isocratically with 5 column volumes of 50 mM sodium HEPES, 0.5 M NaCl, pH 7.0. 1.5 ml and 1.8 ml fractions were collected from two separate batches. IL-4 titers were found for both chromatographies to elute between 300 mM and 500 mM sodium chloride.

The fractions from the S-Sepharose columns containing IL-4 titers were combined for total separate volumes of 9.0 and 10.8 ml. Both volumes were concentrated to 1.9 ml by ultrafiltration using an Amicon YM5 membrane (molecular weight cut-off: 5000). The recovery of protein from this step was about 80%. The concentrated IL-4 solution was applied to a Sephadex G-100 column (1.1 x 58 cm) pre-equilibrated in 50 mM HEPES, 0.4 M NaCl, pH 7.0, and the column was eluted with the same buffer at 0.15 ml/min. A total of 50 fractions (1.0 ml/fraction) was collected and analyzed for IL-4 titers. A peak in biological activity was observed at an apparent molecular weight of 22,000 daltons. The Sephadex G-100 was calibrated for apparent molecular determination with bovine serum albumin (65,000 daltons), carbonic anhydrase (30,000 daltons) and cytochrome C (11,700 daltons).

A fraction from the Sephadex G-100 column containing IL-4 activity was concentrated 3-4 fold in vacuo and was injected onto a Vydac C-4 guard column (4.6 x 20 mm). A linear gradient of 0 to 72% (v/v) acetonitrile in 0.1% (v/v) trifluoroacetic acid (TFA) was produced in 15 minutes at a column temperature of 35°C and a flow rate of 1.0 ml/min. Three peaks resulted that were detected at 214 nm with retention times of 7, 8.2 and 8.7 min. (peaks 1, 2, and 3 of Figure 2, respectively). A 40 µl aliquot of peak 2 (8.2 min. elution time) was lyophilized and redissolved in minimal essential medium containing 10% fetal calf serum. This solution showed a positive TCGF response. A 300 µl aliquot of peak 2 was evaporated to dryness and redissolved in 200 µl of 0.1% (w/v) sodium dodecyl sulfate (SDS). A 2 µl aliquot was diluted in 200 µl of 1% (v/v) TFA and rechromatographed. The HPLC of this sample demonstrated a single peak at 215 nm. Peak 2 material indicated an activity of about 7×10^8 units/mg.

Example III. Production of Unglycosylated Human IL-4 in *Escherichia coli*

An *E. coli* expression vector, denoted TRPC11, was constructed using standard techniques, e.g. as disclosed in Maniatis et al., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, New York, 1982).

The TRPC11 vector was constructed by ligating a synthetic consensus RBS fragment to *Cla*I linkers (ATGCAT) and by cloning the resulting fragments into *Cla*I-restricted pMT11hc (which had been previously modified to contain the *Cla*I site). pMT11hc is a small (2.3 kilobase) high copy, AMP^R, TET^S derivative of pBR322 that bears the *Eco*RI-*Hind*III polylinker region of the π VX plasmid (described by Maniatis et al., cited above). It was modified to contain the *Cla*I site by restricting pMT11hc with *Eco*RI and *Bam*HI, filling in the resulting sticky ends and ligating with *Cla*I linker (CATCGATG), thereby restoring the *Eco*RI and *Bam*HI sites and replacing the *Sma*I site with a *Cla*I site.

One transformant from the TRPC11 construction had a tandem RBS sequence flanked by *Cla*I sites. One of the *Cla*I sites and part of the second copy of the RBS sequence were removed by digesting this plasmid with *Pst*I, treating with *Bal*31 nuclease, restricting with *Eco*RI, and treating with T4 DNA polymerase in the presence of all four deoxynucleotide triphosphates. The resulting 30-40 bp fragments were recovered by polyacrylamide gel electrophoresis and cloned into *Sma*I-restricted pUC12. A 248 bp *E. coli* *trpP*-bearing *Eco*RI fragment derived from pKC101 (described by Nichols et al. in Methods in Enzymology, Vol. 101, pg. 155 (Academic Press, N.Y. 1983)) was then cloned into the *Eco*RI site to complete the TRPC11 construction, which is illustrated in Figure 1.

TRPC11 was employed as a vector for human IL-4 cDNA by first digesting it with ClaI and BamHI, purifying it, and then mixing it with the EcoRV/BamHI fragment of pcD-125 (deposited with the ATCC under accession number 67029) in a standard ligation solution containing 0.1 micromolar of the following synthetic linker:

5' - CG ATG CAC AAG TGC GAT - 3'
TAC GTG TTC ACG CTA

E. coli AB1899 was transformed directly with the ligation solution using the standard calcium chloride procedure, propagated, and plated. Colonies containing the IL-4 cDNA insert were selected using a labeled oligonucleotide probe. The transformants were cultured in L-broth, and IL-4 was expressed constitutively.

Example IV. Purification of Unglycosylated Human IL-4 from Aggregates Produced by *Escherichia coli*

A 1 liter culture of *E. coli* AB1899 (lon⁻) (obtained from Yale University *E. coli* Genetics Center, New Haven, CT) was grown to OD₅₅₀ = 2 (about 1.6 x 10⁹ cells/ml). Cells were harvested by centrifugation at 4500 xg for 15 minutes at 4°C. The pellets were resuspended in 30 ml of 50 mM Tris buffer, pH 8.0, containing 50 mM NaCl, 1mM ethylenediaminetetraacetic acid (EDTA), and 0.1 mM phenylmethylsulfonyl fluoride (PMSF). EDTA and PMSF were added to inhibit protease activity which might degrade the human IL-4 before purification. Next, the cells were sonicated (50 pulses (50%) at 70 watts) and centrifuged at 25,000 xg for 15 minutes at 4°C. The major protein component of the resulting pellet was shown to be IL-4 by comparing the gel band pattern of electrophoretically separated pellet material (which had been solubilized in sodium dodecylsulphate (SDS) and stained with Coomassie Blue) with a negative control.

