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CHEMICAL ABSTRACTS, vol. 88, no. 3, 16th January, 1978, page 432, abstract 20373u Columbus, Ohio, USA WILLIAMS A.F. "Analysis of cell surfaces by xenogeneic myeloma hybrid antibodies: differentiation antigens of rat lymphocytes"
CHEMICAL ABSTRACTS, vol. 90, no. 1, 1st January, 1979, page 412, abstract 4241k Columbus, Ohio, USA HAEMMERLING G.J. et al. "Monoclonal antibodies against murine cell surface antigens: anti-H-2, anti-Ia and anti-T cell antibodies"
UNLISTED DRUGS, vol. 32, no. 5, May 1980, page 65

(10)

Description

Field of the invention

This invention relates generally to new hybrid cell lines and more specifically to hybrid cell lines for production of monoclonal antibody to an antigen found on all normal human helper T cells, to the antibody so produced, to therapeutic and diagnostic compositions and to diagnostic methods employing this antibody.

Description of the prior art

The fusion of mouse myeloma cells to spleen cells from immunized mice by Kohler and Milstein in 1975 [*Nature 256*, 495—497 (1975)] demonstrated for the first time that it was possible to obtain a continuous cell line making homogeneous (so-called "monoclonal") antibody. Since this seminal work, much effort has been directed to the production of various hybrid cells (called "hybridomas") and to the use of the antibody made by these hybridomas for various scientific investigations. See, for example, *Current Topics in Microbiology and Immunology*, Volume 81—"Lymphocyte Hybridomas", F. Melchers, M. Potter, and N. Warner, Editors, Springer-Verlag, 1978, and references contained therein; C. J. Barnstable, *et al., Cell, 14*, 9—20 (May, 1978); P. Parham and W. F. Bodmer, *Nature 276*, 397—399 (November, 1978); *Handbook of Experimental Immunology*, Third Edition, Volume 2, D. M. Wier, Editor, Blackwell, 1978, Chapter 25; and *Chemical and Engineering News*, January 1, 1979, 15—17.

These references simultaneously indicate the rewards and complications of attempting to produce monoclonal antibody from hybridomas. While the general technique is well understood conceptually, there are many difficulties met and variations required for each specific case. In fact, there is no assurance, prior to attempting to prepare a given hybridoma, that the desired hybridoma will be obtained, that it will produce antibody if obtained, or that the antibody so produced will have the desired specificity. The degree of success is influenced principally by the type of antigen employed and the selection technique used for isolating the desired hybridoma.

The attempted production of monoclonal antibody to human lymphocyte cell-surface antigens has been reported only in a few instances. See, for example, *Current Topics in Microbiology and Immunology, ibid*, 66—69 and 164—169. The antigens used in these reported experiments were cultured human lymphoblastoid leukemia and human chronic lymphocytic leukemia cell lines. Many hybridomas obtained appeared to produce antibody to various antigens on all human cells. None of the hybridomas produced antibody against a predefined class of human lymphocytes.

It should be understood that there are two principal classes of lymphocytes involved in the immune system of humans and animals. The first of these (the thymus-derived cell or T cell) is differentiated in the thymus from haemopoietic stem cells. While within the thymus, the differentiating cells are termed "thymocytes". The mature T cells emerge from the thymus and circulate between the tissues, lymphatics, and the bloodstream. These T cells form a large proportion of the pool of recirculating small lymphocytes. They have immunological specificity and are directly involved in cell-mediated immune responses (such as graft rejection) as effector cells. Although T cells do not secrete humoral antibodies, they are sometimes required for the secretion of these antibodies by the second class of lymphocytes discussed below. Some types of T cells play a regulating function in other aspects of the immune system. The mechanism of this proces of cell cooperation is not yet completely understood.

The second class of lymphocytes (the bone marrow-derived cells or B cells) are those which secrete antibody. They also develop from haemopoietic stem cells, but their differentiation is not determined by the thymus. In birds, they are differentiated in an organ analogous to the thymus, called the Bursa of Fabricius. In mammals, however, no equivalent organ has been discovered, and it is thought that these B cells differentiate within the bone marrow.

It is now recognized that T cells are divided into at least several subtypes, termed "helper", "suppressor", and "killer" T cells, which have the function of (respectively) promoting a reaction, suppressing a reaction, or killing (lysing) foreign cells. These subclasses are well understood for murine systems, but they have only recently been described for human systems. See, for example, R. L. Evans, *et al, Journal of Experimental Medicine*, Volume 145, 221—232, 1977; and L. Chess and S. F. Schlossman—"Functional Analysis of Distinct Human T-Cell Subsets Bearing Unique Differentiation Antigens", in *"Contemporary Topics in Immunobiology"*, O. Stutman, Editor, Plenum Press, 1977, Volume 7, 363—379.

The ability to identify or suppress classes or subclasses of T cells is important for diagnosis or treatment of various immunoregulatory disorders or conditions.

For example, certain leukemias and lymphomas have differing prognosis depending on whether they are of B cell or T cell origin. Thus, evaluation of the disease prognosis depends upon distinguishing between these two classes of lymphocytes. See, for example, A. C. Aisenberg and J. C. Long, *The American Journal of Medicine*, 58:300 (March, 1975); D. Belpomme, *et al.*, in *"Immunological Diagnosis of Leukemias and Lymphomas"*, S. Thierfelder, *et al.*, eds, Springer, Heidelberg, 1977, 33—45; and D. Belpomme, *et al., British Journal of Haematology*, 1978, *38*, 85.

Certain disease states (e.g., juvenile rheumatoid arthritis, certain leukemias, and agammaglobulinemia) are associated with an imbalance of T cell subclasses. It has been suggested that autoimmune diseases generally are associated with an excess of "helper" T cells or a deficiency of certain

"suppressor" T cells, while agammaglobulinemia is associated with an excess of certain "suppressor" T cells or a deficiency of "helper" T cells. Malignancies generally are associated with an excess of "suppressor" T cells.

In certain leukemias, excess T cells are produced in an arrested stage of development. Diagnosis may thus depend on the ability to detect this imbalance or excess. See, for example, J. Kersey, *et al.*, "Surface Markers Define Human Lymphoid Malignancies with Differing Prognoses" in Haematology and Blood Transfusion, Volume 20, Springer-Verlag, 1977, 17—24, and references contained therein.

Acquired agammaglobulinemia, a disease state in which no immunoglobulin is produced, comprises at least two distinct types. In type I the failure to produce immunoglobulin is due to an excess of suppressor T cells, while in type II it is due to a lack of helper T cells. In both types, there appears to be no defect or lack in the patients' B cells, the lymphocytes which are responsible for the actual secretion of the antibody; however, these B cells are being either suppressed or "not helped", resulting in greatly decreased or absent immunoglobulin production. The type of acquired agammaglobulinemia may thus be determined by testing for an excess of suppressor T cells or an absence of helper T cells.

On the therapeutic side, there is some suggestion, as yet not definitely proven, that administration of antibodies against the subtype of T cell in excess may have therapeutic benefit in autoimmune disease or malignancies. For example, a helper T cell cancer (certain cutaneous T cell lymphomas and certain T cell acute lymphoblastic leukemias) may be treated by an antibody to a helper T cell antigen. Treatment of autoimmune disease caused by an excess of helper cells may also be accomplished in the same fashion.

Antisera against the entire class of human T cells (so-called antihuman thymocyte globulin or ATG) has been reported useful therapeutically in patients receiving organ transplants. Since the cell-mediated immune response (the mechanism whereby transplants are rejected) depends upon T cells, administration of antibody to T cells prevents or retards this rejection process. See, for example, Cosimi, *et al.*, "Radomized Clinical Trial of ATG in Cadaver Renal Allograft Recipients: Importance of T Cell Monitoring", *Surgery 40*: 155—163 (1976) and references contained therein.

The identification and suppression of human T cell classes and subclasses has previously been accomplished by the use of spontaneous autoantibodies or selective antisera for human T cells obtained by immunizing animals with human T cells, bleeding the animals to obtain serum, and adsorbing the antiserum with (for example) autologous but not allogeneic B cells to remove antibodies with unwanted reactivities. The preparation of these antisera is extremely difficult, particularly in the adsorption and purification steps. Even the adsorbed and purified antisera contain many impurities in addition to the desired antibody, for several reasons. First, the serum contains millions of antibody molecules even before the T cell immunization. Second, the immunization causes production of antibodies against a variety of antigens found on all human T cells injected. There is no selective production of antibody against a single antigen. Third, the titer of specific antibody obtained by such methods is usually quite low, (e.g., inactive at dilutions greater than 1:100) and the ratio of specific to non-specific antibody is less than $1/10^6$.

