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(54) Title: MONOCLONAL ANTIBODIES AGAINST HIV-1 AND VACCINES MADE THEREOF

(57) Abstract

The present invention discloses antibodies which can be used for the manufacture of vaccines for active and/or passive immunization of persons in need of such treatment. The invention also provides for human monoclonal antibodies that are functionally equivalent to the above-mentioned antibodies produced by any one of the cell lines CL1 through CL6 (deposited at the European Collection of Animal Cell Cultures (ECACC) at the PHLS in Porton Down, Salisbury, UK). It is also a goal of the present invention to provide for hybridoma and/or CHO cell lines producing any one of the antibodies disclosed and claimed herein. The invention is further directed to mixtures of the antibodies of the present invention, as well as to methods of using individual antibodies or mixtures thereof for the detection, prevention and/or therapeutical treatment of HIV-1 infections in vitro and in vivo.

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# Monoclonal antibodies against HIV-1 and vaccines made thereof

## Technical field

This invention is in the field of immunology, especially detection, prevention and treatment of HIV-1 infection and AIDS therapy. More particularly, it concerns monoclonal antibodies, drugs and vaccines made from these antibodies and methods based on the use of these antibodies, drugs and vaccines for analytical and/or clinical applications.

## Background of the invention

In the sera of human immunodeficiency virus type 1 (HIV-1) infected patients, anti-virus antibodies can be detected over a certain period after infection without any clinical manifestations of the acquired immunodeficiency syndrome (AIDS). At this state of active immune response, high numbers of antigen-specific B-cells are expected in the circulation. These B-cells are used as fusion partners for the generation of human monoclonal anti-HIV antibodies.

The monoclonal antibodies according to the present invention are produced by known procedures, e.g. as described by R. Kennet et al. in "Monoclonal antibodies and functional cell lines; progress and applications". Plenum Press (New York), 1984.

Further materials and methods applied in the present invention are based on known procedures, e.g. such as described in J. Virol. 67:6642-6647, 1993.

Viable samples of the hybridoma cell lines CL1 to CL6 producing the monoclonal antibodies herein described were deposited at the European Collection of Animal Cell Cultures (ECACC) at the Public Health Laboratory Service (PHLS), Centre for Applied Microbiology and Research, Porton Down, Salisbury SP4 OJG, United Kingdom. They are identified by their accession numbers:

CL1 - Accession No. 90091704 (deposited on 17 September 1990);
CL2 - Accession No. 93091517 (deposited on 15 September 1993);
CL3 - Accession No. 95032235 (deposited on 22 March 1995);
CL4 - Accession No. 95032236 (deposited on 22 March 1995);
CL5 - Accession No. 95032240 (deposited on 22 March 1995); and
CL6 - Accession No. 95032241 (deposited on 22 March 1995).

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The corresponding monoclonal antibodies produced by these cell lines are hereinafter termed MAb CL1, MAb CL2 through MAb CL6, when used in the abbreviated form.

Monoclonal antibodies and in particular human monoclonal antibodies have been widely used in the last few years in order to improve the understanding of HIV-1 neutralization by antibodies released upon immunization with HIV-1 derived immunogens or upon infection in afflicted patients (J. Virol. 62:2107-2114, 1988; Immunology 76:515-534, 1992; J. Virol. 67:6642-6647, 1993; US 5,087,557). Many efforts have been made to overcome the detrimental capability of the HIV-1 virus to rapidly change its morphology under immunological pressure and thereby to escape the capture by antibodies released from a patient's immune system or developed and applied by researchers. As a result thereof, there is presently no reliable antibody-based (nor any other) vaccine for active or passive immunization on the market.

One significant step forward has been made when an antigenic determinant on the smalller subunit gp41 of the HIV-1 envelope glycoprotein gp160 was found (EP 570 357 A2), which corresponds to the amino acid sequence "ELDKWA" located at amino acid position number 662 to 667 of gp41 of HIV-1 isloate BH10. The authors report therein an HIV-1 neutralizing human monoconal antibody specifically binding to said antigenic determinant. The antibody proved to be a powerful tool for biochemical analysis of the binding epitope and its variability. The discovery of the highly conserved state of said gp41-epitope gave rise to the hope of possibly finding a vaccine composition suitable for more reliable prevention of human individuals from HIV-1 infection and/or for more successful therapeutic treatment of infected patients.

The results reported in EP 570 357 A2 motivated the inventors of the present invention to intensify their research activities which finally led them to the novel and inventive findings herein disclosed .

However, in spite of promising results of the art relating to the use of HIV-1 neutralizing monoclonal antibodies, there is at least one major drawback to this sort of approach. It lies in the wide-spread use of laboratory strains of HIV-1 isolates, which have become adapted to lab-conditions and are more or less attenuated and hence only poorly - if at all - representative for the

properties and behaviour of primary HIV-1 isolates. Consequently, promising vaccine compositions drawn against laboratory HIV-1 strains frequently proved non-efficacious when applied against primary HIV-1 isolates, e.g. of blood samples of infected persons (see J. Cohen, Science 262:980-981, 1993).

The second major drawback was and still is the ability of the HIV-1 virus to escape antibody capture by morphological variation, which very often renders the remarkable efforts of the researchers almost useless. Such escape mutants may be characterized by a change of only one or several of the amino acids within one of the targetted antigenic determinants and may occur e.g. as a result of spontaneous or induced mutation.

## Summary of the invention

The present invention therefore discloses antibodies which have been found to overcome the disadvantages of the prior art and which can be used for the manufacture of vaccines for active and/or passive immunization of persons in need of such treatment. The invention also provides for human monoclonal antibodies that are functionally equivalent to the above-mentioned antibodies produced by any one of the cell lines CL1 through CL6. It is also a goal of the present invention to provide for the hybridoma and/or CHO cell lines producing any one of the antibodies disclosed and claimed herein.

The invention is further directed to mixtures of antibodies according to the present invention, as well as to methods of using individual antibodies or mixtures thereof for the prevention and/or therapeutical treatment of HIV-1 infections in vitro and in vivo, and/or for improved detection of HIV-1 infections.

The cell lines CL1 to CL4 produce monoclonal antibodies recognizing HIV-envelope glycoproteins, and in particular specific antigenic determinants of gp160. The antibodies of CL1 and CL4 recognize and bind to an amino acid sequence of gp41/gp160 corresponding to the epitope located at amino acid position number 662 to 667 ("ELDKWA") of gp41 of HIV-1 isloate BH10 (GenBank accession M15654; numbering as described in the Swissprot database entry ENV$HIV10). The monoclonal antibodies of CL2 and CL3 bind to two different antigenic determinants, more particularly to fragments of

gp120/gp160 corresponding to the epitope sequences located at amino acid positions 79 to 184 and 326 to 400 respectively, of processed gp120 of HIV-1 isolate BH10 (GenBank accession M15654; numbering as described in the Swissprot database entry ENV$HIV10).

While the idiotypic antibodies produced by CL1 to CL4 are directed to the capture and neutralization of HIV-1 viruses in vitro and in vivo, the anti-idiotypic antibodies released from CL5 and CL6 take an opposite role, i.e. they mimic the viruses, more particularly they mimic the corresponding antigenic determinant(s) of the HIV-1 viruses. The anti-idiotypic antibodies of CL5 and CL6 are of a nature such that they bind to the idiotypic antibody of CL2 at essentially the same location(s) (antigenic determinants) on gp160 as does the virus itself.

**Detailed description of some embodiments of the invention**

When conducting experiments to find novel anti-HIV-1 antibodies the inventors found human monoclonal antibodies which could be shown to efficiently neutralize HIV-1 in vitro including a variety of primary HIV-1 isolates, such as e.g. primary HIV-1 isolates 92RW009, 92RW021, 92UG037, 92TH014, 92BR030, N70-2, DJ259 (all obtained from WHO network for HIV-1 isolation and characterization), or WYG, WRF, WRB, WSC, WHM (isolated from Austrian patients).

Surprisingly, it turned out that these antibodies recognize and bind to two different antigenic determinants of the glycoprotein gp160 of HIV-1.