After removal of the supernatant, pellet material was resuspended in Tris buffer solution (50 mM Tris, 50 mM NaCl, 1mM EDTA, 0.1mM PMSF, pH 8.0; 9 ml for each gram of pellet material) containing 5M guanidine HCl, 2mM glutathione (reduced form), and 0.2mM glutathione (oxidized form). After approximately 1 hour at room temperature, the solution was diluted 1:9 into a Tris buffer solution, pH 8.0, containing 2mM glutathione (reduced form) and 0.2mM glutathione (oxidized form). Whenever precipitates formed during the dilution, dialysis, or concentration steps, they were removed by centrifugation before proceeding. The entire volume was then dialyzed overnight 3 times against 3 liters of phosphate buffer solution. The dialyzate (i.e. the material retained by the dialysis bag) was concentrated by an Amicon YM5 filter (final concentration 8 mg/ml), and subjected to gel filtration chromatography (column: P30 (BioRad), 1.5 x 90 cm; PBS elution buffer; flow rate: 8 ml/hr). Fractions were collected over 15 minute intervals. Fractions 23-27 were pooled and further analyzed by reverse phase HPLC. Such analysis indicated that the pooled fractions contained 95% pure human IL-4. The yield from the 1 liter culture (OD₅₅₀ of 2) was 2 mg of human IL-4 with a specific activity of 5 x 10⁷ units/mg.

Example V. Production of Hybridoma IC1.11b4.6

A male Lewis rat was immunized intraperitoneally (i.p.) with 1 ml of human IL-4 solution emulsified with 1 ml complete Freund's adjuvant (CFA). The human IL-4 solution consisted of human IL-4 at a concentration of 14 µg/ml in 10 mM Tris-HCl, 0.5 M NaCl, pH 7.4. The human IL-4 was produced in accordance with Examples I and II, and had a specific activity of 2 x 10⁷ units/mg. Two weeks after the initial immunization, the rat was again injected i.p. with 1 ml human IL-4 solution emulsified with 1 ml CFA. Three months after the second injection, the rat was boosted intravenously with 1 ml human IL-4 solution (15 µg). Four days after the booster injection the rat was sacrificed, blood was collected, and the spleen was removed for fusion. Spleen cells were fused with mouse myeloma cells, P3X63-Ag8.653 (ATCC CRL 1580), in a 1:1 ratio using polyethylene glycol (PEG). The cell suspension (3.5 x 10⁵ cells/ml in HAT medium) was distributed into 40 96-well plates. Ten days later hybridoma supernatants were tested for their ability to bind to human IL-4 immobilized directly on microtiter plates (indirect ELISA), or to human IL-4 bound to immobilized polyclonal IgG fraction of rabbit anti-human IL-4. Bound antibody was detected by peroxidase-conjugated goat anti-rat immunoglobulin with a standard protocol. Hybridomas secreting antibodies reacting with IL-4 were cloned by limiting dilution. IC1.11B4.6 was one such hybridoma selected by these procedures. Antibodies from IC1.11B4.6 were determined to be of the IgG_{2a} isotype. The hybridoma can be stored (e.g. -70°C in culture medium with 10% DMSO) and cultured using standard mammalian cell culture techniques

(e.g., RPMI 1640 with 10% fetal bovine serum, supplemented with 1 mM glutamine and 50 mM 2-mercaptoethanol).

Example VI. Production of Hybridoma MP4.25D2.11

A collection of hybridomas was produced and their antibodies were screened for human IL-4 specificity in substantially the same manner as in Example V. The hybridomas of the collection were then screened further for the ability of their antibodies to block the TCGF activity of human IL-4 in a standard in vitro assay (as disclosed in Example II). Of several blocking monoclonals identified, that produced by MP4.25D2.11 was selected as having the highest titer of blocking activity. The antibodies produced by MP4.25D2.11 were determined to be rat IgG₁.

Example VII. Sandwich Assay for Human IL-4

100 μ l of rabbit polyclonal anti-human IL-4 antibody (10 μ g/ml in PBS purified on a protein A affinity column) is adsorbed onto the surface of each well in a 96-well polyvinyl chloride microtiter plate for 2 hrs. at 37°C. (PBS consists of 8.0 g of NaCl, 0.2 g of KH₂PO₄, 2.9 g of Na₂HPO₄·12H₂O, and 0.2 g of KCl, in 1 liter of distilled water. The pH is 7.4.) The plate is washed with PBS-Tween (prepared exactly as PBS, except that 0.5 ml of Tween 20 is added per liter) to remove unbound antibody, and then duplicate serial dilutions (in PBS) of purified *E. coli*-produced human IL-4 are placed in two 12-well rows of wells in order of decreasing IL-4 concentrations, which range from 1000 pg/ml to 15 pg/ml. The following samples were loaded into the remaining wells: (1) culture supernatants from a human T cell clone, e.g. CILy1⁺2⁻/9 (ATCC CRL 8179), (2) culture supernatants of COS 7 cells transfected with pcD-human-IL-4, (3) human serum containing different concentrations of purified COS7-produced IL-4, and (4) samples containing human IL-1 α , IL-2, IL-3, IFN- γ , IFN- α 2b, GM-CSF, and BSF-2. All of the samples were incubated for 2 hours at room temperature. After washing with PBS-Tween, a 1:10 dilution of supernatant from a culture of IC1.11B4.6 was added to each well (100 μ l/well) and was allowed to incubate for 1 hour at room temperature. After incubation, the plate was washed and peroxidase-conjugated goat anti-rat antibody was added and allowed to incubate for 1 hour at room temperature, after which the plate was washed. Next, the peroxidase substrate ABTS was added, and the human IL-4 concentrations were determined by means of optical densities in the wells. The results indicate that the assay can detect mammalian-produced human IL-4 at concentrations as low as 50 pg/ml in human serum, and that the assay does not detect any of the lymphokines listed above.

Table A below and Fig. 3A show the ability of 11B4 F(ab), IgG, and crude supernatant (unpurified antibody) to inhibit binding of ¹²⁵I-HuIL-4 to Daudi cells. All three preparations inhibited binding to a maximum extent of 70%. The control monoclonal antibodies GL117 F(ab), IgG, and crude supernatant did not affect binding. (The control antibodies are to an unrelated antigen of the same idio type.) The concentration of purified 11B4 IgG or F(ab) required to produce 50% inhibition of binding is in the range of 10-100 ng/ml.

Table B below and Fig. 3B show the ability of 25D2.11 F(ab) to inhibit binding in the same assay. This preparation resulted in a 90% inhibition with a 50% maximum effect at 10-15 ng/ml.

In Figs. 3A and 3B the X-axis shows ng/ml (in a log-scale) and the Y-axis shows percent inhibition.

