

INSTRUCTIONS

(a) If Convention application insert "Convention"

(a) Convention

AUSTRIA

Patents Act

609447

(b) Delete one

APPLICATION FOR A (b) STANDARD/~~PETTY~~ PATENT

(c) Insert FULL name(s) of applicant(s)

h/We (c) NISSIN SHOKUHIN KABUSHIKI KAISHA
Trading as NISSIN FOOD PRODUCTS CO. LTD.

(d) Insert FULL address(es) of applicant(s)

of (d) 4-1-1 Nishinakajima,
Yodogawa-ku,
Osaka 532,
Japan

(e) Delete one

hereby apply for the grant of a (e) Standard/~~PETTY~~ Patent for an invention entitled

(f) Insert TITLE of invention

(f) METHODS AND MATERIALS FOR HIV DETECTION AND THERAPY

(g) Insert "complete" or "provisional" or "petty patent"

which is described in the accompanying (g) complete specification.

(Note: The following applies only to Convention applications)

Details of basic application(s)

	Application No.	Country	Filing Date
(h)	016,282	United States of America	19 February 1987
	064,066	United States of America	29 June 1987
	146,371	United States of America	3 February 1988

APPLICATION ACCEPTED AND AMENDMENTS

Address for Service:

PHILLIPS ORMONDE AND FITZPATRICK
Patent and Trade Mark Attorneys
367 Collins Street
Melbourne, Australia 3000

LODGED AT SUB-OFFICE
17 FEB 1988
Melbourne

Dated (i) 17 February 1988

(k)

(j) PHILLIPS ORMONDE & FITZPATRICK
Attorneys for:
NISSIN SHOKUHIN KABUSHIKI KAISHA
Trading as NISSIN FOOD PRODUCTS CO. LTD.

Note: No legalization or other witness required

FEE-STAMP TO VALUE OF

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MAIL OFFICE

PHILLIPS ORMONDE AND FITZPATRICK
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367 Collins Street
Melbourne, Australia

COMMONWEALTH OF AUSTRALIA

DECLARATION CONCERNING THE DEPOSIT, IN RELATION TO A PATENT
APPLICATION OR PATENT, OF A MICRO-ORGANISM WITH AN
INTERNATIONAL DEPOSITARY AUTHORITY

INSTRUCTIONS

Insert FULL name(s)
of applicant(s)

In the matter of the application made by

(a) Nissin Shokuhin Kabushiki Kaisha, trading as Nissin Food
Products Co. Ltd.Insert "of addition
applicable"("applicant(s)") for a patent (b)
invention entitled (c)

for an

Insert TITLE of
invention

METHODS AND MATERIALS FOR HIV DETECTION AND THERAPY

Insert FULL name(s)
and address(es) of
applicant(s)I/We (d) Koki ANDO, 4-1-1 Nishinakajima, Yodogawa-ku,
Osaka 532, Japan

declare as follows:

1. I am/We are the applicant(s) for the invention referred to above.
(or, in the case of an application by a body corporate)1. I am/We are authorized to make this declaration on behalf of the
applicant(s) for the invention referred to above.Insert name of
international
depository authority
Insert date on which
deposit was made2. A micro-organism was deposited with (e) as shown in the
attached paper.
on (f) and accorded the number
(g)Insert file,
accession or
registration number3. The deposit referred to in paragraph 2 was an original deposit
within the meaning of Rule 7.3 of the Budapest Treaty Regulations.

4. Samples of that micro-organism are obtainable from that
international depository authority as provided by:
- (a) such provisions of the Budapest Treaty on the International
Recognition of the Deposit of Micro Organisms for the purposes
of Patent Procedure, and the Regulations annexed to that
Treaty as affected by any amendments made under Article 12 of
that Treaty, as are applicable; and
 - (b) such provisions of the Patents Regulations as are applicable.

5. ~~XXXXXX~~

(or)

~~The applicant for the patent of the invention referred to above~~
~~XXXXXX~~

~~the depositor(s) within the meaning of Article 12 of the Budapest~~
~~Treaty of the deposit referred to in paragraph 2.~~

(or)

The depositor, within the meaning of Article 2 of the Budapest
Treaty, of the deposit referred to in paragraph 2 is (h)

(h) Insert FULL name
and address of
place of business or
residence of depositor

Tsuneya OHNO, a Japanese citizen

639 Fox Court, Ridgewood, Bergen, New Jersey 07450,
U.S.A.

6. ~~XXXXXX~~

(or)

~~I/we have the consent of the depositor referred to above to rely~~

(or)

~~the applicant for the patent for the invention referred to above~~
~~XXXXXX~~

(or)

The applicant for the patent for the invention referred to above
has the consent of the depositor referred to above to rely

on that deposit for the purposes of Section 40 of the Patents Act
1952 in relation to the application for the patent for the
invention referred to above.

(i) Insert PLACE of
signing

Declared at (i) Osaka, Japan

(j) Insert DATE of
signing

Dated (j) March 2, 1988

(k) Signature(s) of
declarant(s)

(k)

Koki Ando

(Koki ANDO, President)

To: The Commissioner of Patents

PHILLIPS ORMONDE & FITZPATRICK
Patent and Trade Mark Attorneys
367 Collins Street
Melbourne, Australia

Lodged by:

(l) Insert name and
address of person
lodging declaration
and name of person
on whose behalf it
is lodged

(1)

(Annexed Paper)

With Regard to the Paragraph 2 in the DECLARATION:

Identification Reference by Depositor:	ATCC Designation
Hybridoma, JT4C8	HB 9385
Hybridoma, JT4C16	HB 9386
Hybridoma, JT4C12	HB 9387

The deposits were received by the American Type Culture Collection (ATCC) on February 18, 1987.

Identification Reference by Depositor:	ECACC Designation
JTI-1F3	87062501
JT1-1F3-E5	87062502

The deposits were received by the European Collection of Animal Cell Cultures on June 25, 1987.

Identification Reference by Depositor:	FERM Designation
Mouse/Mouse Hybridoma cell line JT2-N15	FERM BP-1684
Mouse/Mouse Hybridoma cell line JT1-D7	FERM BP-1685

The deposits were received by the Fermentation Research Institute, Agency of Industrial Science and Technology (FERM) on January 29, 1988.

Declared at Osaka, Japan

Dated *March 2, 1988*

Koki Ando

(Koki ANDO, President)

(12) PATENT ABRIDGMENT (11) Document No. AU-B-11903/88
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 609447

(54) Title
METHODS AND MATERIALS FOR HIV DETECTION AND THERAPY

International Patent Classification(s)
(51)⁴ **C07K 015/12 A61K 039/42 C12N 005/00 C12N 015/00**
C12P 021/08 G01N 033/569 G01N 033/577

(21) Application No. : **11903/88** (22) Application Date : **17.02.88**

(30) Priority Data

(31) Number	(32) Date	(33) Country
016282	19.02.87	US UNITED STATES OF AMERICA
064066	29.06.87	US UNITED STATES OF AMERICA
146371	03.02.88	US UNITED STATES OF AMERICA

(43) Publication Date : **01.09.88**

(44) Publication Date of Accepted Application : **02.05.91**

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(56) Prior Art Documents
AU 42926/85 C12N 7/00
AU 63322/86 C12Q 1/70 C12N 5/00 7/00

(57) Antigen T4 from HIV (AIDS Virus) and antibody against this antigen, as well as methods of detection of HIV.

CLAIM

1. A purified and isolated immunologically active polypeptide capable of specific immunobinding with that portion of the HIV virion which is necessarily interactive with T4 surface proteins during HIV infection of host cells and characterized by the capacity to neutralize infectivity of HIV in vitro and by specific immunoreactivity with an antibody immunospecific for human lymphocyte T4.

2. A polypeptide according to claim 1 in the form of a antibody or chimeric antibody or a fragment thereof.

12. Monoclonal anti-monoclonal-anti-human lymphocyte T4 antibody.

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(10) 609447

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13. Monoclonal anti-OKT4 antibody as hereinbefore defined.
14. Monoclonal anti-OKT4A antibody as hereinbefore defined.

15. A hybridoma cell line capable of producing in the medium of its growth a monoclonal antibody according to claim 12.

33. A purified and isolated HIV protein, characterized by:

- (a) a molecular weight of from about 60,000 to 80,000 as determined by SDS-PAGE; and
- (b) specific immunoreactivity with monoclonal anti-monoclonal-anti-human lymphocyte T4 antibodies.

AUSTRALIA
Patents Act

609447

**COMPLETE SPECIFICATION
(ORIGINAL)**

Class

Int. Class

Application Number:
Lodged:

Complete Specification Lodged:
Accepted:
Published:

Priority

Related Art:

This document contains the
amendments made under
Section 49 and is correct for
printing.

APPLICANT'S REFERENCE: MKM

Name(s) of Applicant(s):

Nissin Shokuhin Kabushiki Kaisha, trading as Nissin Food Products
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Address for Service is:

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Complete Specification for the invention entitled:

METHODS AND MATERIALS FOR HIV DETECTION AND THERAPY

Our Ref : 84810
POF Code: 1522/27545

The following statement is a full description of this invention, including
the best method of performing it known to applicant(s):

"METHODS AND MATERIALS FOR HIV DETECTION AND THERAPY"

This is a continuation-in-part of co-pending U.S. Patent Application Serial No. 064,066, filed June 5 29, 1987, which is in turn a continuation-in-part of U.S. Patent Application Serial No. 016,282, filed February 19, 1987.

BACKGROUND

10

The present invention relates generally to methods and materials useful in the diagnosis and treatment of infection with Human Immunodeficiency Virus (HIV). More particularly, the invention relates to 15 immunologically active polypeptides, preferably antibodies or antibody fragments, and most preferably monoclonal antibodies, which are reactive with idiotypes of antibodies to human lymphocyte T4 protein and also reactive with the HIV virion in a manner allowing for in 20 vitro and in vivo neutralization of HIV infectivity. Moreover, the invention relates to immunologically active polypeptides useful in vaccine compositions for developing protective responses to HIV infection.