Moreover, it appears that the binding target of these antibodies is extraordinarily unique. In a comparative test involving a mixture of 41 different HIV-1 binding antibodies supplied by laboratories from different companies and research institutes it was shown that no one of the other antibodies present in the mixture competed with an antibody of the above-identified group, for instance with the human monoclonal antibody from cell line CL2, for binding to the targetted antigenic fragments of gp120/gp160 corresponding to amino acid sequences 79 to 184 and 326 to 400 of processed gp120 of HIV-1 isolate BH10.

Also, investigations of blood serum and blood plasma of HIV-1 infected patients revealed that antibodies of the CL2 type were not present in the samples tested so far. This finding again emphasizes the uniqueness of these HIV-1 neutralizing human monoclonal antibodies and simultaneously indicates that there might exist an extraordinary potential to combat HIV-1 infection, by using these antibodies in a suitable form for the prophylactic and/or therapeutic treatment of human individuals.

Another embodiment of the present invention comprises antibodies of the CL2 type which have been found to bind to the above-mentioned antigenic determinants of gp120/gp160 only if the determinants remain in a glycosilated form; they do not bind to these antigenic glycopeptide fragments when the fragments are deglycosilated, e.g. by the action of Peptide-N-Glycosidase F (EC 3.2.2.18; hereinafter referred to as "PN Gase F").

Still another embodiment of the invention encompasses human monoclonal antibodies of the CL2 type which are further characterized in that they also specifically bind to a fragment of gp120 produced in the SF9 insect cell/Baculovirus expression system in the absence of tunicamycin, while they do not bind to gp120 fragments expressed in the presence of tunicamycin.Tunicamycin is known for its inhibitory activity toward the glycosilating action of glycosyl transferase in glycoprotein biosynthesis.

Among the antibodies of the CL2 type as disclosed hereinbefore, there are also specimens which inhibit the infection of human lymphocytes by primary HIV-1 isolates such as the ones listed above, as could successfully be demonstrated by the inventors in *in vitro* experiments.

The invention also refers to antibodies of the CL2 type which possess one or more of the above-mentioned properties and can further be characterized by their special interaction with the anti-idiotypic monoclonal antibodies of hybridoma cell lines CL5 and CL6. While they can be bound by one and/or the other of the two anti-idiotypic antibodies CL5 and CL6, at least part of them is bound by anti-idiotypic huMAb produced by CL6 in a way that results in a specific blockade of the capability of the antibody to inhibit the infection of human lymphocytes by primary HIV-1 isolates.

In a further embodiment of the present invention, antibodies of the CL2 type are comprised which show at least one of the above-mentioned features or properties and which - in addition - have been proved to compete for binding to the antigenic determinants of gp120/160 with the antibody produced by hybridoma cell line CL2. The antibodies of this category are therefore - at least functionally - very closely related with the antibody released by CL2, and can be regarded as functional equivalents to it.

Another embodiment of the present invention is directed to the most beneficial human monoclonal antibody produced by hybridoma cell line CL2. This antibody can be used e.g. for passive immunization of HIV-1 infected individuals, but may even be more useful as a biochemical tool for developing vaccines applicable in the prevention and/or therapy of HIV-1 infections in vivo.

An attractive embodiment of the invention comprises the use of recombinant CHO cells for the production of the antibodies of the CL2 type. After successful identification of the antigenic determinants recognized and bound by these antibodies, the inventors also succeeded in transforming the respective genetic information into CHO cells, resulting in a stable cell line CL3, which synthesizes the CL2 type antibodies in a more efficient manner than the hybridoma cell line CL2 itself.

In another embodiment, anti-idiotypic antibodies are disclosed which can specifically bind to idiotypic antibodies of the CL2 type and/or which can interact with at least some of them in a fashion that eliminates their anti-HIV protective capability, i.e. bars them from inhibiting the infection of human lymphocytes by primary HIV-1 isolates. Such anti-idiotypic antibodies are therefore expected to be conformationally related with the HIV-1 viruses in that they probably contain similar or even identical antigenic fragments of a viral glycoprotein, e.g. of gp160.

The antibodies of the following embodiment seem to be very interesting because they are of an anti-idiotypic type and combine the features of the anti-idiotypic antibodies of the previous embodiment with their ability of inducing - upon administration to a mammal, e.g. a human or animal individual - the production and release of anti-HIV-1 antibodies. Optionally, the induced antibodies are of a nature such that they compete for binding to the above

specified antigenic determinants of gp120/160 with at least one antibody of the CL2 type as hereinbefore described in any one of the respective embodiments. A special representative of this group of anti-idiotypic antibodies is the one produced by hybridoma cell line CL6.

While the anti-idiotypic antibodies of the preceding embodiment may be used for active immunization of test animals or HIV-1 endangered and preferably not yet infected persons, the antibodies induced upon such active immunization may serve as components of a vaccine for passive immunization or as subjects of investigation to design and/or synthetically or genetically prepare such antibodies. Optionally, these (idiotypic) antibodies are functional equivalents to the CL2 type antibodies, i.e. they compete with the CL2 type antibodies for binding to the above specified antigenic determinants of gp120/gp160.

In a further - most exiting - embodiment of the invention, the human monoclonal antibodies exhibit strong HIV-1 neutralizing activity and bind to the smaller subunit of gp160, hereinafter referred to as gp41/gp160. Preclinical studies have proved that they are able to significantly reduce - upon intravenous administration to a human HIV-1 infected individual - the level of circulating HIV-1 in the blood serum and/or blood plasma of said individual (see also Example 8 and Fig.6).

Moreover, at least part of these antibodies may be further characterized in that they also compete with an idiotypic antibody produced by hybridoma cell line CL1 for binding to the gp41/gp160 antigenic determinant. Finally, the antibody produced by said cell line CL1 itself can be regarded as an important member of this group of HIV-1 level reducing antibodies.

Similarly to the situation with the CHO cell line CL3 producing CL2 type antibodies, the inventors also succeeded in cloning a recombinant CHO cell line CL4 producing antibodies which compete with the antibody of CL1 for binding to the gp41/gp160 antigenic determinant and hence may be regarded as more or less close equivalents to the CL1 antibody. Such recombinant CHO cell lines are easier to grow and more efficiently employed in the manufacture of the respective antibodies.

Various *in vitro* experiments have proved that the CL2 type antibodies as well as the CL1 type antibodies are able to neutralize a variety of different laboratory and primary HIV-1 isolates including a number of escape mutants, which usually develop upon individual application of any one of these antibodies. It could further be shown, that both antibody types are cross-reactive, i.e. they interact synergistically in that each of them is able to capture the escaped HIV-1 mutants of the other one antibody. Combined in a mixture, they are therefore a powerful tool to combat HIV-1 infections and AIDS. It is one of the intentions of the present invention to provide for a mixture of at least one antibody of the CL1 type and at least one antibody of the CL2 type.

The present invention also refers to a cell line producing any one of the preceedingly described antibodies and in particular to the cell lines CL1 through CL6 identified by their accession numbers as hereinbefore described.

In a further embodiment of the invention peptide fragments are provided which contain at least one of the antigenic determinants of gp41/gp160 and gp120/gp160 as herein described. It is desired that these peptide fragments are of a nature such that they are able to induce an immune response against HIV-1 infection, optionally the production and/or release of HIV-1 neutralizing antibodies, after administration to mammals, e.g. to an animal or a human individual.

In another embodiment, these peptide fragments may be linked to a suitable carrier in order to improve the efficacy of antigen presentation to the immune system. Such carriers may be, for instance, organic polymers including proteins, but any other appropriate and physiologically acceptable carrier may also be used. It may be advantageous in many cases to have the peptide fragments linked to a modified i.e. attenuated and/or recombinant virus such as modified influenza virus or modified hepatitis B virus or to parts of a virus, e.g. to a viral glycoprotein such as e.g. hemagglutinin of influenza virus or surface antigen of hepatitis B virus, in order to increase the immunological response against HIV-1 viruses and/or infected cells.

