

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 normal human monocytes and granulocytes, to the antibody so produced, and to therapeutic and diagnostic compositions and 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 pre-defined class of human lymphocytes.

More recently, the present applicants and others have authored articles disclosing the preparation and testing of hybridomas making antibody to certain T-cell antigens. See, for

example, Reinherz, E. L., *et al.*, *J. Immunol.* 123, 1312—1317 (1979); Reinherz, E. L., *et al.*, *Proc. Natl. Acad. Sci.*, 76, 4061—4065 (1979); and Kung, P. C., *et al.*, *Science*, 206, 347—349 (1979).

Additionally, there has been a recent report of production of an anti-macrophage producing clone. See Springer, *et al.*, *Eur. J. Immunol.*, 9, 301 (1979).

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 process 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, malignancies, 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 and to determine which developmental stage is in excess. See, for example, J. Kerséy, 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; and E. L. Reinherz, et al., *J. Clin. Invest.*, 64, 392-397 (1979).

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. Treatment of diseases (e.g., malignancies or type I acquired agammaglobulinemia) due to an excess of suppressor T cells may be treated by administration of an antibody to a suppressor T cell antigen.

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., "Randomized Clinical Trial of ATG in Cadaver Renal Allograft Recipients: Importance of T Cell Monitoring", *Surgery* 40:155-163 (1976) and references contained therein.

Lymphocytes, however, comprise only one class of leukocyte. The other two classes, granulocytes and monocytes, are also important in the function of the immune systems of humans and animals. In particular, macrophages, which are a type of monocyte, are extensively involved in immune function. For example, although macrophages themselves do not secrete antibody, they have been found necessary for the cooperation of T cells in the production of antibody by B cells.

Macrophages are also required for the generation of cytotoxic T cells and for the occurrence of a proliferative response by T cells in reaction to mitogens such as PHA or Con A. Although there has been speculation about the function of the macrophage in these processes, its precise role is not known. See, for example, E. S. Golub, *The Cellular Basis of the Immune Response*, Sinauer Associates, Sunderland, Massachusetts, 1977, pages 146-158.

The identification and suppression of human T cell and monocyte 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 1/10⁶.

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 invention

There has now been discovered a novel hybridoma (designated OKM1) which is capable of

producing novel monoclonal antibody against an antigen found on normal human peripheral blood monocytes and granulocytes but not on normal human peripheral lymphoid cells (T cells, B cells, or null cells), thymocytes, lymphoblastoid cell lines, or tumor cells of T or B cell lineage.

The antibody so produced is monospecific for a single determinant on normal human peripheral blood monocytes and granulocytes and contains essentially no other antihuman 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 monocyte 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 normal human peripheral blood monocytes and granulocytes.

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 normal human peripheral blood monocytes and granulocytes.

A still further object is to provide compositions for treatment or diagnosis of disease or for identification of monocyte subclasses employing this antibody.

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 normal human peripheral blood monocytes and granulocytes (but not on normal human peripheral lymphoid cells, thymocytes, lymphoblastoid cell lines or tumor cells of T or B cell lineage), 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 purified human mononuclear cells, 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 and E rosette negative human peripheral blood lymphocyte populations. The desired hybridomas were subsequently cloned and characterized. As a result, a hybridoma was obtained which produces antibody (designated OKM1) against an antigen on normal human peripheral blood monocytes.

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 the use of a T cell malignant cell line or purified antigens separated from the cell surface as the antigen were generally unsuccessful.

Both the subject hybridoma and the antibody produced thereby are identified herein by the designation "OKM1" the particular material referred to being apparent from the context. The subject hybridoma was deposited on December 13, 1979, at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, and was given the ATCC accession number CRL 8026.

According to the present invention, there is provided a mouse monoclonal antibody which

- i) reacts with an antigen found on normal human monocytes and granulocytes;
- ii) does not react with any of the normal human peripheral cells in the group consisting of T cells and B cells;
- iii) does not react with normal human thymocytes; and
- iv) does not react with either of lymphoblastoid cell lines and tumour cells of T or B cell lineage,

preferably of class IgG.

Preferably, the antibody exhibits the pattern of reactivity to normal human monocytes shown in Figure 1 and Table I, or exhibits the pattern of reactivity to normal human E⁺ and E⁻ peripheral blood cells shown in Figure 2 and Table II.

The antibody may also exhibit the pattern of reactivity with normal human Ig⁺, E⁻, non-adherent null cells shown in Figure 3, or exhibit the pattern of lytic capacity shown in Figure 4 and Table III.

Preferably, the antibody reacts with leukemia cells from patients with AMML.

Advantageously the antibody defines a monocyte population necessary for a proliferative response to soluble antigens, or defines a monocyte population (OKM1⁺) which is lower than normal levels in primary biliary cirrhosis, acute infectious mononucleosis, and graft versus host reaction, higher than normal levels in multiple sclerosis, myasthenia gravis, and abnormal Hodgkins disease, and normal levels in hyper IgE, early Hodgkins disease, and psoriasis.

Most preferably, the antibody is produced by a hybridoma formed by fusion of cells from a mouse myeloma line and spleen cells from a mouse previously immunized with human mononuclear cells.

The present invention also provides monoclonal antibody produced by hybridoma ATCC CRL 8026 (OKM1), and the hybridoma itself.

According to another aspect of the present invention there is provided a method of producing monoclonal antibody according to the invention which comprises the steps of:

- i) immunizing mice with human mononuclear 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 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.

Alternatively, the selected and cloned hybridoma may be transferred intraperitoneally to mice and the antibody harvested from the malignant ascites or serum from said mice.

The present invention also provides a method of producing monoclonal antibody comprising culturing hybridoma ATCC CRL 8026 in a suitable medium and recovering the antibody from the supernatant above said hybridoma.