In these experiments the HuIL-4 was prepared in Chinese hamster ovary cells and is designated CHO-HuIL-4 in the following Tables:

TABLE A

NEUTRALIZATION OF ¹²⁵ I-CHO-HuIL-4 BINDING TO DAUDI CELLS BY 11B4					
Sample	ng/ml	percent binding	Sample	ng/ml	percent binding
11B4 F(ab)	1	28.1	11B4 IgG	1	0
	10	25.34		10	11.7
	100	54.28		100	71.01
	1000	67.94		500	73.66
	10000	64.56		1000	69.54
11B4 supernatant	10	0	GL117 F(ab)	1	0
	100	45.1		10	2.4
	1000	67.9		100	0
	2500	74.3		1000	3.7
	5000	73.6		10000	5.8
GL117 IgG	1	0	GL117 supernatant	10	7.5
	10	0		100	4.7
	100	1.8		1000	0
	1000	0		2500	5.5
	10000	0		5000	0

TABLE B

NEUTRALIZATION OF ¹²⁵ I-CHO-HuIL-4 BINDING TO DAUDI CELLS BY 25D2.11 F(ab) FRAGMENT			
ng/ml	percent inhibition	ng/ml	percent inhibition
3000	90	15	54.1
1500	90	10	30.75
1000	84	7.5	10.5
60	77	3.0	0
30	67.4		

The descriptions of the foregoing embodiments of the invention have been presented for purpose of illustration and description. They are not intended to be exhaustive or to limit the invention to the precise forms disclosed, and obviously many modifications and variations are possible in the light of the above teaching. The embodiments were chosen and described in order to best explain the principles of the invention and its practical application to thereby enable others skilled in the art to best utilize the invention in various embodiments and with various modifications as are suited to the particular use contemplated. It is intended that the scope of the invention be defined by the claims appended hereto.

Applicants have deposited hybridomas IC1.11B4.6 and MP4.25D2.11 with the American Type Culture Collection, Rockville, MD, USA (ATCC), under accession numbers HB9550 and HB9809 respectively. These deposits were made under conditions as provided under ATCC's agreement for Culture Deposit for Patent Purposes, which assures that the deposits will be made available to the US Commissioner of Patents and Trademarks pursuant to 35 USC 122 and 37 CFR 1.14 and will be made available to the public upon issue of a U.S. patent, and which requires that the deposits be maintained. Availability of the deposited strains is not to be construed as a license to practise the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

Claims

Claims for the following Contracting States : AT, BE, CH, DE, FR, GB, GR, IT, LI, LU, NL, SE

1. The monoclonal antibodies specific for human interleukin-4 produced by hybridomas IC1.11B4.6 and MP4.25D2.11 (ATCC HB 9550 and HB 8909).



TABLE A

NEUTRALIZATION OF ¹²⁵ I-CHO-HuIL-4 BINDING TO DAUDI CELLS BY 11B4					
Sample	ng/ml	percent binding	Sample	ng/ml	percent binding
11B4 F(ab)	1	28.1	11B4 IgG	1	0
	10	25.34		10	11.7
	100	54.28		100	71.01
	1000	67.94		500	73.66
	10000	64.56		1000	69.54
11B4 supernatant	10	0	GL117 F(ab)	1	0
	100	45.1		10	2.4
	1000	67.9		100	0
	2500	74.3		1000	3.7
	5000	73.6		10000	5.8
GL117 IgG	1	0	GL117 supernatant	10	7.5
	10	0		100	4.7
	100	1.8		1000	0
	1000	0		2500	5.5
	10000	0		5000	0

TABLE B

NEUTRALIZATION OF ¹²⁵ I-CHO-HuIL-4 BINDING TO DAUDI CELLS BY 25D2.11 F(ab) FRAGMENT			
ng/ml	percent inhibition	ng/ml	percent inhibition
3000	90	15	54.1
1500	90	10	30.75
1000	84	7.5	10.5
60	77	3.0	0
30	67.4		

The descriptions of the foregoing embodiments of the invention have been presented for purpose of illustration and description. They are not intended to be exhaustive or to limit the invention to the precise forms disclosed, and obviously many modifications and variations are possible in the light of the above teaching. The embodiments were chosen and described in order to best explain the principles of the invention and its practical application to thereby enable others skilled in the art to best utilize the invention in various embodiments and with various modifications as are suited to the particular use contemplated. It is intended that the scope of the invention be defined by the claims appended hereto.

Applicants have deposited hybridomas IC1.11B4.6 and MP4.25D2.11 with the American Type Culture Collection, Rockville, MD, USA (ATCC), under accession numbers HB9550 and HB9809 respectively. These deposits were made under conditions as provided under ATCC's agreement for Culture Deposit for Patent Purposes, which assures that the deposits will be made available to the US Commissioner of Patents and Trademarks pursuant to 35 USC 122 and 37 CFR 1.14 and will be made available to the public upon issue of a U.S. patent, and which requires that the deposits be maintained. Availability of the deposited strains is not to be construed as a license to practise the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

Claims

Claims for the following Contracting States : AT, BE, CH, DE, FR, GB, GR, IT, LI, LU, NL, SE

- The monoclonal antibodies specific for human interleukin-4 produced by hybridomas IC1.11B4.6 and MP4.25D2.11 (ATCC HB 9550 and HB 8909).



2. The hybridomas IC1.11B4.6 and MP4.25D2.11 (ATTC HB 9550 and HB 8909) capable of secreting monoclonal antibodies specific for human interleukin-4.
3. A polypeptide comprising a light chain variable region of a monoclonal antibody defined in Claim 1.
4. A polypeptide comprising a heavy chain variable region of a monoclonal antibody defined in Claim 1.
5. A binding composition specific for human interleukin-4 comprising a heavy chain variable region and a light chain variable region of a monoclonal antibody defined in Claim 1.
6. A method of detecting the presence of human interleukin-4 in a sample suspected of containing human interleukin-4, the method comprising the steps of:
 - providing a first monoclonal antibody specific for a first antigenic determinant on human interleukin-4;
 - providing a second antibody selected from the group consisting of a polyclonal antibody composition specific for human interleukin-4 and a second monoclonal antibody specific for a second antigenic determinant on human interleukin-4, the second antigenic determinant being different from the first antigenic determinant;
 - providing a signal-generating means capable of being operationally associated with either the first monoclonal antibody or the second antibody to produce a signal whose intensity is monotonically related to the amount of either the first monoclonal antibody or the second antibody, respectively;
 - attaching either the first monoclonal antibody or the second antibody to a support means to form an antibody-support conjugate;
 - contacting the sample with the antibody-support conjugate so that human interleukin-4 in the sample binds to the antibody-support conjugate;
 - contacting the first monoclonal antibody operationally associated with the signal generating means with the human interleukin-4 bound to the antibody-support conjugate, when the antibody-support conjugate includes the second antibody; or
 - contacting the second antibody operationally associated with the signal-generating means with the human interleukin-4 bound to the antibody-support conjugate, when the antibody-support conjugate includes the first monoclonal antibody; and
 - measuring the signal generated by the signal-generating means,wherein one of the monoclonal antibodies is that produced by hybridoma IC1.11B4.6 or MP4.25D2.11 (ATTC HB 9550 and HB 8909).
7. The method of claim 6 wherein said antibody-support conjugate includes said first monoclonal antibody and wherein said second antibody is said polyclonal antibody composition specific for human interleukin-4.
8. The method of claim 7 wherein said first monoclonal antibody is the monoclonal antibody produced by hybridoma IC1.11B4.6 or hybridoma MP4.25D2.11.
9. The method of claim 6 wherein said antibody-support conjugate includes said second antibody and wherein said second antibody is said polyclonal antibody composition specific for human interleukin-4.
10. The method of claim 9 wherein said first monoclonal antibody is the monoclonal antibody produced by hybridoma IC1.11B4.6 or hybridoma MP4.25D2.11.
11. A kit for detecting the presence of human interleukin-4 in a sample suspected of containing human interleukin-4, the kit comprising:
 - a first monoclonal antibody specific for a first antigenic determinant on human interleukin-4;
 - a second antibody selected from the group consisting of a polyclonal antibody composition specific for human interleukin-4 and a second monoclonal antibody specific for a second antigenic determinant on human interleukin-4, the second antigenic determinant being different from the first antigenic determinant;
 - a support means; and
 - a signal generating means,wherein one of the monoclonal antibodies is that produced by hybridoma IC1.11B4.6 or