See, for example, the Chess and Schlossman article referred to above (at pages 365 and following) and the Chemical and Engineering News article referred to above, where the deficiencies of prior art antisera and the advantages of monoclonal antibody are described.

Summary of the invention

There has now been discovered a novel hybridoma (designated OKT4) which is capable of producing novel monoclonal antibody against an antigen found on essentially all normal human peripheral helper T cells (about 55% of normal human peripheral T cells). The antibody so produced is monospecific for a single determinant on normal human helper T cells and contains essentially no other anti-human immunoglobulin, in contrast to prior art antisera (which are inherently contaminated with antibody reactive to numerous human antigens) and to prior art monoclonal antibodies (which are not monospecific for a human helper T cell antigen). Moreover, this hybridoma can be cultured to produce antibody without the necessity of immunizing and killing animals, followed by the tedious adsorption and purification steps necessary to obtain even the impure antisera of the prior art.

It is accordingly one object of this invention to provide hybridomas which produce antibodies against an antigen found on essentially all normal human helper T cells.

It is a further aspect of the present invention to provide methods for preparing these hybridomas.

A further object of the invention is to provide essentially homogeneous antibody against an antigen found on essentially all normal human helper T cells.

A still further object is to provide methods for diagnosis of disease employing these antibodies.

Other objects and advantages of the invention will become apparent from the examination of the present disclosure.

In satisfaction of the foregoing objects and advantages, there is provided by this invention a novel hybridoma producing novel antibody to an antigen found on essentially all normal human helper T cells, the antibody itself, and diagnostic and therapeutic compositions employing the antibody. The hybridoma was prepared generally following the method of Milstein and Kohler. Following immunization of mice with normal E rosette positive human T cells, the spleen cells of the immunized mice were fused with cells from a mouse myeloma line and the resultant hybridomas screened for those with supernatants containing antibody which gave selective binding to normal E rosette positive human T cells. The desired hybridomas

were subsequently cloned and characterized. As a result, a hybridoma was obtained which produces antibody (designated OKT4) against an antigen on essentially all normal human helper T cells. Not only does this antibody react with essentially all normal human peripheral helper T cells, but it also does not react with other normal peripheral blood lymphoid cells including non-helper T cells. In addition, the cell surface antigen recognized by this antibody is detected on approximately 80% of normal human thymocytes. Type II acquired agammaglobulinemia patients were detected by OKT4 antibody in a blind test. The subject antibody also reacts with about 55% of Rhesus monkey peripheral T cells.

In view of the difficulties indicated in the prior art and the lack of success reported using malignant cell lines as the antigen, it was surprising that the present method provided the desired hybridoma. It should be emphasized that the unpredictable nature of hybrid cell preparation does not allow one to extrapolate from one antigen or cell system to another. In fact, the present applicants have discovered that using a T cell malignant cell line as the antigen caused formation of hybridomas which did not produce the desired antibody. Attempts to use purified antigens separated from the cell surfaces were also unsuccessful.

Both the subject hybridoma and the antibody produced thereby are identified herein by the designation "OKT4", the particular material referred to being apparent from the context.

A sample of the subject hybridoma OKT-4 was deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, 20852, United States of America, on April 26, 1979, and has been assigned the ATCC number CRL 8002.

The present invention provides mouse monoclonal antibody which (i) reacts with essentially all normal human peripheral helper T cells (being about 55% of all normal human peripheral T cells) but (ii) does not react with any of the normal human peripheral cells in the groups comprising non-helper T cells, B cells, null cells and macrophages.

Preferably the antibody is of class IgG, most preferably of class IgG_2.

The antibody reacts with about 80% of normal human thymocytes and with about 55% of Rhesus monkey peripheral T cells. It preferably fixes complement and defines a T cell population ($OKT4^+$) which is unreactive with anti-TH_2 serum and is only minimally cytotoxic.

Preferably, the antibody does not react with leukemic cells from humans with T cell chronic lymphoblastic leukemia, B cell chronic lymphoblastic leukemia, T cell acute lymphoblastic leukemia, or null cell acute lymphoblastic leukemia.

The antibody may be produced by a hybridoma formed by fusion of spleen cells from a mouse previously immunized with human T cells and cells from a mouse myeloma line and preferably the monoclonal antibody is produced from hybridoma ATCC CRL 8002 (OKT4).

According to another aspect of the present invention, there is provided a hybridoma, which produces an antibody according to the invention, formed by fusion of spleen cells from a mouse previously immunised with humam T cells and cells from a mouse myeloma line.

The hybridoma is preferably ATCC CRL 8002 (OKT4).

According to yet another aspect of the present invention, there is provided a method for preparing an antibody according to the invention which comprises the steps of:

i) immunizing mice with E rosette positive purified human T cells,
ii) removing the spleens from said mice and making a suspension of spleen cells;
iii) fusing said spleen cells with mouse myeloma cells in the presence of a fusion promoter;
vi) diluting and culturing the fused cells in separate wells in a medium which will not support the unfused myeloma cells;
v) evaluating the supernatant in each well containing a hybridoma for the present of an antibody having any of the properties specified above;
vi) selecting and cloning hybridomas producing the desired antibody; and
vii) recovering the antibody from the supernatant above said clones.

Alternately the method comprises the steps of:

i) immunizing mice with E rosette positive purified human T cells;
ii) removing the spleens from said mice and making a suspension of the spleen cells;
iii) fusing said spleen cells with mouse myeloma cells in the presence of a fusion promoter;
iv) diluting and culturing the fused cells in separate wells in a medium which will not support the unfused myeloma cells;
v) evaluating the supernatant in each well containing a hybridoma for the presence of an antibody having any of the properties specified above;
vi) selecting and cloning hybridomas producing the desired antibody;
vii) transferring said clones intraperitoneally into mice; and
viii) harvesting the maglignant ascites or serum from said mice.

According to a further aspect of the present invention, a method of preparing an antibody comprises culturing the hybridoma ATCC CRL 8002 in a suitable medium and recovering the antibody from the supernatant above said hybridoma.

Alternatively the method comprises injecting into a mouse the hybridoma ATCC CRL 8002 and recovering the antibody from the malignant ascites or serum of said mouse.

The preparation and characterization of the hybridoma and the resultant antibody will be better understood by reference to the following description and Examples.

Detailed description of the invention

The method of preparing the hybridoma generally comprises the following steps:

A. Immunizing mice with E rosette positive purified normal human peripheral T cells. While it has been found that female CAF_1 mice are preferred, it is contemplated that other mouse strains could be used. The immunization schedule and T cell concentration should be such as to produce useful quantities of suitably primed splenocytes. Three immunizations at fourteen day intervals with 2×10^7 cells/mouse/injection in 0.2 ml phosphate buffered saline has been found to be effective.

B. Removing the spleens from the immunized mice and making a spleen suspension in an appropriate medium. About one ml of medium per spleen is sufficient. These experimental techniques are well-known.

C. Fusing the suspended spleen cells with mouse myeloma cells from a suitable cell line by the use of a suitable fusion promoter. The preferred ratio is about 5 spleen cells per myeloma cell. A total volume of about 0.5—1.0 ml of fusion medium is appropriate for about 10^8 splenocytes. Many mouse myeloma cell lines are known and available, generally from members of the academic community or various deposit banks, such as the Salk Institute Cell Distribution Center, La Jolla, CA. The cell line used should preferably be of the so-called "drug resistant" type, so that unfused myeloma cells will not survive in a selective medium, while hybrids will survive. The most common class is 8-azaguanine resistant cell lines, which lack the enzyme hypoxanthine guanine phophoribosyl transferase and hence will not be supported by HAT (hypoxanthine, aminopterin, and thymidine) medium. It is also generally preferred that the myeloma cell line used be of the so-called "non-secreting" type, in that it does not itself produce any antibody, although secreting types may be used. In certain cases, however, secreting myeloma lines may be preferred. While the preferred fusion promoter is polyethylene glycol having an average molecular weight from about 1000 to about 4000 (commercially available as PEG 1000, etc.), other fusion promoters known in the art may be employed.

D. Diluting and culturing in separate containers, the mixture of unfused spleen cells, unfused myeloma cells, and fused cells in a selective medium which will not support the unfused myeloma cells for a time sufficient to allow death of the unfused cells (about one week). The dilution may be a type of limiting one, in which the volume of diluent is statistically calculated to isolate a certain number of cells (e.g., 1—4) in each separate container (e.g., each well of a microtiter plate). The medium is one (e.g., HAT medium) which will not support the drug-resistant (e.g., 8-azaguanine resistant) unfused myeloma cell line. Hence, these myeloma cells perish. Since the unfused spleen cells are non-malignant, they have only a finite number of generations. Thus, after a certain period of time (about one week) these unfused spleen cells fail to reproduce. The fused cells, on the other hand, continue to reproduce because they possess the malignant quality of the myeloma parent and the ability to survive in the selective medium of the spleen cell parent.

