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Australia

Patents Act 1990

NOTICE OF ENTITLEMENT

I, John Gordon Hinde, of Spruson & Ferguson, St Martins Tower, 31 Market Street, Sydney, New South Wales 2000, Australia, being the patent attorney for the Applicant/Nominated Person in respect of an application entitled:

Diagnostic Method

state the following:-

The Applicant/Nominated Person has entitlement from the actual inventor(s) as follows:-

The Applicant/Nominated Person is the assignee of the actual inventor(s).

The Applicant/Nominated Person is the applicant of the application(s) listed in the Declaration under Article 8 of the PCT. The basic application(s) listed on the Declaration under Article 8 of the PCT is/are the application(s) first made in a Convention country in respect of the invention.

DATED this Thirteenth day of July 1992

.....

John Gordon Hinde

IRN:

INSTR CODE:

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(57) Claim

1. A method of diagnosing an human immunodeficiency virus infection in a subject comprising:

(a) analyzing, with labelled antibodies, labelled antibody fragments or mixtures thereof, a bodily fluid taken from the subject for proteins present on cell surfaces, said proteins reactive with antibodies directed against prosomal proteins or prosome-like proteins, to obtain a quantitative determination of the amount or percentage of cells displaying the protein on their surface; and

(b) comparing the amount or percentage thus obtained with a standard from a person not infected with human immunodeficiency virus.

9. A method of diagnosing a human immunodeficiency virus infection compri ıg: identifying or isolating cells selected from the group consisting of B4 cells, T4 cells, T8 cells, natural killer cells, T3 lymphocytes, or mixtures of these cells with antibodies directed against these cells; incubating the thus isolated or identified cells with labelled monoclonal antibodies directed against prosomal proteins; and thus analysing proteins present on the cell's surface.

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(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

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(54) Title: DIAGNOSTIC METHOD

(57) Abstract

Disclosed is a method useful in the diagnosis of HIV. The method involves the use of an immunochemical reagent containing one or more antibodies. Such a method would typically involve: obtaining blood from a subject suspected of suffering from HIV; contacting such an immunochemical reagent with the blood; and detecting the presence of immune complexes formed between the antibodies and any peptides reactive therewith present on the cell's surface.

Diagnostic Method.

Technical Field. This invention relates to diagnostic methods useful in the diagnosis of HIV infection.

Background Art: Acquired immune deficiency syndrome ("AIDS") is a disease thought most likely to be caused by the human immunodeficiency virus ("HIV"). The main targets of HIV are two different cells, the T4 lymphocyte and the macrophage. Once infected the T4 lymphocyte population generally begins to decline. Unlike the T4 lymphocytes, the macrophage is not killed by HIV infection but may actually serve as a reservoir for the virus. Gallo & Montagneir "AIDS in 1988", Scientific American, p. 25 (Oct. 1988).

The early diagnosis of persons suffering from HIV infection generally involves using diagnostic tests to determine whether or not a person has antibodies to HIV. Early diagnosis is especially important in HIV infection as it enables the patient to receive optimal medical care from the earliest moments of the disease and to check further spread of the contagion. Redfield & Burke "HIV Infection: The Clinical Picture", Scientific American, p. 70.

European Patent application 219,368 A (corresponding to U. S. Patent Application Serial No. 07/298,791) discloses certain monoclonal antibodies directed against proteins which form constituent parts of the "prosome". Prosomes are cellular particles described in Schmid et al "The prosome: an ubiquitous morphologically distinct RNP particle associated with repressed mRNPs and containing specific ScRNA and a characteristic set of proteins," The EMBO Journal, 3(1), 29-34 (1984).

The prosome has been also variously called the "proteasome" or "mcp" (multicatalytic protease). European Patent application 0 345 750 A2 discloses a "polyfunctional protease", and antibodies thereto. The polyfunctional protease described in this application may be the prosome.

Since early 1990, mouse monoclonal antibodies against prosomal proteins have been commercially available from Organon Teknika nv of Turnhout, Belgium. The commercially available monoclonal antibodies have been ones directed against prosomal proteins p23K, p25K, p27K, p29K (p28K, p33K), p30/33K, and p31K and the anti-p21K ("prosome-like particle"). These antibodies are more thoroughly described in Research Reagent News, (Jan. 1990) available from Organon Teknika nv.

In EP 219,368 A1, it is also disclosed that prosomes may be involved in many physiological processes related to the differentiation of cells and organisms, communication between cells, and to autoimmune disease. Also disclosed is that monoclonal antibodies directed against prosomes can be useful in the diagnosis of, for example, cancer.

In Research Reagent News, disclosed uses for the monoclonal antibodies against the prosomal proteins include the study of the molecular biology of prosomes, the distribution of specific types of prosomes in normal and pathological cells and tissues, messenger and other ribonucleoproteins.

No where is it described or suggested that any of these anti-prosomal proteins monoclonal antibodies might have any possible usefulness in the diagnosis of infectious diseases such as AIDS or HIV infection.

Disclosure of the Invention.

It has now been found that persons infected with HIV have cells (e.g. T4 and T8 lymphocytes as well as B4 cells) with different surface antigens than cells of persons not infected with the virus. Surprisingly, the presence of these surface antigens on the particular cells can be detected with certain labelled anti-prosomal protein monoclonal antibodies (i.e. monoclonal antibodies directed against prosomal proteins p23K, p25K, p27K, p29K (p28K, p33K), p30/33K, and p31K as well as monoclonal antibodies against p21K of the prosome-like particle).

According to a first embodiment of this invention there is provided a method of diagnosing a human immunodeficiency virus infection in a subject comprising:

(a) analysing, with labelled antibodies, labelled antibody fragments or mixtures thereof, a bodily fluid taken from the subject for proteins present on cell surfaces, said proteins reactive with antibodies directed against prosomal proteins or prosome-like proteins, to obtain a quantitative determination of the amount or percentage of cells displaying the protein on their surface; and

(b) comparing the amount or percentage thus obtained with a standard from a person not infected with human immunodeficiency virus.

According to a second embodiment of this invention there is provided a method of diagnosing a human immunodeficiency virus infection comprising: identifying or isolating cells selected from the group consisting of B4 cells, T4 cells, T8 cells, natural killer cells, T3 lymphocytes, or mixtures of these cells with antibodies directed against these cells; incubating the thus isolated or identified cells with labelled monoclonal antibodies directed against prosomal proteins; and thus analysing proteins present on the cell's surface.

According to a third embodiment of this invention there is provided a diagnostic test kit for carrying out the method according to claim 1 comprising: a solid phase on which antibodies directed against cells selected from the group consisting of B4 cells, T4 cells, T8 cells, natural killer cells, T3 lymphocytes, or mixtures of these cells has been immobilised, and a diagnostic fluid comprising labelled antibodies directed against prosomal proteins or prosome-like proteins.

According to a fourth embodiment of this invention there is provided a polypeptide having part or all of the primary structural conformation of the polypeptide depicted in SEQ ID NO:1 characterised by being the product of procaryotic or eucaryotic expression of an exogenous DNA source.

According to a fifth embodiment of this invention there is provided a polypeptide having part or all of the primary structural conformation of the polypeptide depicted in SEQ ID NO:3 characterised by being the product of procaryotic or eucaryotic expression of an exogenous DNA source.

According to a sixth embodiment of this invention there is provided a purified and isolated polypeptide of SEQ ID NO:3.

According to a seventh embodiment of this invention there is provided a DNA sequence for use in securing expression in a procaryotic or eucaryotic host cell of a peptide product having at least part of the primary structure of the polypeptide depicted in SEQ ID NO:1 or SEQ ID NO:3, said DNA sequence selected from the group consisting of:

(a) the DNA sequence set out in SEQ ID NO:1 or SEQ ID NO:3 or their complementary strands;

(b) DNA sequences which hybridise under stringent conditions to the protein coding regions of the DNA sequences defined in (a) or fragments thereof; and

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(c) DNA sequences which, but for the degeneracy of the genetic code, would hybridise to the DNA sequence defined in (a) and (b).

The invention therefore includes a method of diagnosing HIV infection using these anti-prosomal protein and anti-prosome-like particle monoclonal antibodies, no matter what can be used to determine whether T4 cells have been infected even before they are killed by HIV, thus enabling the method to be used as an early diagnostic tool for the diagnosis of HIV infection.

The method typically involves: obtaining body fluid containing cells capable of infection with HIV (eg. identifying the particular cells in question present in the fluid; contacting the thus identified or isolated cells with an immunochemical agent containing one or more of the described monoclonal antibodies; and detecting the presence and relative amount of immune complexes formed between the cells and the monoclonal antibodies reactive therewith present in the blood. These results once obtained can be compared with a "norm" derived from a person not infected with HIV.