MP4.25D2.11 (ATTC HB 9550 and HB 8909).

12. The kit of claim 11 wherein said first monoclonal antibody is the monoclonal antibody produced by hybridoma IC1.11B4.6 or hybridoma MP4.25D2.11 and wherein said second antibody is said polyclonal antibody composition specific for human interleukin-4.
13. The kit of claim 12 wherein said signal generation means comprises an enzyme operationally associated with said first monoclonal antibody, the enzyme being selected from the group consisting of peroxidase, beta-galactosidase, and alkaline phosphatase.
14. An immunopurification process for extracting human interleukin-4 from a sample containing human interleukin-4;
said process including the step of passing the sample through an immunoadsorbent column comprising a monoclonal antibody specific for human interleukin-4;
wherein the monoclonal antibody is that produced by hybridoma IC1.11B4.6 or MP4.25D2.11 (ATTC HB 9550 and HB 8909).
15. The use of a monoclonal antibody as claimed in Claim 1, a polypeptide as claimed in Claim 3 or Claim 4 or a binding composition as claimed in Claim 5 in the manufacture of a therapeutic agent for use as an agonist or antagonist of human IL-4.

Claims for the following Contracting State : ES

1. A method of producing a monoclonal antibody specific for human interleukin-4, or a polypeptide comprising a light chain variable region or a heavy chain variable region thereof, or a binding composition specific for human interleukin-4 comprising a heavy chain variable region and a light chain variable region thereof, which comprises (a) culturing a hybridoma selected from hybridomas IC1.11B4.6 and MP4.25D2.11 (ATTC HB 9550 and HB 8909) or (b) producing said monoclonal antibodies, polypeptides or binding compositions by recombinant means, and if desired generating fragments from the produced products or carrying out chemical reassociation of half molecules.
2. The hybridomas IC1.11B4.6 and MP4.25D2.11 (ATTC HB 9550 and HB 8909) capable of secreting monoclonal antibodies specific for human interleukin-4.
3. A method of detecting the presence of human interleukin-4 in a sample suspected of containing human interleukin-4, the method comprising the steps of:
providing a first monoclonal antibody specific for a first antigenic determinant on human interleukin-4;
providing a second antibody selected from the group consisting of a polyclonal antibody composition specific for human interleukin-4 and a second monoclonal antibody specific for a second antigenic determinant on human interleukin-4, the second antigenic determinant being different from the first antigenic determinant;
providing a signal-generating means capable of being operationally associated with either the first monoclonal antibody or the second antibody to produce a signal whose intensity is monotonically related to the amount of either the first monoclonal antibody or the second antibody, respectively;
attaching either the first monoclonal antibody or the second antibody to a support means to form an antibody-support conjugate;
contacting the sample with the antibody-support conjugate so that human interleukin-4 in the sample binds to the antibody-support conjugate;
contacting the first monoclonal antibody operationally associated with the signal generating means with the human interleukin-4 bound to the antibody-support conjugate, when the antibody-support conjugate includes the second antibody; or
contacting the second antibody operationally associated with the signal-generating means with the human interleukin-4 bound to the antibody-support conjugate, when the antibody-support conjugate includes the first monoclonal antibody; and
measuring the signal generated by the signal-generating means, wherein one of the monoclonal antibodies is that produced by hybridoma IC1.11B4.6 or MP4.25D2.11 (ATTC HB 9550 and HB 8909).



4. The method of Claim 3 wherein said antibody-support conjugate includes said first monoclonal antibody and wherein said second antibody is said polyclonal antibody composition specific for human interleukin-4.
5. The method of Claim 4 wherein said first monoclonal antibody is the monoclonal antibody produced by hybridoma IC1.11B4.6 or hybridoma MP4.25D2.11
6. The method of Claim 3 wherein said antibody-support conjugate includes said second antibody and wherein said second antibody is said polyclonal antibody composition specific for human interleukin-4.
7. The method of Claim 6 wherein said first monoclonal antibody is the monoclonal antibody produced by hybridoma IC1.11B4.6 or hybridoma MP4.25D2.11.
8. A kit for detecting the presence of human interleukin-4 in a sample suspected of containing human interleukin-4, the kit comprising:
 - a first monoclonal antibody specific for a first antigenic determinant on human interleukin-4;
 - a second antibody selected from the group consisting of a polyclonal antibody composition specific for human interleukin-4 and a second monoclonal antibody specific for a second antigenic determinant on human interleukin-4, the second antigenic determinant being different from the first antigenic determinant;
 - a support means; and
 - a signal generating means,
 wherein one of the monoclonal antibodies is that produced by hybridoma IC1.11B4.6 or MP4.25D2.11 (ATTC HB 9550 and HB 8909).
9. The kit of Claim 8 wherein said first monoclonal antibody is the monoclonal antibody produced by hybridoma IC1.11B4.6 or hybridoma MP4.25D2.11 and wherein said second antibody is said polyclonal antibody composition specific for human interleukin-4.
10. The kit of Claim 9 wherein said signal generation means comprises an enzyme operationally associated with said first monoclonal antibody, the enzyme being selected from the group consisting of peroxidase, beta-galactosidase, and alkaline phosphatase.
11. An immunopurification process for extracting human interleukin-4 from a sample containing human interleukin-4;
 - said process including the step of passing the sample through an immunoabsorbent column comprising a monoclonal antibody specific for human interleukin-4;
 - wherein the monoclonal antibody is that produced by hybridoma IC1.11B4.6 or MP4.25D2.11 (ATTC HB 9550 and HB 8909).