E. Evaluating the supernatant in each container (well) containing a hybridoma for the presence of antibody to E rosette positive purified human T cells.

F. Selecting (e.g., by limiting dilution) and cloning hybridomas producing the desired antibody.

Once the desired hybridoma has been selected and cloned, the resultant antibody may be produced in one of two ways. The purest monoclonal antibody is produced by *in vitro* culturing of the desired hybridoma in a suitable medium for a suitable length of time, followed by recovery of the desired antibody from the supernatant. The suitable medium and suitable length of culturing time are known or are radially determined. This *in vitro* technique produces essentially monospecific monoclonal antibody, essentially free from other specific antihuman immunoglobulin. There is a small amount of other immunoglobulin present since the medium contains xenogeneic serum (e.g., fetal calf serum). However, this *in vitro* method may not produce a sufficient quantity or concentration of antibody for some purposes, since the concentration of monoclonal antibody is only about 50 µg/ml.

To produce a much greater concentration of slightly less pure monoclonal antibody, the desired hybridoma may be injected into mice, preferably syngenic or semi-syngenic mice. The hybridoma will cause formation of antibody-producing tumors after a suitable incubation time, which will result in a high concentration of the desired antibody (about 5—20 mg/ml) in the bloodstream and peritoneal exudate (ascites) of the host mouse. Although these host mice also have normal antibodies in their blood and ascites, the concentration of these normal antibodies is only about 5% of the monoclonal antibody concentration. Moreover, since these normal antibodies are not antihuman in their specificity, the monoclonal antibody obtained from the harvested ascites or from the serum is essentially free of any contaminating antihuman immunoglobulin. This monoclonal antibody is high titer (active at dilutions of 1:50,000 or higher) and high ratio of specific to non-specific immunoglobulin (about 1/20). Immunoglobulin produced incorporating the κ light myeloma chains are non-specific, "nonsense" peptides which merely dilute the monoclonal antibody without detracting from its specificity.

Example I

Production of monoclonal antibodies

A. Immunization and somatic cell hybridization

Female CAF_1 mice (Jackson Laboratories; 6—8 weeks old) were immunized intraperitoneally with 2×10^7 E rosette purified T cells in 0.2 ml of phosphate buffered saline at 14-day intervals. Four days after the third immunization, spleens were removed from the mice, and a single cell suspension was made by pressing the tissue through a stainless steel mesh.

Cell fusion was carried out according to the procedure developed by Kohler and Milstein. 1×10^8 splenocytes were fused in 0.5 ml of a fusion medium comprising 35% polyethylene glycol (PEG 1000) and 5% dimethylsulfoxide in RPMI 1640 medium (Gibco, Grand Island, NY) with 2×10^7 P3X63Ag8U1 myeloma cells supplied by Dr. M. Scharff, Albert Einstein College of Medicine, Bronx, NY. These myeloma cells secrete IgG_1 κ light chains.

B. Selection and growth of hybridoma

After cell fusion, cells were cultured in HAT medium (hypoxanthine, aminopterin, and thymidine) at 37°C with 5% CO_2 in a humid atmosphere. Several weeks later, 40 to 100 µl of supernatant from cultures containing hybridomas were added to a pellet of 10^6 peripheral lymphocytes separated into E rosette positive (E^+) and E rosette negative (E^-) populations, which were prepared from blood of healthy human donors as described by Mendes (*J. Immunol. 111*: 860, 1973). Detection of mouse hybridoma antibodies binding to these cells was determined by radioimmunoassay and/or indirect immunofluorescence. In the first method, the cells were initially reacted with 100 µl of affinity-purified ^{125}I goat-anti-mouse IgG (10^6 cpm/µg; 500 µg/µl). (Details of iodination of goat-anti-mouse IgG were described by Kung, *et al., J. Biol. Chem. 251* (8): 2399), 1976). Alternatively, cells incubated with culture supernatants were stained with a fluorescinated goat-anti-mouse IgG (G/M FITC) (Meloy Laboratories, Springfield, VA; F/p=2.5) and the fluorescent antibody-coated cells were subsequently analyzed on the Cytofluorograf FC200/4800A (Ortho Instruments, Westwood, MA) as described in Example III. Hybridoma cultures containing antibodies reacting specifically with E^+ lymphocytes (T cells) were selected and cloned. Subsequently, the clones were transferred intraperitoneally by injecting 1×10^7 cells of a given clone (0.2 ml volume) into CAF_1 mice primed with 2,6,10,14-tetramethylpentadecane, sold by Aldrich Chemical Company under the name Pristine. The malignant ascites from these mice were then used to characterize lymphocytes as described below in Example II. The subject hybrid antibody OKT4 was demonstrated by standard techniques to be of IgG_2 subclass and to fix complement.

Example II

Characterization of OKT4 reactivity

A. Isolation of lymphocyte populations

Human peripheral blood mononuclear cells were isolated from healthy volunteer donors (ages 15—40) by Ficoll-Hypaque density gradient centrifugation (Pharmacia Fine Chemicals, Piscataway, NJ) following the technique of Boyum, *Scand. J. Clin. Lab. Invest. 21* (Suppl. 97) 77, 1968). Unfractionated mononuclear cells were separated into surface Ig^+ (B) and Ig^- (T plus Null) populations by Sephadex G-200 anti-$F(ab')_2$ column chromatography as previously described by Chess, *et al., J. Immunol. 113*: 1113 (1974). T cells were recovered by E rosetting the Ig^- population with 5% sheep erythrocytes (Microbiological Associates, Bethesda, MD). The rosetted mixture was layered over Ficoll-Hypaque and the recovered E^+ pellet treated with 0.155M NH_4Cl (10 ml per 10^8 cells). The T cell population so obtained was <2% EAC rosette positive and >95% E rosette positive as determined by standard methods. In addition, the non-rosetting Ig^- (Null cell) population was harvested from the Ficoll interface. This latter population was <5% E^+ and ≤2% sIg^+. The surface Ig^+ (B) population was obtained from the Sephadex G-200 column following elution with normal human gamma globulin as previously described. This population was >95% surface Ig^+ and <5% E^+.

Normal human macrophages were obtained from the mononuclear population by adherence to polystyrene. Thus, mononuclear cells were resuspended in final culture media (RPMI 1640, 2.5 mM HEPES [4-(2-hydroxyethyl)-1-piperazinepropane sulfonic acid] buffer, 0.5% sodium-bicarbonate, 200 mM L-glutamine, and 1% penicillin-streptomycin, supplemented with 20% heat-inactivated human AB serum) at a concentration of 2×10^6 cells and incubated in plastic petri dishes (100×20 mm) (Falcon Tissue Culture Dish; Falcon, Oxnard, CA) at 37°C overnight. After extensive washing to remove non-adherent cells, the adherent population was detached by brisk washing with cold serum-free medium containing 2.5 mM EDTA and occasional scraping with the rubber tip of a disposable syringe plunger. Greater than 85% of the cell population was capable of ingesting latex particles and had morphologic characteristics of monocytes by Wright-Giemsa staining.

B. Normal thymus

Normal human thymus gland was obtained from patients aged two months to 14 years undergoing corrective cardiac surgery. Freshly obtained portions of the thymus gland were immediately placed in 5% fetal calf serum in medium 199 (Gibco), finely minced with forceps and scissors, and subsequently made into single cell suspensions by being pressed through wire mesh. The cells were next layered over

Ficoll-Hypaque and spun and washed as previously described in section A above. The thymocytes so obtained were >95% viable and ≥90% E rosette positive.

C. Cell lines

Epstein-Barr Virus (EBV) transformed B cell lines from four normal individuals (Laz 007, Laz 156, Laz 256, and SB) and T cell lines CEM, HJD-1, Laz 191, and HM1 established from leukemic patients were provided by Dr. H. Lazarus, Sidney Farber Cancer Institute, Boston, MA.

D. T acute lymphoblastic leukemia (T-ALL) cells and T chronic lymphatic leukemia (T-CLL) cells

Leumekia cells were obtained from 12 patients with T-ALL. These individuals' cells had previously been determined to be of T cell lineage by their spontaneous rosette formation with sheep erythrocytes (>20% E^+) and reactivity with T cell specific hetero-antisera, anti-HTL (anti-B.K.) and A99, as previously described by Schlossman, *et al., Proc. Nat. Acad. Sci. 73*: 1288 (1976). Tumor cells from three individuals were reactive (TH_2^+) with rabbit and/or equine anti-TH_2 while cells from the remaining nine were non-reactive (TH_2^-). Leukemic cells from two patients with TH_2^- T-CLL were also utilized. Both acute and chronic T cell leukemia cells were cryopreserved in −196°C vapor phase liquid nitrogen in 10% dimethylsulfoxide and 20% AB human serum until the time of surface characterization. The tumor populations analyzed were >90% blasts by Wright-Giemsa morphology in all instances.