The state of the art with respect to the epidemiology and immunology of the causative agent of AIDS 25 in humans is well summarized in: Laurence, "The Immune System in AIDS", Scientific American, December, 1985, pp. 84-93; Gallo, "The First Human Retrovirus", Scientific American, December, 1986, pp. 88-98; Gallo, 30 "The AIDS Virus", Scientific American, January, 1987, pp. 47-56; Levy et al., Science, 225, 840-842 (1984); "Mobilizing Against AIDS", Institute of Medicine, National Academy of Sciences, Harvard University Press (Cambridge, Mass. 1986); and, Lane et al., Ann. Rev. 35 Immunol., 3, pp. 477-500 (1985). The role of T4 surface glycoprotein (sometimes referred to as "CD4" protein or

determinant) of human T lymphocytes in infection by HIV has been extensively studied as represented by Dalgleish et al., Nature, 312, pp. 763-767 (1984); Klatzman et al., Science, 312, 767-768 (1986); Klatzman et al., Science, 225, pp. 59-62 (1984); McDougal et al., J.Immunol., 135, pp. 3151-3162 (1985); and, Maddon et al., Cell, 47, pp. 333-348 (1986). See also, Marrack et al., "The T Cell and Its Receptor", Scientific American, February 1986, pp. 36-45; and, McDougal et al., Science, 231, 382-385 (1986).

It has recently been projected that soluble forms of CD4 may have therapeutic utility in treatment of HIV infection. See, Fisher et al., Nature, 331, 76-77 (1988); Hussey et al., Ibid, at pp. 78-81; Deen et al., Ibid, at pp. 82-83; and Trauneker et al., Ibid, at pp. 84-86 all of which relate to in vitro neutralization of HIV infectivity by soluble CD4. Among the potential drawbacks to the projected use of soluble CD4 therapeutic agents is the known reactivity of CD4 with class II major histocompatibility complex ("MHC") molecules present on the surface of other immune cells including B cells, macrophages and monocytes, leading to the suggestion that CD4 may need to be modified (e.g., by truncation) prior to attempted therapeutic use.

Numerous reports appear in the literature relating to the potential of antibodies to neutralize infectivity of HIV in vitro and in vivo and specifically to attempts at active immunization for the purpose of developing protective immunity. See, e.g., Matthews et al., Proc. Nat'l. Acad. Sci. (USA), 83, pp. 9709-9713 (1986); Norman, "AIDS Therapy: A New Push For Clinical Trials", Science, 230, pp. 1355-1358 (1985) and prior articles in this series; Newmark, Nature, 324, pp. 304-305 (1986); and notes appearing in Scientific American, February, 1987, at pages 86-88 under the heading, "AIDS: Hope...And Warnings", and in New Scientist,

December 18, 1986, page 7, under the heading "Can Protein T Thwart The AIDS Virus...?". See also, Mitsuya et al., Nature, 325, 773-778 (1987); Kennedy et al., Science, 231, 1556-1559 (1986); Chanh et al., EMBO Journal, 5(11), 3065-3071 (1986); Chanh et al., Eur. J. Immunol., 16, 1465-1468 (1986); Putney et al., Science, 234, 1392-1395 (1986); and, Matsushita et al., Abstract W.3.2, p.106, "III International Conference on Acquired Immunodeficiency Syndrome (AIDS), June 1-5, 1987.

- 10 Of interest to the background of the present invention are the published results of investigation into the immunological role of anti-idiotypes. See, e.g., Kennedy et al., "Anti-Idiotypes and Immunity", Scientific American, July, 1986, pp. 48-56; Jerne, "The
15 Immune System", Scientific American, July, 1973, pp. 52-60; Marx, "Making Antibodies Without The Antigens", Science, 228, pp. 162-165 (1985); Finberg et al., CRC Critical Reviews in Immunology, 7, 269-284 (1987); and Kennedy et al., Science, 232, pp. 220-223 (1986). See,
20 also, Norman, supra, relating to a potential correlation between anti-HIV-immunotherapy and production of anti-idiotypic antibodies to the HIV surface proteins.

Of particular interest to the background of the present invention is the work reported by McDougal
25 et al., J. Immunology, 137, 2937-2944 (1986) wherein it was noted that: "...rabbit anti-idiotypic sera raised against each of four candidate CD4 monoclonal antibodies [OKT4A, OKT4D, OKT4F and Leu3a (sometimes referred to as "anti-Leu3a")] did not react with [HIV] virus or inhibit
30 virus binding to CD4⁺ T cells." This notation should be compared with the recent oral presentations of Ronald C. Kennedy at the 7th Annual DNA/Hybridoma Congress, San Francisco, March 1-4, 1987, as reported in Bio/Technology, 5, 421-422 (1987), and at the III
35 International Conference on Acquired Immunodeficiency Syndrome (AIDS), Washington D.C. June 1-5, 1987, (see,

Abstract TH.9.5) as reported in New Scientist June 11, 1987 at page 26. In these presentations the development of polyclonal antisera against anti-T4 antibodies was noted, as was the capacity of such antisera to recognize HIV and partially neutralize HIV infectivity in vitro. The latter presentation also mentioned preparation of monoclonal anti-idiotypic antibody and this development is also described in Chanh et al., Proc. Nat'l. Acad. Sci. (USA), 84, 3891-3895 (June, 1987).

10 There continues to exist a substantial need in the art for new methods and materials for diagnosis for the presence of HIV particles and HIV-infected cells in biological fluid and tissue specimens and also a substantial need for new means for effecting in vivo neu-
15 tralization of the infectivity of HIV and the development of vaccination procedures conferring immunological protection against HIV infection.

BRIEF SUMMARY

20 The present invention provides purified and isolated immunologically active polypeptides, preferably antibodies or chimeric antibodies or fragments thereof, and most preferably monoclonal antibodies, which are
25 reactive with idiotypes of antibodies (preferably monoclonal antibodies) to human lymphocyte T4 protein. Such products of the invention are capable of specific immunobinding with that portion of the HIV virion which is necessarily interactive with T4 surface proteins during
30 infection by HIV of host cells such as human T lymphocytes and cells of the human nervous system. These products of the invention may be characterized, inter alia, by their strain independent capacity to neutralize infectivity of HIV in vitro, by their specific reac-
35 tivity with HIV protein having a molecular weight of from about 60,000 to about 80,000 as determined by SDS-

PAGE, and by their non-reactivity with class II major histocompatibility molecules associated with human immune cell surfaces.

In one of its aspects, therefore, immunologically active products are generated which "respond" through specific immunobinding to HIV particles and to the surfaces of HIV-infected cells, even though they are generated without direct immunological reference to such surface proteins.

10 Purified and isolated polypeptides according to the invention are conspicuously suitable for use in assay procedures for the detection and/or quantification of HIV in biological fluids wherein the detection procedure is based upon immunobinding between HIV particles
15 and reactive polypeptides (e.g., antibodies) of the invention. Moreover, antibodies, chimeric antibodies and fragments thereof developed according to the invention are selectively immunoreactive with the surfaces of HIV-infected cells and thus provide useful reagents for
20 detection of HIV-infected cells in fluid and tissue samples and for segregation of HIV-infected cells from cell populations including both infected and non-infected cells. As such, products of the invention will be useful in diagnostic and therapeutic procedures
25 involving separation and/or selective destruction of HIV-infected cells.

Purified and isolated immunologically active materials of the invention are also conspicuously suitable for use in anti-HIV treatment of animals, especially humans, susceptible to infection with HIV. According to one such method, immunologically effective amounts of, e.g., monoclonal antibodies of the invention, are administered to a patient either already infected with HIV or to a patient at risk of infection with HIV to
35 develop passive immunity with respect to HIV infection. According to another method, cells infected with

HIV are subjected to, e.g., in vitro segregation from non-infected patient cells and the latter may be returned to the patient.

As set forth in the following detailed description, antibody-related polypeptides of the present invention are preferably obtained by means of initial development of mono-specific antibodies (preferably monoclonal antibodies) to the human lymphocyte T4 glycoprotein (the CD4 determinant) followed by preparation of
10 antibodies (preferably monoclonal antibodies) to the T4 idiotypic region of antibodies formed in the initial development step.

Chimeric antibodies and fragments thereof and especially bi-specific antibodies are also products
15 within the contemplation of the present invention, as are antibody-related products produced in microbial hosts, (e.g., procaryotic and eucaryotic cells in culture) which hosts are transformed or transfected with DNA sequences encoding the desired polypeptide pro-
20 ducts.

As one example, with structural information in hand concerning the idiotypic regions of antibodies of the invention, it becomes possible to employ procaryotic and eucaryotic hosts such as E. coli, yeast, insect, and
25 mammalian cells in culture to produce useful antibody fragments (such as fab' and f(ab')₂ fragments). Moreover, it is within the contemplation of the invention that chimeric antibodies (e.g. mouse/human antibodies) may be prepared using transformed mouse myeloma cells or
30 hybridoma cells (especially heavy chain deletion mutant cells) as production hosts. Hybridoma cell producing bi-specific antibodies having diagnostic and therapeutic uses are contemplated. Recombinant methods may also be applied to the production of HIV subunit
35 vaccine materials. For example, monoclonal antibodies of the invention are expected to be extremely well suit-

ed for the screening of expression products of HIV DNA in transformed or transfected vial or procaryotic hosts, allowing isolation of DNA encoding all or part of the amino acid sequence of naturally occurring immunologi-
5 cally significant HIV proteins (including glycopro-
teins). In suitable hosts, the presence of such DNA may allow for high level production of vaccine materials.

In a preferred form, the invention provides antibody-related polypeptides characterized as mono-
10 clonal anti-monoclonal-anti-human lymphocyte T4 anti-
bodies. Especially preferred are monoclonal anti-OKT4
and anti-OKT4A antibodies, ^{terms used herein the claims and description} both of which are reactive
with 60-80 Kd HIV proteins. Presently most preferred
are monoclonal anti-OKT4A antibodies which have substan-
15 tial capacity for in vitro neutralization of HIV infec-
tivity of multiple HIV strains and are participative in
complement-mediated cytolysis of HIV infected cells.