Alternatively, hybridoma ATCC CRL 8026 is injected into a mouse and the antibody is recovered 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 normal human peripheral blood mononuclear cells. While it has been found that female BALB/cJ mice are preferred, it is contemplated that other mouse strains could be used. The immunization schedule and mononuclear 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 phosphoribosyl 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 nonmalignant, 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 or antibody to E rosette positive purified or E rosette negative human peripheral blood mononuclear 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 readily 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 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 BALB/cJ mice (Jackson Laboratories; 6–8 weeks old) were immunized intraperitoneally with 2×10^7 rosette purified human mononuclear 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 P3×63Ag8UI myeloma cells supplied by Dr. M Scharff, Albert Einstein College of Medicine, Bronx, NY. These myeloma cells secrete IgG₁ 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% CO₂ 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 mononuclear cells 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 indirect immunofluorescence. Cells incubated with culture supernatants were stained with a fluoresceinated goat-anti-mouse IgG (G/M FITC) (Meloy Laboratories, Springfield, VA; F/p=2.5) and 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 mononuclear cells were selected and cloned twice by limiting dilution methods in the presence of feeder cells. Subsequently, the clones were transferred intraperitoneally by injecting 1×10^7 cells of a given clone (0.2 ml volume) into BALB/cJ 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 mononuclear cells as described below in Example II. The monoclonal antibody produced by one of these clones, which reacted with a fraction of both E⁺ and E⁻ cells, was termed OKM1. The subject hybrid antibody OKM1 was demonstrated by standard techniques to be of IgG_{2a} subclass.

Example II

Characterization of OKM1 reactivity

A. Isolation of mononuclear cell 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.

Adherent cells were obtained by a one hour incubation of mononuclear cells in polystyrene dishes (100×20 mm tissue culture dishes) (Falcon, Oxnard, CA) in RPMI 1640 (Grand Island Biological Company, Grand Island, NY) supplemented with 20% pooled human AB serum. After removal of the non-adherent fraction, adherent cells were detached by incubation at 4°C in serum-free MEM, 2.5 mM EDTA, followed by gentle scraping with the rubber tip of a disposable syringe plunger.

Mononuclear cells were separated into E⁺ and E⁻ populations by rosetting with sheep erythrocytes and differential centrifugation over Ficoll-Hypaque. Rosetting was accomplished by centrifugation of the mononuclear cells/sheep erythrocyte mixture for five minutes at 800 rpm and incubating for one hour at 4°C. The erythrocyte: lymphocyte ratio was 40:1. The E⁺ cells were recovered by lysis of the sheep erythrocytes with a 0.85% ammonium chloride solution.

Null cells were prepared by similar rosetting on the surface immunoglobulin negative (slg⁻) population obtained by Sephadex G-200 anti-human (F(ab')₂) chromatography as previously described by Chess, *et al.*, *J. Immunol.*, 113:1113

(14)

(1974). Polymorphonuclear leukocytes were obtained by lysis of the erythrocytes present in the pellet formed during Ficoll Hypaque centrifugation of peripheral blood.

B. Isolation of thymocytes

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 of T lineage and T acute lymphoblastic leukemia cells

T cell lines CEM and HSB-2, and B cell lines Laz 156 and Laz 388 were provided by Dr. H. Lazarus (Sidney Farber Cancer Institute, Boston, MA). Leukemic cells were obtained from patients with the diagnosis of acute myelomonocytic leukemia (AMML; 5 patients) and acute myeloblastic leukemia (AML; 8 patients). Tumor populations were cryopreserved at -196°C vapor phase liquid nitrogen with 10% DMSO and 20% AB human serum until the time of surface characterization.

Example III

Cytofluorographic analysis and cell separation

Cytofluorographic analysis of monoclonal antibodies with all cell populations was performed by indirect immunofluorescence with fluorescein-conjugated goat anti-mouse IgG (G/M FITC) (Mely Laboratories) utilizing a Fluorescence Activated Cell Sorter (FACS-I) (Becton, Dickinson, Mountain View, CA) or a Cytofluorograf FC200/4800A (Ortho Instruments). In brief, 1×10^6 cells were treated with 0.15 ml OKM1 at a 1:500 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 twice. Cells were then analyzed on the FACS-I and the intensity of fluorescence per cell was recorded on a pulse height analyzer. Background staining was obtained by substituting a 0.15 ml aliquot of 1:500 ascites from a BALB/cJ mouse intraperitoneally injected with a non-producing hybrid clone.

In experiments involving antibody and complement mediated lympholysis, mononuclear cells were cultured overnight following selective lysis and then subsequently analyzed on the Cytofluorograf or FACS.

A. Cytotoxicity studies

Sensitization cultures for cell-mediated lympholysis (CML) were established by the following procedure. Five $\times 10^6$ target cells were treated with 0.2 ml ^{51}Cr sodium chromate (292 $\mu\text{Ci/ml}$) (New

England Nuclear, Boston, MA) and incubated for 90 minutes at 37°C. After two washes, the cells were diluted to $2 \times 10^5/\text{ml}$ in media containing 10% FCS. Twenty μl of labeled cells were distributed in conical microplate wells with 20 μl of serial dilutions of hybridoma antibody. Following a one hour incubation at 4°C, 20 μl fresh rabbit serum (1:10 dilution) were added to the wells as a source of complement. The plates were incubated at 37°C for one hour. One hundred forty μl of media were then added to the wells and the plates were spun at 400 g for 10 minutes. One hundred μl of supernatant were removed from each well and counted on a gamma scintillation counter (Packard Instrumentation Company, Downer's Grove, IL). Specific ^{51}Cr release was calculated using the following formula:

$$\% \text{ specific } ^{51}\text{Cr release} = \frac{\text{Exp} - \text{SR}}{\text{MR} - \text{SR}} \times 100$$

where Exp=mean of the observed triplicate, SR=spontaneous release from the cells incubated with complement alone, and MR=maximum release obtained by treating the cells with the detergent Triton X (1% solution)

Lysis of larger numbers of cells for subsequent proliferative assays was done by resuspending 20 to 50×10^6 mononuclear cells in 1 ml of OKM1 at a 1:250 dilution and incubating at 4°C for one hour.

Subsequently, 0.1 ml fresh rabbit serum was added and the cells further incubated at 37°C for one hour. After three washes, the cells were counted and their viability was assessed by Trypan blue exclusion.