Monoclonal antibodies directed against the anti-prosomal proteins p27K and p33K can be raised by using certain polypeptides or fragments or derivatives thereof. These polypeptides have generally all or part of the primary structural conformation of the polypeptides shown in SEQ ID NO:1 or SEQ ID NO:3 and may be produced by the procaryotic or eucaryotic expression of an exogenous DNA source.

The invention therefore also includes the DNA sequence for use in securing expression in a procaryotic or eucaryotic host cell of a peptide product having at least part of the primary structure of the polypeptide depicted in SEQ. ID NO:1 or SEQ ID NO:3. Generally such a DNA sequence will be selected from the group consisting of the DNA sequence set out in SEQ ID NO:1 or SEQ ID NO:3 or their complementary strands; DNA sequences which hybridise under stringent conditions to the protein coding regions of these DNA sequences or fragments thereof; and DNA

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sequences which, but for the degeneracy of the genetic code, would hybridize to those DNA sequences.

The invention thus includes the procaryotic or eucaryotic host cell transformed or transfected with such a DNA sequence, and the biologically functional circular plasmid or other DNA vector including such a DNA sequence.

The invention also includes the use of antibodies directed against prosomal proteins to analyze surface antigens present on a cell's surface for use, in example, phenotyping the cell. For this aspect of the invention the profiles presented in FIGs. 1 - 8 are used to identify an unknown white blood cell.

Brief Description of the Figures

FIG. 1 is a graph depicting the percentage of T4 lymphocytes in blood reacting with various labelled anti-prosomal protein monoclonal antibodies in both non-HIV infected ("temoins") and HIV infected subjects.

FIG. 2 is a graph depicting the number of T4 lymphocytes per cubic millimeter of subject blood reacting with various labelled anti-prosomal protein monoclonal antibodies in both non-HIV infected ("temoins") and HIV infected subjects.

FIG. 3 is a graph depicting the percentage of natural killer cells ("NK") in blood reacting with various labelled anti-prosomal protein monoclonal antibodies in both non-HIV infected ("temoins") and HIV infected subjects.

FIG. 4 is a graph depicting the number of NK per mm^3 of subject blood reacting with various labelled anti-prosomal protein monoclonal antibodies in both non-HIV infected ("temoins") and HIV infected subjects.

FIG. 5 is a graph depicting the percentage of T3 cells in blood reacting with various labelled anti-prosomal protein monoclonal antibodies in both non-HIV infected ("temoins") and HIV infected subjects.

FIG. 6 is a graph depicting the number of T3 cells per mm^3 of subject blood reacting with various labelled

anti-prosomal protein monoclonal antibodies in both non-HIV infected ("temoins") and HIV infected subjects.

FIG. 7 is a graph depicting the percentage of T8 cells in blood reacting with various labelled anti-prosomal protein monoclonal antibodies in both non-HIV infected ("temoins") and HIV infected subjects.

FIG. 8 is a graph depicting the percentage of B4 cells in blood reacting with various labelled anti-prosomal protein monoclonal antibodies in both non-HIV infected ("temoins") and HIV infected subjects.

FIG. 9 depicts the cDNA encoding a protein of the instant invention, and the amino acid sequence of that protein (the human p27K prosomal protein).

FIG. 10 depicts the cDNA encoding another protein of the instant invention, and the amino acid sequence of that protein (the human p33K prosomal protein).

Best Mode of the Invention

I. The Polypeptides -

A polypeptide or peptide is a linear series of amino acids connected one to the other by peptide bonds between the α-amino and carboxy groups of adjacent amino acids. Preferred polypeptides for use in producing antibodies for use in the invention are depicted in SEQ ID NO:1 and SEQ ID NO:3.

The protein or polypeptide may be derived from whole prosomes which are found intracellularly, separately in the blood, and on cell membrane surfaces.

The preparation of the peptides or fragments thereof according to the invention may be by recombinant DNA methods or one of the known organic chemical methods for peptide synthesis.

Organic chemical methods are especially useful in the synthesis of smaller peptide fragments having the desired activity (e.g. the ability to be used to raise antibodies). Once the sequence of the fragment is known, the synthesis is rather straightforward for an ordinarily skilled peptide chemist. Organic chemical

methods for peptide synthesis include coupling the required amino acids by means of a condensation reaction in either a homogenous phase or with the aid of a solid phase as disclosed by Merrifield. See, e.g. Merrifield, J. Am. Chem. Society, 85, 2149 (1963) and Wang, J. Am. Chem. Soc., 95, 1328 (1974).

Different solid supports and different strategies are known for the solid phase synthesis see, e.g. Barany and Merrifield in The Peptides, Analysis, Synthesis, Biology, Vol. 2, E. Gross and J. Meienhofer, eds., (Acad. Press, N.Y., 1980), Kneib-Cordonier and Mullen Int. J. Peptide Protein Res., 30, 705-739 (1987) and Fields and Noble Int. J. Peptide Protein Res., 35, 161-214 (1990).

Recombinant DNA methods involve the preparation of the desired peptide or fragment thereof by means of expressing recombinant polynucleotide with a polynucleotide sequence which codes for one or more of the peptides in question in a suitable microorganism as host. Generally the process involves introducing into a cloning vehicle (e.g. a plasmid, phage DNA, or other DNA sequence able to replicate in a host cell) a DNA sequence, such as that depicted in FIGs. 1 or 2; introducing said cloning vehicle into a suitable eucaryotic or procaryotic host cell; and culturing the host cell thus transformed. Typical host cells include bacteria such as E. coli, Pseudomonas, and Bacillus subtilis. Eukaryotes such as various strains of yeast and mammalian cell lines are also very useful.

Once the peptides have been made, fragments useful in the invention can also be determined. Suitable immunochemically active peptide fragments can be found according to means of the method described in Patent Application WO 86/06487 to Geysen et al; Geysen et al, Proc. Natl. Acad. Sci., (81) 3998-4002, (1984); and Geysen et al, J. Immunol Meth., (102) 259-274 (1987) based on the "pep-scan" method. In this method a series of overlapping peptides corresponding with partial sequences of the complete peptide under consideration

are synthesized and their reactivity with the relevant antibodies is investigated.

Of course the fragments or peptides do not have to be exactly as depicted in SEQ ID NO:1 and SEQ ID NO:3. It is self-evident that one or more amino acids in the peptides may be substituted with other amino acids or amino acid analogues or derivatives so long as the immunochemical activity is not significantly adversely affected.

Furthermore functional derivatives of the peptides may also be used. Such functional derivatives include acid addition salts (such as hydrochloric, hydrobromic, fumaric, phosphoric, ascorbic, tartaric, citric, lactic, maleic, palmitic, and other well-known acids) of the peptides; amides of the peptides and especially the C-terminal amides; esters and especially C-terminal esters; and N-acyl derivatives, especially N-terminal acyl derivatives and in particular N-acetyl derivatives. All functional derivatives are considered "polypeptides", "peptides" and "peptide fragments" according to the invention.

Similarly, substitutions can be made in the nucleic acid sequences of SEQ ID NO:1 and SEQ ID NO:3. The degeneracy of the genetic code permits substitution of bases in a codon resulting in another codon, but still coding for the same amino acid. For example, the codon for the amino acid glutamic acid is both GAG and GAA. Consequently it is clear that for the expression of a polypeptide with the amino acid sequence shown in SEQ ID NO:1 or SEQ ID NO:3, or an antigenic fragment thereof, use can be made of a nucleic acid sequence with such an alternative codon composition or compositions different from the nucleic acid sequence shown in the figures.

Also included within the scope of the invention is a nucleic acid sequence which hybridizes under stringent conditions iwth a nucleic acid sequence shown in SEQ ID NO:1 or SEQ ID NO:3. These hybridizable nucleic acid sequences display substantial homology with a nucleic acid sequence shown in SEQ ID NO:1 or 3, or a fragment

thereof, but may comprise nucleotide substitutions, mutations, insertions, deletions, inversions, etc. and encode a protein or polypeptide which is functionally equivalent to a prosomal protein or protein from a prosome-like particle. The resulting functionally equivalent protein, polypeptide or fragment will not be identical with a protein shown in SEQ ID NO:1, SEQ ID NO:3, or a fragment thereof, but will have corresponding antigenic properties.

II. Antibodies-

Antibodies are preferably used to isolate or identify the cells of the patient to be studied (i.e. a patient's B4 cells, T4 cells, T8 cells, natural killer cells, T3 lymphocytes, or mixtures of these cells). Such antibodies are preferably monoclonal antibodies or monospecific polyclonal (e.g. affinity purified) antibodies, although polyclonal antibodies and antibody fragments may also be used. Monoclonal antibodies directed against the various cells are commercially available from companies such as Ortho Diagnostics or Coultronics of Paris, France, or may be made. For example, antibodies against natural killer cells are described in WO 8903396 (claiming priority from U.S. Serial No. 109,730 filed on 16 October 1987).