In another aspect, the present invention pro-
vides, for the first time, hybridoma cell lines which
20 produce "anti-idiotypic" monoclonal antibodies specifi-
cally immunoreactive with a monoclonal antibody to human
lymphocyte T4 protein in an antigen/antibody reaction.
Illustratively, the present invention provides new mur-
ine-derived hybridoma cell lines, JT4C8, JT4C12 and
25 JT4C16, JT1-1F3, JT1-1F3-E5, JT1-1D7 and JT2-N15, each
of which produces, as a component of the supernatant of
its growth in culture, a monoclonal antibody specifi-
cally reactive with anti-T4 idiotypic and moreover reac-
tive with HIV virion proteins in a manner expected to
30 allow for both in vitro and in vivo neutralization of
HIV infectivity.

Hybridoma cell line JT4C8 was received for
deposit with the American Type Culture Collection,
Rockville, Maryland, at the facilities of the United
35 States Department of Agriculture, Plum Island, New York
on February 18, 1987, with A.T.C.C. Accession No.



HB9385. Hybridoma cell line JT4C12 was received for deposit with the American Type Culture Collection, Rockville, Maryland, at the facilities of the United States Department of Agriculture, Plum Island, New York
5 on February 18, 1987, with A.T.C.C. Accession No.

HB9387. Hybridoma cell line JT4C16 was received for deposit with the American Type Culture Collection, Rockville, Maryland, at the facilities of the United States Department of Agriculture, Plum Island, New York
10 on February 18, 1987, with A.T.C.C. Accession No.

HB9386. Hybridoma cell line JT1-1F3 was received for deposit at the European Collection of Animal Cell Cultures, Salisbury, Wiltshire, U.K. on June 25, 1987, with ECACC Accession No. 87062501. Hybridoma cell line
15 JT1-1F3-E5 was received for deposit at the European Collection of Animal Cell Cultures, Salisbury, Wiltshire, U.K. on June 25, 1987 with ECACC Accession No. 87062502. Hybridoma cell line JT1-1D7 was received for deposit by the Fermentation Research Institute,

20 Ibaragi-ken, Japan on January 29, 1988 with the Accession No. FERM BP-1685. Hybridoma cell line JT2-N15 was received for deposit by the Fermentation Research Institute, Ibaragi-ken, Japan on January 29, 1988 with the Accession No. FERM BP-1684.

25 In still another of its aspects, the present invention provides for production of HIV subunit vaccine materials by means of well-known affinity purification methodologies whereby HIV protein fractions (especially those in the molecular weight range of from about 60,000
30 to about 80,000 and most especially about 65,000-67,000) are isolated through use of a selective immunoabsorbants prepared using antibodies of the invention.

Numerous aspects and advantages of the present invention will be apparent upon consideration of the
35 illustrative examples and descriptions of practice of the invention in the following detailed description

thereof and the drawing, wherein: Figures 1, 2 and 3 graphically represent the results of immunoreactivity testing of antibodies of the invention with HIV and non-infected cell proteins; Figures 4 and 5 provide immunoblot assay results involving antibodies of the invention and HIV proteins; and Figures 6A through 6E provide photographic results of immunofluorescent staining assays on infected and uninfected cells employing antibodies of the invention.

10

DETAILED DESCRIPTION

The following examples illustrate practice of the invention in the production of a number of hybridoma cell lines including JT4C8, JT4C12, JT4C16, JT1-1F3, JT1-1F3-E5, JT1-1D7 and JT2-N15 the isolation therefrom of monoclonal antibodies to anti-CD4, and the amplification and characterization of such monoclonal antibodies.

More particularly, Example 1 is directed to stimulation of a murine host toward production of antibodies to a commercially available anti-T4 monoclonal antibody, "OKT4", the fusion of spleen cells with myeloma cells, the screening, cloning and growth of hybridoma cells, and the isolation of monoclonal antibodies therefrom. Example 2 relates to the characterization of monoclonal antibodies so produced by fluorescent immunoassay, by Western blot assay for reactivity with HIV protein, and by screening for capacity to effect in vitro neutralization of HIV infectivity.

Example 3 relates to a first procedure for development

of hybridoma cell lines capable of providing in the medium of their growth monoclonal antibodies to the commercially available antibody "OKT4A". Example 4 relates to characterization of monoclonal antibodies so produced by means of immunofluorescence assay, Western blot assay, in vitro neutralization assay, ELISA assay, and fluorescent cell staining assays. Example 5 relates to a second procedure for development of hybridoma cell lines capable of providing in the medium of their growth monoclonal antibodies to the commercially available antibody "OKT4A" and to characterization of monoclonal antibodies so produced by means of Western blot assay, and in vitro neutralization assay.

15 EXAMPLE 1

According to one aspect of the practice of the invention, hybrid tumor cell lines are produced using standard immunological techniques such as described in 20 Oi and Herzenberg, Selected Methods Cell Immunology, 351-372 (1979) and Godding, "Antibody Production By Hybridomas", J.Immunol.Meth., 39, pp. 285-308 (1980). Spleen cells from mice, hyperimmunized with monoclonal anti-T4 are fused with a mouse myeloma cell line in the 25 presence of polyethylene glycol. The supernatant from growth of each "hybridoma" cell culture is tested for the presence of the desired antibody activity. Selected hybridoma cells are cloned to propagate cell lines which produce an antibody in their growth culture supernatant, 30 which antibody has highly specific anti-anti-T4 activity.

A. Immunization

BALB/C mice each were subject to splenic injection with monoclonal anti-human lymphocyte T4 antibody (OKT4, Ortho Diagnostics, Rahway, N.J.).

One milligram of the lyophilized OKT4 material was brought up in 1 ml of distilled water. Dialysis was employed to remove and replace the original phosphate buffer with 50mM MES buffer (Sigma Chemicals), pH 6.0.

- 5 The material was then subjected to high pressure liquid chromatography separation on FPLC® apparatus (Pharmacia, Laboratory Separation Division, Piscataway, N.J. 08854). Separation was carried out using a Mono Q column under recommended procedures except that the salt gradient was changed to 0 to 0.5 M NaCl. Aliquots (20 µl) of each 0.5 ml fraction were assayed for activity using freshly collected human lymphocytes (10^5 cells per ml). More specifically, cells and HPLC fractions were mixed and centrifuged. The cells were then washed several times and resuspended in phosphate buffered saline (PBS). 5 µg rabbit anti-mouse IgG labelled with FITC was incubated with the cells. Following centrifugation, the cell pellet was resuspended in PBS and results were read using a fluorometric cell counter.
- 10
- 20 Fractions displaying highest activity were pooled, analyzed on SDS-PAGE and were revealed to be greater than 90% pure.

- Each of 4 mice was initially given a splenic injection totalling approximately 100 µl of inoculant (approximately 1.5 µg OKT4 per mouse). Fourteen and twenty-eight days later the mice were each given booster injections of 100 µl inoculant.
- 25

- Four days after the final booster, the mice were sacrificed and spleens were removed aseptically and placed in petri dishes (on ice) containing Dulbecco's Modified Eagle's Medium (Gibco). The spleens were trimmed of fat and connective tissue, passed through 100 gauge stainless steel mesh. The resulting individual spleen cells were pelleted by centrifugation for 10 minutes at 1000 rpm. The cell pellet was washed twice with media (as above) and was resuspended in RPMI 1640
- 30
- 35

and the cell concentration was determined by counting in a hemocytometer in the presence of 0.2% trypan blue.

Mouse myeloma cells NS1/1.Ag4.1 derived from Balb/c strain, were grown in RPMI 1640 medium containing 15% heat-inactivated horse serum (Pel-Freeze). The cells were pelleted by centrifugation at 1000 rpm for 10 minutes and washed with RPMI 1640 containing no antibiotic. The cell concentration was determined by counting after resuspension in the same medium.

10 Spleen and NS1/1 cells were combined in a ratio of 4:1 and centrifuged at 1000 rpm for 10 minutes. The supernatant fluid was aspirated away and cell fusion was conducted at 37°C using polyethylene glycol (PEG) 1500, molecular weight 500-600. The procedure was
15 carried out with constant gentle stirring by addition of the following, at the times indicated: 1.0 ml of 50% PEG in RPMI 1640 added over one minute, with one minute stirring, 1.0 ml RPMI 1640 containing 15% horse serum over one minute; 1.0 ml of RPMI 1640 containing 15%
20 horse serum added over one minute; and 8 µl RPMI 1640 containing 15% horse serum added over 2 minutes.

The resulting fused cells were centrifuged at 1000 rpm for 10 minutes, resuspended in RPMI 1640 containing 15% horse serum and also containing penicillin G
25 and streptomycin at 100 units and 100 mg per ml, respectively. The cells were plated at 0.1 ml per well in 96-well plates previously equilibrated in 5% CO₂. Plates were incubated overnight at 37°C in 5% CO₂.

On day two, each well received 0.1 ml of HAT
30 medium (13.6 µg/ml hypoxanthine 0.176 µg/ml aminopterin and 3.88 µg/ml thymidine) in RPMI 1640 containing 15% horse serum. This medium selectively allows spleen cell NS1/1 hybrids to survive, screening out unfused NS1/1 cells or those fused to other cells. Unfused primary
35 spleen cells from adult mice will not survive in culture for more than a few days.

On days 3, 4, 6, 9 and 12, 0.1 ml of medium was removed from each well, and 0.1 ml of fresh HAT in RPMI 1640 containing 15% horse serum was added. On days 15, 19, 23 and 27, 0.1 ml of medium was removed from 5 each well, and was replaced with 0.1 ml of RPMI 1640 containing 15% horse serum and only hypoxanthine and thymidine in the same concentration as above. On day 28, culture supernatants from all wells were screened for detection of antibody specific for OKT4.