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, FR, GB, GR, IT, LI, LU, NL, SE

1. Für Human-Interleukin-4 spezifische monoklonale Antikörper, welche von den Hybridomen IC1.11B4.6 und MP4.25D2.11 (ATTC HB 9550 und HB 8909) produziert wurden.
2. Die Hybridome IC1.11B4.6 und MP4.25D2.11 (ATTC HB 9550 und HB 8909), welche zum Sekretieren monoklonaler Antikörper, die für Human-Interleukin-4 spezifisch sind, befähigt sind.
3. Polypeptid, welches eine variable Domäne der leichten Kette eines in Anspruch 1 definierten monoklonalen Antikörpers enthält.
4. Polypeptid, welches eine variable Domäne der schweren Kette eines in Anspruch 1 definierten monoklonalen Antikörpers enthält.
5. Für Human-Interleukin-4 spezifische Bindungsstruktur, welche eine variable Domäne der schweren Kette und eine variable Domäne der leichten Kette eines in Anspruch 1 definierten monoklonalen Antikörpers umfaßt.



6. Verfahren zum Nachweis der Anwesenheit von Human-Interleukin-4 in einer Probe, von welcher vermutet wird, daß sie Human-Interleukin-4 enthält, umfassend die Schritte des Bereitstellens eines ersten monoklonalen Antikörpers, welcher für eine erste antigene Determinante auf Human-Interleukin-4 spezifisch ist;
 - 5 Bereitstellen eines zweiten Antikörpers, welcher aus der Gruppe ausgewählt ist, welche aus einer für Human-Interleukin-4 spezifischen polyklonalen Antikörperzusammensetzung und einem zweiten, für eine zweite antigene Determinante auf Human-Interleukin-4 spezifischen monoklonalen Antikörper besteht, wobei die zweite antigene Determinante von der ersten antigenen Determinante verschieden ist;
 - 10 Bereitstellen eines signalerzeugenden Mittels, welches dazu befähigt ist, entweder mit dem ersten monoklonalen Antikörper oder dem zweiten Antikörper unter Erzeugen eines Signals funktionell verbunden zu sein, dessen Intensität mit der Menge entweder des ersten monoklonalen Antikörpers oder des zweiten Antikörpers gleichförmig Bezug hat;
 - Anbringen entweder des ersten monoklonalen Antikörpers oder des zweiten Antikörpers an ein Trägermittel unter Bilden eines Antikörper-Träger-Konjugats;
 - 15 Zusammenbringen der Probe mit dem Antikörper-Träger-Konjugat, so daß das Human-Interleukin-4 in der Probe an das Antikörper-Träger-Konjugat bindet;
 - Zusammenbringen des ersten, mit dem signalerzeugenden Mittel funktionell verbundenen monoklonalen Antikörpers mit dem an das Antikörper-Träger-Konjugat gebundenen Human-Interleukin-4, wenn das Antikörper-Träger-Konjugat den zweiten Antikörper enthält; oder
 - 20 Zusammenbringen des zweiten, mit dem signalerzeugenden Mittel funktionell verbundenen Antikörpers mit dem an das Antikörper-Träger-Konjugat gebundenen Human-Interleukin-4, wenn das Antikörper-Träger-Konjugat den ersten monoklonalen Antikörper enthält; und
 - Messen des durch das signalerzeugende Mittel erzeugten Signals, wobei einer der monoklonalen Antikörper der von den Hybridomen IC1.11B4.6 oder MP4.25D2.11 (ATTC HB 9550 und HB 8909) produzierte ist.
 - 25
7. Verfahren des Anspruchs 6, bei welchem das Antikörper-Träger-Konjugat den ersten monoklonalen Antikörper einschließt und bei welchem der zweite Antikörper die für Human-Interleukin-4 spezifische polyklonale Antikörper-Zusammensetzung ist.
- 30 8. Verfahren des Anspruchs 7, bei welchem der erste monoklonale Antikörper der von dem Hybridom IC1.11B4.6 oder Hybridom MP4.25D2.11 produzierte Antikörper ist.
9. Verfahren des Anspruchs 6, bei welchem das Antikörper-Träger-Konjugat den zweiten Antikörper einschließt und bei welchem der zweite Antikörper die für Human-Interleukin-4 spezifische polyklonale Antikörper-Zusammensetzung ist.
- 35 10. Verfahren des Anspruchs 9, bei welchem der erste monoklonale Antikörper der von dem Hybridom IC1.11B4.6 oder dem Hybridom MP4.25D2.11 produzierte Antikörper ist.
- 40 11. Kit zum Nachweis der Anwesenheit von Human-Interleukin-4 in einer Probe, von welcher vermutet wird, daß sie Human-Interleukin-4 enthält, umfassend
 - einen ersten monoklonalen Antikörper, welcher für eine erste antigene Determinante auf Human-Interleukin-4 spezifisch ist;
 - 45 einen zweiten Antikörper, welcher aus der Gruppe ausgewählt ist, welche aus einer für Human-Interleukin-4 spezifischen polyklonalen Antikörperzusammensetzung und einem zweiten, für eine zweite antigene Determinante auf Human-Interleukin-4 spezifischen monoklonalen Antikörper besteht, wobei die zweite antigene Determinante von der ersten antigenen Determinante verschieden ist;
 - ein Trägermittel und
 - 50 ein signalerzeugendes Mittel,
 - wobei einer der monoklonalen Antikörper der von den Hybridomen IC1.11B4.6 oder MP4.25D2.11 (ATTC HB 9550 und HB 8909) produzierte ist.
12. Kit des Anspruchs 11, worin der erste monoklonale Antikörper der von dem Hybridom IC1.11B4.6 oder dem Hybridom MP4.25D2.11 produzierte monoklonale Antikörper ist und worin der zweite Antikörper die für Human-Interleukin-4 spezifische polyklonale Antikörper-Zusammensetzung ist.
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13. Kit des Anspruchs 12, worin das signalerzeugende Mittel ein mit dem ersten monoklonalen Antikörper funktionell verbundenes Enzym umfaßt, wobei das Enzym aus der aus Peroxidase, Beta-Galaktosidase und alkalischer Phosphatase bestehenden Gruppe ausgewählt ist.