It is also an important goal of the present invention to provide for the manufacture of a reliable vaccine to prevent people from HIV-1 infection and/or to treat patients with already manifest HIV-1 infections in the course

of a therapy. Vaccines based on at least one of the idiotypic antibodies of the CL2 and CL1 groups can be applied for active immunization in the prophylaxis and therapy of higher animals including man. Convincing evidence could be provided for the reduction of the HIV-1 level in the plasma and serum of a seropositive patient in the course of a therapeutic treatment in a preclinical study (cf. Example 8 and Fig.6). Also, the preventive potency of the idiotypic antibodies of cell line CL1 was demonstrated in an impressive SCID-mouse trial as well as in a chimpanzee experiment. Neither the antibody-treated mice nor the chimpanzees developed HIV-1 infection upon challenge with live HIV-1 virus, while the animals in the untreated control groups became infected.

The use of at least one anti-idiotypic antibody as hereinbefore described for the manufacture of a vaccine for active immunization can help to successfully combat HIV-1 infection. The anti-idiotypic antibodies - as well as the drugs and vaccines derived therefrom - may primarily be used for the preventive treatment of HIV-1 endangered people and are optimally applied prior to getting into contact with HIV-1 virus. Due to their unique paratope characteristics they may also be administered to already infected patients in order to stimulate the immune system to release the corresponding - and possibly even more powerful - HIV-1 neutralizing antibodies. They may be either directly administered to a person or in combination with at least one suitable carrier and/or additive as usual in the art, and/or along with additional drugs. The anti-idiotypic antibodies may, however, also serve as "model templates" for the design and construction of e.g. fusion proteins carrying their respective antigenic determinant(s) (paratopes).

It might be preferable in many cases to combine an individual antibody or a mixture of at least two different antibodies with an immunoserum and/or an antibiotic, in order to further improve the benefit of an antibody vaccine manufactured accordingly.

In other cases it might be advantageous to use at least one of the herein specified antigenic peptide fragments of gp41/gp160 and gp120/gp160 to substitute the anti-idiotypic antibodies in the corresponding vaccines and drugs. Therefore the present invention also relates to said peptide fragments and to the use thereof for the manufacture of drugs and/or vaccines applicable in the prophylactic and/or therapeutic treatment of HIV-1 endangered or HIV-1 infected people. The fragments are optionally applied as

fusion proteins, wherein they are linked to a suitable carrier which might be a recombinant or attenuated virus or a part of a virus such as, e.g. the hemagglutinin of influenza virus or the surface antigen of hepatitis B virus, or another suitable carrier for efficient presentation of the antigenic site(s) to the immune system. The antigenic fragments might, however, also be directly applied for the prevention and/or treatment of HIV-1 infections in human individuals, e.g. persons belonging to one of the high-risk groups of HIV-1 endangered people including medical and scientific staff dealing with HIV-1 viruses and /or infected individuals.

The idiotypic antibodies referred to herein may further be used for the detection and/or determination of HIV-1 infected cells and/or HIV-1 viruses, either as individual antibodies or as an antibody cocktail. Similarly, one or more of the anti-idiotypic antibodies and/or the above-specified peptide fragments can successfully be applied to detect and/or determine anti-HIV-1 antibodies binding to the viruses or to HIV-1 infected cells. Both the idiotypic and anti-idiotypic antibodies of the present invention may therefore be prepared and arranged for an analytical testing procedure and/or for a commercially utilizable test kit.

Finally, it is also a part of the invention to offer a method of treating HIV-1 infected persons in need of such treatment, and to provide for a method of preventing people from becoming HIV-1 infected. Patients with manifest HIV-1 infections may therapeutically be treated with a vaccine comprising at least one of the idiotypic antibodies of the CL2 and the CL1 type, preferably a mixture thereof. However, in some cases it might be preferable to administer at least one of the anti-idiotypic antibodies and/or antigenic peptide fragments in order to induce additional - possibly even more powerful - antibodies to neutralize the viruses and to reduce the HIV-1 levels in the blood of infected patients.

The vaccine based on antibodies and/or antigenic peptide fragments may further comprise suitable i.e. physiologically acceptable carriers and further additives as usually applied in the art. The patients may be adminstered a dose of approximately 1 to 10 µg/kg body weight, preferably by intravenous injection once a day. For less threatening cases or long-lasting therapies the dose may be lowered to 0.5 to 5 µg/kg body weight. The treatment may be

repeated in periodic intervals, e.g. two to three times per day, or in daily or weekly intervals, depending on the status of the infection.

Vaccines according to the present invention may comprise any one of the idiotypic or anti-idiotypic antibodies or any one of the peptide fragments disclosed herein, either alone or in combination with suitable carriers and/or linked to carrier molecules. In some cases, e.g. where HIV-1 infection is acute and/or has already considerably progressed, it might be preferable to apply a mixture of idiotypic antibodies, while in other cases it might be more beneficial to apply a mixture of anti-idiotypic antibodies and/or - preferably carrier-linked - gp160 peptide fragments. It is recommended to apply a dose of 0.5 to 10 µg/kg body weight of antibody or carrier-linked gp160 peptide fragments, administered once to three times a day and possibly repeated in periodic intervals, e.g. weekly, monthly or yearly intervals, depending on the status of HIV-1 infection or the estimated threat of an individual of getting HIV infected.

**Brief description of the figures**

Fig.1 shows the reactivity of a human monoclonal antibody released by hybridoma cell line CL2 with glycoprotein subunit gp120 of gp160 of HIV-1;

Fig.2 shows the reactivity of a CL2 antibody with the glycosilated and deglycosilated form of gp160 of HIV-1;

Fig.3 shows the reactivity of a CL2 antibody with a recombinant gp120 in the presence and absence of tunicamycin;

Fig.4 shows the blocking effect of anti-idiotypic antibodies toward HIV-1 neutralizing antibodies of the CL2 cell line in a p24 antigen ELISA;

Fig.5 shows the uniqueness of antibodies of the CL2 cell line among a multitude of different anti-HIV antibodies, as characterized by the binding pattern of two different anti-idiotypic antibodies;

Fig.6 shows HIV-1 neutralizing efficacy of antibodies of cell line CL1 after in vivo application to a human patient.

In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting this invention in any respect.

Example 1 (Fig.1): Reactivity of GST/HIV-gp120 fusion proteins with antibodies from cell line CL2

The binding characteristics of human monoclonal antibody produced by CL2 (referred to hereinafter as MAb CL2) to the HIV-1 envelope glycoprotein gp120:

Overlapping gp120 fragments were fused to Glutathion S-tranferase (GST) and expressed using the insect cell/baculovirus system. Cell lysates of SF9 cells infected with recombinant baculovirus clones expressing different GST-gp120 fragments were first tested for their production level of GST. Lysates of GST-gp120 fusion proteins were then analysed in order to determine the binding affinity of MAb CL2. OD values of Ab CL2 given in the figure correspond to different rgp120 fragments. Microtiter plates were precoated with 2 µg/ml glutathion, cell lysates were added and incubated for 1 hour followed by an incubation step of 1 hour with 1 µg/ml MAb CL2 and detection with horse radish peroxidase conjugated anti-human IgG. The optical densities of the cell lysates corresponding to an equal amount of the GST fusion protein are shown.

As can be seen in Fig.1 GST-fusion-protein containing fragment 1 corresponds to amino acids 1-95 of processed gp120 of the BH10 isolate of HIV-1, fragment 2 corresponds to amino acids 79-184 of gp120, fragment 3 to amino acids 170-279, fragment 4 to amino acids 264-354, fragment 5 to amino acids 326-400 and fragment 6 to amino acids 384-481.

Example 2 (Fig.2): Antibody binding to deglycosylated $gp160_{HIV\ MN}$

For N-deglycosilation protein samples (500 ng recombinant $gp160_{HIV\ MN}$) were boiled 10 min. in denaturation buffer (0.5% SDS, 1% β-Mercapto-ethanol). Then 1/10 volume of each 10x enzyme reaction buffer and 10% NP-40 was added. This reaction mixture was incubated with 2000 U of PNGaseF (Boehringer Mannheim) for 12 hours at 37°C. Polyacrylamid gel electrophoresis was performed on 10-20% Tris/Glycin gels. After protein blotting, identical membranes were incubated with 5 µg/ml MAb CL2 (panel A), and 5 µg/ml MAb CL1 (panel B) as control.