10 A fluorescent-linked immunosorbent assay ("FLISA") was utilized for the detection of hybridomas producing antibodies to OKT4. Wells of 96-well plates were coated overnight at 37°C with 100 µl of carbonate-bicarbonate solution containing 3.0 µg/ml of rabbit 15 anti-mouse IgGFc. Wells were washed ten times with PBS containing 0.05% Tween 20. The wells were blocked for 1 hour at 37°C with 20% horse sera. Blocking agent was removed by two washings with PBS/Tween 20 as above.

Twenty microliter aliquots of culture fluid 20 from each hybridoma well and 80 µl PBS were incubated in the coated wells at room temperature for 1 hour and then at 4°C overnight. The wells were washed ten times with PBS containing 0.05% Tween 20 and blocked with 150 µl/well mouse serum (8 µg/ml), incubated for 3 hours 25 at 37°C and then 2 hours at 4°C.

100 µl of FITC-labelled OKT4 (0.3 µg/ml) was incubated in each well overnight and in the dark at 4°C. Wells were washed 10 times with PBS/Tween 20 solution as above. To each well was added 200 µl of 30 5×10^{-5} N NaOH and 5.6×10^{-4} N NH_4OH solution. The plates were maintained at room temperature for 15 minutes and then shaken for 1 minute. After transfer to Titertek Microtitration 1 x 8 plates, the wells were fluorometrically (excitation 490 nm; emission 530 nm) 35 using a Corona Electric model MTP 100F microplate reader.

Of the 2710 wells screened, 19 were significantly positive for reaction with OKT4. The 19 positive clones were designated JT4C1 through JT4C19.

Formal cloning of hybridoma cells obtained from the positive wells was conducted by diluting the cells into additional wells at a ratio such that there was approximately 1 cell per 3 wells. Generally, formal cloning from an active well produced formal clones which appeared to be subclones of the same hybridoma cell but in several instances revealed different clone populations over three generations. Subclones from the same original well were named with the parent number and an additional number (e.g., clones obtained from well JT4C7 were labeled JT4C7-1, JT4C7-2, etc.).

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EXAMPLE 2

In order to characterize the antibodies produced by the positive clones described in Example 1, tests were conducted to determine relative affinity of monoclonal antibodies the original immunogen, the antibodies were also screened by Western blot analysis for immunoreactivity with HIV virion proteins, and the antibodies were screened for capacity to neutralize infectivity of HIV virus.

25

A. Relative Affinity Titrations

A fluorescent-linked assay was carried out according to the same general procedure employed for antibody screening in Example 1, except that various dilutions of rabbit anti-mouse IgG Fc were deposited in test wells. Fluorescence results are set out in Table 1 for antibodies derived from clones JT4C1 through JT4C13 and JT4C16. Fluorescence results for various subclones of JT4C7 are set out in Table 2.

35

The data in Table 1 indicate that antibodies of clones JT4C1, JT4C2 and JT4C4 are of relatively high affinity. Antibodies from clones JT4C11 and JT4C13 are of relatively lower affinity, with the antibodies of the 5 remaining clones tested being of an intermediate affinity.

Correspondingly, Table 2 reveals that of the subclones of JT4C7, clones JT4C7-12 and JT4C7-9 are respectively of the highest and lowest relative affinity 10 in this test procedure.

15

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1 2 3 4 5 6 7

TABLE 1

Concentration Anti-Mouse Antibody ($\mu\text{g/ml}$)	0 (Blank)	1	2	Clone No.		5	6	7
				3	4			
10	-27	2882	2799	2511	2913	2529	2435	2601
3	271	877	803	823	938	723	688	763
1	37	201	200	236	128	178	133	199
0.3	24	31	17	106	37	99	109	142
0.1	205	14	-1	123	23	49	53	103
0.03	392	21	-82	76	68	66	55	120
0.01	301	15	23	105	54	88	46	85
0.003	17	-8	-43	86	-14	116	42	115

Concentration Anti-Mouse Antibody ($\mu\text{g/ml}$)	8	9	10	Clone No.		13	16
				11	12		
10	2395	2523	2463	1003	2281	1151	2203
3	617	751	716	405	830	497	620
1	84	98	112	92	215	192	272
0.3	38	88	76	101	397	185	263
0.1	19	49	61	16	117	70	157
0.03	43	43	125	76	104	107	202
0.01	-51	40	103	31	80	60	198
0.003	-27	29	126	26	51	65	136

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TABLE 2

Concentration Anti-Mouse Antibody ($\mu\text{g/ml}$)

Subclone No.	3.0	1.5	0.75	0.37	0.18	0.09	0.045	0.022	0.011	0.005	0.002
JT4C7-3	2364	1004	399	337	267	259	217	206	246	215	161
JT4C7-12	2480	914	376	298	278	262	232	200	249	230	202
JT4C7-8	2420	869	351	291	236	214	235	201	217	188	208
JT4C7-9	2238	924	366	270	289	214	250	211	214	245	251
JT4C7-6	2441	864	344	250	234	210	243	210	203	239	235
JT4C7-4	2372	869	369	275	249	243	233	204	228	227	229
JT4C7-11	2330	828	378	290	268	246	224	211	245	228	227
HAT Medium	786	475	248	306	241	237	245	200	220	242	272

B. Western Blot Analysis

Antibodies derived from all nineteen positive clones identified in Example 1 were assayed by Western blot analysis for immunoreactivity with proteins of the HIV virion. More specifically, HTLV-IIIB particles were disrupted with SDS (0.1%) and dithiothriitol (0.003M) and the material was placed on a 7.5% polyacrylamide gel, the gel was electrophoresed using standard procedures and materials were transferred to nitrocellulose filter paper. Filters were initially incubated with 20% heat inactivated horse serum in PBS for 1 hour and then washed with PBS. Filters were then incubated with antibody supernatants of each clone for 3 hours at room temperature with gentle shaking and then overnight at 4°C with gentle shaking. After washing with PBS/Tween 20, and re-blocking with 20% horse serum as above for one hour, 10 µg of peroxidase labelled rabbit anti-mouse IgG was added and the mixture was incubated at room temperature for 4 hours. After 10 washings with PBS/Tween 20, color was developed by standard means. Antibodies derived from clones JT4C8, JT4C12 and JT4C16 all strongly reacted with HIV protein having a molecular weight of about 60,000 to 80,000. The ability of these antibodies to immunoreact with HIV protein that played no part in their generation is strongly predictive of the capacity of these antibodies to effect neutralization of HIV in vitro and in vivo.

Isotype analysis of the above-noted antibodies reactive in Western blot procedures revealed that JT4C12 and JT4C16 antibodies were of the IgG₃ isotype.

C. HIV Neutralization

Hybridoma culture supernatants were tested for HIV neutralization capacity in the following manner. Three day growth supernatants were diluted 1:5 in complete medium [500 ml RPMI 1640; 6 ml 100 x Penicillin/-

Streptomycin; 6 ml 100 x L-glutamine; 100 ml FCS; and, 1.2 ml Polybrene Stock (1 mg/ml)]. 200 μ l of the medium-diluted sample was added to all but two wells of a 24 well microtiter plate and two wells received an equal quantity of medium alone. Additional medium was added to one of the "medium-only" wells and each remaining well received 200 μ l of high titer HIV virus stock. Plates were sealed in plastic bags, incubated for 1-1½ hours at 4°C and allowed to return to 17°C upon standing for about 15 minutes. H9 cells were incubated in complete medium for 30 minutes at 37°C at a density of 1×10^6 cells/ml, then centrifuged and resuspended in fresh complete medium at a density of 5×10^6 cell/ml. 200 μ l of the cell suspension was added to each well (bringing the total volume to 600 μ l) and the plates were incubated for 1 hour at 37°C, whereupon 150 μ l was transferred to a duplicate plate containing 2.0 ml of fresh complete medium per well. Cultures were incubated at 37°C in a CO₂ incubator. After 4 days, the cultures were split and fresh complete medium was added. At day 7 of incubation samples were prepared for neutralization screening by IFA and Reverse Transcriptase procedures. [See, Guroff et al., Nature, 316, 72-74 (1985); Matthews et al., Proc. Nat'l. Acad. Sci. (USA), 83, 9709-9713 (1986); and Poiesz et al., Proc. Nat'l. Acad. Sci. (USA), 77, 7415-7419 (1980)]. In a first neutralization screening procedure, the results were essentially negative or inconclusive but in a second procedure commenced concurrently with the running of the first, antibodies from 12 of 15 tested clones in the JT4C1-19 series displayed neutralizing activity in the IFA or RT test or both and 5 of 6 antibodies of the JT4C7 subclones tested displayed neutralization characteristics. All neutralization was "partial" in comparison to human AIDS (HTLV-III_B) patient serum which displayed 100 percent neutralization in these assays.

EXAMPLE 3

The general hybridoma forming and screening procedures of Example 1 were repeated using the commercially available monoclonal anti-human lymphocyte T4 antibody designated OKT4A (Ortho Diagnostics, Rahway, N.J.) which was purified using a Mono S (rather than Mono Q) column. The specific immunization procedure varied slightly from that of Example 1 in that the initial injection was intraperitoneal and involved approximately 15 µg of purified antibody. The second intraperitoneal inoculation was seven days later and consisted of approximately 10 µg of purified antibody. Thirteen days later, the final booster of about 5 µg of antibody was administered by splenic injection. Of 2090 wells screened, 34 were significantly positive for reaction with OKT4A, with 10 of these displaying high activity (fluorescence values of about 1000 or more against a "background" of approximately 200). These ten positive clones were designated JT1-1D11, JT1-1F3, JT1-1G2, JT1-6E12, JT1-6F12, JT2-8E9, JT3-2C4, JT3-5A11, JT3-6D9, AND JT3-6E8.

EXAMPLE 4

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In order to characterize the antibodies produced by the positive clones described in Example 3, tests were conducted essentially as described in Example 2. Briefly, supernatant antibodies were reactive on the Western blot assay with HIV protein having a molecular weight of from about 60,000 to about 80,000. The assays predominantly indicated reaction with an approximately 67,000 molecular weight protein, with some antibodies showing reactivity with a 78,000 molecular weight species. Significantly no reactivity was noted with 41 Kd or 120 Kd fractions usually characterized as the

major immuologically significant HIV envelope glycoproteins. Of the antibodies displaying the strongest reaction, two (JT1-6E12 and JT2-8E9) were of an IgM isotype and the antibody of clone JT1-1F3 was of the IgG₁ isotype. Set out below in Table 3 are the results of the fluorescent-linked assay for employing the JT1-1F3 antibody.