Example III

Cytofluorographic analysis and cell separation

Cytofluorographic analysis of all cell populations was performed by indirect immunofluorescence with fluorescein-conjugated goat-anti-mouse IgG (G/M FITC) (Meloy Laboratories) on a Cytofluorograf FC200/4800A (Ortho Instruments). In brief, 1—2×10^6 cells were treated with 0.15 ml OKT4 at a 1:1000 dilution, incubated at 4°C for 30 minutes, and washed twice. The cells were then reacted with 0.15 ml of a 1:40 dilution G/M FITC at 4°C for 30 minutes, centrifuged, and washed three times. These cells were then analyzed on the Cytofluorograf and the intensity of fluorescence per cell recorded on a pulse height analyzer. A similar pattern of reactivity was observed at a dilution of 1:50,000, but further dilution caused loss of reactivity. Background staining was obtained by substituting a 0.15 ml aliquot of 1:1000 ascites from a Balb/cJ mouse intraperitoneally immunized with a non-producing hybrid clone.

In experiments designed to separate $OKT4^+$ and $OKT4^-$ cells, 100×10^6 unfractionated mononuclear cells or thymocytes were labeled with 4 ml of a 1:1000 dilution of OKT4 and developed with G/M FITC. An identical staining approach was utilized to prepare human T cells isolated as in Example IIA above. Utilizing a fluorescence activated cell sorter (FACS-I) (Becton-Dickinson, Mountain View, CA), lymphocytes were separated into $OKT4^+$ and $OKT4^-$ populations. Post sort viability was >95% by Trypan blue exclusion in all instances. Purity of all separated populations was ≥95%.

Example IV

Analysis of FACS separated $OKT4^+$ and $OKT4^-$ subsets with equine anti-TH_2

$OKT4^+$ and $OKT4^-$ T cells were separated on FACS and placed in culture at 2×10^6 cells per ml in RPMI 1640 (Grand Island Biological Company), containing 20% human AB serum, 1% penicillin-streptomycin, 200 mM L-glutamine, 25 mM HEPES buffer (Microbiological Associates), and 0.5% sodium bicarbonate. After 24 hours in a 5% CO_2 humid atmosphere at 37°C, 1—2×10^6 cells of each population were reacted with equine anti-TH_2 and stained with fluorescein-conjugated IgG fraction rabbit anti-horse Ig (Cappel Laboratories, Downington, PA) as described by Reinherz and Schlossman, *J. Immunol. 122*: 1335—1341 (1979). Background staining was determined by substituting normal horse IgG for specific antibody and staining as above.

Example V

Functional studies

A. Proliferative studies.

The mitogenic response of the unseparated and FACS-fractionated lymphoid cells was tested in microculture to optimal and suboptimal doses of Concanavalin A (Con A) (Calbiochem, La Jolla, CA) and Phytohemagglutinin (PHA) (Burroughs-Wellcome Company, Greenville, NC). Alloantigen proliferative response was measured concurrently for these same populations using mitomycin c treated Laz 156, an EBV transformed human B lymphoblastoid cell line, as a stimulus. Proliferation to tetanus toxoid (Massachusetts Department of Public Health Biological Laboratories, Boston, MA) was tested utilizing 10 µg/ml final concentration. Herpes-Zoster antigen was kindly provided by Dr. John Zaia (Harvard Medical School, Boston, MA) and utilized at a 1:6 dilution. Five percent macrophages obtained in the manner described above were added to all populations at the initiation of *in vitro* cultures. Mitogen stimulated cultures were pulsed after four days with 0.2 µCi of ^{3}H-thymidine (^{3}H-TdR) (1.9 Ci/mM specific activity) (Schwartz-Mann, Division of Becton, Dickinson, Orangeburg, NY) and harvested 18 hours later on a MASH II apparatus (Microbiological Associates). ^{3}H-TdR incorporation was measured in a Packard Scintillation Counter (Packard Instrument Company, Downer's Grove, IL). Background ^{3}H-TdR incorporation was

obtained by substituting media for mitogen. Soluble antigen and alloantigen stimulated cultures were pulsed after five days with ^{3}H-TdR for 18 hours, harvested, and counted as above.

B. Cytotoxicity studies

Sensitization cultures for cell-mediated lympholysis (CML) were established by placing unfractionated T cells, FACS-separated OKT4$^+$ and OKT4$^-$ T cell subsets, or different ratios of recombined OKT4$^+$ and OKT4$^-$ T cells with mitomycin-treated stimulator cells all at 2×10^6 cells per ml in multiple microtitre plate wells. At the end of five days, non-viable cells were removed by Ficoll-Hypaque centrifugation. These unfractionated and fractionated T cell populations were then added to ^{51}Cr sodium chromate labeled target cells and specific chromium release determined after a six-hour cell incubation. In other experiments, unfractionated T cells were sensitized with mitomycin-treated stimulator cells as above and then fractionated into OKT4$^+$ and OKT4$^-$ T cell subsets on FACS after five days in MLC and specific chromium release determined. Percent cycotoxicity was determined with the following formula:

$$\frac{^{51}\text{Cr released by experiment} - ^{51}\text{Cr released spontaneously}}{^{51}\text{Cr released by freeze-thaw} - ^{51}\text{Cr released spontaneously}} \times 100$$

All samples were performed in triplicate and results expressed as the mean. Spontaneous release was less than 20% of maximal lysis in all cases.

Brief description of the drawings

Figure 1 shows the fluorescence pattern obtained on the Cytofluorograf after reaching normal human peripheral T cells with OKT4 at a 1:1000 dilution and G/M FITC. For comparison, results with monoclonal antibodies OKT1 and OKT3 are shown under equivalent conditions in Figures 1—5.

Figure 2 shows the fluorescence pattern obtained on the Cytofluorograf after reacting human thymocytes with OKT4 and G/M FITC.

Figure 3 shows the fluorescence pattern obtained on the Cytofluorograf after reacting leukemic cells from B cell chronic lymphoblastic leukemia patients with OKT4 and G/M FITC.

Figure 4 shows the fluorescence pattern obtained on the Cytofluorograf after reacting the human T cell line HJD-1 with OKT4 and G/M FITC.

Figure 5 shows the fluorescence pattern obtained on the Cytofluorograf after reacting the human T cell line CEM with OKT4 and G/M FITC.

Figure 6 shows the results of reaction of T cell populations with equine anti-TH_2 serum.

Figure 7 shows the cytotoxic capacity of unfractionated T cells and T cell subsets. Percent specific lysis is shown on the ordinate and effector/target (E/T) ratio is shown on the abscissa.

The production of the hybridoma and the production and characterization of the resulting monoclonal antibody were conducted as described in the above Examples. Although large quantities of the subject antibody were prepared by injecting the subject hybridoma intraperitoneally into mice and harvesting the malignant ascites, it is clearly contemplated that the hybridoma could be cultured *in vitro* by techniques well-known in the art and the antibody removed from the supernatant.

As shown in Figure 1, approximately 55% of the human peripheral blood T cell population of a given normal individual is reactive with OKT4, whereas the entire B cell, null cell, and macrophage populations isolated from the same individual are unreactive with OKT4. Similar results were obtained on populations of lymphocytes from fifteen other normal individuals. The monoclonal antibody is thus characterized in that it is reactive with an antigen contained on the surface of approximately 55% of normal human peripheral T cells, while being unreactive with any antigens on the surface of the other three cell types discussed above. As will be discussed below, the OKT4$^+$ portion of the human peripheral T cell population is the subclass of helper T cells. This differential reactivity is one test by which the subject antibody OKT4 may be detected and distinguished from other antibodies.

As shown in Figure 2, approximately 80% of normal human thymocytes from a six-month old infant are reactive with OKT4. Similar results (about 80% reactivity) were obtained using six additional thymus specimens from normal individuals two months to 19 years of age. The pattern of reactivity in Figure 2 provides a second method of detecting the subject antibody OKT4 and distinguishing it from other antibodies.

As shown in Figure 3, the subject antibody is unreactive with leukemic cells from B cell chronic lymphoblastic leukemia.

A further characterization of the subject antibody OKT4 is shown by the reactivity to various human T cell lines illustrated in Figures 4 and 5. As can be seen, the reactivity of the subject antigen to human T cell lines was heterogeneous, being strong for the line CEM, and nonexistent for the line HDJ-1. There was also no reactivity with the cell lines Laz 191 and HMI. This differential reactivity of OKT4 to various readily-available human T cell lines provides yet another method of characterizing and describing the subject antibody.