B. Proliferative response assay

The proliferative response to soluble antigens was measured as previously described. Cells were brought up at a concentration of 2×10^6 viable cells/ml in RPMI 1640 supplemented with 20% human AB serum and cultured in microtiter plates in the presence of tetanus toxoid (TT) (Massachusetts Department of Public Health Biological Laboratories, Boston, MA), purified protein derivative (PPD) (National Institutes of Health, Bethesda, MD), or mumps CF antigen (Microbiological Associates, Walkersville, MD). The cultures were pulsed after five days with 0.2 μCi of [H-methyl] thymidine (^3H TdR) (1.9 $\mu\text{Ci/mM}$ specific activity) (Schwarz-Mann, Orangeburg, NY) and harvested 18 hours later on a MASH 11 apparatus (Microbiological Associates). ^3H -TdR incorporation was measured in a Packard Liquid Scintillation Counter (Packard Instrumentation Company). Each experimental group was assayed in triplicate and the results are expressed as mean counts per minute (cpm) $\pm$ the standard error of the mean.

Brief description of the drawings

Figure 1 shows the fluorescence pattern obtained on the FACS after reacting unfractionated, nonadherent, and adherent normal

human peripheral blood mononuclear cells from donor #1 with OKM1 at a 1:500 dilution and G/M FITC. Background fluorescence staining was obtained by incubating each population with a 1:500 dilution of ascitic fluid from a mouse injected with a non-producing clone.

Figure 2 shows the fluorescence obtained on the FACS after reacting normal human E⁺ and E⁻ peripheral blood mononuclear cells with OKM1 and G/M FITC.

Figure 3 shows the fluorescence pattern obtained on the FACS after reacting normal human Ig⁻, E⁻, nonadherent null cells with OKM1 and G/M FITC.

Figure 4 shows the lytic capacity of OKM1 and complement on human mononuclear cells.

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.

Peripheral blood mononuclear cells of nine normal donors were separated into adherent and nonadherent populations on polystyrene dishes, and each fraction was analyzed by indirect immunofluorescence on the FACS. The percentages of cells specifically labeled by OKM1 are given in Table I. The mean of positive cells was 27% for unfractionated cells, 18% for nonadherent cells, and 78% for adherent cells. The FACS fluorescence profiles of the three populations for donor #1 are illustrated in Figure 1. As seen in Figure 1A, the fluorescence pattern of OKM1 on unfractionated mononuclear cells is bimodal. A two dimensional mapping of the same population showed that the cells brightly stained by OKM1 are also larger. A population of smaller cells with a weaker fluorescence intensity was also detected. Most of these smaller cells are found in the nonadherent fraction (Figure 1B). Adherent cells, in contrast, contain the brightly stained larger population (Figure 1C). This reactivity of OKM1 with adherent populations suggested that the mononuclear cell recognized by the antibody was a monocyte and provides one test by which the subject antibody may be detected and distinguished from other antibodies.

The study of lymphoid and myeloid cells of different origins gave further evidence for this specificity. OKM1 was unreactive with B cell lines (Laz 156, and Laz 388), T cell lines (CEM and HSB 2), and three human thymocyte preparations. Moreover, three T-CLL, six B-CLL, three T-ALL, and six non T-ALL tumor cells were also negative. In contrast, OKM1 reacted strongly with 44 to 82% of the cells of five acute myelomonocytic leukemia (AMML) samples and with about 35% of the cells in two out of eight acute myeloblastic leukemia (AML) cases. Two myeloid lines, K562

(Lozzio, *et al.*, *Blood* 45, 321 (1975) and HL 60 (Collins, *et al.*, *Nature*, 270 347 (1977)) were negative. Isolated polymorphonuclear leukocytes from four normal donors were brightly labeled.

These results were consistent with the notion of granulocytes and monocytes being closely related cells of common origin. In addition, the data obtained with AML cells seemed to indicate that OKM1 reacted preferentially with monocytic cells with the myeloid lineage. This pattern of reactivity provides an additional test by which the subject antibody may be detected and distinguished from other antibodies.

The staining pattern observed on unfractionated, adherent, and nonadherent mononuclear cells suggested that the monocyte population defined by the antibody was heterogeneous with respect to size and adherent properties. As surface Ia antigens have been described on subpopulations of monocytes, the above cell fractions (donor #1) were tested with a monoclonal anti-Ia antibody, OKI1, in order to characterize further the phenotype of the cells identified by OKM1. The percentage of OKI1⁺ cells in the nonadherent and adherent fractions were 9% and 80% respectively. As Ia bearing B lymphocytes account for some of the staining found with OKI1 in nonadherent populations, it appeared that the small, nonadherent cell recognized by OKM1 is mainly Ia⁻. In contrast, the large, adherent OKM1⁺ monocytes carry surface Ia determinants.

The distribution of OKM1 cells within E⁺ and E⁻ populations is shown in Table II. Although most of the labeled cells were found in the E⁻ population, up to 22% (mean, 13%) positive cells were present in the E⁺ fraction. FACS fluorescence profiles for E⁻ and E⁺ cells are shown in Figure 2. E⁻ cells had a bimodal staining pattern due to the presence of the large and small cells described above. The fluorescence intensity on the E⁺ population was more homogeneous. The positive cells found in this fraction were of a small size and largely Ia⁻ (mean reactivity with OKI1, 3%). This pattern of reactivity is a further test by which the subject antibody may be detected and distinguished from other antibodies.

A representative FACS histogram of OKM1 reactivity with the Ig⁻, E⁻, nonadherent cell population is illustrated in Figure 3. Eighty-four percent of the cells were stained with OKM1 and 11% with OKI1. Thus, these studies indicated that the OKM1⁺, Ia⁻ nonadherent cell accounted surprisingly for most of the Null cell population. This pattern of reactivity is a still further test by which the subject antibody may be detected and distinguished from other antibodies.