TABLE 3

10

Concentration Anti-

Mouse Antibody

(μ g/ml)

Blank

Control

(HAT)

JT1-1F3

15

20 μ g/ml

4

754

1093

10

-37

738

1010

5

-43

756

899

2.5

-56

759

908

1.25

-2

454

634

20

0.625

-38

291

325

0.313

23

315

310

0.157

11

340

331

Neutralization studies carried out concurrently with those of Example 2(C) also indicated initial negative neutralization results and, in the second trial, only slight evidence of neutralization capacity on the RT assay. Nonetheless, the IgG₁-secreting JT1-1F3 clone was selected for ascites amplification and further antibody ELISA screening for reactivity with HIV protein.

In the first ELISA screen, varying concentrations of HTLV-III_B protein (respectively, concentrations of 40, 20, 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078, 0.039 and 0.020 μ g/ml) were coated onto microtiter plates, blocked (20% horse serum/PBS, 37°C, 2 hours),

and dried overnight. Culture supernatant (20 μ l with 80 μ l PBS) or HAT medium control was added as a first antibody. After one hour of incubation at room temperature and storage at 4°C overnight, peroxidase conjugated rabbit anti-mouse IgG (0.3 μ g/ml in 5% horse serum/PBS) was added as the second antibody. After 2 hours of incubation at room temperature, substrate was added (O-phenylenediamine, 0.5 mg/ml in McIlvan's Buffer, pH 5.5; 10 ml of 3% hydrogen peroxide). After 20 min. of incubation at room temperature, the reaction was stopped with 50 μ l 0.4 M sulfuric acid and absorbance was read at λ 492-610. The results of the test are graphically represented in Figure 1 and indicate that HIV was detectable at levels of 1.25 μ g and also that reactivity progressively increased with up to 40 μ g of virus.

The ELISA procedure was repeated on plates initially coated with varying concentrations of uninfected H9 cell membrane preparations [10^6 , 6×10^5 , 4×10^5 , 2×10^5 , 6×10^4 , 4×10^4 , 2×10^4 , 1×10^4 , 6×10^3 , 4×10^3 , 2×10^3 cells/ml]. The results of this test procedure are graphically represented in Figure 2 and indicate no reactivity with non-infected preparations.

Finally, the HIV protein ELISA was repeated using the same varying concentrations of protein from the HTLV-IIIB HIV variant, the Haitian HIV variant designated AL1212C (obtained from Dr. David Hall, Massachusetts General Hospital, Boston, MA) and the African HIV variant designated 906 (obtained from Dr. Jerome Groopman, New England Deaconess Hospital, Boston, Massachusetts). As graphically illustrated in Figure 3, the JT1-1F3 antibody recognized a common epitope of all three HIV variants.

Ascites fluid derived JT1-1F3 antibody was then tested for in vitro neutralization activity as indicated by the RT assay described above and by syncytium

induction. In this procedure a comparison was made between varying levels of JTI-1F3 antibody (ascites fluid IgG fraction), a negative control (ascites fluid/pristane) and a positive control in the form of 5 serum from an HTLV-IIIB-infected patient. According to this procedure, HIV strains IIIB, AL1212C and 906 were incubated with Mab JR1-1F3, control ascites or neutralizing human serum (in a 1:5 dilution in phosphate buffered saline) for 90 minutes at 4°C. H9 cells 10 (5×10^6) were then added to each well and incubated for 1 hour at 37°C. Aliquots (150 μ l) were removed from each well and added to 2.0 ml fresh medium. The cultures were split 1:1 on day 4. Reverse transcriptase activity and syncytium induction were monitored on day 4 15 and day 7. The day 7 results of this procedure are provided in Table 4, wherein the relative syncytium induction is indicated as follows: (-), 0/200 cells; (+/-), 1-5/ 200 cells; (+), >10% cells; (++) , >25% of cells; and (+++) , >50% of cells.

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TABLE 4

JT1-1F3 NEUTRALIZATION

	<u>HIV = HTLV-III B</u>		<u>HIV = AL1212C</u>		<u>HIV = 906</u>	
	<u>RT Activity/ % Neutralization</u>	<u>Syncitium Induction</u>	<u>RT Activity/ % Neutralization</u>	<u>Syncitium Induction</u>	<u>RT Activity/ % Neutralization</u>	<u>Syncitium Induction</u>
1. Non-infected H9	5100/-	-	3420/-	-	4800/-	-
2. HIV	135150/0	+++	317280/0	+++	627510/0	+++
3. HIV + 1mg/ml Ab	5730/100	-	12030/96.2	-	56970/90.9	-
4. HIV + 600 µg/ml Ab	50700/62.4	-	130380/58.9	+/-	431670/31.2	-
5. HIV + 300 µg/ml Ab	78330/42.0	++	240030/24.3	+	497850/20.6	+/-
6. HIV + 50 µg/ml Ab	132780/1.7	+++	198690/37.4	+++	647670/0	++
7. HIV + Ascites (control)	168000/0	+++	281100/11.4	+++	765000/0	+++
8. HIV + Human Serum	5460/100	-	197130/37.8	++	688680/0	+++

The results shown in Table 4 clearly demonstrate in vitro neutralizing activity for the JT1-1F3 antibody versus all three HIV variants tested, in contrast to the neutralizing activity for infected patient serum, which was completely variant-specific, indicating recognition by the monoclonal antibody of a significant type-common epitope.

The molecular weight of the JT1-1F3 antibody reactive species was determined by immunoblotting.

10 Purified HTLV-IIIB (5 and 10 µg) was analyzed by SDS-PAGE and Coomassie blue/silver staining (Fig. 4, Lanes B and C). Similar aliquots were analyzed by SDS-PAGE, transferred to nitrocellulose paper and analyzed for reactivity with JT1-1F3 antibody. A single
15 reactive species was detectable at approximately 67 kd (Fig. 4, Lanes E and F).

Reactivity of JT1-1F3 antibody with the HTLV-IIIB isolate was compared to that with other HIV stains. Similar patterns of reactivity by ELISA were
20 obtained with the HTLV-IIIB, ALI2I2C and 906 strains. Furthermore, Western blot analysis with JT1-1F3 antibody and each of the three HIV strains revealed reactivity with antigens of similar molecular weight (Fig. 5). These findings indicate that MAb JT1-1F3 reacts with
25 related or identical antigens detectable in divergent HIV strains.

The above findings with JT1-1F3 antibody suggested that it might also be useful in detecting HIV virus-infected cells. In this regard, the binding of
30 JT1-1F3 antibody (ascites fluid IgG fraction) to uninfected and HIV-infected H9 cells was monitored. The extent of JT1-1F3 finding was determined by a fluoresceinated rabbit anti-mouse IgG. As shown in Figure 6A, there was little if any detectable binding of antibody
35 to uninfected H9 cells. In contrast, focal and diffuse binding of the antibody was detectable with HTLV-IIIB-

infected H9 cells (Fig. 6B). Similar findings were obtained when using H9 cells infected with the 906 and AL1212C strains (Fig. 6C and D). This approach has been extended to hematopoietic cells from a patient with
5 AIDS. Mononuclear cells were collected from peripheral blood of the AIDS patient by Ficoll-Hypaque separation and examined for reactivity with JT1-1F3. As shown in Figure 6E, a focal and diffuse immunofluorescent staining pattern was detected with these cells that was
10 similar to the findings obtained with HIV-infected H9 cells.

It is noteworthy that screening of the JT1-1F3 antibody for reactivity with cells possessing class II major histocompatibility ("MHC") surface components
15 (i.e., human B cell line MD1 and normal peripheral blood mononuclear cells) reveal no substantial reactivity.

Subcloning of the JT1-1F3 resulted in the selection of subclones JT1-1F3-E5 and JT1-1D7 as respectively producing progressively higher levels of IgG₁
20 antibody than JT1-1F3. Comparative neutralization assay data for JT1-1F3, JT1-1F3-E5 and JT1-1D7 is set out in Table 5 below. Reactivity within the ELISA assay format for the antibodies produced by these three hybridomas is set out in Table 6 below.

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TABLE 5

NEUTRALIZATION

	HIV = HTLV-III B	HIV = AL1212C	HIV = 906
	RT Activity/ % Neutralization	RT Activity/ % Neutralization	RT Activity/ % Neutralization
<u>JT1-1F3</u>			
1. Non-infected H9	464/-	455/0	494/0
2. HIV	10976/0	58131/0	127317/0
3. HIV + 4 mg/ml Ab	5823/49.0	15754/73.5	27212/78.9
4. HIV + 2 mg/ml Ab	8561/23.0	47571/18.3	156653/0
5. HIV + 400 µg/ml Ab	7183/36.1	515261/11.5	1803291/0
<u>JT1-1F3-E5</u>			
1. Non-infected H9	126/0	121/-	126/-
2. HIV	10990/0	33501/0	16213/0
3. HIV + 2.9 mg/ml Ab	NT*	9281/72.6	NT
4. HIV + 1.9 mg/ml Ab	NT	11559/65.7	-
5. HIV + 1 mg/ml Ab	552/96.1	17967/46.5	2253/86.8
6. HIV + 500 µg/ml Ab	775/94.0	NT	3894/76.6
<u>JT1-1D7</u>			
1. Non-infected H9	214/0		
2. HIV	10998/0		
3. HIV + 500 µg/ml Ab	177/100		
4. HIV + 250 µg/ml Ab	1549/87.6		

* NT = Not Tested

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TABLE 6

Comparison of 3 Hybridoma on ELISA

<u>HIV-IIIB Viral Protein</u>	<u>JT1-1F3</u>	<u>JT1-1E5</u>	<u>JT1-1D7</u>	<u>Control (Culture Media)</u>
10	1.337	1.111	1.392	0.031
5	0.914	0.887	1.118	0.023
2.5	0.996	0.831	1.081	0.022
1.25	0.499	0.278	0.523	0.011
0.63	0.221	0.122	0.176	0.020
0.31	0.070	0.075	0.060	0.015
0.16	0.048	0.049	0.036	0.004
0.08	0.027	0.027	0.031	0.012

EXAMPLE 5

The hybridoma forming and screening procedures of Examples 1 and 3 were again repeated using OKT4A, with the following variations in the immunization procedure. The initial immunization involved intraperitoneal injection of 10 µg of Mono S purified OKT4A antibody; the second intraperitoneal injection (7 µg) was given seventeen days later; and the final, 7 µg intravenous dose was administered 18 days later. After fusion and screening, an IgG₁-producing positive clone, designated JT2-N15 was selected for further study. Like JT1-1F3 and its subclones JT1-1F3-E5 and JT1-1D7, clone JT2-N15 produced a monoclonal antibody which was reactive on the Western blot assay with HIV protein having a molecular weight of about 65-67,000. Culture media from growth of JT2-N15 was positive in the ELISA assay and the results in a preliminary neutralization assay with respect to HIV-IIIB infectivity are set out in Table 7 below.