Antibodies against prosomal proteins for use in diagnostic kits and methods according to the invention are preferably monoclonal antibodies directed against the various prosomal proteins. Polyclonal antibodies and modified polyclonal antibodies may also be used however.

Monoclonal antibodies produced against the proteins can be produced by biologically pure cell lines of immortalized antibody-producing cells. Immortalized antibody producing cells can be obtained according to any of the various methods which are known in the art, and generally include the steps: 1) inducing suitable cells such as lymphocytes to produce specific antibodies (for example by injecting an immunogen, such as the

polypeptide of SEQ ID NO:1 or SEQ ID NO:3), 2) immortalizing those cells, and 3) selecting clones out of these cells which produce antibodies of the desired specificity and affinity. For example, one method would be that of Kohler and Millstein, Nature, vol. 256, 495-497 (1975). This method comprises immunizing mice with the particular peptide (e.g. that of SEQ ID NO:1 or SEQ ID NO:3), isolating spleen cells and fusing these with mouse myeloma cells to obtain hybridomas. Of course animals other than mice could be used as well.

All of the specifically identified antibodies (i.e. those directed against the following proteins: p21K, p23K, p25K, p27K, p29K (p28K, p33K), p30/33K, and p31K) are commercially available from Organon Teknika nv. Deposits of cell lines producing monoclonal antibodies against the p27K and p31K have been made with the Collection Nationale de Cultures des Microorganismes of the Pasteur Institute under numbers I-588 and I-589. The antibodies may be likewise made as described in EP 219,368 A1 (corresponding to U. S. Patent Application Serial No. 07/298,791).

For use in studying a subject's T4 cells to determine whether or not the subject is infected with HIV, anti-p27K antibody ($p = 0.01$) and anti-p30-33K antibody ($p = 0.001$), are preferred (FIGs. 1 and 2). However, anti-p29K ($p = 0.01$) and anti-p23K antibody ($p = 0.001$) may also be used (FIG. 1).

Monoclonal antibodies against any of the following anti-prosomal proteins may be used to study a subject's natural killer cells to determine whether or not the subject has been infected with HIV: p25K, p29K (p28K, p33K), p30/33K, and p31K or the similar p21K (FIGs. 3 & 4).

Monoclonal antibodies against any of the following anti-prosomal proteins may be used to study a subject's T3 lymphocytes to determine whether or not the subject has been infected with HIV: p23K, p25K, p29K (p28K, p33K), p30/33K, and p31K or the similar p21K (FIGs. 5 &

6). Preferred antibodies are those directed against the p21K, p23K, p29K, p30-33K, and the p31K.

Although monoclonal antibodies against any of the anti-prosomal proteins (p23K, p25K, p27K, p29K (p28K, p33K), p30/33K, and p31K) or the similar anti-p21K (from a "prosome-like particle") may be used to study a subject's T8 cells to determine whether or not the subject is infected with HIV, anti-p27K antibody and anti-p30-33K antibody are preferred (FIG. 7).

For use in studying a subject's B4 cells to determine whether or not the subject is infected with HIV, the anti-p23K antibody is preferred (FIG. 8).

III. Diagnostic Methods-

In a preferred diagnostic method according to the invention, directly or indirectly labelled (e.g. with phycoerythrin) antibodies directed against the various cells (e.g. the T3 lymphocyte, T4 lymphocyte, T8 cell, B4 cell or natural killer cell) are used to identify or isolate these cells in a subject's blood containing the cells. The cell-antibody complexes are then incubated with antibodies directed against prosomal proteins also labelled, e.g with a fluorescent compound. The amount of cells thus labelled are measured or counted, and the measurement is compared with a norm or standard measurement.

Several devices can be used with the method.

As used herein an "immunochemical reagent" means that the antibodies displaying the appropriate selectivity have been bound to a suitable support or have been provided with a labelling substance. Supports typically used include the inner wall of a microtest well or a cuvette, a tube or capillary, a membrane filter, test strip, or the surface of a particle such as a latex particle, bead, erythrocyte, dye sol, metal (e.g. gold) sol or metal compound as sol particle. Labelling substances typically used include various radioactive isotopes, fluorescent compounds, enzymes, dye sols or metal compounds used as sol particles.

In one method of detecting the presence of HIV infection in a test fluid containing the described cells (e.g. a bodily fluid such as blood, lymph or plasma of a human thought to have been exposed to HIV), an immunochemical reagent of the invention is bought into contact with the test fluid. After which the presence of immune complexes formed between the antibodies and the surface antigens of the described cells in the test fluid is measured for the presence of an immunochemical reaction. The immunochemical reaction is preferably a sandwich reaction (using labelled antibody), an agglutination reaction (using also sols), a competition reaction, or an inhibition reaction.

In one type of immunoassay, antibodies directed against the cell or cells to be studied are attached to a solid phase, for example the inside of a test tube. The bodily fluid containing the desired cells are then added to the tube so as to bind with the antibody. To the tube coated with the antigen-antibody complex is added an immunological reagent containing a known amount of the appropriate anti-prosomal protein antibody or antibodies labelled with a fluorescent compound. The amount of fluorescence is thus measured, and compared with a norm.

Other diagnostic test kits are known to those skilled in the art which may be adapted for use in the instant invention. For example, immunological diagnostic test kits include radio-immunoassay or enzyme immunoactivity assay ("EIA") as described in U.S. Patent Reissue No. 32,696 to Schuurs et al. A light scattering immunoassay system is disclosed in EP 0,254,430 A2 to Ortho Diagnostic Systems, Inc. (corresponding to U.S. Serial No. 879,236 filed 26 June 1986). Diagnostic test kits using two monoclonal antibodies of high binding affinity are described in U. S. Patent Nos. 4,376,110 and 4,486,530 to Hybritech Inc.

A method of phenotyping a white blood cell comprises incubating the cell with labelled antibodies reactive with a prosomal protein; and analyzing the cell's surface by the means of the activity of the label for the presence of proteins reactive with the labeled antibody.

EXAMPLE I

DOUBLE MARKING IMMUNOLOGY

The various cells to be analyzed were identified and isolated according to the following procedure. Monoclonal antibodies labelled with phycoerythrin ("PE") were introduced into 0.1 milliliters (ml) aliquots of blood and allowed to incubate for 10 minutes at room temperature. The monoclonal antibodies used in the various aliquots were anti-CD3 (T3-PE), anti-CD4 (T4-PE), anti-CD8 (T8-PE), anti-CD2 (11-PE), anti-CD19 (B4-PE), anti-NK (NK-PE). The red blood cells were lysed and fixed for passage by a flow cytometer QPREP (formic acid / formaldehyde - Coultronics France of Paris, France) to fix the cell membrane to ensure that it is not destroyed during preparation.

EXAMPLE II

DOUBLE MARKING IMMUNOLOGY

Mouse serum was incubated for 4 to 5 hours at 4 degrees centigrade. It was washed once with concentrated PBS. It was then mixed with monoclonal antibodies directed against prosomal proteins, incubated, and allowed to cool to room temperature after 30 minutes. It was again washed with concentrated PBS one time. Goat anti-mouse fluorescein-isothiocyanate ("GAM-FITC") was then added in order to reveal the monoclonal antibodies against the prosomal proteins (30 minute incubation time at room temperature). It was again washed with concentrated PBS one time. It was then resuspended in 0.5 ml of concentrated PBS one time. Analysis of any fluorescence was made with CFM (flux cytometry).

EXAMPLE III

Using the procedures of EXAMPLEs I and II, the blood of 20 people who were HIV positive (2 of which were suffering from AIDS) was compared with the blood of a group of non-infected individuals. The results of comparing the subjects' (HIV-1) sera are graphically illustrated in FIGs. 1 through 8.

EXAMPLE IV

SEQ ID NO:1 depicts a 266 amino acid polypeptide along with the DNA coding therefor. The cDNA encoding this protein was isolated from a Hela cell cDNA expression library ((lambda)gt11), using a monoclonal antibody directed against the p27k prosomal protein (Organon Teknika). The insert was subcloned in pTZ18R plasmid vector for sequencing. The cDNA lacks approximately fifty nucleotides at the 5′ end (as indicated by Northern blot analysis). The length of the DNA is 980 nucleotides, with a coding region of 798 nucleotides (266 amino acids).

EXAMPLE V

SEQ ID NO:3 depicts a 269 amino acid polypeptide along with the DNA coding therefor. The cDNA encoding this protein was isolated from a Hela cell cDNA expression library ((lambda)gt11) using a monoclonal antibody (62A32) directed against the p33K prosomal protein. The insert was subcloned in pTZ18R plasmid vector for sequencing. Sequencing of the insert in both directions revealed an open reading frame of 810 nucleotides and a full length sequence with a large 5′ leader. The length of the DNA is 1264 nucleotides, with a coding region of 810 nucleotides (269 amino acids).