Complement mediated lysis assays were performed on several of the populations described above. Serial dilutions of OKM1 were tested on unfractionated, nonadherent, and adherent cells. Specific ⁵¹Cr release values are shown in Figure 4. There was a good correlation between the percentages found by indirect immunofluorescence (donor #5, Table I) and complement mediated lysis for each population.

E⁺ and E⁻ cells were also studied and the data obtained are presented in Table III. Again, the specific ⁵¹Cr release results corresponded to the labeling found by indirect immunofluorescence (donors #8, #9, and #10).

The effect of OKM1 and complement pretreatment on a known monocyte dependent T cell function, i.e., proliferation to soluble antigens, was studied. Mononuclear cells from a normal donor were incubated with OKM1 antibody (1:250 dilution) in the presence of complement. Graded amount of autologous adherent cells were added to this pre-treated population prior to triggering with soluble antigens. As shown in Table IV, OKM1 and complement treated mononuclear cells did not proliferate in response to soluble antigens, while the addition of 1% adherent cells could restore their proliferative response. As incubation with OKM1 antibodies in the absence of complement did not affect this T cell function, the effect seen in the presence of complement could be attributed to specific killing of monocytes within the mononuclear cell population.

It should be noted that, when 10 to 20% adherent cells were added to OKM1 pretreated cells, both the background and the antigen induced proliferation were increased when compared to the untreated population.

The effect of OKM1 on CML is also a test by which the subject antibody may be detected and distinguished from other antibodies.

Table V shows the relationship between levels of peripheral mononuclear cells and various disease states. These relationships may be used for diagnostic purposes (e.g., to detect acute infectious mononucleosis) by analyzing the blood sample of an individual suspected of having one of these disease states to determine the levels of mononuclear cells. For therapeutic use,

administration of the appropriate monoclonal antibody to a patient with an elevated mononuclear cell level will decrease or eliminate the excess. The relationships shown in Table V are a further way in which OKM1 antibody may be detected and distinguished from other antibodies.

Other monoclonal antibody producing hybridomas prepared by the present applicants (designated OKT1, OKT3, OKT4, and OKT5) are described and claimed in the following European patent application Nos. 80300829.1, 80301356.4, 80301357.2 and 80303272.1. Still other monoclonal antibody producing hybridomas prepared by the present applicants (designated OKT6, OKT8, OKT9 and OKT10) are described and claimed in European patent applications Nos. 80304347.0, 80304348.8, 80304349.6, and 80304350.4.

A further monoclonal antibody producing hybridoma prepared by the present applicants (designated OKT11) is described and claimed in a European patent application filed on even date herewith and entitled:

Hybrid Cell Line for Producing Antibody for a Human T cell Antigen, Antibody, and Methods, and numbered 81300047.8.

These applications are incorporated herein by reference.

According to the present invention there are provided a hybridoma capable of producing antibody against an antigen found on normal human monocytes, a method for producing this hybridoma, monoclonal antibody against an antigen found on normal human monocytes, methods for producing the antibody, and compositions for treatment or diagnosis and methods of diagnosis of disease or identification of monocyte subclasses employing this antibody.

TABLE I
Percentage of cells reactive with OKM1 by indirect immunofluorescence

Donor	Percentage of positive cells		
	F/H ^a	F/H Non adherent	Adherent
1	32.3	20.4	80.6
2	28.2	23.6	83.4
3	20.4	17.5	67.6
4	20.6	16.1	74.4
5	26.1	16.5	82.7
6	28.1	20.2	74.2
7	30.5	ND	84.8
8	27.7	ND	80.7
9	28.7	11.2	76.0
Mean	26.9	17.9	78.2

^aMononuclear cells prepared by centrifugation on Ficoll-Hypaque gradient.

TABLE II
Percentage of cells reactive with OKM1 in E rosette positive
and E rosette negative populations

Donor	Percentage of positive cells	
	E ⁺	E ⁻
1	18.2	64.2
2	12.1	51.2
3	13.8	47.9
4	9.7	67.9
5	14.2	41.5
6	22.2	69.0
7	11.5	51.2
8	10.6	58.6
9	9.4	55.6
10	12.8	68.5
Mean	13.5	57.5

TABLE III
OKM1 reactivity by complement mediated lysis on E rosette positive
and E rosette negative populations

Antibody dilution	Percent specific ⁵¹ Cr release		
	Donor #8	Donor #9	Donor #10
10 ⁻²	11.2±3	9.8±2	12.5±1
10 ⁻³	7.8±2	10.7±2	11.7±2
10 ⁻⁴	4.4±2	9.0±2	11.2±2
10 ⁻⁵	1.5±1	6.7±1	5.5±2
	E ⁻		
10 ⁻²	71.5±4	68.7±5	74.3±5
10 ⁻³	73.0±3	63.3±7	70.6±4
10 ⁻⁴	67.0±5	57.3±4	70.9±3
10 ⁻⁵	56.7±3	52.9±3	60.2±5

TABLE IV
Effect of pre-treatment of mononuclear cells with OKM1 and C' on antigen induced proliferative responses*
³H-thymidine incorporation

Cell population	Media	TT	PPD	Mumps
F/H	385±88	1,767±425	5,063±1,010	19,094±3,139
F/H OKM1+C'	144±10	142±12	162±23	254±4
F/H OKM1+C'				
+1% Adherent cells	190±51	1,358±209	5,777±1,013	7,269±1,090
+10% Adherent cells	1,425±259	4,128±921	16,412±2,747	38,558±1,680
+20% Adherent cells	1,944±665	5,926±1,429	13,182±3,007	40,065±5,810
Adherent cells	192±69	261±186	224±95	804±42

*All cultures done in triplicate. Results expressed as mean counts per minute ± standard error of the mean

^bTT (Tetanus Toxoid): 10 g/ml

^cPPD (Purified Protein Derivative): 10 g/ml

^dMumps: 1/20 dilution

TABLE V
Peripheral mononuclear cell levels in disease states
Mononuclear cell levels