TABLE 7

Neutralization of HIV-IIIB By JT2-N15

	<u>RT Activity and</u> <u>% Neutralization</u>
1. Non-infected H9	157/-
2. HIV	12194/0
3. HIV + 1.5 mg/ml Ab	343/98.5
4. HIV + 750 µg/ml Ab	568/96.6

In further screening procedures for neutralizing activity of anti-idiotypic antibodies propagated by the ascites method, it has been preliminarily determined that the following protocol generates the most active ascites preparation. Five to eight week old mice are "primed" with 0.5 to 1.0 ml Pristane and two weeks later injected with 2 to 8×10^6 (preferably about 5×10^6) hybridoma cells. Collection of ascites fluids commenced two weeks after inoculation and the initial fluids collected (about 3-5 ml) displayed the highest neutralization activity. A second collection of ascites fluid carried out three days later produced from 8 to 10 ml of fluid having lesser activity. A third collection from surviving animals generally provided 3-5 ml of fluid which, at times, was substantially less active than either of the materials from the first and second collection. Neutralization data shown for JT1-1F3 in Tables 4 and 5 was based on ascites pooled from three collections whereas data for JT1-1F3-E5, JT1-1D7 and JT2-N15 in Tables 5 and 7 was based on ascites materials pooled from first and second collections only.

While the foregoing illustrative examples have been directed to procedures involving the commercially available monoclonal antibody preparations OKT4 and OKT4A, other commercial antibodies such as Leu3a (Becton-Dickenson, Immunocytometry Systems, Mountain View, California, 94039) are expected to be equally suitable for use in generating anti-idiotypic antibodies according to the invention. Equally suitable are non-commercial antibodies (preferably monoclonal antibodies) prepared by known hybridoma techniques using human T cells, human T lymphoblast cells which express the T4 glycoprotein and recombinant-produced human T4 protein isolates as the initial immunogen. Moreover, while antibodies produced by JT1-1F3 and its subclones and JT2-N15 are of the IgG₁ isotype, it is expected that

antibodies of differing isotypes will be equally useful. Antibodies of the IgG₂ isotype, for example, may be more useful in procedures involving complement-mediated cytolytic reactions.

5 Confirmation of the operability of the procedures of the present invention is provided by the reports of Chanh et al., P.N.A.S. (USA), 84, 3891-3895 (June, 1987) wherein it is reported that monoclonal antibody to Leu3a (designated HF1.7) was capable of in
10 vitro neutralization of HIV-IIIB infectivity. However, neutralizing activity of HF1.7 has been characterized as "weak" [see Weiss, Nature, 331, p. 15 (January, 1988)] and has not been demonstrated to extend to strains other than HIV-IIIB. Moreover, unlike the anti-OKT4 and OKT4A
15 monoclonal antibodies of the foregoing examples, the anti-Leu3a antibody of Chanh et al. is not indicated as reactive with any HIV-derived protein other than gp120 as shown in Figure 4 at page 3894 of Chanh et al., supra. [See also, Dalglish et al., The Lancet, ii,
20 1047-1050, November 7, 1987, relating to polyclonal anti-Leu3a antibodies.]

While the foregoing examples relate to murine-derived hybridoma cell preparations, it is within the contemplation of the invention to generate and employ
25 hybrid hybridomas (e.g., mouse/human) and especially human/human hybridomas prepared, for example, in a manner consistent with Borrebaeck, TIBTECH, June, 1986, pp. 147-153; Abrams et al., Methods in Enzymology, 121, pp. 107-119 (1986); Kozbor et al., Methods in
30 Enzymology, 121, pp. 120-140 (1986); Suresh et al., Methods in Enzymology, 121, pp. 210-228 (1986); and Masuho et al., Biochem. & Biophys. Res. Comm., 135(2), pp. 495-500 (1986). See, also, Klausner, "'Single Chain' Antibodies Become a Reality", Bio/Technology, 4,
35 1041-42 (1986), Klausner, "Stage Set For 'Immunological Star Wars'", Bio/Technology, 5, 867-868 (1987) and Marx,

"Antibodies Made To Order", Science, 229, 455-456 (1985).

It will be readily understood, therefore, that the above specific illustrative methodologies for the
5 production of hybridomas and the identification and isolation of monoclonal antibodies are not intended to be restrictive of the scope of practice of the invention. Numerous alternative methodologies exist for achieving the same results as demonstrated, for example,
10 by articles appearing in Methods in Enzymology, Vol. 121, "Immunological Techniques, Part I", Langone et al., eds., Academic Press, Inc. (New York, 1986).

It is also within the contemplation of the invention to develop immunologically active polypeptides
15 for use in diagnostic and therapeutic methods of the invention by means of expression of DNA sequences encoding therefor in suitably transformed or transfected procaryotic and eucaryotic host cells in culture.

Anti-HIV therapeutic methods of the invention
20 will be understood to comprise the administration of effective amounts of antibodies or antibody fragments of the invention to a patient infected with HIV or at risk of HIV infection in order to generate passive immunity involving neutralizing infectivity of HIV in vivo. In
25 this regard, combination of products of the invention with immunologically acceptable diluents, adjuvants and carriers is contemplated in order to form immunologically effective anti-HIV therapeutic compositions.

Anti-HIV therapeutic methods of the invention
30 within the contemplation of the invention also comprehend active immunization using biologically active HIV protein fractions reactive with the monoclonal anti-monoclonal anti-human lymphocytes. Such products may be obtained directly from virus preparations by well known
35 affinity purification processes involving forming immunological reaction mixtures between HIV proteins and

antibodies of the invention, followed by isolation of the desired protein. As one example, the 60,000 to 80,000 molecular weight HIV protein recognized by the JTI-1F3 in procedures of Example 4 is a prime candidate
5 for vaccine use. The same is expected to be true for recombinant expression products based on HIV DNA which can be immunologically identified (and/or purified) by means of antibodies of the invention.

Diagnostic methods of the invention wherein
10 polypeptide products are employed to detect and quantify HIV particles in biological fluids such as blood are expected to form an essential part in preliminary screening for patients who would benefit from passive immunization according to the invention and in the moni-
15 toring therapeutic regimens of the invention.

The finding that antibodies of the invention are selectively reactive with the surfaces of HIV-infected cells is indicative of a variety of diagnostic and therapeutic utilities including histological screen-
20 ing of tissue (e.g., lymphatic cells) and fluid (e.g., blood) samples for detection of HIV infection, possibly at early stages of infection not readily detectable by assays based on screening for antibodies. Recognition of infected cells by antibodies of the invention allows
25 for segregation of such cells from cell populations comprising both infected and non-infected cells. It is thus contemplated that blood of AIDS patients may be subjected to extracorporeal treatment to remove or selectively kill infected lymphocytes through use of
30 antibodies of the invention. Moreover, within the contemplation of the invention is in vivo treatment with antibodies of the invention coupled with supplementation of circulating complement to effect cytolysis of infected cells. Support for the operability of such a
35 therapeutic protocol is provided by preliminary positive results of tests for the capacity of JTI-1D7 antibodies

to participate in the in vitro complement dependent cytolysis of HIV infected H9 cells.

Selective reactivity properties of antibodies make them good candidates for in vivo drug or toxin delivery to infected cells and use in development of bi-specific antibodies which will include double determinants allowing for, e.g., "focusing" effector T cell activity. See, e.g., Stearz et al., Proc. Nat'l. Acad. Sci. (USA), 83, 1453-1457 (1986).

10 Based on the fact that the present invention has its foundation in the immunological characteristics of T4 protein -- which is believed to provide the common receptor for HIV infection by all variants (e.g., AL1212C, 906, ARC, LAV, HTLV-IIIRF, HTLV-IIIB) -- rather
15 than any specific HIV variant, it is expected that diagnostic and therapeutic methods of the invention will be applicable to detection and treatment of infection involving all HIV variants. Use of polypeptides of the invention as diagnostic and research tools is expected
20 to provide additional information into the nature of the interaction between HIV and host cells. As one example, monoclonal antibodies of the invention will be useful in identifying the precise primary, secondary and tertiary structural conformation of region(s) of surface proteins
25 of HIV and host cells which are specifically and necessarily interactive in the recognition and association processes involved in HIV infection of cells. This information, in turn, would allow for generation of immunologically active materials of the invention
30 through use of synthetic and recombinant-produced peptide immunogens.

The foregoing detailed description has been given for clearness of understanding only and no unnecessary limitations should be understood therefrom
35 inasmuch as numerous modifications and variations will be expected to occur to those skilled in the art.

~~WHAT IS CLAIMED IS:~~ THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A purified and isolated immunologically
active polypeptide capable of specific immunobinding
5 with that portion of the HIV virion which is necessarily
interactive with T4 surface proteins during HIV infec-
tion of host cells and characterized by the capacity to
neutralize infectivity of HIV in vitro and by specific
immunoreactivity with an antibody immunospecific for
10 human lymphocyte T4.