In Fig.2 lanes 1 to 3 display the following:
lanes 1: untreated $gp160_{HIV\ MN}$;

lanes 2: gp160 $_{HIV\ MN}$ conditioned for PNGaseF treatment without enzyme;
lanes 3: gp160 $_{HIV\ MN}$ PNGaseF treated;
molecular weight markers are indicated in kDa.

It can be seen in Fig.2 that MAb CL2 does not bind to gp160 after the deglycosilating action of PNGaseF (panel A, lane 3), while MAb CL1 binds to the PNGaseF treated gp160 (panel B, lane 3).

Example 3 (Fig.3): Reactivity of recombinant GST/HIVgp120 fusion protein with MAb CL2 and anti-GST antiserum in the presence and absence of tunicamycin (TM) .

SF9 insect-cells were infected with either wildtype baculovirus or GST-gp120 expressing recombinant baculovirus. 5 hours after infection tunicamycin was added to a final concentration of 5 $\mu$g/ml. Cells were harvested after 48 hours and lysed. Anti-GST reactivity and MAb CL2 reactivity was tested by ELISA. Baculovirus infected cell-lysates (obtained from $1x10^7$ cells/ml) were transferred to microtiter plates, which were precoated with 2 $\mu$g/ml glutathione and incubated for 1.5 hours. GST-fusion protein or gp120 was detected by GST-antiserum (diluted 1:2000) or MAb 2G12 (1.5 $\mu$g/ml), respectively and horse radish peroxidase conjugated anti-mouse/anti-human IgG. The absorbance was determined at 492nm.

As can be seen in Fig.3 MAb CL2 does not bind to the gp120 fusion protein in the precence of tunicamycin, whereas anti-GST does, although at a decreased level.

Example 4: Neutralization capacity of MAb CL2 for primary HIV-1 isolates

A PBMC based neutralization assay was performed as described by Purtscher et al. (1994) by pre-incubating virus with serial antibody dilutions for 1h at 37°C and subsequent infection of fresh PHA stimulated PBMC prepared from HIV-negative donor buffy-coat cells. Neutralization capacity was estimated after 7 to 12 days by comparing the amounts of p24 antigen produced by the cells in the presence or in the absence of antibody.

Table 1: Summary of neutralization capacity of MAb CL2 in neutralization assays using PBMC.

| primary isolate | subtype clade | neutralizing capacity |
|---|---|---|
| 92RW009 | A | + + + |
| 92RW021 | A | + + + |
| 92UG037 | A | + + + |
| 92TH014 | B | + + + |
| 92BR030 | B | + + + |
| N70-2 | B | + |
| DJ259 | C | + |
| WYG | unkown | + + + |
| WRF | unkown | + + + |
| WRB | unkown | + + + |
| WSC | unkown | + |
| WHM | unkown | + |

Key: + + + 90% neutralization at a conc. of < 1 µg/ml
+ + 90% neutralization at a conc. between 1 to 50 µg/ml
+ 50% neutralization at a conc. below 50 µg/ml

Example 5 (Fig.4): Syncytia inhibition assay / Anti-idiotype blocking

An anti-idiotype (Ab2) blocking assay was performed to show whether the anti-idiotypic antibodies Ab2 block the neutralization capacity of MAb CL2 by binding to the neutralizing paratope of MAb CL2. The syncytia inhibition concentrations ($EC_{50}$) of MAbs CL1 and CL2 in the absence of anti-idiotypic antibodies were 2.0 and 8.8 µg/ml, respectively (the HIV-1 isolate RF was used). The addition of anti-idiotypic antibodies to MAb CL2 revealed, that the antibodies M1A3 and M4C12 did not alter the neutralizing capacity, but when M1G1 (= anti-idiotypic antibody produced by CL6) was incubated with MAb CL2 a significant impairment could be observed (Table 2). The syncytia inhibiting capacity of MAb CL1, which is directed against gp41, should not be affected by the anti-idiotypic antibodies tested. No syncytia inhibition was observed with anti-idiotypic antibodies alone at a concentration of 100 µg/ml as well as with MAb 3D6, which was used as a non-neutralizing control.

Table 2: $EC_{50}$ of MAb CL2 and MAb CL1 in the presence of M1G1, M1A3 or M4C12

| MAb | alone | anti-idiotype added (µg/ml) | | |
|---|---|---|---|---|
| | | + M1G1 (= MAb CL6) | + M1A3 (=MAb CL5) | + M4C12 |
| | | 100 | 100 | 100 |
| MAb CL2 | 2,02 | 5,26 | 2,63 | 0,66 |
| MAb CL1 | 8,83 | 7,43 | 8,83 | 6,25 |

Anti-idiotypic antibodies were diluted to 200 µg/ml and MAbs CL2 and CL1 (as control antibody) were diluted to 10 µg/ml in medium. 50 µl of serial two-fold dilutions of MAbs CL2 and CL1 were prepared starting at 100 µg/ml in four replicates. 50 µl of anti-idiotypic antibody (200 µg/ml) were added to each well and pre-incubated for 1h at 37°C in the incubator. As virus inoculum the HIV-1 isolate RF was diluted to approximately $10^2$ - $10^3$ $TCID_{50}$/ml and 50 µl of the virus suspension was added to each well. After an incubation of 1h at 37°C, 50 µl of AA-2 cell suspension ($10^6$ cells per ml; see CHAFFEE et al. 1988, J.Exp.Med. 168:605-621) was added to each well. The cells were then cultivated during 5 days at 37°C and 5% $CO_2$ , followed by microscopical evaluation of syncytia formation. Occurrence of at least one syncytium per well was recorded as an indication of HIV-1 infection. The 50% effective concentration ($EC_{50}$) was calculated by the method of Reed and Muench (1938).

All wells of one dilution step were then pooled and p24 was determined quantitatively in a p24 antigen ELISA. The measured p24 values were plotted against the MAb concentration. The results thereof can be seen in Fig.4 which displays the production of antigen p24 in cultures containing MAb CL2 (graph A) and MAb CL1 (graph B) with different amounts of anti-idiotypic antibodies.

Example 6 (Fig.6): Reactivity of the anti-idiotypic antibodies with different anti-gp160 antibodies

A panel of human anti-gp160 antibodies and pooled serum of HIV-1 positive individuals (HIVIG) were incubated on gp160-coated microtiter plates in the presence of M1G1 (=MAb CL6) and M1A3 (=MAb CL5), to prove the reactivity of the anti-idiotypes. Fig. 5, graphs A and B show the MAb CL2-specific binding of M1G1 and M1A3. Both anti-idiotypic antibodies were only reactive with MAb CL2 and not with other tested human antibodies (MAb CL1 and 5F3 and HIVIG are representative examples of human anti-HIV-1 antibodies).

Fig. 5, graphs A and B: 96-microtiter plates were coated with 2 µg/ml gp160 (Immuno AG, Vienna). Starting dilution of the human monoclonal antibody samples began at a concentration of about 200 ng/ml and HIVIG was prediluted 1:100. Eight dilutions of the human antibodies were preformed in $2^n$ steps. M1G1 and M1A3 were used at a concentration of 1 µg/ml. The human and murine antibody dilutions were transferred to the test plate and simultaneously incubated for 1 h. Then peroxidase-conjugated goat anti-mouse IgG was applied to the plate. After 1 h of incubation staining solution was added to each well; the absorbances were read at 492 nm against 620 nm.

Example 7: Immune selection experiments with HIV-1 molecular clone cl82.