Figure 6 illustrates the reactivity of OKT4 separated subsets with anti-TH_2. About 25% of the unfractionated T cell population is reactive with anti-TH_2. In contrast, the OKT4$^+$ population contains no

cells which are reactive with anti-TH_2, while the OKT4$^-$ population is predominantly TH_2^+ and contains all the TH_2^+ cells found in the unfractionated T cell population. This indicated that the TH_2^+ and the OKT4$^+$ subsets are reciprocal and distinct from one another.

Figure 7 illustrates the cytotoxic capacity of unfractionated, OKT4$^+$, and OKT4$^-$ T cells. The OKT4$^+$ population is only minimally cytotoxic, while the degree of killing mediated by the OKT4$^-$ population is greater than for the unfractionated T cell population. The differential reactivities of OKT4$^+$ and OKT4$^-$ T cell populations shown in Figures 6 and 7 provide a further means of characterizing the subject antibody.

The results illustrated in Figures 1—5 are summarized and augmented with additional data in Table I below. The Table compares monoclonal antibodies produced by hybridomas designated OKT1, OKT3, and OKT4 (which last is the subject of the present application). Hybridomas OKT1 and OKT3 are described in our co-pending European applications Nos. 80300829.1 and 80301357.2 (publication numbers 0 017 381 and 0 018 795) respectively. In addition to the data in Figures 1—5, Table I also demonstrates that OKT4 is unreactive with normal human peripheral B cells, null cells and macrophages, as well as leukemic cells from T cell and null cell acute lymphoblastic leukemia patients and EBV transformed B cell lines. In contrast to OKT1, OKT4 does not react with leukemic cells from T cell chronic lymphoblastic leukemia patients.

Functional studies were conducted on lymphoid populations which had been separated on a fluorescence activated cell separator (FACS). The results of these studies are shown in Tables II through IV below and provide further support for the previously-described characterization of the subject monoclonal antibody.

In these studies, an unfractionated T cell population was treated with a 1:1000 dilution of OKT4 and G/M FITC and separated on the FACS in OKT4$^+$ and OKT4$^-$ subsets. Given the purity of the populations obtained (greater than or equal to 95%), 5% macrophages were added to the separated populations prior to *in vitro* culture. The unfractionated T cell population and isolated OKT4$^+$ and OKT4$^-$ T cell subsets were then stimulated with PHA Con A, soluble antigens, and alloantigens to assess their *in vitro* proliferative responses.

The proliferative response of the unfractionated T cell populations to PHA and Con A is shown in Table II. A maximal proliferative response by the unfractionated T cell population is obtained with 1 µg of PHA per 10^6 cells with diminished responses occurring at 0.5 µg and 0.1 µg of PHA per 10^6 cells. Treatment of the unfractionated T cells with OKT4 and goat-mouse FITC without subsequent fractionation did not alter the proliferative response. In contrast, differences in response to PHA were obtained with the separated OKT4$^+$ and OKT4$^-$ T cell subset. The OKT4$^+$ population of cells responded to all doses of PHA in a fashion similar to the unseparated T cell population. However, the proliferative response of OKT4$^-$ cells was significantly less at all doses of PHA tested. Furthermore, at a dose of PHA of 0.1 µg per 10^6 cells, the OKT4$^-$ T cells did not proliferate at all, whereas the OKT4$^+$ T cell subset and unfractionated cells were still responsive. The proliferative response of these subsets to Con A, on the other hand, was similar and the two subsets of cells could not be distinguished from one another or the unfractionated T cell population.

The responses to alloantigen in MLC and to soluble antigens were next examined. As shown in Table III, the unfractionated T cell population, the unfractionated T cell population treated with OKT4 and G/M FITC, and both the OKT4$^+$ and OKT4$^-$ T cell subsets responded in a similar fashion in MLC against Laz 156. It should be noted, however, that the OKT4$^-$ cells from two of six individuals tested, although proliferating significantly in MLC, incorporated less ^{3}H-TdR than their respective OKT4$^+$ subset (data not shown). In contrast, proliferative responses to soluble antigens provided the clearest distinction between the subsets. In all cases tested, the OKT4$^+$ T cell subset proliferated well to the soluble antigens, tetanus toxoid and Herpes-Zoster, whereas the OKT4$^-$ T cell subset was virtually unresponsive.

Table IV shows that the OKT4$^-$ subset of T cells cannot generate much, if any, cytolysis when it is sensitized alone in MLC. Thus, although the OKT4$^-$ T cells became cytotoxic/effectors in the unfractionated allosensitized T cell population, by itself it could not be induced to mediate CML despite its response in MLC. Moreover, when the OKT4$^+$ population was sensitized in MLC in the absence of the OKT4$^-$ T cells, they could mediate a moderate, but significant, lysis in MLC. However, the recombined mixture of OKT4$^+$ and OKT4$^-$ T cells effected a maximal cytolysis, not unlike that of the unseparated T cell population. These findings demonstrate that the OKT4$^-$ subset cannot effect a maximal cytotoxic response alone, but requires help from the OKT4$^+$ population. This T-T interaction is analogous to the T cell help provided by the TH_2^- T cell subset to the TH_2^+ T cell subset in generating maximal cytotoxicity.

According to the present invention there are provided a hybridoma capable of producing antibody against an antigen found on essentially all normal human helper T cells, a method for producing this hybridoma, monoclonal antibody against an antigen found on essentially all human helper T cells, methods for producing the antibody compositions for treatment or diagnosis and methods of diagnosis of disease employing this antibody.

Although only a single hybridoma producing a single monoclonal antibody against human helper T cell antigen is described, it is contemplated that the present invention encompasses all monoclonal antibodies exhibiting the characteristics described herein. It was determined that the subject antibody OKT4 belongs to the subclass IgG_2, which is one of four subclasses of murine IgG. These subclasses of immunoglobulin G differ from one another in the so-called "fixed" regions, although an antibody to a specific antigen will have a so-called "variable" region which is functionally identical regardless of which subclass of immunoglobulin G it belongs to. That is, a monoclonal antibody exhibiting the characteristic

described herein may be of subclass IgG_1, IgG_2a, IgG_2b, or IgG_3, or of classes IgM, IgA, or other known Ig classes. The differences among these classes or subclasses will not affect the selectivity of the reaction pattern of the antibody, but may affect the further reaction of the antibody with other materials, such as (for example) complement or anti-mouse antibodies. Although the subject antibody is specifically IgG_2, it is contemplated that antibodies having the patterns of reactivity illustrated herein are included within the subject invention regardless of the immunoglobulin class or subclass to which they belong.

Further included within the subject invention are methods for preparing the monoclonal antibodies described above employing the hybridoma technique illustrated herein. Although only one example of a hybridoma is given herein, it is contemplated that one skilled in the art could follow the immunization, fusion, and selection methods provided herein and obtain other hybridomas capable of producing antibodies having the reactivity characteristics described herein. Since the individual hybridoma produced from a known mouse myeloma cell line and spleen cells from a known species of mouse cannot be further identified except by reference to the antibody produced by the hybridoma, it is contemplated that all hybridomas producing antibody having the reactivity characteristics described above are included within the subject invention, as are methods for making this antibody employing the hybridoma.

Further aspects of the invention are methods of diagnosis of disease employing the monoclonal antibody OKT4 or any other monoclonal antibody exhibiting the pattern of reactivity provided herein. The subject antibody may be used to detect type II acquired agammaglobulinemia by reacting a T cell composition from an individual with OKT4 antibody. Helper T cell absence or deficiency will be indicated by the presence of less than 55% of the total peripheral T cell population which reacts with OKT4. This test may also be used to detect helper T cell defects or excesses generally. Treatment of helper T cell cancers may be accomplished by administration of a therapeutically effective amount of OKT4 antibody to an individual in need of such treatment. By selective reaction with helper T cell antigen, the effective amount of OKT4 antibody will reduce the excess of helper T cells, thus ameliorating the effects of the helper T cell malignancy. Autoimmune disease caused by an excess of helper T cells may also be treated by administration of a therapeutically effective amount of OKT4 antibody to an individual in need of such treatment. Diagnostic and therapeutic compositions comprising effective amounts of OKT4 antibody in admixture with diagnostically or pharmaceutically acceptable carriers, respectively, are also included within the present invention.

TABLE I
Monoclonal antibody reactivity and properties.