Disease state	OKT3 ⁺	OKT4 ⁺	OKT5	OKT8	OKT6	OKT11	OKM1
Primary Biliary Cirrhosis (2)	N	+	—	—	—	++	—
Multiple Sclerosis (advanced disease) (8)	—	N	—	—	—	—	+
Myasthenia Gravis (early untreated) (3)	O	O	O	O	O	—	+
Acute Graft vs Host (3)	O to —	—	O	+	+	+	—
Acquired Agammaglobulinemia Type I			+				
Type II		O					
Hyper IgE (4)	—	N	O to —	O to —	—	+	N
Acute Infectious Mononucleosis (4)*	+	O to —	++	++	O	++	—
Hodgkins Disease Stages I & II	N	N	N	N	O	O	N
Stages III & IV	—	N	N	N	O	O	++
Psoriasis (3/5)	N	+ to ++	N	N	O	N	N

N=within normal limits

+=above normal

—=below normal

O=absent

++=greatly above normal

—=greatly below normal

*these levels return to normal about one week prior to the disappearance of clinical symptoms
The numbers in parentheses indicate the number of patients evaluated.

Although only a single hybridoma producing a single monoclonal antibody against a human monocyte 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 OKM1 belongs to the subclass IgG_{2a}, 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 a subclass IgG₁, IgG_{2a}, IgG_{2b}, or IgG₃, 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_{2a}, 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 techniques 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 OKM1 or any other monoclonal antibody exhibiting the pattern of reactivity provided herein. The subject antibody may be used to detect and study monocyte differentiation. Moreover, the subject antibody may be employed to diagnose disease states as shown in Table V. These techniques may be employed using OKM1 antibody alone or in combination with other antibodies (e.g., OKT3—OKT11). Patterns of reactivity with a panel of antibodies to T cells and T cell subsets and/or monocyte subsets will allow more precise detection of certain disease states than is possible using prior diagnostic methods. As an example, AMML and (to a lesser extent)

AML may be detected by reacting leukemia cells from individuals with OKM1 antibody.

Treatment of disease states (e.g., malignancies such as AMML) manifesting themselves as an excess of OKM1⁺ cells may be accomplished by administration of a therapeutically effective amount of OKM1 antibody to an individual in need of such treatment. By selective reaction with OKM1⁺ antigen, the effective amount of OKM1 antibody will reduce the excess of OKM1⁺ cells; thus ameliorating the effects of the excess.

Diagnostic and therapeutic compositions comprising effective amounts of OKM1 antibody in admixture with diagnostically or pharmaceutically acceptable carriers, respectively, are also included within the present invention.

Claims

1. A mouse monoclonal antibody which:
 - i) reacts with an antigen found on normal human monocytes and granulocytes;
 - ii) does not react with any of the normal human peripheral cells in the group consisting of T cells and B cells;
 - iii) does not react with normal human thymocytes; and
 - iv) does not react with either of lymphoblastoid cell lines and tumour cells of T or B cell lineage.
2. Monoclonal antibody according to claim 1, of class IgG.
3. Monoclonal antibody according to claim 1 or claim 2, which is produced by a hybridoma formed by fusion of cells from a mouse myeloma line and spleen cells from a mouse previously immunized with human mononuclear cells.
4. Monoclonal antibody according to any one of claims 1 to 3, which exhibits the pattern of reactivity to normal human monocytes shown in Figure 1 and Table I.
5. Monoclonal antibody according to any one of claims 1 to 4, which exhibits the pattern of reactivity with normal human E⁺ and E⁻ peripheral blood cells shown in Figure 2 and Table II.
6. Monoclonal antibody according to any one of claims 1 to 5, which exhibits the pattern of reactivity with normal human Ig⁺, E⁻, non-adherent null cells shown in Figure 3.
7. Monoclonal antibody according to any one of claims 1 to 6, which exhibits the pattern of lytic capacity shown in Figure 4 and Table III.
8. Monoclonal antibody according to any one of claims 1 to 7, which reacts with leukemia cells from patients with AMML.
9. Monoclonal antibody according to any one of claims 1 to 8, which defines a monocyte population necessary for a proliferative response to soluble antigens.
10. Monoclonal antibody according to any one of claims 1 to 9, which defines a monocyte population (OKM1⁺) which is lower than normal levels in primary biliary cirrhosis, acute infectious

Although only a single hybridoma producing a single monoclonal antibody against a human monocyte 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 OKM1 belongs to the subclass IgG_{2a}, 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 sub-class of immunoglobulin G it belongs to. That is, a monoclonal antibody exhibiting the characteristic described herein may be a subclass IgG₁, IgG_{2a}, IgG_{2b}, or IgG₃, 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_{2a}, 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 techniques 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 OKM1 or any other monoclonal antibody exhibiting the pattern of reactivity provided herein. The subject antibody may be used to detect and study monocyte differentiation. Moreover, the subject antibody may be employed to diagnose disease states as shown in Table V. These techniques may be employed using OKM1 antibody alone or in combination with other antibodies (e.g., OKT3—OKT11). Patterns of reactivity with a panel of antibodies to T cells and T cell subsets and/or monocyte subsets will allow more precise detection of certain disease states than is possible using prior diagnostic methods. As an example, AMML and (to a lesser extent)

AML may be detected by reacting leukemia cells from individuals with OKM1 antibody.

Treatment of disease states (e.g., malignancies such as AMML) manifesting themselves as an excess of OKM1⁺ cells may be accomplished by administration of a therapeutically effective amount of OKM1 antibody to an individual in need of such treatment. By selective reaction with OKM1⁺ antigen, the effective amount of OKM1 antibody will reduce the excess of OKM1⁺ cells, thus ameliorating the effects of the excess.

Diagnostic and therapeutic compositions comprising effective amounts of OKM1 antibody in admixture with diagnostically or pharmaceutically acceptable carriers, respectively, are also included within the present invention.

Claims

1. A mouse monoclonal antibody which:

- i) reacts with an antigen found on normal human monocytes and granulocytes;
- ii) does not react with any of the normal human peripheral cells in the group consisting of T cells and B cells;
- iii) does not react with normal human thymocytes; and
- iv) does not react with either of lymphoblastoid cell lines and tumour cells of T or B cell lineage.