Table 3:

| Selection conditions | | | |
|---|---|---|---|
| MAb | µg/ml | virus emerged | $EC_{50}$ >50 µg/ml |
| MAb CL1 | 25 | no | |
| | 6,3 | no | |
| | 1,6 | yes | + |
| | 0,8 | yes | + |
| MAb CL2 | 25 | no | |
| | 6,3 | yes | + |
| | 1,6 | yes | + |
| | 0,8 | ND | |
| MAb CL1 + CL2 (Mixture: 50/50) | 25 | no | |
| | 6,3 | no | |
| | 1,6 | no | |
| | 0,8 | 1) | - |
| | 0,4 | 2) | - |

ND, not done

1) neutralization resistant to MAb CL2; Mixture and MAb CL1 still neutralizing;

2) neutralization resistant to MAb CL1; Mixture and MAb CL2 still neutralizing.

Example 8 (Fig.6): Course of p24 production in cultures with serum samples from an HIV-1 infected individual before and after treatment with 3 doses of MAb CL1.

Serum was incubated with PHA-stimulated PBMC form healthy, HIV-negative blood donors. Culture supernatant was changed 1:2 twice weekly. Once per week fresh PHA-stimulated PBMC were added to the culture. The culture was monitored for 5 weeks.

Fig. 6 shows the increase in syncytia formation of cultured serum samples taken from the patient before the administration of MAb CL1 (triangles) and the impressive neutralization of the patient's HIV-1 infection upon administration of MAb CL1, as displayed by the horizontal line at the zero level of p24 production.

Example 9: In vivo prevention of HIV-1 infection in a chimpanzee experiment

4 chimpanzees have been selected for testing the in vivo neutralization in a co-laboration with the Merck-research center in West Point, Pennsylvania 19486, USA.

Prior to the in vivo test, CD4 positiv primary T cells (PBMC's) have been isolated from each chimpanzee to test the permissiveness of in vitro infection with the primary HIV-1 isolate, clade B. For all *in vitro* tests conventional procedures as described in M. Purtscher et.al., Aids Research and Human Retroviruses, Vol. 10, Nr. 12, 1994, Mary Ann Liebert, Inc., Publ., have been used. The CD4 PBMC of all four chimpanzees were permissive to viral propagation in vitro. This was to prove that an in vivo infection should be successful.

Two of the chimpanzees were infused with the monoclonal antibody CL1 (PHLS Deposit No. 90091704). For the intravenous infusion of the antibodies 300 ml of solution containing approx. 1 mg/per mililiter stabilized in 1% human serum albumine, at pH value 7 were applied per animal. Two other animals were only treated with human serum albumine.

All four chimpanzees were challenged one day after treatment with the primary HIV-1 isolate by intraveneous injection of 3 chimpanzee infective

doses of the virus. All four animals were routinely controlled for HIV-1 infection for a period of four months so far.

Result: The two chimpanzees who have been treated with antibody CL1 showed no signs of HIV-1 infection; they have been prevented from infection. Both control animals i.e. those treated only with human serum albumine became HIV-1 positive.

Example 10: In vivo prevention of HIV-1 infection in a SCID-mouse trial

Another experiment to prove the in-vivo neutralization of MAb CL1 has been carried out in collaboration with Transgene, Strasbourg, France.

The genes encoding the heavy and light chains of MAb CL1 have been supplied to Transgene to genetically manipulate mouse fibroblasts (3T3) using standard technologies of genetic engineering. The transformed mouse fibroblasts producing MAb CL1 were propagated in vitro on GOREDEX® fibres to form cell pellets. The cell pellets were then applied under the skin of SCID mice to form organelles within these mice in a way to release the MAb CL1 into the blood stream.

The SCID-mice were reconstituted using conventional procedures with the human white blood cell system in order to give an animal model suitable to be infected by HIV-1.

Those SCID-mice having a level of MAb CL1 higher than 2 micrograms of antibody were protected against a challenge with HIV-1 IIIB, whereas those having a lower level of antibody per ml in the serum showed a significant delay of infection. SCID-mice treated otherwise in an anologous way and having no MAb CL1 in their serum were all infected.

**Claims**

1. HIV-1 neutralizing human monoclonal antibody, characterized in that it binds to two different antigenic determinants of HIV-1.

2. Antibody according to claim 1, characterized in that the antigenic determinants are fragments of gp160 and correspond to amino acid sequences 79 to 184 and 326 to 400 of processed gp120 of HIV-1 isolate BH 10 (numbering according to the Swissprot database entry ENV$HIV10).

3. Antibody according to claim 1 or 2, characterized in that it does not bind to PN Gase F treated (=deglycosilated) form of said antigenic determinants.

4. Antibody according to any one of the preceding claims, characterized in that it specifically binds to a fragment of gp120 produced in the SF9 insect cell/Baculovirus expression system in the absence of tunicamycin, while it does not bind in the presence of tunicamycin.

5. Antibody according to any one of the preceding claims, characterized in that it inhibits the infection of human lymphocytes by primary HIV-1 isolates *in vitro*.

6. Antibody according to claim 5, characterized in that its capability of inhibiting the infection of human lymphocytes by primary HIV-1 isolates is specifically blocked by a monoclonal antibody produced by a cell line CL6 deposited at PHLS Porton Down, Salisbury, United Kingdom, under Accession No. 95032241.

7. Antibody according to any one of the preceding claims, characterized in that it is specifically bound by a monoclonal antibody produced by at least one of the cell lines CL5 (Accession No. 95032240) and CL6 (Accession No. 95032241; deposited at PHLS Porton Down, Salisbury, United Kingdom).

8. Antibody according to any one of the preceding claims, characterized in that it competes for binding to said antigenic determinants with an antibody produced by hybridoma cell line CL2 deposited at PHLS Porton Down, Salisbury, United Kingdom, under Accession No. 93091517.

9. Antibody according to any one of claims 1 to 7, characterized in that it is produced by hybridoma cell line CL2 deposited at PHLS Porton Down, Salisbury, United Kingdom, under Accession No. 93091517.

10. Antibody according to any one of claims 1 to 8, characterized in that it is produced by a recombinant CHO cell line CL3 deposited at PHLS Porton Down, Salisbury, United Kingdom, under Accession No. 95032235.

11. A monoclonal antibody, characterized in that it specifically binds to an antibody according to claim 6 or 7 and/or bars an antibody according to claim 6 from inhibiting the infection of human lymphocytes by primary HIV-1 isolates.

12. Antibody according to claim 11, characterized in that it induces - after administration to an animal or a human individual - HIV-1 neutralizing antibodies, which optionally compete with an antibody of any one of claims 1 to 10 for binding to said antigenic determinants of HIV-1.

13. Antibody according to claim 11 or 12, characterized in that it is produced by a hybridoma cell line CL 6 deposited under Accession No. 95032241 at PHLS Porton Down, Salisbury, United Kingdom.

14. Antibody according to claim 11, characterized in that it is produced by a hybridoma cell line CL 5 deposited under Accession No. 95032240 at PHLS Porton Down, Salisbury, United Kingdom.

15. An antibody induced upon active immunization of an animal or human individual with an antibody according to any one of claims 11 to 14, and optionally competing with any one of the HIV-1 neutralizing antibodies of claims 1 to 10 for binding to antigenic determinants of HIV-1.

16. An HIV-1 neutralizing human monoclonal antibody binding to gp41/gp160 of HIV-1, characterized in that it significantly reduces - upon intravenous administration to a human HIV-1 infected individual - the level of circulating HIV-1 in the blood serum and/or blood plasma of said individual.

17. An antibody according to claim 16, characterized in that it competes with an antibody produced by hybridoma cell line CL1 deposited at PHLS

Porton Down, Salisbury, United Kingdom, under Accession No. 90091704, for specific binding to gp41/gp160 of HIV-1.

18. An antibody according to claim 16, characterized in that it is produced by a hybridoma cell line CL1 deposited at PHLS Porton Down, Salisbury, United Kingdom, under Accession No. 90091704.

19. An antibody according to claim 16, characterized in that it competes with an antibody produced by a recombinant CHO cell line CL 4 deposited under Accession No. 95032236 at PHLS Porton Down, Salisbury, United Kingdom.

20. An antibody according to any one of claims 16 to 19, characterized in that it prevents an HIV-1 challenged individual from becoming HIV-1 infected in vivo, as evidenced in a SCID mouse and a chimpanzee animal test model.