EXAMPLE VI

The protein of EXAMPLE IV (i.e. that of SEQ ID NO:1) is used to produce monoclonal antibodies against the p27K prosomal protein. The method comprises immunizing mice with the peptide, isolating spleen cells from the mice and fusing these with mouse myeloma cells to obtain hybridomas. The hybridomas are screened for those producing antibodies against the p27K protein.

EXAMPLE VII

Monoclonal antibodies against the polypeptide of EXAMPLE V (i.e. the protein of SEQ ID NO:3) are produced in a similar manner as that described in EXAMPLE VI.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: PRO-SOMA SARL
(B) STREET: 9, Rue Larrey
(C) CITY: Paris
(E) COUNTRY: France
(F) POSTAL CODE: F 75 005

(ii) TITLE OF INVENTION: Diagnostic Method

(iii) NUMBER OF SEQUENCES: 4

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 980 nucleotides
(B) TYPE: nucleotide
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: human

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Hela cell cDNA expression library (lambda)gt11

(ix) FEATURE:

(B) LOCATION: 4..804

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

```
CGG TGC CTG GTG CGG GAG CTA CGG GGC CCA GGG ATT GTG TTT AAA GTA    48
    Cys Leu Val Arg Glu Leu Arg Gly Pro Gly Ile Val Phe Lys Val
     1               5                  10                  15

GTG CTT CTA CCA ACA TGT CCC GTG GTT CCA GCG CCG GTT TTG ACC GCC    96
Val Leu Leu Pro Thr Cys Pro Val Val Pro Ala Pro Val Leu Thr Ala
                20                  25                  30

ACA TTA CCA TTT TTT TCA CCC GAG GGT CGG CTC TAC CAA GTA GAA TAT   144
Thr Leu Pro Phe Phe Ser Pro Glu Gly Arg Leu Tyr Gln Val Glu Tyr
            35                  40                  45

GCT TTT AAG GCT ATT AAC CAG GGT GGC CTT ACA TCA GTA GCT GTC AGA   192
Ala Phe Lys Ala Ile Asn Gln Gly Gly Leu Thr Ser Val Ala Val Arg
        50                  55                  60

GGG AAA GAC TGT GCA GTA ATT GTC ACA CAG AAG AAA GTA CCT GAC AAA   240
Gly Lys Asp Cys Ala Val Ile Val Thr Gln Lys Lys Val Pro Asp Lys
    65                  70                  75

TTA TTG GAT TCC AGC ACA GTG ACT CAC TTA TTC AAG ATA ACT GAA AAC   288
Leu Leu Asp Ser Ser Thr Val Thr His Leu Phe Lys Ile Thr Glu Asn
80                  85                  90                  95
```

```
ATT GGT TGT GTG ATG ACC GGA ATG ACA GCT GAC AGC AGA TCC CAG GTA   336
Ile Gly Cys Val Met Thr Gly Met Thr Ala Asp Ser Arg Ser Gln Val
                100                 105                 110

CAG AGG GCA CGC TAT GAG GCA GCT AAC TGG AAA TAC AAG TAT GGC TAT   384
Gln Arg Ala Arg Tyr Glu Ala Ala Asn Trp Lys Tyr Lys Tyr Gly Tyr
            115                 120                 125

GAG ATT CCT GTG GAC ATG CTG TGT AAA AGA ATT GCC GAT ATT TCT CAG   432
Glu Ile Pro Val Asp Met Leu Cys Lys Arg Ile Ala Asp Ile Ser Gln
        130                 135                 140

GTC TAC ACA CAG AAT GCT GAA ATG AGG CCT CTT GGT TGT TGT ATG ATT   480
Val Tyr Thr Gln Asn Ala Glu Met Arg Pro Leu Gly Cys Cys Met Ile
    145                 150                 155

TTA ATT GGT ATA GAT GAA GAG CAA GGC CCT CAG GTA TAT AAG TGT GAT   528
Leu Ile Gly Ile Asp Glu Glu Gln Gly Pro Gln Val Tyr Lys Cys Asp
160                 165                 170                 175

CCT GCA GGT TAC TAC TGT GGG TTT AAA GCC ACT GCA GCG GGA GTT AAA   576
Pro Ala Gly Tyr Tyr Cys Gly Phe Lys Ala Thr Ala Ala Gly Val Lys
                180                 185                 190

CAA ACT GAG TCA ACC AGC TTC CTT GAA AAA AAA GTG AAG AAG AAA TTT   624
Gln Thr Glu Ser Thr Ser Phe Leu Glu Lys Lys Val Lys Lys Lys Phe
            195                 200                 205

GAT TGG ACA TTT GAA CAG ACA GTG GAA ACT GCA ATT ACA TGC CTG TCT   672
Asp Trp Thr Phe Glu Gln Thr Val Glu Thr Ala Ile Thr Cys Leu Ser
        210                 215                 220

ACT GTT CTA TCA ATT GAT TTC AAA CCT TCA GAA ATA GAA GTT GGA GTA   720
Thr Val Leu Ser Ile Asp Phe Lys Pro Ser Glu Ile Glu Val Gly Val
    225                 230                 235

GTG ACA GTT GAA AAT CCT AAA TTC AGG ATT CTT ACA GAA GCA GAG ATT   768
Val Thr Val Glu Asn Pro Lys Phe Arg Ile Leu Thr Glu Ala Glu Ile
240                 245                 250                 255

GAT GCT CAC CTT GTT GCT CTA GCA GAG AGA GAC TAAACATTGT CGTTAGTTTA 821
Asp Ala His Leu Val Ala Leu Ala Glu Arg Asp
                260                 265

CCAGATCCGT GATGCCACTT ACCTGTGTGT TTGGTAACAA CAAACAAACA TCATGGAGGT 881

CCCTGGATTG AAAAAGGAGC CTCTCCCACT CCTCCTACCA CCGAAGTGGT TAGGACTCTA 941

TATAAATAAA AACAAGGCTT TTGGAAAAAA AAAAAAAAA                        980
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 266 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: human

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

Cys Leu Val Arg Glu Leu Arg Gly Pro Gly Ile Val Phe Lys Val
1 5 10 15

Val Leu Leu Pro Thr Cys Pro Val Val Pro Ala Pro Val Leu Thr Ala
20 25 30

Thr Leu Pro Phe Phe Ser Pro Glu Gly Arg Leu Tyr Gln Val Glu Tyr
35 40 45

Ala Phe Lys Ala Ile Asn Gln Gly Gly Leu Thr Ser Val Ala Val Arg
50 55 60

Gly Lys Asp Cys Ala Val Ile Val Thr Gln Lys Lys Val Pro Asp Lys
65 70 75

Leu Leu Asp Ser Ser Thr Val Thr His Leu Phe Lys Ile Thr Glu Asn
80 85 90 95

Ile Gly Cys Val Met Thr Gly Met Thr Ala Asp Ser Arg Ser Gln Val
100 105 110

Gln Arg Ala Arg Tyr Glu Ala Ala Asn Trp Lys Tyr Lys Tyr Gly Tyr
115 120 125

Glu Ile Pro Val Asp Met Leu Cys Lys Arg Ile Ala Asp Ile Ser Gln
130 135 140

Val Tyr Thr Gln Asn Ala Glu Met Arg Pro Leu Gly Cys Cys Met Ile
145 150 155

Leu Ile Gly Ile Asp Glu Glu Gln Gly Pro Gln Val Tyr Lys Cys Asp
160 165 170 175

Pro Ala Gly Tyr Tyr Cys Gly Phe Lys Ala Thr Ala Ala Gly Val Lys
180 185 190

Gln Thr Glu Ser Thr Ser Phe Leu Glu Lys Lys Val Lys Lys Lys Phe
195 200 205

Asp Trp Thr Phe Glu Gln Thr Val Glu Thr Ala Ile Thr Cys Leu Ser
210 215 220

Thr Val Leu Ser Ile Asp Phe Lys Pro Ser Glu Ile Glu Val Gly Val
225 230 235

Val Thr Val Glu Asn Pro Lys Phe Arg Ile Leu Thr Glu Ala Glu Ile
240 245 250 255

Asp Ala His Leu Val Ala Leu Ala Glu Arg Asp
260 265

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1264 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: human

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Hela cell cDNA expression library (lambda)gt11

(ix) FEATURE:
(B) LOCATION: 328..1137

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