5 14. Immunaufreinigungsverfahren zum Extrahieren von Human-Interleukin-4 aus einer Human-Interleukin-4 enthaltenden Probe, umfassend die Schritte des Leitens der Probe durch eine Immunadsorbenssäule, welche einen für Human-Interleukin-4 spezifischen monoklonalen Antikörper enthält, wobei der monoklonale Antikörper der von den Hybridomen IC1.11B4.6 oder MP4.25D2.11 (ATTC HB 9550 und HB 8909) produzierte ist.

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15. Verwendung eines in Anspruch 1 beanspruchten monoklonalen Antikörpers, eines in Anspruch 3 oder Anspruch 4 beanspruchten Polypeptids oder einer in Anspruch 5 beanspruchten Bindungsstruktur bei der Herstellung eines therapeutischen Mittels zur Verwendung als Human-IL-4-Agonist oder -Antagonist.

15 **Patentansprüche für folgenden Vertragsstaat : ES**

1. Verfahren zum Herstellen eines auf Human-Interleukin-4 spezifischen monoklonalen Antikörpers oder eines Polypeptids, welches eine variable Domäne der leichten Kette oder eine variable Domäne der schweren Kette desselben enthält, oder einer auf Human-Interleukin-4 spezifischen Bindungsstruktur, welche eine variable Domäne der schweren Kette und eine variable Domäne der leichten Kette desselben enthält, welches (a) das Kultivieren eines aus den Hybridomen IC1.11B4.6 und MP4.25D2.11 (ATTC HB 9550 und HB 8909) ausgewählten Hybridoms oder (b) das Erzeugen der monoklonalen Antikörper, Polypeptide oder Bindungsstrukturen durch rekombinante Mittel und gewünschtenfalls das Erzeugen von Fragmenten aus den hergestellten Produkten oder das Durchführen einer chemischen Wiedervereinigung von Molekülhälften umfaßt.

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2. Die Hybridome IC1.11B4.6 und MP4.25D2.11 (ATTC HB 9550 und HB 8909), welche zum Sekretieren auf Human-Interleukin-4 spezifischer monoklonaler Antikörper befähigt sind.

30 3. Verfahren zum Nachweis der Anwesenheit von Human-Interleukin-4 in einer Probe, von welcher vermutet wird, daß sie Human-Interleukin-4 enthält, umfassend die Schritte des Bereitstellens eines ersten monoklonalen Antikörpers, welcher für eine erste antigene Determinante auf Human-Interleukin-4 spezifisch ist;

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Bereitstellen eines zweiten Antikörpers, welcher aus der Gruppe ausgewählt ist, welche aus einer für Human-Interleukin-4 spezifischen polyklonalen Antikörperzusammensetzung und einem zweiten, für eine zweite antigene Determinante auf Human-Interleukin-4 spezifischen monoklonalen Antikörper besteht, wobei die zweite antigene Determinante von der ersten antigenen Determinante verschieden ist;

Bereitstellen eines signalerzeugenden Mittels, welches dazu befähigt ist, entweder mit dem ersten monoklonalen Antikörper oder dem zweiten Antikörper unter Erzeugen eines Signals funktionell verbunden zu sein, dessen Intensität mit der Menge entweder des ersten monoklonalen Antikörpers oder des zweiten Antikörpers gleichförmig Bezug hat;

Anbringen entweder des ersten monoklonalen Antikörpers oder des zweiten Antikörpers an ein Trägermittel unter Bilden eines Antikörper-Träger-Konjugats;

Zusammenbringen der Probe mit dem Antikörper-Träger-Konjugat, so daß das Human-Interleukin-4 in der Probe an das Antikörper-Träger-Konjugatbindet;

Zusammenbringen des ersten, mit dem signalerzeugenden Mittel funktionell verbundenen monoklonalen Antikörpers mit dem an das Antikörper-Träger-Konjugat gebundenen Human-Interleukin-4, wenn das Antikörper-Träger-Konjugat den zweiten Antikörper enthält; oder

Zusammenbringen des zweiten, mit dem signalerzeugenden Mittel funktionell verbundenen Antikörpers mit dem an das Antikörper-Träger-Konjugat gebundenen Human-Interleukin-4, wenn das Antikörper-Träger-Konjugat den ersten monoklonalen Antikörper enthält; und

Messen des durch das signalerzeugende Mittel erzeugten Signals, wobei einer der monoklonalen Antikörper der von den Hybridomen IC1.11B4.6 oder MP4.25D2.11 (ATTC HB 9550 und HB 8909) produzierte ist.

4. Verfahren des Anspruchs 3, bei welchem das Antikörper-Träger-Konjugat den ersten monoklonalen Antikörper einschließt und bei welchem der zweite Antikörper die für Human-Interleukin-4 spezifische



polyklonale Antikörper-Zusammensetzung ist.

5. Verfahren des Anspruchs 4, bei welchem der erste monoklonale Antikörper der von dem Hybridom IC1.11B4.6 oder MP4.25D2.11 produzierte monoklonale Antikörper ist.
6. Verfahren des Anspruchs 3, bei welchem das Antikörper-Träger-Konjugat den zweiten Antikörper einschließt und bei welchem der zweite Antikörper die für Human-Interleukin-4 spezifische polyklonale Antikörper-Zusammensetzung ist.
7. Verfahren des Anspruchs 6, bei welchem der erste monoklonale Antikörper der von dem Hybridom IC1.11B4.6 oder Hybridom MP4.25D2.11 produzierte Antikörper ist.
8. Kit zum Nachweis der Anwesenheit von Human-Interleukin-4 in einer Probe, von welcher vermutet wird, daß sie Human-Interleukin-4 enthält, umfassend
 - einen ersten monoklonalen Antikörper, welcher für eine erste antigene Determinante auf Human-Interleukin-4 spezifisch ist;
 - einen zweiten Antikörper, welcher aus der Gruppe ausgewählt ist, welche aus einer für Human-Interleukin-4 spezifischen polyklonalen Antikörperzusammensetzung und einem zweiten, für eine zweite antigene Determinante auf Human-Interleukin-4 spezifischen monoklonalen Antikörper besteht, wobei die zweite antigene Determinante von der ersten antigenen Determinante verschieden ist;
 - ein Trägermittel und
 - ein signalerzeugendes Mittel,
 - wobei einer der monoklonalen Antikörper der von den Hybridomen IC1.11B4.6 oder MP4.25D2.11 (ATTC HB 9550 und HB 8909) produzierte ist.
9. Kit des Anspruchs 8, worin der erste monoklonale Antikörper der von dem Hybridom IC1.11B4.6 oder dem Hybridom MP4.25D2.11 produzierte monoklonale Antikörper ist und worin der zweite Antikörper die für Human-Interleukin-4 spezifische polyklonale Antikörper-Zusammensetzung ist.
10. Kit des Anspruchs 9, worin das signalerzeugende Mittel ein mit dem ersten monoklonalen Antikörper funktionell verbundenes Enzym umfaßt, wobei das Enzym aus der aus Peroxidase, Beta-Galaktosidase und alkalischer Phosphatase bestehenden Gruppe ausgewählt ist.
11. Immunaufläuterungsverfahren zum Extrahieren von Human-Interleukin-4 aus einer Human-Interleukin-4 enthaltenden Probe, umfassend die Schritte des Leitens der Probe durch eine Immunadsorbenssäule, welche einen für Human-Interleukin-4 spezifischen monoklonalen Antikörper enthält, wobei der monoklonale Antikörper der von den Hybridomen IC1.11B4.6 oder MP4.25D2.11 (ATTC HB 9550 und HB 8909) produzierte ist.