21. A mixture of HIV-1 neutralizing, human monoclonal antibodies, characterized in that it comprises at least one of the antibodies of claims 1 to 10 and at least one antibody according to claims 15 to 20.

22. A mixture according to claim 21, characterized in that it prevents *in vitro* an escape of HIV-1 mutants usually developing upon individual application of any one of said antibodies of the mixture.

23. A monoclonal antibody producing cell line, characterized in that it produces a monoclonal antibody according to any one of claims 1 to 20.

24. A cell line according to claim 23, characterized in that it is selected from the group consisting of cell line CL1 (Accession No. 90091704), CL2 (Accession No. 93091517), CL3 (Accession No. 95032235), CL4 (Accession No. 95032236), CL5 (Accession No. 95032240), CL6 (Accession No. 95032241), all deposited at PHLS Porton Down, Salisbury, United Kingdom, and progeny of said hybridomas.

25. A peptide fragment containing at least one of the antigenic determinants according to claim 1 or 2.

26. Peptide fragment according to claim 25, characterized in that it induces an immune response against HIV-1, optionally the release of HIV-1 neutralizing antibodies, after administration to an animal or human individual.

27. Peptide fragment according to claim 25 or 26, characterized in that it is linked to a suitable carrier to form a fusion protein.

28. Peptide fragment according to claim 27, characterized in that the carrier is a virus or part of a virus, optionally the hemagglutinin of influenza virus or the surface antigen of hepatitis B virus.

29. A vaccine for the prevention and/or therapeutic treatment of HIV-1 infection, characterized in that it comprises at least one antibody according to any one of claims 1 to 20 and/or at least one peptide fragment according to any one of claims 25 to 28, and optionally at least one suitable carrier and/or further additives.

30. Vaccine according to claim 29, characterized in that it contains the antibodies and/or peptide fragments in an effective dose, preferably in a dose appropriate for administration of 0.5 to 10 µg/kg of body weight.

31. Use of at least one monoclonal antibody according to any one of claims 1 to 20, for the manufacture of a vaccine for the prevention and/or treatment of HIV-1 infections in vivo.

32. Use according to claim 31, wherein the at least one antibody is applied in a mixture with a conventional immunoserum and/or an antibiotic.

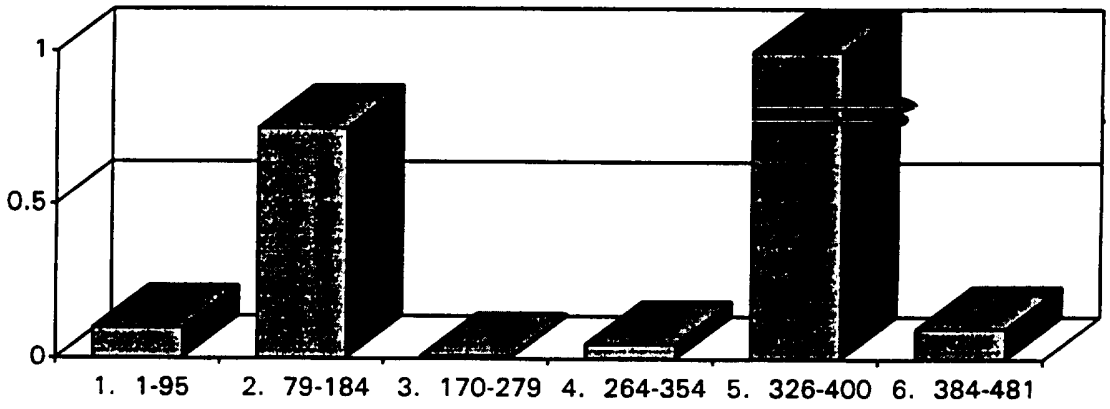
33. Use according to claim 31 or 32, wherein at least two different antibodies are applied.

34. Use of at least one antibody according to any one of claims 1 to 10 and 15 to 20, for the detection and/or determination of HIV-1 infections and/or infected cells in samples of human blood and/or serum.

35. Use of at least one antibody according to any one of claims 11 to 14 and/or at least one antigenic peptide fragment according to claims 25 to 28, for the detection and/or determination of anti-HIV-1 antibodies.

36. Use of at least one peptide fragment according to any one of claims 25 to 28 for the manufacture of a vaccine for active immunization of human individuals against HIV-1 infection.

Fig. 1




GST/HIV-gp 120 fragments

FIG. 2

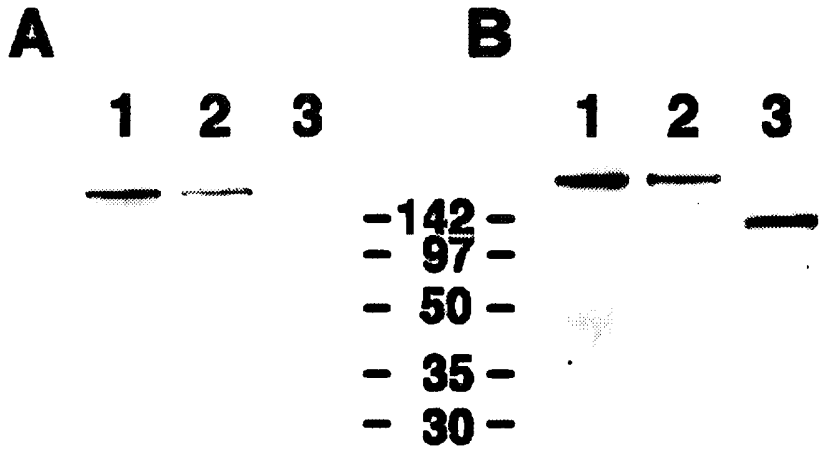


Fig. 3

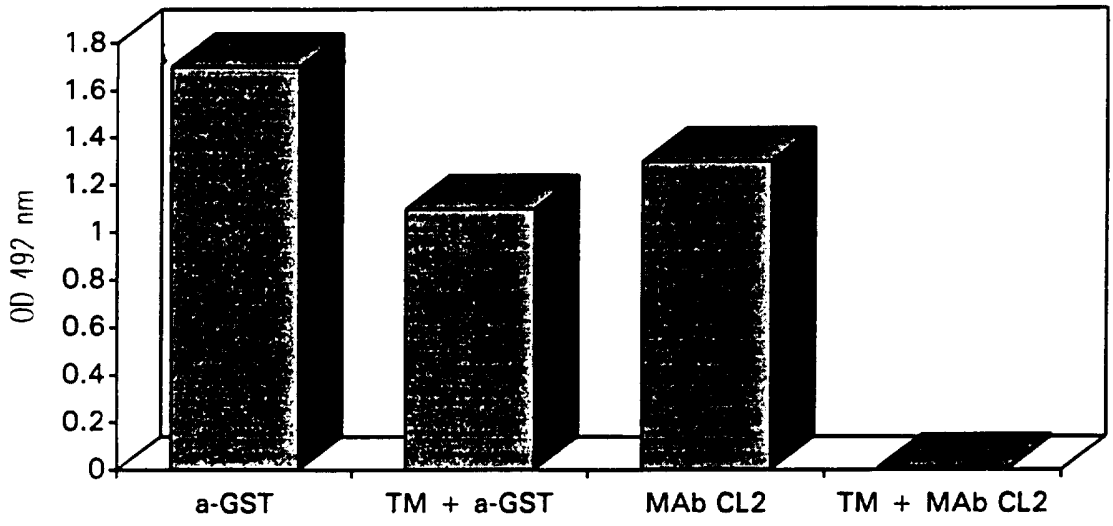
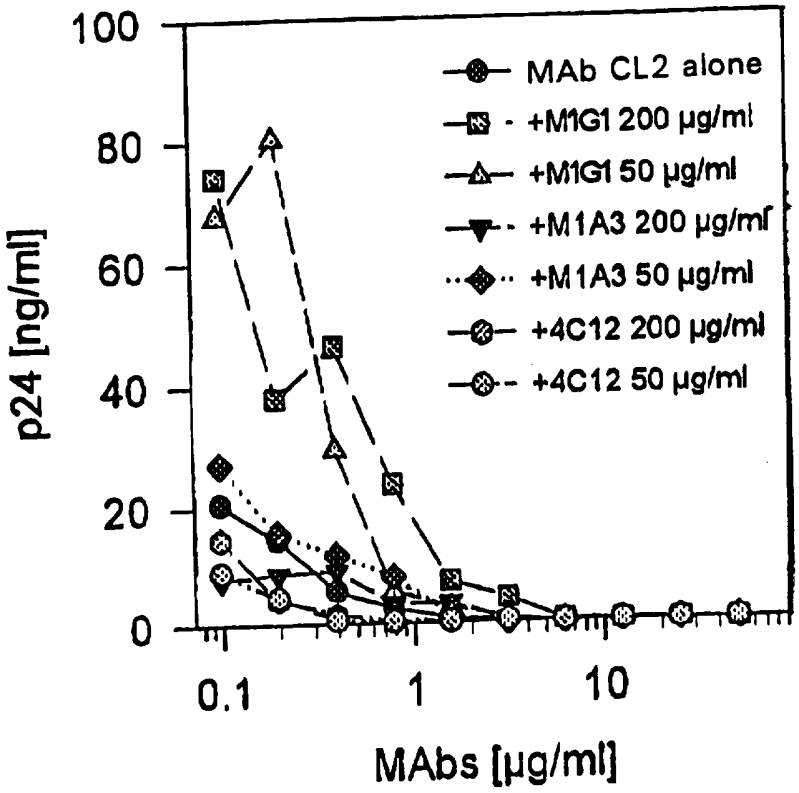