```
CGCGAGCCGG GACCACGCCG ACCCAGCGTG CCCAGGCCGA GGAAAGCGCG GCGGCGGCAG      60

TCCGAAGACC CACCGGACTG AAAGAGAAGG ACGAGGTCAT CTTCGGACGG GAGGGGCAAG     120

CCAGCCATCC TGGGACCCCA GGCGTGCAGG TTCTCTTTGA GGGTATTCCA CCCTGCAAAA     180

AGCATGTATT CATGGTCAGC TCTCAGCAAG GCCAGTAGCA GAGTGGTAAA GGCCTTGGCC     240

CTCCAAGGCT GGGAAAAGAC AATGACAAGT CAAATCCAGA CCTATGTTGG ATGTTGGTCT     300

ACTAGGTGAC TGTCTCCTGG AAATGTT ATG CAG CTC AGC AAG GTG AAG TTT CGA      354
                              Met Gln Leu Ser Lys Val Lys Phe Arg
                                1               5

AAT CAC TAT GAC AAT GAT GTC ACT GTT TGG ACC GCC CAG GGC AGG ATT        402
Asn Gln Tyr Asp Asn Asp Val Thr Val Trp Thr Ala Gln Gly Arg Ile
 10                  15                  20                  25

CAT CAA ATT GAA TAT GCA ATG GAA GCT GTT AAA CAA GGT TCA GCC ACA        450
His Gln Ile Glu Tyr Ala Met Glu Ala Val Lys Gln Gly Ser Ala Thr
                 30                  35                  40

GTT GGT CTG AAA TCA AAA ACT CAT GCA GTT TTG GTT GCA TTG AAA AGG        498
Val Gly Leu Lys Ser Lys Thr His Ala Val Leu Val Ala Leu Lys Arg
             45                  50                  55

GCG CAA TCA GAG CTT GCA GCT CAT CAG AAA AAA ATT CTC CAT GTT GAC        546
Ala Gln Ser Glu Leu Ala Ala His Gln Lys Lys Ile Leu His Val Asp
         60                  65                  70

AAC CAT ATT GGT ATC TCA ATT GCG GGG CTT ACT GCT GAT GCT AGA CTG        594
Asn His Ile Gly Ile Ser Ile Ala Gly Leu Thr Ala Asp Ala Arg Leu
     75                  80                  85

TTA TGT AAT TTT ATG CGT CAG GAG TGT TTG GAT TCC AGA TTT GTA TTC        642
Leu Cys Asn Phe Met Arg Gln Glu Cys Leu Asp Ser Arg Phe Val Phe
 90                  95                  100                 105
```

```
GAT AGA CCA CTG CCT GTG TCT CGT CTT GTA TCT CTA ATT GGA AGC AAG      690
Asp Arg Pro Leu Pro Val Ser Arg Leu Val Ser Leu Ile Gly Ser Lys
                110                 115                 120

ACC CAG ATA CCA ACA CAA CGA TAT GGC CGG AGA CCA TAT GGT GTT GGT      738
Thr Gln Ile Pro Thr Gln Arg Tyr Gly Arg Arg Pro Tyr Gly Val Gly
            125                 130                 135

CTC CTT ATT GCT GGT TAT GAT GAT ATG GGC CCT CAC ATT TTC CAA ACC      786
Leu Leu Ile Ala Gly Tyr Asp Asp Met Gly Pro His Ile Phe Gln Thr
        140                 145                 150

TGT CCA TCT GCT AAC TAT TTT GAC TGC AGA GCC ATG TCC ATT GGA GCC      834
Cys Pro Ser Ala Asn Tyr Phe Asp Cys Arg Ala Met Ser Ile Gly Ala
    155                 160                 165

CGT TCC CAA TCA GCT CGT ACT TAC TTG GAG AGA CAT ATG TCT GAA TTT      882
Arg Ser Gln Ser Ala Arg Thr Tyr Leu Glu Arg His Met Ser Glu Phe
170                 175                 180                 185

ATG GAG TGT AAT TTA AAT GAA CTA GTT AAA CAT GGT CTG CGT GCC TTA      930
Met Glu Cys Asn Leu Asn Glu Leu Val Lys His Gly Leu Arg Ala Leu
                190                 195                 200

AGA GAG ACG CTT CCT GCA GAA CAG GAC CTG ACT ACA AAG AAT GTT TCC      978
Arg Glu Thr Leu Pro Ala Glu Gln Asp Leu Thr Thr Lys Asn Val Ser
            205                 210                 215

ATT GGA ATT GTT GGT AAA GAC TTG GAG TTT ACA ATC TAT GAT GAT GAT     1026
Ile Gly Ile Val Gly Lys Asp Leu Glu Phe Thr Ile Tyr Asp Asp Asp
        220                 225                 230

GAT GTG TCT CCA TTC CTG GAA GGT CTT GAA GAA AGA CCA CAG AGA AAG     1074
Asp Val Ser Pro Phe Leu Glu Gly Leu Glu Glu Arg Pro Gln Arg Lys
    235                 240                 245

GCA CAG CCT GCT CAA CCT GCT GAT GAA CCT GCA GAA AAG GCT GAT GAA     1122
Ala Gln Pro Ala Gln Pro Ala Asp Glu Pro Ala Glu Lys Ala Asp Glu
250                 255                 260                 265

CCA ATG GAA CAT TAAGTGATAA GCCAGTCTAT ATATGTATTA TCAAATATGT        1174
Pro Met Glu His

AAGAATACAG GCACCACATA CTGATGACAA TAATCTATAC TTTAACCAAA AGTTGCAGAG  1234

TGGTGGAATG CTATTTTTTA GGAATCAGTC                                   1264
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 269 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: human

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

Met Gln Leu Ser Lys Val Lys Phe Arg
1 5

Asn Gln Tyr Asp Asn Asp Val Thr Val Trp Thr Ala Gln Gly Arg Ile
10 15 20 25

His Gln Ile Glu Tyr Ala Met Glu Ala Val Lys Gln Gly Ser Ala Thr
30 35 40

Val Gly Leu Lys Ser Lys Thr His Ala Val Leu Val Ala Leu Lys Arg
45 50 55

Ala Gln Ser Glu Leu Ala Ala His Gln Lys Lys Ile Leu His Val Asp
60 65 70

Asn His Ile Gly Ile Ser Ile Ala Gly Leu Thr Ala Asp Ala Arg Leu
75 80 85

Leu Cys Asn Phe Met Arg Gln Glu Cys Leu Asp Ser Arg Phe Val Phe
90 95 100 105

Asp Arg Pro Leu Pro Val Ser Arg Leu Val Ser Leu Ile Gly Ser Lys
110 115 120

Thr Gln Ile Pro Thr Gln Arg Tyr Gly Arg Arg Pro Tyr Gly Val Gly
125 130 135

Leu Leu Ile Ala Gly Tyr Asp Asp Met Gly Pro His Ile Phe Gln Thr
140 145 150

Cys Pro Ser Ala Asn Tyr Phe Asp Cys Arg Ala Met Ser Ile Gly Ala
155 160 165

Arg Ser Gln Ser Ala Arg Thr Tyr Leu Glu Arg His Met Ser Glu Phe
170 175 180 185

Met Glu Cys Asn Leu Asn Glu Leu Val Lys His Gly Leu Arg Ala Leu
190 195 200

Arg Glu Thr Leu Pro Ala Glu Gln Asp Leu Thr Thr Lys Asn Val Ser
205 210 215

Ile Gly Ile Val Gly Lys Asp Leu Glu Phe Thr Ile Tyr Asp Asp Asp
220 225 230

Asp Val Ser Pro Phe Leu Glu Gly Leu Glu Glu Arg Pro Gln Arg Lys
235 240 245

Ala Gln Pro Ala Gln Pro Ala Asp Glu Pro Ala Glu Lys Ala Asp Glu
250 255 260 265

Pro Met Glu His

Claims

What is claimed is:

1. A method of diagnosing an human immunodeficiency virus infection in a subject comprising:
(a) analyzing, with labelled antibodies, labelled antibody fragments or mixtures thereof, a bodily fluid taken from the subject for proteins present on cell surfaces, said proteins reactive with antibodies directed against prosomal proteins or prosome-like proteins, to obtain a quantitative determination of the amount or percentage of cells displaying the protein on their surface; and
(b) comparing the amount or percentage thus obtained with a standard from a person not infected with human immunodeficiency virus.

2. The method of claim 1 wherein said antibodies or antibody fragments are selected or derived from the group of antibodies directed against prosomal proteins p23K, p25K, p27K, p29K (p28K, p33K), p30/33K, and p31K or the prosomal-like particle p21K.

3. The method of claim 2 wherein the cells are the subject's B4 cells and the antibody is an anti-p23K monoclonal antibody.

4. The method of claim 2 wherein the cells are the subject's T4 cells and the antibody is an anti-p27K antibody raised by use of the protein identified in SEC ID NO:2, or an antigenic fragment thereof.

5. The method of claim 1 wherein the antibody is an antibody raised by use of the protein identified in SEC ID NO:4, or an antigenic fragment thereof.