	Monoclonal antibodies		
	OKT1	OKT3	OKT4
% Reactivity With:			
Peripheral T-cells (10 samples)	>95%	>95%	55%
Peripheral B-cells (10 samples)	<2%	<2%	<2%
Peripheral Null cells (10 samples)	<2%	<2%	<2%
Thymocytes* (8 samples)	5—10%	5—10%	80%
Reactivity With:			
T-chronic lymphatic Leukemia (3 cases)	+	+(1); −(2)	−
T-acute lymphatic Leukemia (8 cases)	−	−	−
Null acute lymphatic Leukemia (15 cases)	−	−	−
B-chronic lymphatic Leukemia (6 cases)	+(4); −(2)	−	−
B-cell lines+ (4)	−	−	−
T-cell lines+ HJD-1	+	(±)	−
CEM	+	−	+
Laz 191	+	−	−
HMI	+	−	−
IgG Subclass	IgG_1	IgG_2	IgG_2
Complement fixation	−	+	+

*From patients aged 2 months to 18 years

+Obtained from Dr. H. Lazarus, Sidney Farber Cancer Center. B cell lines Laz 256, 156, 007 and SB obtained by Epstein-Barr virus transformation of human peripheral B cells and HJD-1, CEM, Laz 191, and HMI established from leukemic patients.

TABLE II
Mitogen responsiveness of unfractionated and monoclonal antibody separated T cell subsets

Proliferative stimulus	T cells	T cells treated with OKT4 and G/M FITC	$OKT4^+$ T cells	$OKT4^-$ T cells
PHA (1 µg/10^6 cells)	98,876±3,061	99,780±5,615	109,643±11,043	23,841±890
PHA (0.5 µg/10^6 cells)	18,082±3,588	17,423±2,623	15,202±603	3,185±492
PHA (0.1 µg/10^6 cells)	4,215±386	4,317±523	4,999±677	72±11
Con A (250 µg/10^6 cells)	199,310±14,317	192,158±14,258	159,560±2,619	174,992±20,179
Con A (125 µg/10^6 cells)	100,726±8,864	92,633±6,780	97,003±9,089	82,199±6,154
Con A (50 µg/10^6 cells)	50,673±6,155	52,317±7,813	49,329±4,110	38,842±6,735
Media Control	63±2	57±6	167±8	69±12

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TABLE III
Proliferative response of unfractionated T cells and monoclonal antibody separated T cell subsets to soluble antigens and alloantigens

Proliferative stimulus	/ T cells	T cells treated with OKT4 and G/M FITC	OKT4$^+$ T cells	OKT4$^-$ T cells
		Experiment #1		
Laz 156$_m$	150,304±6,966	149,810±7,630	173,061±4,336	167,087±5,228
Tetanus Toxoid	18,046±271	19,947±3,214	19,660±2,348	516±69
Herpes-Zoster	47,413±3,208	40,774±4,305	55,785±10,608	188±129
Media	166±22	180±35	220±10	106±13
		Experiment #2		
Laz 156$_m$	67,265±3,466	68,399±6,154	97,615±4,361	67,483±4,792
Tetanus Toxoid	16,853±1,033	18,597±1,643	17,222±911	206±23
Herpes-Zoster	23,629±3,677	22,889±1,956	25,951±2,609	169±25
Media	167±43	289±57	298±41	31±8

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TABLE IV
Evidence for T-T interactions in the generation of maximal cytotoxicity in cml

Responder population sensitized to Laz 156_m	E/T ratio:	% Specific lysis+ Experiment #1 20:1	5:1	Experiment #2 20:1	5:1
Unfractionated T Cells		40+	—	23	11.9
Unfractionated T Cells treated with OKT4+G/M FITC		44	24	25	13
$OKT4^-$ T Cells		6	3	10.9	6.7
$OKT4^+$ T Cells		26	14	18.3	12.3
$OKT4^+$ and $OKT4^-$ T Cells++		48	31	31	16

+=S.R. less than 20% in all cases and S.D. less than or equal to 10% at all E/T ratios. Lysis by unsensitized T cells was less than or equal to 3% for all populations tested.
++=$OKT4^+$ and $OKT4^-$ recombined at 1:1 ratios.

Claims

1. Mouse monoclonal antibody which (i) reacts with essentially all normal human peripheral helper T cells (being about 55% of all normal human peripheral T cells) but (ii) does not react with any of the normal human peripheral cells in the group comprising non-helper T cells, B cells, null cells and macrophages.
2. A monoclonal antibody according to claim 1, of class IgG.
3. A monoclonal antibody according to claim 2, of class IgG_2.
4. A monoclonal antibody according to any one of claims 1 to 3, which reacts with about 80% of normal human thymocytes.
5. A monoclonal antibody according to any one of claims 1 to 4, which does not react with leukemic cells from humans with T cell chronic lymphoblastic leukemia, B cell chronic lymphoblastic leukemia, T cell acute lymphoblastic leukemia, or null cell acute lymphoblastic leukemia.
6. A monoclonal antibody according to any one of claims 1 to 5, which reacts with about 55% of Rhesus monkey peripheral T cells.
7. A monoclonal antibody according to any one of claims 1 to 6 which fixes complement.
8. A monoclonal antibody according to any one of claims 1 to 7, which defines a cell population ($OKT4^+$) which is unreactive with anti-TH_2 serum and is only minimally cytotoxic.
9. A monoclonal antibody according to any one of claims 1 to 8 which is produced by a hybridoma formed by fusion of spleen cells from a mouse previously immunized with human T cells and cells from a mouse myeloma line.
10. Monoclonal antibody which is produced from hybridoma ATCC CRL 8002.
11. A hybridoma which produces a monoclonal antibody according to any one of claims 1 to 9, formed by fusion of spleen cells from a mouse previously immunised with human T cells and cells from a mouse myeloma line.
12. Hybridoma ATCC CRL 8002.
13. A method for preparing a monoclonal antibody according to any one of claims 1 to 9 which comprises the steps of:

i) immunizing mice with E rosette positive purified human T cells;
ii) removing the spleens from said mice and making a suspension of spleen cells;
iii) fusing said spleen cells with mouse myeloma cells in the presence of a fusion promoter;
iv) diluting and culturing the fused cells in separate wells in a medium which will not support the unfused myeloma cells;
v) evaluating the supernatant in each well containing a hybridoma for the presence of an antibody having the properties specified in any one of claims 1 to 9;
vi) selecting and cloning hybridomas producing the desired antibody; and
vii) recovering the antibody from the supernatant above said clones.

14. A method for preparing a monoclonal antibody according to any one of claims 1 to 9, which comprises the steps of:

i) immunizing mice with E rosette positive purified human T cells;
ii) removing the spleens from said mice and making a suspension of the spleen cells;

TABLE IV
Evidence for T-T interactions in the generation of maximal cytotoxicity in cml

Responder population sensitized to Laz 156_m	E/T ratio:	% Specific lysis+ Experiment #1 20:1	5:1	Experiment #2 20:1	5:1
Unfractionated T Cells		40+	—	23	11.9
Unfractionated T Cells treated with OKT4+G/M FITC		44	24	25	13
$OKT4^-$ T Cells		6	3	10.9	6.7
$OKT4^+$ T Cells		26	14	18.3	12.3
$OKT4^+$ and $OKT4^-$ T Cells++		48	31	31	16

+=S.R. less than 20% in all cases and S.D. less than or equal to 10% at all E/T ratios. Lysis by unsensitized T cells was less than or equal to 3% for all populations tested.
++=$OKT4^+$ and $OKT4^-$ recombined at 1:1 ratios.

Claims

1. Mouse monoclonal antibody which (i) reacts with essentially all normal human peripheral helper T cells (being about 55% of all normal human peripheral T cells) but (ii) does not react with any of the normal human peripheral cells in the group comprising non-helper T cells, B cells, null cells and macrophages.
2. A monoclonal antibody according to claim 1, of class IgG.
3. A monoclonal antibody according to claim 2, of class IgG_2.
4. A monoclonal antibody according to any one of claims 1 to 3, which reacts with about 80% of normal human thymocytes.
5. A monoclonal antibody according to any one of claims 1 to 4, which does not react with leukemic cells from humans with T cell chronic lymphoblastic leukemia, B cell chronic lymphoblastic leukemia, T cell acute lymphoblastic leukemia, or null cell acute lymphoblastic leukemia.
6. A monoclonal antibody according to any one of claims 1 to 5, which reacts with about 55% of Rhesus monkey peripheral T cells.
7. A monoclonal antibody according to any one of claims 1 to 6 which fixes complement.
8. A monoclonal antibody according to any one of claims 1 to 7, which defines a cell population ($OKT4^+$) which is unreactive with anti-TH_2 serum and is only minimally cytotoxic.
9. A monoclonal antibody according to any one of claims 1 to 8 which is produced by a hybridoma formed by fusion of spleen cells from a mouse previously immunized with human T cells and cells from a mouse myeloma line.
10. Monoclonal antibody which is produced from hybridoma ATCC CRL 8002.
11. A hybridoma which produces a monoclonal antibody according to any one of claims 1 to 9, formed by fusion of spleen cells from a mouse previously immunised with human T cells and cells from a mouse myeloma line.
12. Hybridoma ATCC CRL 8002.
13. A method for preparing a monoclonal antibody according to any one of claims 1 to 9 which comprises the steps of:

i) immunizing mice with E rosette positive purified human T cells;
ii) removing the spleens from said mice and making a suspension of spleen cells;
iii) fusing said spleen cells with mouse myeloma cells in the presence of a fusion promoter;
iv) diluting and culturing the fused cells in separate wells in a medium which will not support the unfused myeloma cells;
v) evaluating the supernatant in each well containing a hybridoma for the presence of an antibody having the properties specified in any one of claims 1 to 9;
vi) selecting and cloning hybridomas producing the desired antibody; and
vii) recovering the antibody from the supernatant above said clones.