2. Monoclonal antibody according to claim 1, of class IgG.

3. Monoclonal antibody according to claim 1 or claim 2, which is produced by a hybridoma formed by fusion of cells from a mouse myeloma line and spleen cells from a mouse previously immunized with human mononuclear cells.

4. Monoclonal antibody according to any one of claims 1 to 3, which exhibits the pattern of reactivity to normal human monocytes shown in Figure 1 and Table I.

5. Monoclonal antibody according to any one of claims 1 to 4, which exhibits the pattern of reactivity with normal human E⁺ and E⁻ peripheral blood cells shown in Figure 2 and Table II.

6. Monoclonal antibody according to any one of claims 1 to 5, which exhibits the pattern of reactivity with normal human Ig⁺, E⁻, non-adherent null cells shown in Figure 3.

7. Monoclonal antibody according to any one of claims 1 to 6, which exhibits the pattern of lytic capacity shown in Figure 4 and Table III.

8. Monoclonal antibody according to any one of claims 1 to 7, which reacts with leukemia cells from patients with AMML.

9. Monoclonal antibody according to any one of claims 1 to 8, which defines a monocyte population necessary for a proliferative response to soluble antigens.

10. Monoclonal antibody according to any one of claims 1 to 9, which defines a monocyte population (OKM1⁺) which is lower than normal levels in primary biliary cirrhosis, acute infectious



mononucleosis, and graft versus host reaction, higher than normal levels in multiple sclerosis, myasthenia gravis, and abnormal Hodgkins disease, and normal levels in hyper IgE, early Hodgkins disease, and psoriasis.

11. Monoclonal antibody which is produced by hybridoma ATCC CRL 8026 (OKM1).

12. A hybridoma, for producing a monoclonal antibody according to any one of claims 1 to 10, formed by fusion of spleen cells from a mouse previously immunized with human mononuclear cells and cells from a mouse myeloma line.

13. Hybridoma ATCC CRL 8026 (OKM1).

14. A method for preparing monoclonal antibody according to any one of claims 1 to 10, which comprises the steps of:

- i) immunizing mice with human mononuclear 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 10;
- vi) selecting and cloning hybridomas producing the desired antibody; and
- vii) recovering the antibody from the supernatant above said clones.

15. A method for preparing monoclonal antibody according to any one of claims 1 to 10, which comprises the steps of:

- i) immunizing mice with human mononuclear 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 the properties specified in any one of claims 1 to 10;
- 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.

16. A method of preparing monoclonal antibody, which comprises culturing hybridoma ATCC CRL 8026 in a suitable medium and recovering the antibody from the supernatant above said hybridoma.

17. A method of preparing monoclonal anti-

body, which comprises injecting into a mouse hybridoma ATCC CRL 8026 and recovering the antibody from the malignant ascites or serum of said mouse.

18. A diagnostic composition of matter for detection of OKM1⁺ cell excess or deficiency comprising, in admixture with a diagnostically acceptable carrier, an amount of the antibody of claim 10 or claim 11 effective to detect OKM1⁺ cell excess or deficiency.

19. A therapeutic composition of matter comprising, in admixture with a pharmaceutically acceptable carrier, an amount of the antibody of claim 10 or claim 11 effective to reduce the amount of OKM1⁺ cells in an individual having an excess of said OKM1⁺ cells.

20. A method for detection of a deficiency or excess of OKM1⁺ cells in an individual which comprises reacting a monocyte preparation from said individual with a diagnostically effective amount of the antibody of claim 10 or claim 11 or a composition of claim 18, and measuring the percentage of the monocyte population which reacts with said antibody.

21. Use, in a method of diagnosis, of an individual of a monoclonal antibody in accordance with claim 10 or claim 11, or of a composition in accordance with claim 18.

22. Monoclonal antibody according to claim 10 or claim 11, or a composition according to claim 19, for use in treating an OKM1⁺ cell excess in an individual in need of such treatment.

23. A method for preparing a composition according to claim 18 or claim 19 comprising admixing with a diagnostically or pharmaceutically-acceptable carrier a diagnostically or therapeutically-effective amount of the antibody according to claim 10 or claim 11.

Patentansprüche

1. Monoklonaler Maus-Antikörper

- i) mit einem Antigen reagiert, das auf normalen humanen Monocyten und Granulozyten findet;
- ii) nicht mit irgendeiner der normalen humanen peripheren Zellen (z.B. T-Zellen und B-Zellen) bestehender Gewebe reagiert;
- iii) nicht mit normalen humanen Tumorzellen reagiert; und
- iv) weder mit lymphoblastischen Zellen noch mit Tumorzellen der T- oder B-Zellen reagiert.

2. Monoklonaler Antikörper der Klasse IgG.

3. Monoklonaler Antikörper der Klasse IgG, welcher durch Fusion von Zellen einer Maus-Myelom-Linie und von zuvor mit humanen T-Zellen immunisierten Maus gezeugt wird.

4. Monoklonaler Antikörper der Klasse IgG, der Ansprüche 1 bis 3, welcher in



Tabelle I dargestellte Reaktivitätsmuster gegenüber normalen humanen Monocyten aufweist.

5. Monoklonaler Antikörper nach einem der Ansprüche 1 bis 4, welcher das in Figur 2 und in Tabelle II dargestellte Reaktivitätsmuster mit normalen humanen E^+ - und E^- -peripheren Blutzellen aufweist.

6. Monoklonaler Antikörper nach einem der Ansprüche 1 bis 5, welcher das in Figur 3 dargestellte Reaktivitätsmuster mit normalen humanen Ig^- , E^- , nichtadherierenden Null-Zellen aufweist.

7. Monoklonaler Antikörper nach einem der Ansprüche 1 bis 6, welcher das in Figur 4 und in Tabelle III dargestellte Muster von lytischer Kapazität aufweist.

8. Monoklonaler Antikörper nach einem der Ansprüche 1 bis 7, welcher mit Leukämie-Zellen von Patienten mit AMML reagiert.