6. The method of claim 1 wherein the cells are the subject's T8 cells and the antibodies are monoclonal antibodies against any of the anti-prosomal proteins p23K, p25K, p27K, p29K (p28K, p33K), p30/33K, and p31K or the anti-p21K.

7. The method of claim 1 wherein the cells are the subject's natural killer cells and the antibodies are monoclonal antibodies directed against the following proteins p25K, p29K, (p28K, p33K), p30/33K, and p31K or the similar p21K.

8. The method of claim 1 wherein the cells are the subject's T3 lymphocytes and the antibodies are antibodies against any of the following anti-prosomal proteins: p23K, p25K, p29K (p28K, p33K), p30/33K, and p31K or the p21K protein.

9. A method of diagnosing a human immunodeficiency virus infection comprising: identifying or isolating cells selected from the group consisting of B4 cells, T4 cells, T8 cells, natural killer cells, T3 lymphocytes, or mixtures of these cells with antibodies directed against these cells; incubating the thus isolated or identified cells with labelled monoclonal antibodies directed against prosomal proteins; and thus analysing proteins present on the cell's surface.

10. A diagnostic test kit when used for carrying out the method according to claim 1 comprising: a solid phase on which antibodies directed against cells selected from the group consisting of B4 cells, T4 cells, T8 cells, natural killer cells, T3 lymphocytes, or mixtures of these cells has been immobilised, and a diagnostic fluid comprising labelled antibodies directed against prosomal proteins or prosome-like proteins.

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11. A polypeptide having part or all of the primary structural conformation of the polypeptide depicted in SEQ ID NO:1 characterized by being the product of procaryotic or eucaryotic expression of an exogenous DNA source.

12. A polypeptide having part or all of the primary structural conformation of the polypeptide depicted in SEQ ID NO:3 characterized by being the product of procaryotic or eucaryotic expression of an exogenous DNA source.

13. A purified and isolated polypeptide of SEQ ID NO:1 or SEQ ID NO:3.

14. A DNA sequence for use in securing expression in a procaryotic or eucaryotic host cell of a peptide product having at least part of the primary structure of the polypeptide depicted in SEQ ID NO:1 or SEQ ID NO:3, said DNA sequence selected from the group consisting of:

(a) the DNA sequence set out in SEQ ID NO:1 or SEQ ID NO:3 or their complementary strands;

(b) DNA sequences which hybridize under stringent conditions to the protein coding regions of the DNA sequences defined in (a) or fragments thereof; and

(c) DNA sequences which, but for the degeneracy of the genetic code, would hybridize to the DNA sequences defined in (a) and (b).

15. A biologically functional circular plasmid or viral DNA vector including a DNA sequence according to claim 14.

16. A procaryotic or eucaryotic host cell transformed or transfected with a DNA sequence according to claim 14.

17. A method of diagnosing a human immunodeficiency virus infection in a subject which method is according to claim 1, substantially as hereinbefore described with reference to the Examples.

Dated 15 June, 1994
Pro-Soma S.A.R.L.

Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

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FIG. 1

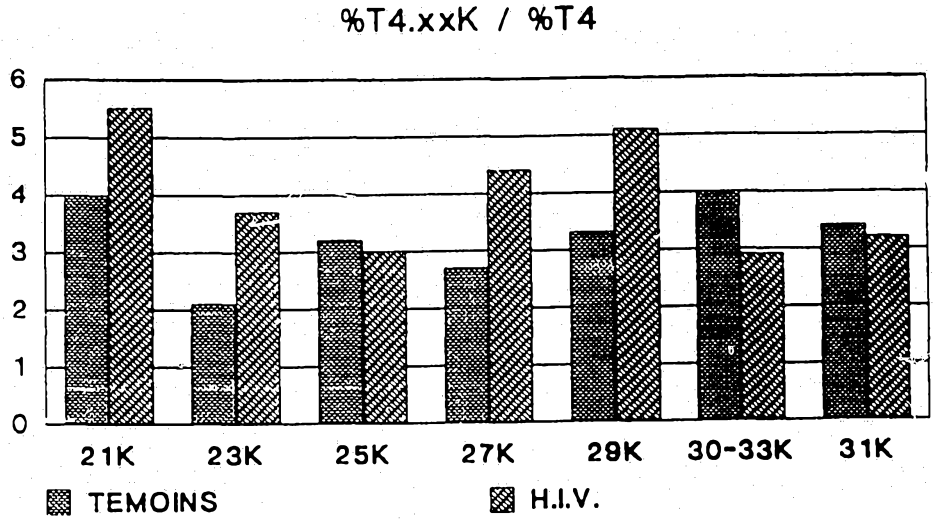

%T4.xxK / %T4
6
5
4
3
2
1
0
21K
23K
25K
27K
29K
30-33K
31K
TEMOINS
H.I.V.