2. A polypeptide according to claim 1 in the
form of a antibody or chimeric antibody or a fragment
thereof.
15

3. A polypeptide according to claim 1 which
is specifically immunoreactive with OKT4 antibody as
hereinbefore defined.

4. A polypeptide according to claim 1 which
20 is specifically immunoreactive with OKT4A antibody.

5. A polypeptide according to claim 1 in the
form of an antibody or chimeric antibody or a fragment
thereof specifically immunoreactive with HIV protein
25 having a molecular weight of from about 60,000 to about
80,000 as determined by SDS-PAGE.

6. A polypeptide according to claim 5 which
is specifically immunoreactive with HIV protein having a
30 molecular weight of about 65,000 to 67,000 as determined
by SDS-PAGE.

7. A polypeptide according to claim 1 which
is immunologically nonreactive with class II histo-
35 compatibility molecules.



8. A polypeptide according to claim 1 in the form of a murine-derived antibody or antibody fragment.

9. A polypeptide according to claim 1 in the form of a human antibody or antibody fragment.

10. A polypeptide according to claim 8 or 9 in the form of a monoclonal antibody or monoclonal antibody fragment.

10

11. A polypeptide according to claim 10 in the form of a double determinant, bi-specific monoclonal antibody.

15

12. Monoclonal anti-monoclonal-anti-human lymphocyte T4 antibody.

13. Monoclonal anti-OKT4 antibody as hereinbefore defined.

20

14. Monoclonal anti-OKT4A antibody as hereinbefore defined.

15. A hybridoma cell line capable of producing in the medium of its growth a monoclonal antibody according to claim 12.

25

16. A hybridoma cell line according to claim 15 and selected from the group consisting of JT4C8, JT4C12, JT4C16, JT1-1F3, JT1-1F3-E5, JT1-1D7 and JT2-N15.

30

17. A hybrid hybridoma cell line capable of producing in the medium of its growth a polypeptide according to claim 11.

35



18. An anti-HIV treatment method comprising administering to an animal susceptible to infection with HIV an immunologically effective amount of a polypeptide according to claim 1.

19. An anti-HIV treatment method comprising administering to an animal susceptible to infection with HIV an immunologically effective amount of an antibody according to claim 12.

20. A protective HIV virus infection immune response eliciting composition comprising an immunologically effective amount of a polypeptide according to claim 1 and a pharmaceutically acceptable carrier..

21. A protective HIV virus infection immune response eliciting composition comprising an immunologically effective amount of an antibody according to claim 12 and a pharmaceutically acceptable carrier..



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~~18. An anti-HIV treatment method comprising~~
administering to an animal susceptible to infection with
HIV an immunologically effective amount of a polypeptide
according to claim 1.

5

19. An anti-HIV treatment method comprising
administering to an animal susceptible to infection with
HIV an immunologically effective amount of an antibody
according to claim 12.

10

20. A vaccine composition for use in develop-
ing a protective immune response to infection by HIV
virus comprising an immunologically effective amount of
a polypeptide according to claim 1.

15

21. A vaccine composition for use in develop-
ing a protective immune response to infection by HIV
virus comprising an immunologically effective amount of
~~an antibody according to claim 12.~~

20

22. In an assay procedure for detection
and/or quantification of HIV in a biological fluid based
upon an immunological reaction between HIV and a immuno-
logically reactive polypeptide capable of specific im-
munobinding to HIV, the improvement comprising employing
a polypeptide according to claim 1 as the immunologi-
cally reactive polypeptide.

25

23. In an assay procedure for detection
and/or quantification of HIV in a biological fluid based
upon an immunological reaction between HIV and an anti-
body capable of specific immunobinding to HIV, the
improvement comprising employing an antibody according
to claim 12.

30

35



24. An assay procedure for detection and/or quantification of HIV-infected host cells in a fluid or tissue sample, said method comprising: forming an immunological reaction mixture of said sample with a polypeptide according to claim 1 and detecting the presence of immunobinding of said polypeptide to surfaces of infected cells.

25. An assay procedure for detection and/or quantification of HIV-infected host cells in a fluid or tissue sample, said method comprising: forming an immunological reaction mixture of said sample with an antibody according to claim 12 and detecting the presence of immunobinding of said antibody to surfaces of infected cells.

26. An assay according to claim 25 wherein said detection step comprises determination of a detectable label bound to said antibody.

27. An assay according to claim 25 wherein said detection step comprises determination of a detectable label bound to an anti-antibody added to said reaction mixture.

28. An assay according to claim 25 wherein said antibody is selected from the group consisting of anti-OKT4 antibody ^{as hereinbefore defined} and anti-OKT4A antibody ^{as hereinbefore defined}.

29. A method for segregating HIV-infected cells in a population of infected and non-infected cells, said method comprising forming an immunological reaction mixture of said cell population and a polypeptide according to claim 1 and segregating infected from non-infected cells on the basis of the selective binding of said polypeptide to infected cells.



30. A method for segregating HIV-infected cells in a population of infected and non-infected cells, said method comprising forming an immunological reaction mixture of said cell population and an antibody according to claim 12 and segregating infected from non-infected cells on the basis of the selective binding of said antibody to infected cells.

31. A method for isolating HIV proteins from a fluid sample, said method comprising contacting said sample with a polypeptide according to claim 1 to form an immunological reaction mixture including said protein and said polypeptide and isolating said protein from said reaction mixture.

32. A method for isolating HIV proteins from a fluid sample, said method comprising contacting said sample with a antibody according to claim 12 to form an immunological reaction mixture including said protein and said polypeptide and isolating said protein from said reaction mixture.

33. A purified and isolated HIV protein characterized by:

- (a) a molecular weight of from about 60,000 to 80,000 as determined by SDS-PAGE; and
- (b) specific immunoreactivity with monoclonal anti-monoclonal-anti-human lymphocyte T4 antibodies.

34. A purified and isolated HIV protein according to claim 33 and characterized by a molecular weight of about 65,000 to 67,000 as determined by SDS-PAGE.

35. An HIV protein according to claim 33 further characterized by the capacity to stimulate an in vivo immunological response protective against HIV infection in a susceptible host.

5 36. An HIV protein according to claim 33 further characterized as a product of affinity purification employing an immunoabsorbant comprising monoclonal anti-monoclonal-anti-human lymphocyte T4 antibodies.

37. A protective HIV virus infection immune response eliciting composition comprising an immunologically effective amount of an HIV protein according to claim 33 and an immunologically acceptable diluent, adjuvant or carrier.

15 38. A protective HIV virus infection immune response eliciting composition comprising the expression product of a host cell transformed or transfected with DNA encoding part or all of an HIV protein having a molecular weight of from about 60,000 to 80,000 as determined by SDS-PAGE and which product specifically immunoreactive with monoclonal anti-monoclonal-anti-human lymphocyte T4 antibodies.

20 39. A microbial host cell transformed or transfected with a DNA sequence encoding a polypeptide according to claim 1.

25 40. A microbial host cell transformed or transfected with a DNA sequence encoding a polypeptide according to claim 33.

41. A purified isolated immunologically active polypeptide as claimed in claim 1 substantially as hereinbefore described with reference to any one of the examples.

30 42. A monoclonal antibody as claimed in claims 12-15 substantially as hereinbefore described with reference to



any one of the examples.

43. A purified and isolated HIV protein as claimed in claim 33 substantially as hereinbefore described with reference to any one of the examples.

5 44. A vaccine composition as claimed in claim 38 substantially as hereinbefore described with reference to any one of the examples.