OD 492 nm
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a-GST
TM + a-GST
MAb CL2
TM + MAb CL2
rec. GST/HIVgp120

Fig. 4

A


100
80
60
40
20
0
p24 [ng/ml]
MAb CL2 alone
+M1G1 200 µg/ml
+M1G1 50 µg/ml
+M1A3 200 µg/ml
+M1A3 50 µg/ml
+4C12 200 µg/ml
+4C12 50 µg/ml
0.1
1
10
MAbs [µg/ml]

B

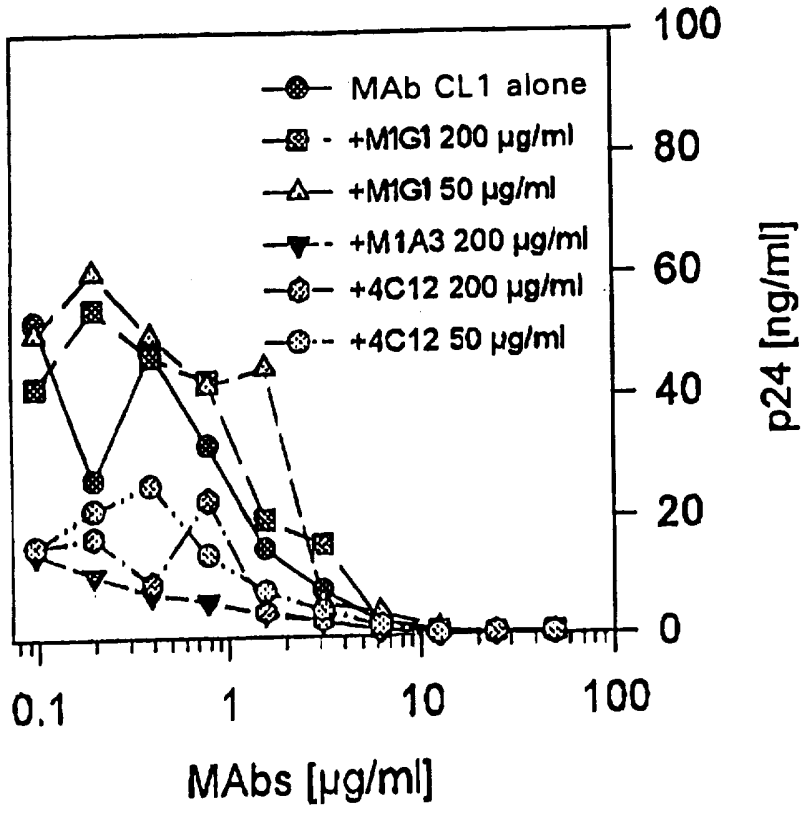
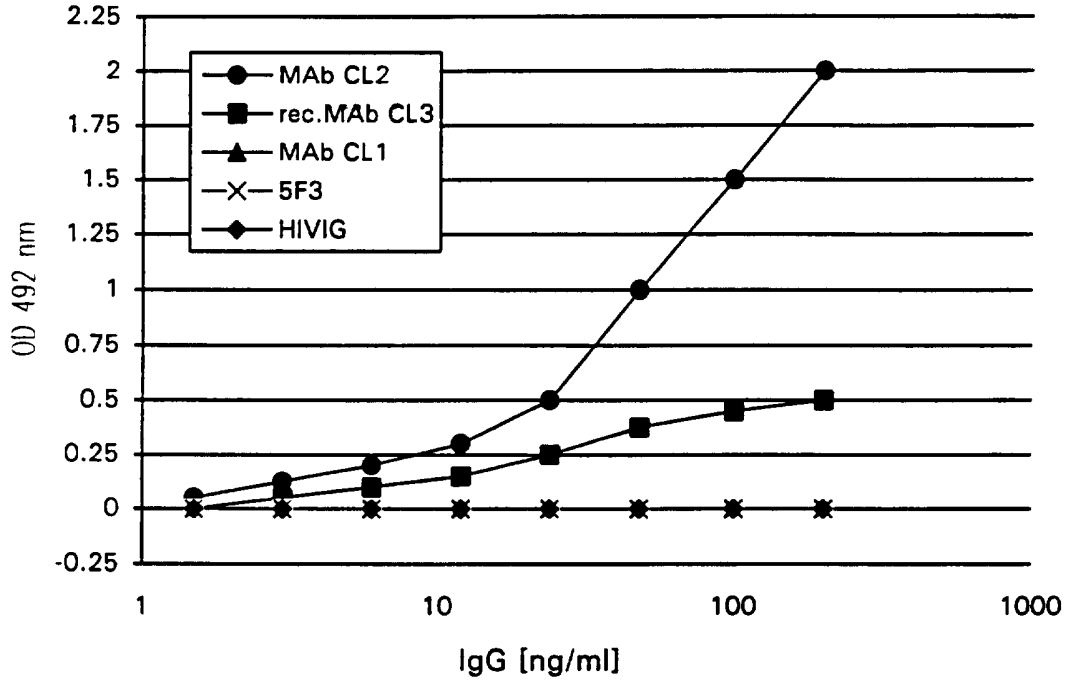

100
80
60
40
20
0
p24 [ng/ml]
MAb CL1 alone
+M1G1 200 µg/ml
+M1G1 50 µg/ml
+M1A3 200 µg/ml
+4C12 200 µg/ml
+4C12 50 µg/ml
0.1
1
10
100
MAbs [µg/ml]

Fig. 5

A


2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
-0.25
OD 492 nm
MAb CL2
rec.MAb CL3
MAb CL1
5F3
HIVIG
1
10
100
1000
IgG [ng/ml]