9. Monoklonaler Antikörper nach einem der Ansprüche 1 bis 8, welcher eine Monocyten-Population definiert, die für eine proliferative Reaktion auf lösliche Antigene erforderlich ist.

10. Monoklonaler Antikörper nach einem der Ansprüche 1 bis 9, welcher eine Monocyten-Population (OKM 1⁺) definiert, welche niedriger ist als normale Gehalte in primärer Gallen-zirrhose, akuter infektiöser Mononukleosis und Implantat-gegen-Wirt-Reaktion, höher ist als normale Gehalte in Multipler Sklerosis, Myasthenia gravis und abnormaler Hodgkins-Krankheit, und normale Gehalte aufweist in Hyper IgE, früher Hodgkins-Krankheit und Psoriasis.

11. Monoklonaler Antikörper, welcher von einem Hybridom ATCC CRL 8026 (OKM1) gebildet wird.

12. Ein Hybridom, zur Herstellung eines monoklonalen Antikörpers nach einem der Ansprüche 1 bis 10, ausgebildet durch Fusion von Zellen von einer zuvor mit humanen mononukleären Zellen immunisierten Maus und von Zellen aus einer Maus-Myelom-Linie.

13. Das Hybridom ATCC CRL 8206 (OKM1).

14. Ein Verfahren zur Herstellung von monoklonalem Antikörper nach einem der Ansprüche 1 bis 10, umfassend die Stufen:

- i) Immunisieren von Mäusen mit humanen mononukleären 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 Fusions-promotors;
- iv) Verdünnen und Vermehren der vereinigten Zellen in getrennten Vertiefungen in einem Medium, welches die nicht-vereinigten Myelom-Zellen 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 10 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.

15. Ein Verfahren zur Herstellung von monoklonalem Antikörper nach einem der Ansprüche 1 bis 10, umfassend die Stufen:

- i) Immunisieren von Mäusen mit humanen mononukleären 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 Fusions-promotors;
- iv) Verdünnen und Vermehren der vereinigten Zellen in getrennten Vertiefungen in einem Medium, welches die nicht-vereinigten Myelom-Zellen 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 10 angegebenen Eigenschaften;
- vi) Auswählen und Klonen von Hybridomen, welche den gewünschten Antikörper bilden;
- vii) Überführen der genannten Klone auf intraperitonealem Wege in Mäuse; und
- viii) Ernten der malignen Asciten oder von Serum aus den genannten Mäusen.

16. Ein Verfahren zur Herstellung von monoklonalem Antikörper, welches ein Vermehren des Hybridoms ATCC CRL 8026 in einem geeigneten Medium und das Gewinnen des Antikörpers aus dem Überstand über dem genannten Hybridom umfaßt.

17. Ein Verfahren zur Herstellung von monoklonalem Antikörper, welches ein Injizieren des Hybridoms ATCC CRL 8026 in eine Maus und das Gewinnen des Antikörpers aus den malignen Asciten oder aus dem Serum der genannten Maus umfaßt.

18. Eine diagnostische Zusammensetzung zur Feststellung eines OKM1⁺-Zellen-Überschusses oder -Mangels, umfassend, im Gemisch mit einem diagnostisch annehmbaren Träger, eine zur Feststellung eines OKM1⁺-Zellen-Überschusses oder -Mangels wirksamen Menge des Antikörpers nach Anspruch 10 oder 11.

19. Eine therapeutische Zusammensetzung, enthaltend, im Gemisch mit einem pharmazeutisch annehmbaren Träger, eine zur Verminderung der Menge an OKM1⁺-Zellen in einem Individuum mit einem Überschuß an den genannten OKM1⁺-Zellen wirksame Menge des Antikörpers nach Anspruch 10 oder 11.

20. Ein Verfahren zur Feststellung eines Mangels oder eines Überschusses an OKM1⁺-Zellen in einem Individuum, umfassend ein Reagieren einer Monocyten-Zusammensetzung des genannten Individuums mit einer diagnostisch wirksamen Menge des Antikörpers nach Anspruch 10 oder





11, oder mit einer Zusammensetzung nach Anspruch 18 un Messen des Prozentsatzes der Monocyten-Population, die mit dem Antikörper reagiert.

21. Verwendung eines monoklonalen Antikörpers nach Anspruch 10 oder 11 oder einer Zusammensetzung nach Anspruch 18 in einem Diagnoseverfahren, das an einer von einem Individuum erhaltenen Monocytenzusammensetzung ausgeführt wird.

22. Monoklonaler Antikörper nach Anspruch 10 oder 11 oder eine Zusammensetzung nach Anspruch 19, zur Anwendung in der Behandlung eines OKM1⁺-Zellenüberschusses in einem Individuum, das eine solche Behandlung benötigt.

23. Ein Verfahren zur Herstellung einer Zusammensetzung nach Anspruch 18 oder 19, umfassend das Vermischen einer diagnostisch oder therapeutisch wirksamen Menge eines Antikörpers nach Anspruch 10 oder 11 mit einem diagnostisch oder pharmazeutisch annehmbaren Träger.

Revendications

1. Un anticorps monoclonal de souris qui:

- i) réagit avec un antigène présent sur les monocytes et les granulocytes humains normaux;
- ii) ne réagit avec aucune des cellules périphériques humaines normales du groupe constitué par les cellules T et les cellules B;
- iii) ne réagit pas avec les thymocytes humains normaux; et
- iv) ne réagit ni avec les lignées des cellules lymphoblastoïdes ni les cellules tumorales des lignées cellulaires T ou B.

2. Anticorps monoclonal selon la revendication 1 appartenant à la fraction IgG.

3. Anticorps monoclonal selon la revendication 1 ou la revendication 2 qui est produit par un hybridome formé par fusion de cellules d'une lignée de myélome de souris et de cellules spléniques d'une souris préalablement immunisée avec des cellules mononucléaires humaines.

4. Anticorps monoclonal selon l'une quelconque des revendications 1 à 3, qui présente le profil de réactivité vis-à-vis des monocytes humains normaux illustrée par la figure 1 et le tableau I.