FIG. 2

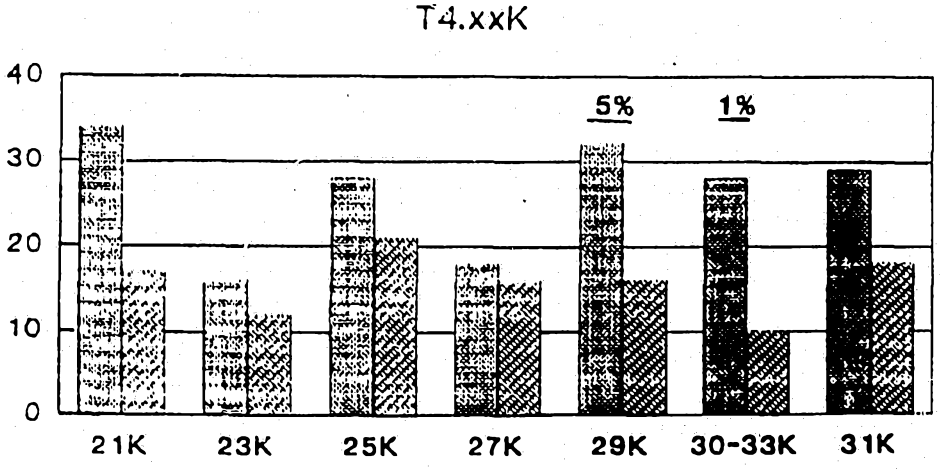

T4.xxK
40
30
20
10
0
5%
1%
21K
23K
25K
27K
29K
30-33K
31K

FIG. 3

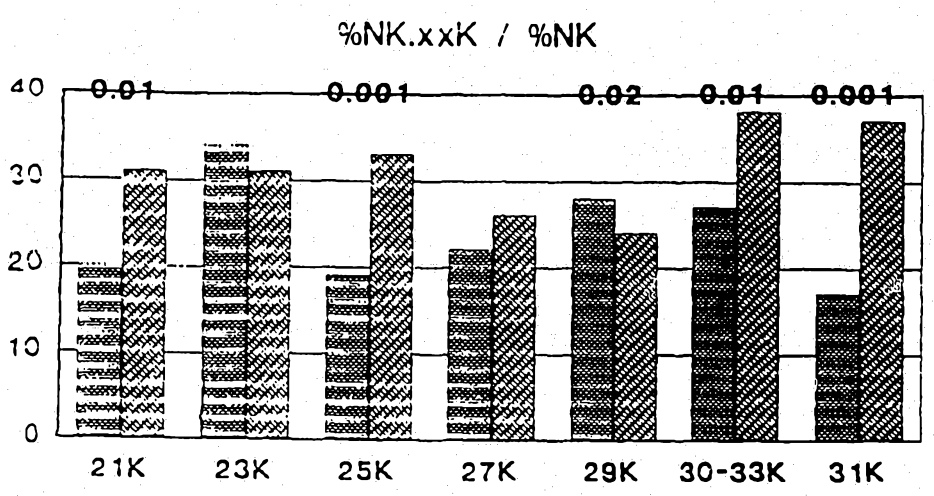

%NK.xxK / %NK
40
30
20
10
0
0.01
0.001
0.02
0.01
0.001
21K
23K
25K
27K
29K
30-33K
31K

FIG. 4

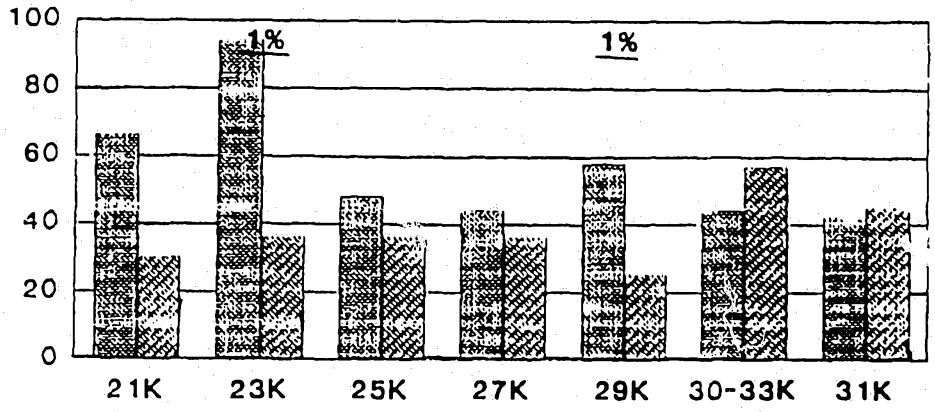

NK.xxK
100
80
60
40
20
0
1%
1%
21K
23K
25K
27K
29K
30-33K
31K

FIG. 5

%T3.xxK / %T3

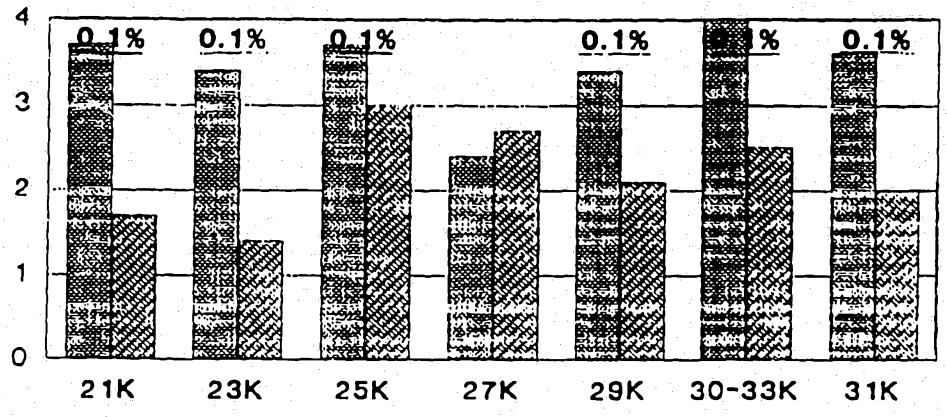


FIG. 6

T3.xxK

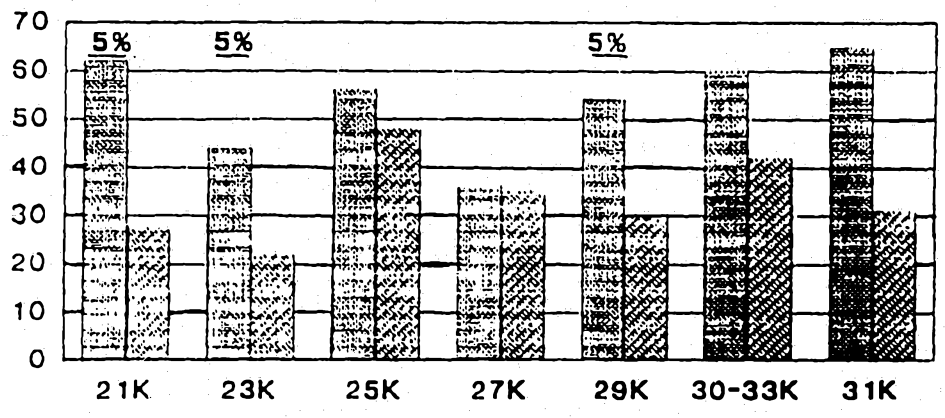


FIG. 7

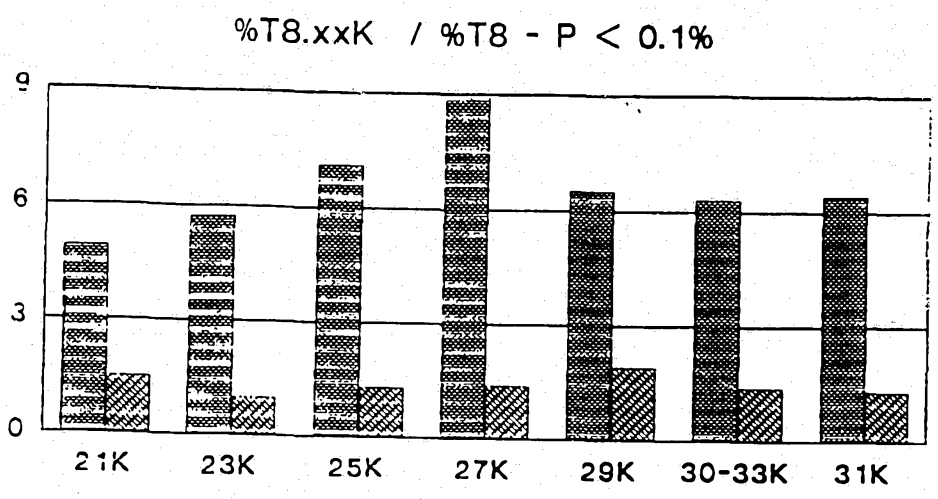
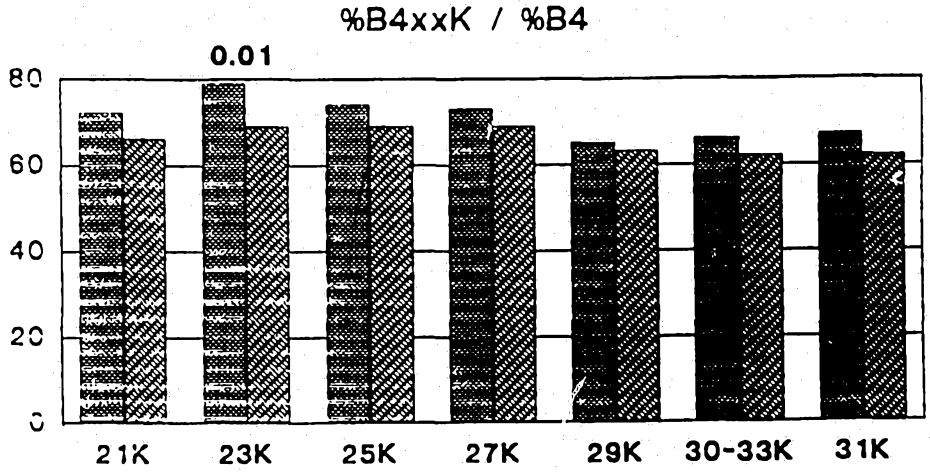

%T8.xxK / %T8 - P < 0.1%
9
6
3
0
21K
23K
25K
27K
29K
30-33K
31K

FIG. 8


%B4xxK / %B4
0.01
80
60
40
20
0
21K
23K
25K
27K
29K
30-33K
31K

SUBSTITUTE SHEET

Figure 9 (1)

```
CGG TGC CTG GTG CGG GAG CTA CGG GGC CCA GGG ATT GTG TTT AAA GTA     48
    Cys Leu Val Arg Glu Leu Arg Gly Pro Gly Ile Val Phe Lys Val
     1               5                  10                  15

GTG CTT CTA CCA ACA TGT CCC GTG GTT CCA GCG CCG GTT TTG ACC GCC     96
Val Leu Leu Pro Thr Cys Pro Val Val Pro Ala Pro Val Leu Thr Ala
                20                  25                  30

ACA TTA CCA TTT TTT TCA CCC GAG GGT CGG CTC TAC CAA GTA GAA TAT    144
Thr Leu Pro Phe Phe Ser Pro Glu Gly Arg Leu Tyr Gln Val Glu Tyr
            35                  40                  45

GCT TTT AAG GCT ATT AAC CAG GGT GGC CTT ACA TCA GTA GCT GTC AGA    192
Ala Phe Lys Ala Ile Asn Gln Gly Gly Leu Thr Ser Val Ala Val Arg
        50                  55                  60

GGG AAA GAC TGT GCA GTA ATT GTC ACA CAG AAG AAA GTA CCT GAC AAA    240
Gly Lys Asp Cys Ala Val Ile Val Thr Gln Lys Lys Val Pro Asp Lys
 65                  70                  75

TTA TTG GAT TCC AGC ACA GTG ACT CAC TTA TTC AAG ATA ACT GAA AAC    288
Leu Leu Asp Ser Ser Thr Val Thr His Leu Phe Lys Ile Thr Glu Asn
 80                  85                  90                  95

ATT GGT TGT GTG ATG ACC GGA ATG ACA CCT GAC AGC AGA TCC CAG GTA    336
Ile Gly Cys Val Met Thr Gly Met Thr Ala Asp Ser Arg Ser Gln Val
                100                 105                 110

CAG AGG GCA CGC TAT GAG GCA GCT AAC TGG AAA TAC AAG TAT GGC TAT    384
Gln Arg Ala Arg Tyr Glu Ala Ala Asn Trp Lys Tyr Lys Tyr Gly Tyr
            115                 120                 125

GAG ATT CCT GTG GAC ATG CTG TGT AAA AGA ATT GCC GAT ATT TCT CAG    432
Glu Ile Pro Val Asp Met Leu Cys Lys Arg Ile Ala Asp Ile Ser Gln
        130                 135                 140
```