40 Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, DE, FR, GB, GR, IT, LU, NL, SE

1. Anticorps monoclonaux spécifiques de l'interleukine-4 humaine, produits par les hybridomes IC1.11B4.6 et MP4.25D2.11 (ATTC HB 9550 et HB 8909)
2. Hybridomes IC1.11B4.6 et MP4.25D2.11 (ATTC HB 9550 et HB 8909), capables de sécréter des anticorps monoclonaux spécifiques de l'interleukine-4 humaine.
3. Polypeptide comprenant une région variable à chaîne légère d'un anticorps monoclonal selon la revendication 1.
4. Polypeptide comprenant une région variable à chaîne lourde d'un anticorps monoclonal selon la revendication 1.
5. Composition de liaison spécifique de l'interleukine-4 humaine, comprenant une région variable à chaîne lourde et une région variable à chaîne légère d'un anticorps monoclonal selon la revendication 1.



6. Procédé pour déceler la présence de l'interleukine-4 humaine dans un échantillon dont on suppose qu'il contient l'interleukine-4 humaine, procédé qui comprend les étapes consistant :
 - à fournir un premier anticorps monoclonal spécifique d'un premier déterminant antigénique se trouvant sur l'interleukine-4 humaine ;
 - à fournir un deuxième anticorps choisi parmi l'ensemble comprenant une composition d'anticorps polyclonal spécifique de l'interleukine-4 humaine et un deuxième anticorps monoclonal spécifique d'un deuxième déterminant antigénique se trouvant sur l'interleukine-4 humaine, le deuxième déterminant antigénique étant différent du premier déterminant antigénique ;
 - à fournir un moyen générateur de signaux, pouvant être opérationnellement associé avec le premier anticorps monoclonal ou avec le deuxième anticorps, pour produire un signal dont l'intensité est reliée d'une manière monotone à la quantité respectivement du premier anticorps monoclonal ou du deuxième anticorps ;
 - à fixer le premier anticorps monoclonal ou le deuxième anticorps à un moyen de support pour former un conjugué anticorps-support ;
 - à mettre en contact l'échantillon avec le conjugué anticorps-support, de façon que l'interleukine-4 humaine se trouvant dans l'échantillon se fixe au conjugué anticorps-support ;
 - à mettre en contact le premier anticorps monoclonal opérationnellement associé au moyen générateur de signaux, avec l'interleukine-4 humaine fixée au conjugué anticorps-support, quand le conjugué anticorps-support comprend le deuxième anticorps, ou
 - à mettre en contact le deuxième anticorps opérationnellement associé au moyen générateur de signaux avec l'interleukine-4 humaine fixée au conjugué anticorps-support quand le conjugué anticorps-support comprend le premier anticorps monoclonal ; et
 - à mesurer le signal produit par le signal générateur de signaux, l'un des anticorps monoclonaux étant celui qui est produit par l'hybridome IC1.11B4.6 ou MP4.25D2.11 (ATTC HB9550 et HB 8909).
7. Procédé selon la revendication 6, dans lequel le conjugué anticorps-support comprend le premier anticorps monoclonal, le deuxième anticorps étant la composition d'anticorps polyclonal spécifique de l'interleukine-4 humaine.
8. Procédé selon la revendication 7, dans lequel le premier anticorps monoclonal est l'anticorps monoclonal produit par l'hybridome IC1.11B4.6 ou l'hybridome MP4.25D2.11.
9. Procédé selon la revendication 6, dans lequel le conjugué anticorps-support comprend le deuxième anticorps, et dans lequel le deuxième anticorps est la composition d'anticorps polyclonal spécifique de l'interleukine-4 humaine.
10. Procédé selon la revendication 9, dans lequel le premier anticorps monoclonal est l'anticorps monoclonal produit par l'hybridome IC1.11B4.6 ou l'hybridome MP4.25D2.11.
11. Trousse destinée à détecter la présence de l'interleukine-4 humaine dans un échantillon dont on suppose qu'il contient de l'interleukine-4 humaine, la trousse comprenant :
 - un premier anticorps monoclonal spécifique d'un premier déterminant antigénique se trouvant sur l'interleukine-4 humaine ;
 - un deuxième anticorps choisi parmi l'ensemble comprenant une composition d'un anticorps polyclonal spécifique de l'interleukine-4 humaine et d'un deuxième anticorps monoclonal spécifique d'un deuxième déterminant antigénique sur l'interleukine-4 humaine, le deuxième déterminant antigénique étant différent du premier déterminant antigénique ;
 - un moyen de support ; et
 - un moyen générateur de signaux, où l'un des anticorps monoclonaux est celui qui est produit par l'hybridome IC1.11B4.6 ou MP4.25D2.11 (ATTC HB 9550 et HB 8909).
12. Trousse selon la revendication 11, dans laquelle le premier anticorps monoclonal est l'anticorps monoclonal produit par l'hybridome IC1.11B4.6 ou l'hybridome MP4.25D2.11, et dans laquelle le deuxième anticorps est la composition d'anticorps polyclonal spécifique de l'interleukine-4 humaine.



13. Trousse selon la revendication 12, dans laquelle le moyen générateur de signaux comprend une enzyme opérationnellement associée au premier anticorps monoclonal, l'enzyme étant choisie parmi l'ensemble comprenant la peroxydase, la bêta-galactosidase et la phosphatase alcaline.