5. Anticorps monoclonal selon l'une quelconque des revendications 1 à 4 qui présente le profil de réactivité avec les cellules sanguines périphériques humaines normales E⁺ et E⁻ illustré par la figure 2 et le tableau II.

6. Anticorps monoclonal selon l'une quelconque des revendications 1 à 5 qui présente le profil de réactivité avec les cellules nulles non adhérentes humaines normales Ig⁻, E⁻ illustré par la figure 3.

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

profil de capacité lytique illustré par la figure 4 et le tableau III.

8. Anticorps monoclonal selon l'une quelconque des revendications 1 à 7 qui réagit avec les cellules leucémiques de malades atteints de leucémie myélomonocytaire aiguë.

9. Anticorps monoclonal selon l'une quelconque des revendications 1 à 8 qui détermine une population de monocytes nécessaire à la réaction de prolifération à des antigènes solubles.

10. Anticorps monoclonal selon l'une quelconque des revendications 1 à 9 qui détermine une population de monocytes (OKM1⁺) qui est à une concentration inférieure à la normale dans la cirrhose biliaire primitive, la mononucléose infectieuse aiguë et la réaction du greffon contre l'hôte, supérieure à la concentration normale dans la sclérose en plaques, la myasthénie grave et la maladie de Hodgkin anormale, et à une concentration normale dans l'hyper IgE, la maladie de Hodgkin à son début et le psoriasis.

11. Anticorps monoclonal qui est produit par l'hybridome ATCC CRL 8026 (OKM1).

12. Un hybridome pour la production d'un anticorps monoclonal selon l'une quelconque des revendications 1 à 10 formé par fusion de cellules spléniques d'une souris préalablement immunisée avec des cellules mononucléaires humaines, et des cellules d'une lignée de myélome de souris.

13. Hybridome ATCC CRL 8026 (OKM1).

14. Un procédé pour la préparation d'un anticorps monoclonal selon l'une quelconque des revendications 1 à 10, qui comprend les stades de:

- i) immunisation de souris avec des cellules mononucléaires humaines;
- ii) prélèvement des rates desdites souris et formation d'une suspension des cellules spléniques;
- iii) fusion desdites cellules spléniques avec des cellules de myélome de souris en présence d'un promoteur de fusion;
- iv) dilution et culture des cellules fusionnées dans des cupules séparées dans un milieu qui n'entretient pas les cellules de myélome non fusionnées;
- v) évaluation du surnageant dans chaque cupule contenant un hybridome pour déterminer la présence d'un anticorps ayant les propriétés spécifiées dans l'une quelconque des revendications 1 à 10;
- vi) sélection et clonage des hybridomes produisant l'anticorps désirée; et
- vii) récupération de l'anticorps dans le surnageant au-dessus desdits clones.

15. Un procédé pour la préparation d'un anticorps monoclonal selon l'une quelconque des revendications 1 à 10 qui comprend les stades de:

- i) immunisation de souris avec des cellules mononucléaires humaines;
- ii) prélèvement des rates desdites souris et



- formation d'une suspension des cellules spléniques;
- iii) fusion desdites cellules spléniques avec des cellules de myélome de souris en présence d'un promoteur de fusion;
 - iv) dilution et culture des cellules fusionnées dans des cupules séparées dans un milieu qui n'entretient pas les cellules de myélome non fusionnées;
 - v) évaluation du surnageant dans chaque cupule contenant un hybridome pour déterminer la présence d'un anticorps ayant les propriétés spécifiées dans l'une quelconque des revendications 1 à 10;
 - vi) sélection et clonage des hybridomes produisant l'anticorps désirée;
 - vii) transfert intrapéritonéal desdits clones à des souris; et
 - viii) récolte du liquide d'ascite maligne ou du sérum desdites souris.

16. Un procédé pour la préparation d'un anticorps monoclonal qui comprend la culture de l'hybridome ATCC CRL 8026 dans un milieu approprié et la récupération de l'anticorps dans le surnageant au-dessus dudit hybridome.

17. Un procédé pour la préparation d'un anticorps monoclonal qui comprend l'injection à une souris de l'hybridome ATCC CRL 8026 et la récupération de l'anticorps à partir du liquide d'ascite maligne ou du sérum de ladite souris.

18. Une composition diagnostique pour la détection d'un excès ou d'un défaut de cellules OKM1⁺ comprenant en mélange avec un support convenant au diagnostic d'une quantité de l'anticorps de la revendication 10 ou de la revendication 11

efficace pour détecter un excès ou un défaut de cellules OKM1⁺.

19. Une composition thérapeutique comprenant en mélange avec un support acceptable en pharmacie une quantité de l'anticorps de la revendication 10 ou de la revendication 11 efficace pour réduire la quantité des cellules OKM1⁺ chez un sujet présentant un excès desdites cellules OKM1⁺.

20. Un procédé pour la détection d'un défaut ou d'un excès de cellules OKM1⁺ chez un sujet comprenant la réaction d'une composition de monocytes dudit sujet avec une quantité efficace pour le diagnostic de l'anticorps de la revendication 10 ou de la revendication 11 ou d'une composition de la revendication 18, et la mesure du pourcentage de la population de monocytes qui réagit avec ledit anticorps.

21. L'emploi dans un procédé de diagnostic effectué sur une composition de monocytes provenant d'un sujet, d'un anticorps monoclonal selon la revendication 10 ou la revendication 11 ou d'une composition selon la revendication 18.

22. Anticorps monoclonal selon la revendication 10 ou la revendication 11 ou une composition selon la revendication 19 pour l'emploi dans le traitement d'un excès de cellules OKM1⁺ chez un sujet nécessitant un tel traitement.

23. Un procédé pour la préparation d'une composition selon la revendication 18 ou la revendication 19 comprenant le mélange avec un support acceptable pour le diagnostic ou en pharmacie d'une quantité efficace pour le diagnostic ou en thérapeutique d'un anticorps selon la revendication 10 ou la revendication 11.

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