14. A method for preparing a monoclonal antibody according to any one of claims 1 to 9, which comprises the steps of:

i) immunizing mice with E rosette positive purified human T cells;
ii) removing the spleens from said mice and making a suspension of the spleen cells;

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iii) fusing said spleen cells with mouse myeloma cells in the presence of a fusion promoter;
iv) diluting and culturing the fused cells in separate wells in a medium which will not support the unfused myeloma cells;
v) evaluating the supernatant in each well containing a hybridoma for the presence of an antibody having the properties specified in any one of claims 1 to 9;
vi) selecting and cloning hybridomas producing the desired antibody;
vii) transferring said clones intraperitoneally into mice; and
viii) harvesting the malignant ascites or serum from said mice.

15. A method of preparing a monoclonal antibody, which comprises culturing hybridoma ATCC CRL 8002 in a suitable medium and recovering the antibody from the supernatant above said hybridoma.

16. A method of preparing a monoclonal antibody, which comprises injecting into a mouse hybridoma ATCC CRL 8002 and recovering the antibody from the malignant ascites or serum of said mouse.

17. The antibody of any one of claims 1 to 10 for use in the detection of helper T cell deficiency or excess in an individual.

18. The antibody of claim 17, wherein the helper T cell deficiency is type II acquired aggammaglobulinemia.

19. The antibody of any one of claims 1 to 10 for use in the treatment of helper T cell cancer in an individual.

20. The antibody of claim 19, wherein the helper T cell cancer in a cutaneous T cell lymphoma or a T cell acute lymphoblastic leukemia.

21. The antibody of any one of claims 1 to 10, for use in the treatment of autoimmune diseases caused by an excess of helper T cells in an individual in need of such treatment.

22. A diagnostic composition of matter, for detection of helper T cell deficiency comprising, in admixture with a diagnostically acceptable carrier, an amount of an antibody according to any one of claims 1 to 10 effective to detect helper T cell excess or deficiency.

23. A therapeutic composition of matter comprising, in admixture with a pharmaceutically acceptable carrier, an amount of an antibody according to any one of claims 1 to 10 effective to reduce the amount of helper T cells in an individual having an excess of said helper T cells.

24. A method for preparing a diagnostic composition according to claim 22 comprising mixing an amount of an antibody according to any one of claims 1 to 10 effective to detect a helper T cell excess or deficiency with a diagnostically acceptable carrier.

25. A method for preparing a therapeutic composition according to claim 23, comprising mixing an amount of an antibody according to any one of claims 1 to 10 effective to reduce the amount of helper T cells in an individual having an excess of said helper T cells with a pharmaceutically acceptable carrier.

Revendications

1. Anticorps monoclonal de souris qui (i) reagit avec essentiellement toutes les cellules T helper périphériques humaines normales (constituant environ 55% de toutes les cellules T périphériques humaines normales) mais (ii) ne réagit avec aucune des cellules périphériques humaines normales dans le groupe comprenant les cellules T non helper, les cellules B, les cellules nulles et les macrophages.

2. Anticorps monoclonal selon la revendication 1, de la classe IgG.

3. Anticorps monoclonal selon la revendication 2, de la classe IgG_2.

4. Anticorps monoclonal selon l'une quelconque des revendications 1 à 3, qui réagit avec environ 80% de thymocytes humains normaux.

5. Anticorps monoclonal selon l'une quelconque des revendications 1 à 4, qui ne réagit pas avec les cellules leucemiques d'êtres humains atteints de leucémie lymphoblastique chronique à cellules T, de leucémie lymphoblastique chronique à cellules B, de leucémie lymphoblastique aiguë à cellules T, ou de leucémie lymphoblastique aigué à cellules nulles.

6. Anticorps monoclonal selon l'une quelconque des revendications 1 à 5, qui réagit avec environ 55% de cellules T périphériques de singe Rhésus.

7. Anticorps monoclonal selon l'une quelconque des revendications 1 à 6 qui fixe le complément.

8. Anticorps monoclonal selon l'une quelconque des revendications 1 à 7, qui définit une population cellulaire ($OKT4^+$) qui est non réactive avec le sérum anti-TH_2 et est le moins possible cytotoxique.

9. Anticorps monoclonal selon l'une quelconque des revendications 1 à 8 qui est produit par un hybridome formé par fusion de cellules de rate d'une souris antérieurement immunisee avec des cellules T humaines et des cellules provenant d'une lignée de myélome de souris.

10. Anticorps monoclonal qui est produit à partir de l'hybridome ATCC CRL 8002.

11. Hybridome qui produit un anticorps monoclonal selon l'une quelconque des revendications 1 à 9, formé par fusion de cellules de rate d'une souris antérieurement immunisée avec des cellules T humaines et des cellules provenant d'une lignée de myélome de souris.

12. L'hybridome ATCC CRL 8002.

13. Procédé de préparation d'un anticorps monoclonal selon l'une quelconque des revendications 1 à 9 qui comprend les étapes consistant à:

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i) immuniser des souris avec des cellules T humaines purifiees positives à la rosette E;
ii) enlever la rate desdites souris et faire une suspension de cellules de rate;
iii) fusionner lesdites cellules de rate avec des cellules de myélome de souris en présence d'un promoteur de fusion;
iv) diluer et cultiver les cellules fusionnées dans des réservoirs séparés dans un milieu qui n'entretient pas les cellules de myélome non fusionnées;
v) évaluer dans le surnageant de chaque réservoir contenant un hybridome la présence d'un anticorps ayant les propriétés spécifiées dans l'une quelconque des revendications 1 à 9;
vi) choisir et cloner les hybridomas produisant l'anticorps désiré; et
vii) recueillir l'anticorps à partir du surnageant au-dessus desdits clones.

14. Procédé de preparation d'un anticorps monoclonal selon l'une quelconque des revendications 1 à 9, comprenant les etapes consistant à:

i) immuniser des souris avec des cellules T humaines purifiees positives à la rosette E;
ii) enlever la rate des dites souris et faire une suspension des cellules de rate;
iii) fusionner lesdites cellules de rate avec des cellules de myelome de souris en présence d'un promoteur de fusion;
iv) diluer et cultiver les cellules fusionnées dans des réservoirs séparés dans un milieu qui n'entretient pas les cellules de myélome non fusionnées;
v) évaluer dans le surnageant de chaque réservoir contenant un hybridome la présence d'un anticorps ayant les proprietés spécifiées dans l'une quelconque des revendications 1 à 9;
vi) choisir et cloner les hybridomes produisant l'anticorps désiré;
vii) transférer lesdits clones par voie intrapéritonéale à des souris; et
viii) recueillir l'ascite ou sérum malin desdites souris.

15. Procédé de préparation d'un anticorps monoclonal, dans lequel on cultive l'hybridome ATCC CRL 8002 dans un milieu approprié et on recueille l'anticorps à partir du surnageant au-dessus dudit hybridome.

16. Procedé de préparation d'un anticorps monoclonal, dans lequel on injecte à une souris l'hybridome ATCC CRL 802 et on recueille l'anticorps à partir de l'ascite ou serum malin de ladite souris.

17. Anticorps de l'une quelconque des revendications 1 à 10 pour application dans la détection d'une déficience ou d'un exces en cellules T helper chez un individu.

18. Anticorps de la revendication 17, où la déficience en cellules T helper est l'agammaglobulinémie acquise de type II.

19. Anticorps de l'une quelconque des revendications 1 à 10 pour application dans le traitement du cancer des cellules T helper chez un individu.

20. Anticorps de la revendication 19, où le cancer des cellules T helper est un lymphome des cellules T cutanées ou une leucémie lymphoblastique aigue des cellules T.

21. L'anticorps de l'une quelconque des revendications 1 à 10 pour application dans le traitement des maladies autoimmunes causées par un excès de cellules T helper chez un individu nécessitant un tel traitement.

22. Composition diagnostique pour la détection d'un excès ou d'un déficience en cellules T helper comprenant, mélangée à un support diagnostiquement acceptable, une quantité d'un anticorps selon l'une quelconque des revendications 1 à 10 efficace pour détecter un excès ou une déficience en cellules T helper.