SUBSTITUTE SHEET

Figure 9 (2)

```
GTC TAC ACA CAG AAT GCT GAA ATG AGG CCT CTT GGT TGT TGT ATG ATT   480
Val Tyr Thr Gln Asn Ala Glu Met Arg Pro Leu Gly Cys Cys Met Ile
    145                 150                 155

TTA ATT GGT ATA GAT GAA GAG CAA GGC CCT CAG GTA TAT AAG TGT GAT   528
Leu Ile Gly Ile Asp Glu Glu Gln Gly Pro Gln Val Tyr Lys Cys Asp
160                 165                 170                 175

CCT GCA GGT TAC TAC TGT GGG TTT AAA GCC ACT GCA GCG GGA GTT AAA   576
Pro Ala Gly Tyr Tyr Cys Gly Phe Lys Ala Thr Ala Ala Gly Val Lys
                180                 185                 190

CAA ACT GAG TCA ACC AGC TTC CTT GAA AAA AAA GTG AAG AAG AAA TTT   624
Gln Thr Glu Ser Thr Ser Phe Leu Glu Lys Lys Val Lys Lys Lys Phe
            195                 200                 205

GAT TGG ACA TTT GAA CAG ACA GTG GAA ACT GCA ATT ACA TGC CTG TCT   672
Asp Trp Thr Phe Glu Gln Thr Val Glu Thr Ala Ile Thr Cys Leu Ser
        210                 215                 220

ACT GTT CTA TCA ATT GAT TTC AAA CCT TCA GAA ATA GAA GTT GGA GTA   720
Thr Val Leu Ser Ile Asp Phe Lys Pro Ser Glu Ile Glu Val Gly Val
    225                 230                 235

GTG ACA GTT GAA AAT CCT AAA TTC AGG ATT CTT ACA GAA GCA GAG ATT   768
Val Thr Val Glu Asn Pro Lys Phe Arg Ile Leu Thr Glu Ala Glu Ile
240                 245                 250                 255

GAT GCT CAC CTT GTT GCT CTA GCA GAG AGA GAC TAAACATTGT CGTTAGTTTA 821
Asp Ala His Leu Val Ala Leu Ala Glu Arg Asp
                260                 265

CCAGATCCGT GATGCCACTT ACCTGTGTGT TTGGTAACAA CAAACAAACA TCATGGAGGT 881

CCCTGGATTG AAAAAGGAGC CTCTCCCACT CCTCCTACCA CCGAAGTGGT TAGGACTCTA 941

TATAAATAAA AACAAGGCTT TTGGAAAAAA AAAAAAAAA                        980
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Figure. 10

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CGCGAGCCGGGACCACGCCGACCCAGCGTGCCCAGGCCGAGGAAAGCGCGGCGGCGGCAGTCCGAAGACCCA   72

CCGGACTGAAAGAGAAGGACGAGGTCATCTTCGGACGGGAGGGGCAAGCCAGCCATCCTGGGACCCCAGGCG  144

TGCAGGTTCTCTTTGAGGGTATTCCACCCTGCAAAAAGCATGTATTCATGGTCAGCTCTCAGCAAGGCCAGT  216

AGCAGAGTGGTAAAGGCCTTGGCCCTCCAAGGCTGGGAAAAGACAATGACAAGTCAAATCCAGACCTATGTT  288

GGATGTTGGTCTACTAGGTGACTGTCTCCTGGAAATGTT ATG CAG CTC AGC AAG GTG AAG TTT   351
                                          M   Q   L   S   K   V   K   F     8

CGA AAT CAG TAT GAC AAT GAT GTC ACT GTT TGG ACC GCC CAG GGC AGG ATT CAT   405
 R   N   Q   Y   D   N   D   V   T   V   W   T   A   Q   G   R   I   H    26

CAA ATT GAA TAT GCA ATG GAA GCT GTT AAA CAA GGT TCA GCC ACA GTT GGT CTG   459
 Q   I   E   Y   A   M   E   A   V   K   Q   G   S   A   T   V   G   L    44

AAA TCA AAA ACT CAT GCA GTT TTG GTT GCA TTG AAA AGG GCG CAA TCA GAG CTT   513
 K   S   K   T   H   A   V   L   V   A   L   K   R   A   Q   S   E   L    62

GCA GCT CAT CAG AAA AAA ATT CTC CAT GTT GAC AAC CAT ATT GGT ATC TCA ATT   567
 A   A   H   Q   K   K   I   L   H   V   D   N   H   I   G   I   S   I    80

GCG GGG CTT ACT GCT GAT GCT AGA CTG TTA TGT AAT TTT ATG CGT CAG GAG TGT   621
 A   G   L   T   A   D   A   R   L   L   C   N   F   M   R   Q   E   C    98

TTG GAT TCC AGA TTT GTA TTC GAT AGA CCA CTG CCT GTG TCT CGT CTT GTA TCT   675
 L   D   S   R   F   V   F   D   R   P   L   P   V   S   R   L   V   S   116

CTA ATT GGA AGC AAG ACC CAG ATA CCA ACA CAA CGA TAT GGC CGG AGA CCA TAT   729
 L   I   G   S   K   T   Q   I   P   T   Q   R   Y   G   R   R   P   Y   134

GGT GTT GGT CTC CTT ATT GCT GGT TAT GAT GAT ATG GGC CCT CAC ATT TTC CAA   783
 G   V   G   L   L   I   A   G   Y   D   D   M   G   P   H   I   F   Q   152

ACC TGT CCA TCT GCT AAC TAT TTT GAC TGC AGA GCC ATG TCC ATT GGA GCC CGT   837
 T   C   P   S   A   N   Y   F   D   C   R   A   M   S   I   G   A   R   170

TCC CAA TCA GCT CGT ACT TAC TTG GAG AGA CAT ATG TCT GAA TTT ATG GAG TGT   891
 S   Q   S   A   R   T   Y   L   E   R   H   M   S   E   F   M   E   C   188

AAT TTA AAT GAA CTA GTT AAA CAT GGT CTG CGT GCC TTA AGA GAG ACG CTT CCT   945
 N   L   N   E   L   V   K   H   G   L   R   A   L   R   E   T   L   P   206

GCA GAA CAG GAC CTG ACT ACA AAG AAT GTT TCC ATT GGA ATT GTT GGT AAA GAC   999
 A   E   Q   D   L   T   T   K   N   V   S   I   G   I   V   G   K   D   224

TTG GAG TTT ACA ATC TAT GAT GAT GAT GAT GTG TCT CCA TTC CTG GAA GGT CTT  1053
 L   E   F   T   I   Y   D   D   D   D   V   S   P   F   L   E   G   L   242

GAA GAA AGA CCA CAG AGA AAG GCA CAG CCT GCT CAA CCT GCT GAT GAA CCT GCA  1107
 E   E   R   P   Q   R   K   A   Q   P   A   Q   P   A   D   E   P   A   260

GAA AAG GCT GAT GAA CCA ATG GAA CAT TAA  GTGATAAGCCAGTCTATATATGTATTATCAA  1168
 E   K   A   D   E   P   M   E   H   *                                    270

ATATGTAAGAATACAGGCACCACATACTGATGACAATAATCTATACTTTAACCAAAAGTTGCAGAGTGGTGG  1240

AATGCTATTTTTTAGGAATCAGTC                                                 1264
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INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 91/01[illegible]5

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 G01N33/573; G01N33/53; G01N33/68; G01N33/569
G01N33/574; C07K15/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	G01N ; C07K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	EP,A,0 219 368 (CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE) 22 April 1987 cited in the application *See whole document*	1-16
A	EP,A,0 345 750 (OTSUKA PHARMACEUTICAL COMPANY LTD) 13 December 1989 cited in the application see page 8, line 6 - line 12 see page 13, line 11	1-16
A	RESEARCH REAGENT NEWS vol. 1, 1 January 1990, TURNHOUT BELBIUM pages 1 - 4; ANONYMOUS: 'Anti-prosome mouse monoclonal antibodies.' cited in the application *See whole article*	1-16

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° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
29 JANUARY 1992	06.02.92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Van Bohemen Ch G

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	BIOLOGY OF THE CELL vol. 67, no. 3, 1989, FRANCE page 22A; J.P. BUREAU ET AL.: 'Identification of prosome proteins in human t lymphocyte populations by flow cytometry.' See whole abstract. ---	10

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. EP 9101945 SA 52194

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 29/01/92

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0219368	22-04-87	FR-A- 2586814	06-03-87
		AU-B- 596605	10-05-90
		AU-A- 6189486	05-03-87
		EP-A- 0434670	26-06-91
		JP-A- 62130699	12-06-87
		US-A- 5059521	22-10-91
EP-A-0345750	13-12-89	JP-A- 1309686	14-12-89