DATED: 25 January 1991

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David B Fitzpatrick



FIG. 1

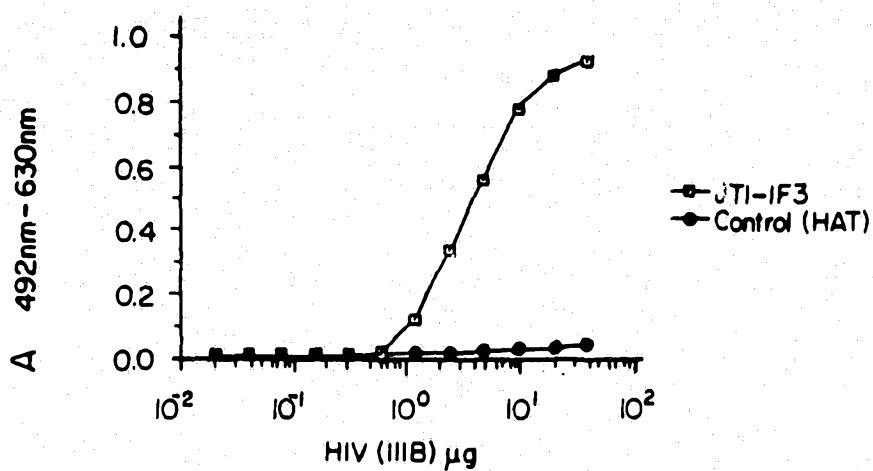


FIG. 2

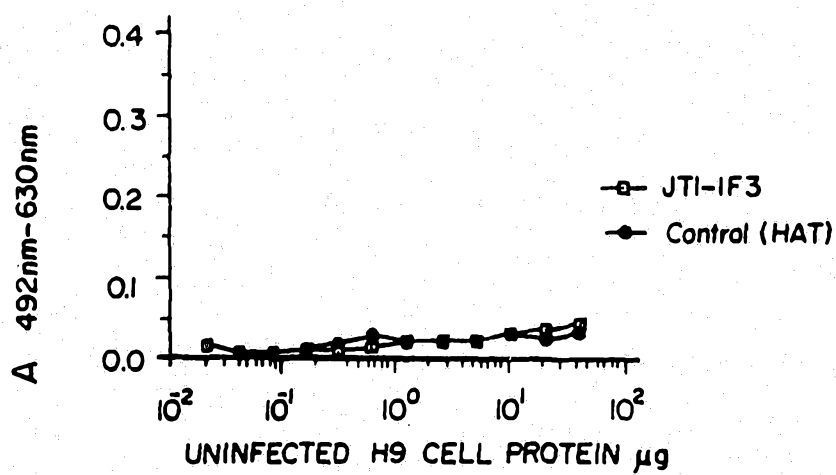
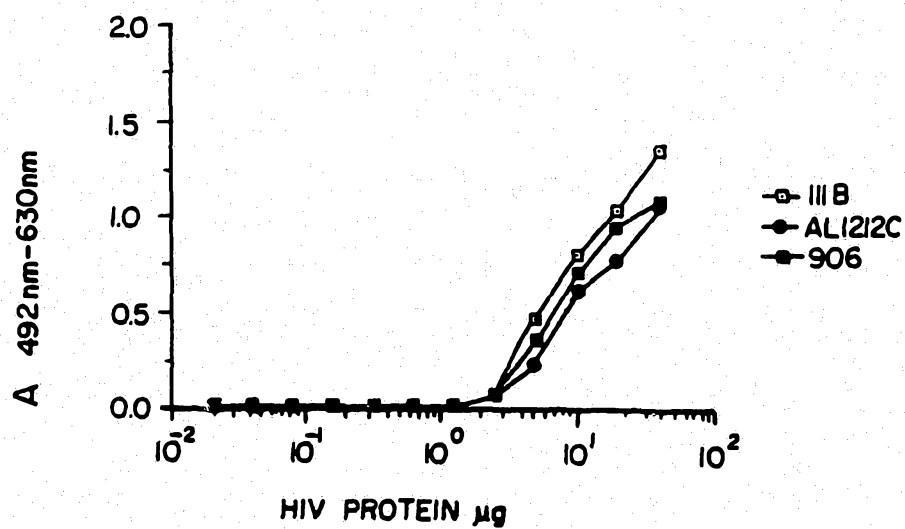


FIG. 3



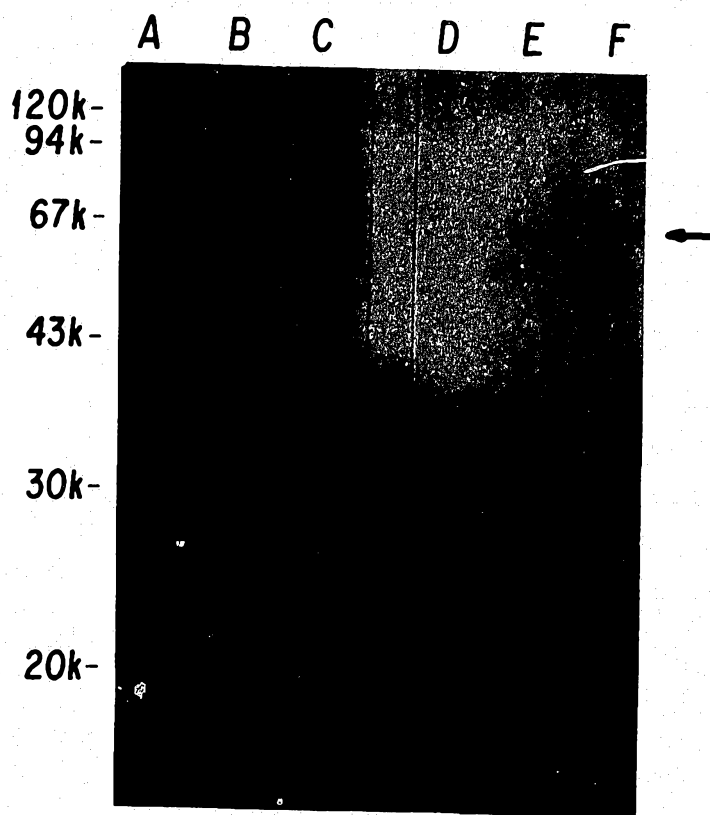


FIG. 4

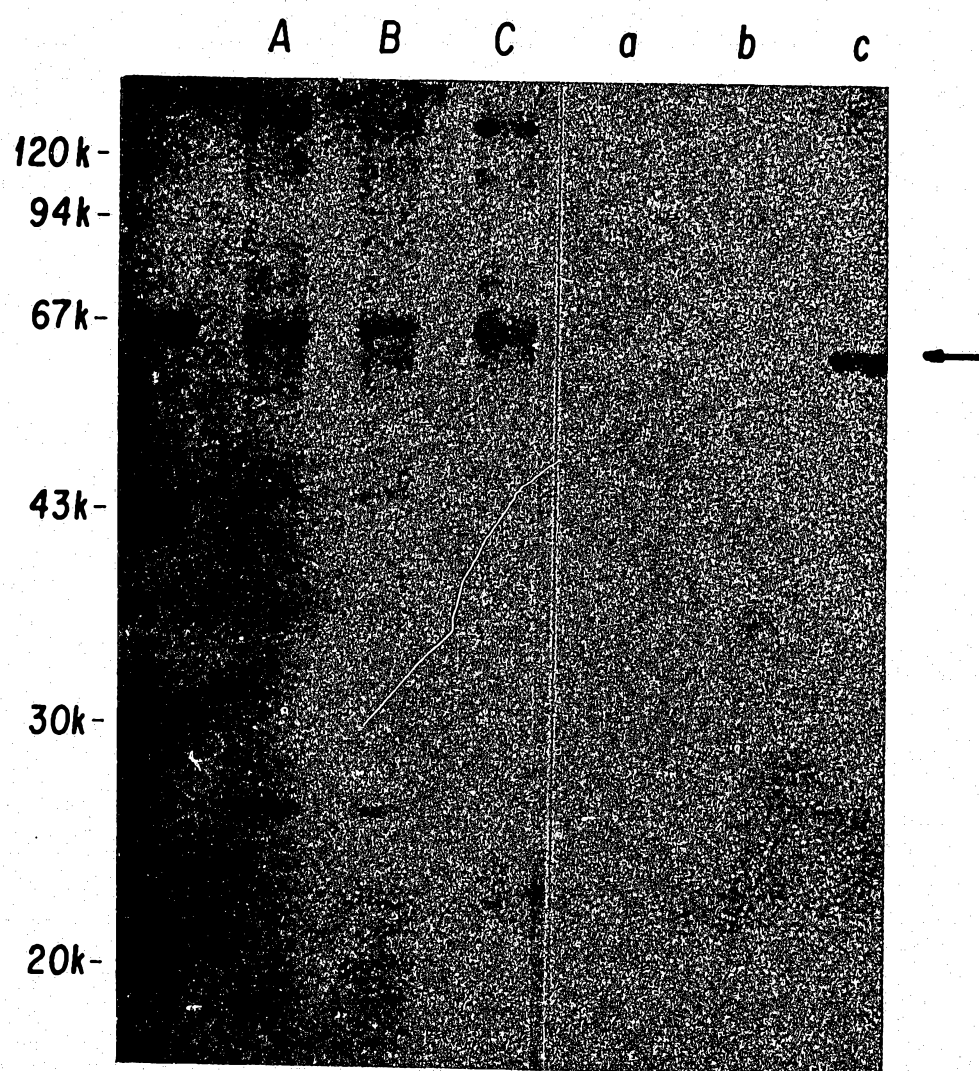


FIG. 5

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FIG. 6A



FIG. 6B

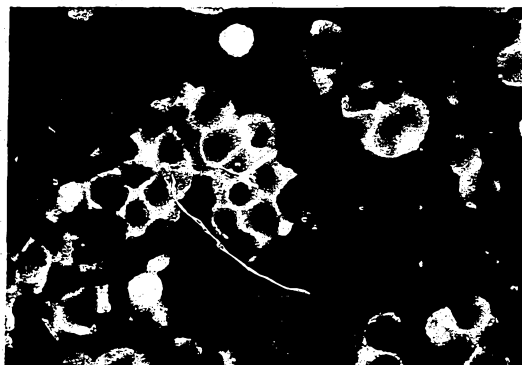


FIG. 6C

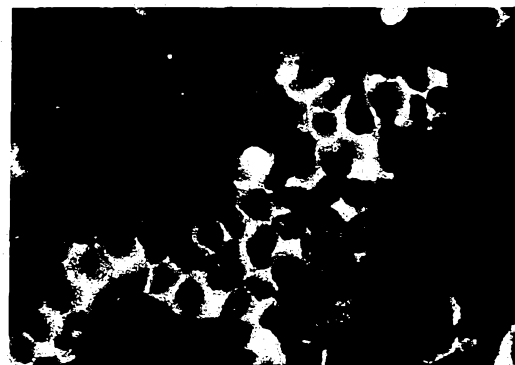


FIG. 6D

2 3 00 11003

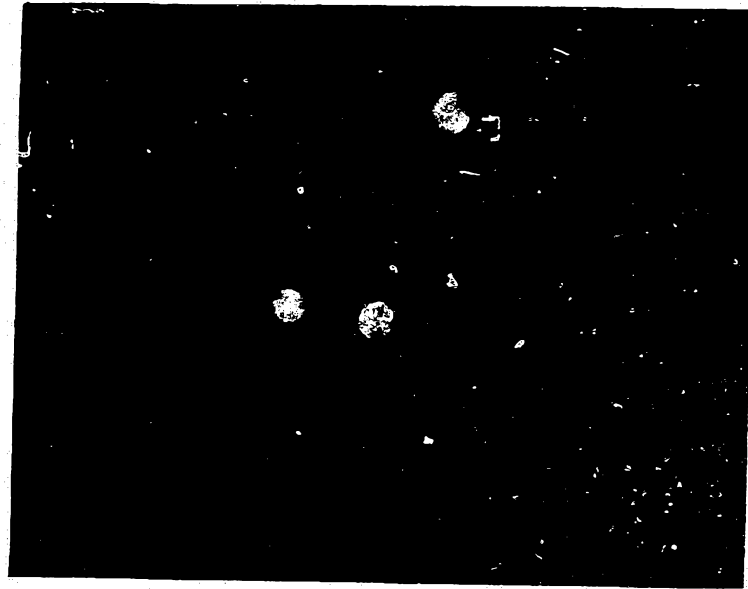


FIG. 6E