5 14. Procédé d'immunopurification destiné à extraire l'interleukine-4 humaine d'un échantillon contenant l'interleukine-4 humaine, procédé qui comprend les étapes consistant à faire passer l'échantillon à travers une colonne d'un immuno-adsorbant comprenant un anticorps monoclonal spécifique de l'interleukine-4 humaine ;
 10 où l'anticorps monoclonal est celui qui est produit par l'hybridome IC1.11B4.6 ou MP4.25D2.11 (ATTC HB 9550 et HB 8909).

15 15. Utilisation d'un anticorps monoclonal selon la revendication 1, d'un polypeptide selon la revendication 3 ou la revendication 4 ou d'une composition de liaison selon la revendication 5, pour fabriquer un agent thérapeutique destiné à être utilisé comme agoniste ou antagoniste de l'IL-4 humaine.

Revendications pour l'Etat contractant suivant : ES

1. Procédé pour produire un anticorps monoclonal spécifique de l'interleukine-4 humaine, ou un polypeptide comprenant une région variable à chaîne légère ou une région variable à chaîne lourde de cet anticorps, ou encore une composition de liaison spécifique de l'interleukine-4 humaine comprenant une région variable à chaîne lourde et une région variable à chaîne légère de cette dernière, qui consiste :
 20 (a) à cultiver un hybridome choisi parmi les hybridomes IC1.11B4.6 et MP4.25D2.11 (ATTC HB 9550 et HB 8909), ou
 25 (b) à produire les anticorps monoclonaux, les polypeptides ou les compositions de liaison par des moyens recombinants et, si on le désire, à produire des fragments à partir des produits obtenus, ou à mettre en oeuvre une réassociation chimique des demi-molécules.

2. Hybridomes IC.11B4.6 et MP4.25D2.11 (ATTC HB 9550 et HB 8909) capables de sécréter des anticorps monoclonaux spécifiques de l'interleukine-4 humaine.

30 3. Procédé pour détecter la présence de l'interleukine-4 humaine dans un échantillon dont on suppose qu'il contient l'interleukine-4 humaine, procédé qui comprend les étapes consistant :

à fournir un premier anticorps monoclonal spécifique d'un premier déterminant antigénique se trouvant sur l'interleukine-4 humaine ;

35 à fournir un deuxième anticorps choisi parmi l'ensemble comprenant une composition d'anticorps polyclonal spécifique de l'interleukine-4 humaine et un deuxième anticorps monoclonal spécifique d'un deuxième déterminant antigénique se trouvant sur l'interleukine-4 humaine, le deuxième déterminant antigénique étant différent du premier déterminant antigénique ;

40 à fournir un moyen générateur de signaux, pouvant être opérationnellement associé avec le premier anticorps monoclonal ou avec le deuxième anticorps, pour produire un signal dont l'intensité est reliée d'une manière monotone à la quantité respectivement du premier anticorps monoclonal ou du deuxième anticorps ;

à fixer le premier anticorps monoclonal ou le deuxième anticorps à un moyen de support pour former un conjugué anticorps-support ;

45 à mettre en contact l'échantillon avec le conjugué anticorps-support, de façon que l'interleukine-4 humaine se trouvant dans l'échantillon se fixe au conjugué anticorps-support ;

à mettre en contact le premier anticorps monoclonal opérationnellement associé au moyen générateur de signaux, avec l'interleukine-4 humaine fixée au conjugué anticorps-support, quand le conjugué anticorps-support comprend le deuxième anticorps, ou

50 à mettre en contact le deuxième anticorps opérationnellement associé au moyen générateur de signaux avec l'interleukine-4 humaine fixée au conjugué anticorps-support quand le conjugué anticorps-support comprend le premier anticorps monoclonal ; et

à mesurer le signal produit par le signal générateur de signaux,
 55 l'un des anticorps monoclonaux étant celui qui est produit par l'hybridome IC1.11B4.6 ou MP4.25D2.11 (ATTC HB9550 et HB 8909).

4. Procédé selon la revendication 3, dans lequel le conjugué anticorps-support comprend le premier anticorps monoclonal, le deuxième anticorps étant la composition d'anticorps polyclonal spécifique de



l'interleukine-4 humaine.

5. Procédé selon la revendication 4, dans lequel le premier anticorps monoclonal est l'anticorps monoclonal produit par l'hybridome IC1.11B4.6 ou l'hybridome MP4.25D2.11.

6. Procédé selon la revendication 3, dans lequel le conjugué anticorps-support comprend le deuxième anticorps, et dans lequel le deuxième anticorps est la composition d'anticorps polyclonal spécifique de l'interleukine-4 humaine.

7. Procédé selon la revendication 6, dans lequel le premier anticorps monoclonal est l'anticorps monoclonal produit par l'hybridome IC1.11B4.6 ou l'hybridome MP4.25D2.11.

8. Trousse destinée à détecter la présence de l'interleukine-4 humaine dans un échantillon dont on suppose qu'il contient de l'interleukine-4 humaine, la trousse comprenant :

- un premier anticorps monoclonal spécifique d'un premier déterminant antigénique se trouvant sur l'interleukine-4 humaine ;

- un deuxième anticorps choisi parmi l'ensemble comprenant une composition d'un anticorps polyclonal spécifique de l'interleukine-4 humaine et d'un deuxième anticorps monoclonal spécifique d'un deuxième déterminant antigénique sur l'interleukine-4 humaine, le deuxième déterminant antigénique étant différent du premier déterminant antigénique ;

un myen de support ; et

un moyen générateur de signaux,

où l'un des anticorps monoclonaux est celui qui est produit par l'hybridome IC1.11B4.6 ou MP4.25D2.11 (ATTC HB 9550 et HB 8909).

9. Trousse selon la revendication 8, dans laquelle le premier anticorps monoclonal est l'anticorps monoclonal produit par l'hybridome IC1.11B4.6 ou l'hybridome MP4.25D2.11, et dans laquelle le deuxième anticorps est la composition d'anticorps polyclonal spécifique de l'interleukine-4 humaine.

10. Trousse selon la revendication 9, dans laquelle le moyen générateur de signaux comprend une enzyme opérationnellement associée au premier anticorps monoclonal, l'enzyme étant choisie parmi l'ensemble comprenant la peroxydase, la bêta-galactosidase et la phosphatase alcaline.

11. Procédé d'immunopurification destiné à extraire l'interleukine-4 humaine d'un échantillon contenant l'interleukine-4 humaine, procédé qui comprend les étapes consistant à faire passer l'échantillon à travers une colonne d'un immuno-adsorbant comprenant un anticorps monoclonal spécifique de l'interleukine-4 humaine ;

où l'anticorps monoclonal est celui qui est produit par l'hybridome IC1.11B4.6 ou MP4.25D2.11 (ATTC HB 9550 et HB 8909).