B

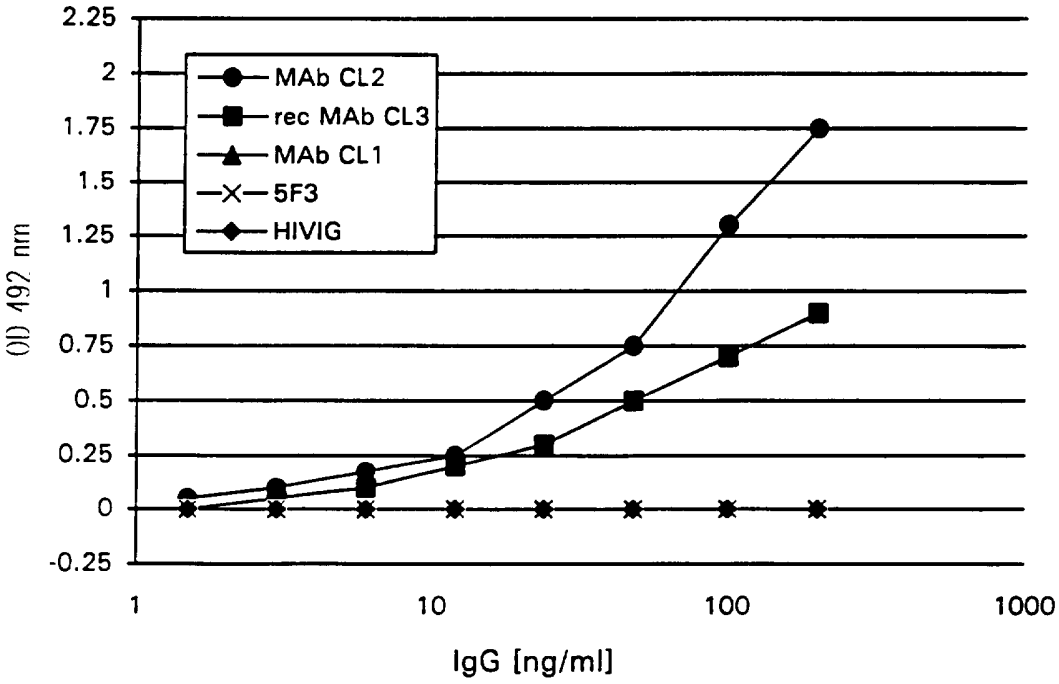
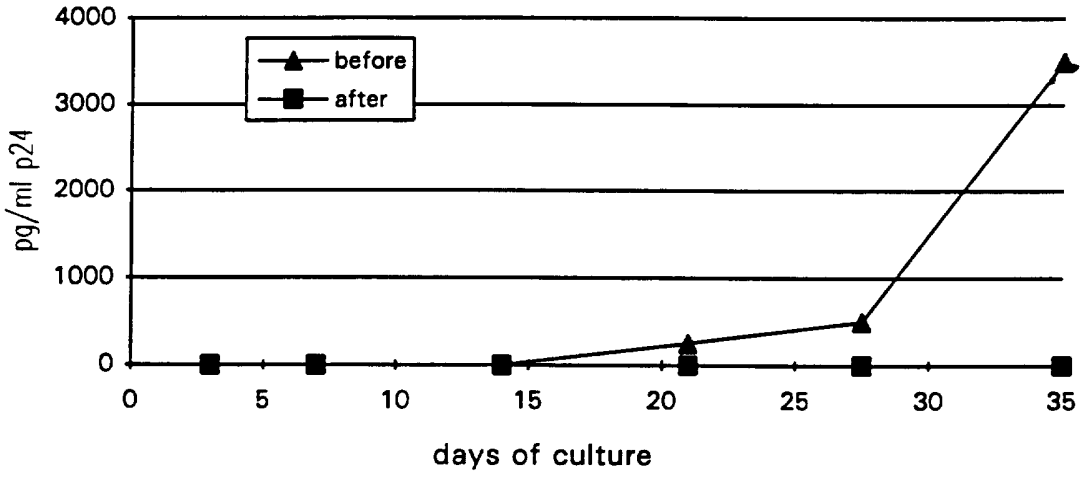

2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
-0.25
OD 492 nm
MAb CL2
rec MAb CL3
MAb CL1
5F3
HIVIG
1
10
100
1000
IgG [ng/ml]

**Fig. 6**


4000
3000
2000
1000
0
pg/ml p24
before
after
0
5
10
15
20
25
30
35
days of culture

# INTERNATIONAL SEARCH REPORT

Interr al Application No
PCT/EP 95/01481

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K16/10 C07K16/42 C12N5/24 C07K14/16 C07K19/00
A61K39/44 A61K39/21 G01N33/577 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | JOURNAL OF VIROLOGY, vol. 67, no. 11, November 1993 WASHINGTON, DC, USA, pages 6642-6647, T. MUSTER ET AL. 'A conserved neutralizing epitope on gp41 of human immunodeficiency virus type 1.' cited in the application see abstract --- -/-- | 16-19, 23,24,34 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"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 when the document is taken alone

"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

1

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 24 November 1995 | 29.12.95 |
| Name and mailing address of the ISA<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax (+ 31-70) 340-3016 | Authorized officer<br>Nooij, F |

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# INTERNATIONAL SEARCH REPORT

Intern. al Application No
PCT/EP 95/01481

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | AIDS RESEARCH AND HUMAN RETROVIRUSES, vol. 10, no. 12, December 1994 NEW YORK, NY, USA, pages 1651-1658, M. PURTSCHER ET AL. 'A broadly neutralizing human monoclonal antibody against gp41 of human immunodeficiency virus type 1.' cited in the application see abstract --- | 16-19, 23,24,34 |
| X | JOURNAL OF VIROLOGY, vol. 65, no. 1, January 1991 WASHINGTON, DC, USA, pages 489-493, D. HO ET AL. 'Conformational epitope on gp120 important in CD4 binding and human immunodeficiency virus type 1 neutralization identified by a human monoclonal antibody.' see the whole document --- | 1-3,5-8, 15, 23-26, 29-31, 34-36 |
| X | ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, vol. 646, 27 December 1991 NEW YORK, NY, USA, pages 212-219, F. RÜKER ET AL. 'Expression of a human monoclonal anti-HIV-1 antibody in CHO cells.' see the whole document --- | 16-19, 23,24,34 |
| X | MOLECULAR IMMUNOLOGY, vol. 31, no. 15, October 1994 OXFORD, GB, pages 1149-1160, J. BAGLEY ET AL. 'Structural characterization of broadly neutralizing human monoclonal antibodies against the CD4 binding site of HIV gp120.' see abstract --- | 1,2,5-8, 15,23,24 |
| X | JOURNAL OF VIROLOGY, vol. 69, no. 1, January 1995 WASHINGTON, DC, USA, pages 122-130, J. MOORE ET AL. 'A human monoclonal antibody to a complex epitope in the V3 region of gp120 of human immunodeficiency virus type 1 has broad reactivity within and outside clade B.' see the whole document --- -/-- | 1,2,5-8, 15,23, 24,34 |

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Inter al Application No
PCT/EP 95/01481

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO,A,89 04370 (CL-PHARMA AG) 18 May 1989<br>see examples<br>see claims | 16-19, 23,24,34 |
| A | A. BUCHACHER ET AL., IN 'Vaccines 92' 1992 , COLD SPRING HARBOR LABORATORY PRESS , COLD SPRING HARBOR, USA<br>see page 191 - page 195 | 16-19, 23,24 |
| A | EP,A,0 570 357 (H. KATINGER) 18 November 1993<br>cited in the application<br>see the whole document | 16-19,34 |
| A | WO,A,95 07354 (POLYMUN SCIENTIFIC IMMUNOBIOLOGISCHE FORSCHUNG GMBH) 16 March 1995<br>see examples<br>see claims | 16-19,34 |
| A | EP,A,0 503 916 (IDEC PHARMACEUTICALS CORPORATION) 16 September 1992<br>see examples<br>see claims | 11-15 |

1

# INTERNATIONAL SEARCH REPORT

Ir ational application No.
PCT/EP 95/01481

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 35
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although this claim (partially, as far as an in vivo method is concerned) is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. claims 1-15 and partially 21-24 and 29-35
2. claims 16-20 and partially 21-24 and 29-34
3. claims 25-28,36 and partially 29,30,35

See additional sheet PCT/ISA/210

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [X] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. HIV-1-neutralizing human monoclonal antibodies binding to two different antigenic determinants of HIV-1, and human monoclonal antibodies binding to said HIV-1-neutralizing monoclonal antibodies. Cell lines producing them. Use of said monoclonal antibodies in a vaccine and for detection purposes.

2. HIV-1-neutralizing human monoclonal antibodies binding to gp41/gp160 of HIV-1. Cell lines producing them. Use of said monoclonal antibodies in a vaccine and for detection purposes.

3. Peptide fragment containing at least one of the two different antigenic determinants of HIV-1. Use of said peptide fragment, optionally in a fusion protein, for active immunization (as a vaccine.

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter ıal Application No
PCT/EP 95/01481

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