23. Composition thérapeutique comprenant, mélangée à un support pharmaceutiquement acceptable, une quantité d'un anticorps selon l'une quelconque des revendications 1 à 10 efficace pour réduire la quantité de cellules T helper chez un individu ayant un excès desdites cellules T helper.

24. Procédé pour préparer une composition diagnostique selon la revendication 22 dans lequel on mélange une quantité d'un anticorps selon l'une quelconque des revendications 1 à 10 efficace pour détecter un excès ou une déficience en cellules T helper avec un support diagnostiquement acceptable.

25. Procédé pour préparer une composition thérapeutique selon la revendication 23, dans lequel on mélange une quantité d'un anticorps selon l'une quelconque des revendications 1 à 10 efficace pour réduire la quantite de cellules T helper chez un individu ayant un excès desdites cellules T helper avec un support pharmaceutiquement acceptable.

Patentansprüche

1. Monoklonaler Maus-Antikörper, welcher (i) mit im wesentlichen allen normalen humanen peripheren Helfer-T-Zellen reagiert (etwa 55% aller normalen humanen peripheren T-Zellen), aber (ii) nicht mit irgendwelchen der normalen humanen peripheren Zellen in der Nichthelfer-T-Zellen, B-Zellen, Null-Zellen und Macrophagen umfassenden Gruppe reagiert.

2. Monoklonaler Antikörper nach Anspruch 1 der Klasse IgG.

3. Monoklonaler Antikörper nach Anspruch 2 der Klasse IgG_2.

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4. Monoklonaler Antikörper nach einem der Ansprüche 1 bis 3, welcher mit etwa 80% normalen humanen Thymocyten reagiert.

5. Monoklonaler Antikörper nach einem der Ansprüche 1 bis 4, welcher nicht mit leukämischen Zellen von Menschen mit chronischer T-Zellen-lymphoblastischer Leukämie, chronischer B-Zellen-lymphoblastischer Leukämie, akuter T-Zellen-lymphoblastischer Leukämie oder akuter Null-Zellen-lymphoblastischer Leukämie reagiert.

6. Monoklonaler Antikörper nach einem der Ansprüche 1 bis 5, welcher mit etwa 55% von peripheren Rhesusaffen-T-Zellen reagiert.

7. Monoklonaler Antikörper nach einem der Ansprüche 1 bis 6, welcher Komplement fixiert.

8. Monoklonaler Antikörper nach einem der Ansprüche 1 bis 7, welcher eine Zellenpopulation (OKT 4^+) definiert, die mit anti-TH_2-Serum nicht reaktiv ist und nur minimal cytotoxisch ist.

9. Monoklonaler Antikörper nach einem Ansprüche 1 bis 8, welcher durch ein Hybridom gebildet wird, das durch Fusion von Milzzellen einer Maus, die zuvor mit humanen T-Zellen immunisiert worden ist, und Zellen aus einer Maus-Myelom-Linie ausgebildet ist.

10. Monoklonaler Antikörper, welcher voneinem Hybridom ATCC CRL 8002 gebildet wird.

11. Ein Hybridom, welches einen monoklonalen Antikörper gemäß einem der Ansprüche 1 bis 9 produziert, gebildet durch Fusion von Milzzellen einer zuvor mit humanen T-Zellen immunisierten Maus und Zellen aus einer Maus-Myeloma-Linie.

12. Das Hybridom ATCC CRL 8002.

13. Ein Verfahren zur Herstellung eines monoklonalen Antikörpers gemäß einem der Ansprüche 1 bis 9, umfassend die Stufen:

i) Immunisieren von Mäusen mit E-Rosetten-positiv gereinigten humanen T-Zellen;
ii) Entfernen der Milz aus den genannten Mäusen und Bereiten einer Suspension von Milzzellen;
iii) Fusion der genannten Milzzellen mit Maus-Myelom-Zellen in Gegenwart eines Fusionspromotors;
iv) Verdünnen und Vermehren der vereinigten Zellen in getrennten Vertiefungen in einem Medium, welches die nichtvereinigten Myelomzellen nicht erhält;
v) Auswerten des Überstandes in jeder Vertiefung, die ein Hybridom enthält, hinsichtlich des Vorliegens eines Antikörpers mit den in einem der Ansprüche 1 bis 9 angegebenen Eigenschaften;
vi) Auswählen und Klonen von Hybridomen, welche den gewünschten Antikörper bilden; und
vii) Gewinnen des Antikörpers aus dem Überstand über den genannten Klonen.

14. Ein Verfahren zur Herstellung eines monoklonalen Antikörpers gemäß einem der Ansprüche 1 bis 9, umfassend die Stufen:

i) Immunisieren von Mäusen mit E-Rosetten-positiv gereinigten humanen T-Zellen;
ii) Entfernen der Milz aus den genannten Mäusen und Bereiten einer Suspension von Milzzellen;
iii) Fusion der genannten Milzzellen mit Maus-Myelom-Zellen in Gegenwart eines Fusionspromotors;
iv) Verdünnen und Vermehren der vereinigten Zellen in getrennten Vertiefungen in einem Medium, welches die nichtvereinigten Myelomzellen nicht erhält;
v) Auswerten des Überstandes in jeder Vertiefung, die ein Hybridom enthält, hinsichtlich des Vorliegens eines Antikörpers mit den in einem der Ansprüche 1 bis 9 angegebenen Eigenschaften;
vi) Auswählen und Klonen von Hybridomen, welche den gewünschten Antikörper bilden;
vii) Überführen der genannten Klone auf intraperitonealem Wege in Mäuse; and
viii) Ernten der malignen Asciten oder von Serum aus den gennanten Mäusen.

15. Ein Verfahren zur Herstellung eines monoklonalen Antikörpers, welches ein Vermehren des Hybridoms ATCC CRL 8002 in einem geeigneten Medium und das Gewinnen des Antikörpers aus dem Überstand über den genannten Hybridom umfaßt.

16. Ein Verfahren zur Herstellung eines monoklonalen Antikörpers, welches ein Injizieren des Hybridoms ATCC CRL 8002 in eine Maus und das Gewinnen des Antikörpers aus den malignen Asciten oder dem Serum dieser Maus umfaßt.

17. Der Antikörper nach einem der Ansprüche 1 bis 10 zur Anwendung in der Feststellung von Helfer-T-Zellen-Mangel oder -Überschuß in einem Individuum.

18. Der Antikörper nach Anspruch 17, worin der Helfer-T-Zellen-Mangel eine erworbene Agammaglobulinämie Typ II ist.

19. Der Antikörper nach einem der Anspruche 1 bis 10 zur Verwendung in der Behandlung von Helfer-T-Zellen-Krebs in einem Individuum.

20. Der Antikörper nach Anspruch 19, worin der Helfer-T-Zellen-Krebs ein T-Zellen-Hautlymphom oder eine akute T-Zellen-lymphoblastique Leukämie ist.

21. Der Antikörper nach einem der Ansprüche 1 bis 10 zur Anwendung in der Behandlung von Autoimmunkrankheiten, die durch einen Überschuß von Helfer-T-Zellen in einem eine solche Behandlung benötigenden Individuum verursacht sind.

22. Eine diagnostische Zusammensetzung zur Feststellung von Helfer-T-Zellen-Überschuß oder -Mangel, umfassend, im Gemisch mit einem diagnostisch annehmbaren Träger, eine zur Feststellung von

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Helfer-T-Zellen-Überschuß oder -Mangel wirksame Menge eines Antikörpers nach einem der Ansprüche 1 bis 10.

23. Eine therapeutische Zusammensetzung, enthaltend, im Gemisch mit einem pharmazeutisch annehmbaren Träger, eine zur Verminderung der Menge von Helfer-T-Zellen in einem Individuum mit einem Überschuß an den genannten Helfer-T-Zellen wirksame Menge an einem Antikörper gemäß einem der Ansprüche 1 bis 10.

24. Ein Verfahren zur Bereitung einer diagnostischen Zusammensetzung gemäß Anspruch 22, umfassend das Vermischen einer zur Feststellung eines Helfer-T-Zellen-Überschusses oder -Mangels wirksamen Menge eines Antikörpers gemäß einem der Ansprüche 1 bis 10 mit einem diagnostisch annehmbaren Träger.

25. Ein Verfahren zur Herstellung einer therapeutischen Zusammensetzung gemäß Anspruch 23, umfassend das Vermischen einer zur Verminderung der Menge von Helfer-T-Zellen in einem Individuum mit einem Überschuß an den genannten Helfer-T-Zellen wirksamen Menge eines Antikörpers nach einem der Ansprüche 1 bis 10 mit einem pharmazeutisch annehmbaren Träger.

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