Peptides are described which elicit antibodies, preferably including secretory antibodies secreted by mucosal surfaces, that show neutralizing activity against different strains and clinical isolates of HIV-1 and that inhibit the fusion of cells caused by HIV-1, which peptides are composed of a sequence of seven or eight amino acids corresponding to the ones on gp160 of the HIV isolate BH10 at the position 662 to 668 (ELDKKWS) or at the position 661 to 668 (ELDKKWS) respectively, preferably including peptides being composed of the sequence ELDNWAS, ELNKWAS, LELDNWAS or LELNKWAS, or that are genetically encoded by nucleotide sequences corresponding to said amino acid sequences, or by sequences hybridizing to said nucleotide sequences, or by sequences deduced from said nucleotide sequences by degeneration. Preferably, the peptides substitute one or more parts of the amino acid sequence of a viral protein or are inserted into antigenic sites of a viral protein, e.g. by fusion of the respective nucleotide sequences, and subsequent expression of the fusion genes is carried out in a biological expression system.
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Peptides that elicit neutralizing antibodies against genetically divergent HIV-1 strains

The present invention relates to peptides which elicit neutralizing antibodies against different strains and clinical isolates of HIV-1, as well as to the antibodies elicited therewith, and also to the preparation of peptide-carrier combinations that efficiently present these peptides to the immune system. More particularly, this invention relates to the development of a vaccine against HIV-1.

The acquired immunodeficiency syndrome (AIDS) is the late stage clinical manifestation of a long term persistent infection with human immunodeficiency virus type 1 (HIV-1). Immune responses directed at the virus and at virus-infected cells during the persistent infection usually fail to mediate the resolution of the infection. Vaccines may elicit immune responses that can prevent the establishment of a persistent infection or even prevent the progression to AIDS. Most vaccine strategies against HIV-1 are directed at its surface glycoprotein gp160 which is made up of gp120 and gp41 and is responsible for binding the virus to the cellular receptor CD4 and for triggering subsequent fusion activity.

However, in context with gp160, several phenomena were observed that argue against the use of whole gp160 or gp120 as an immunogen. In vitro experiments show that synergism between gp120 and gp120-specific antibodies block human T-cell activation (Mittler et al., Science (1989)245:1380). In addition, a number of antigenic domains on gp160 are known to induce antibodies that enhance HIV-1 infection (Jiang et al., J. Exp. Med. (1991) 174:1557).

The use of synthetic peptides as immunogens offers a number of advantages. The antibodies elicited by synthetic peptides have a predetermined specificity, and in the case of viruses, they can be selected to represent structures on the surface.
of virions. The synthetic polypeptides are interesting also in that they can induce antibody responses not seen under normal conditions. For example, it is possible to induce neutralizing antibodies that have a broader reactivity than antibodies induced by whole proteins (Green et al., Cell (1982) 28:477).

In addition, a peptide containing part of the V3 loop of gp120 from the HIV-1 isolate HIV-1 IIIb was shown to induce antibodies that protected chimpanzees against virus challenge with the same HIV-1 isolate (Emini et al., Nature (1992) 355:728).

Because synthetic peptides themselves have poor immunogenicity, they have to be coupled to molecules that provide an adjuvant effect such as tetanus toxoid or keyhole limpet hemocyanin (Bittle et al., Nature (1982) 298:30). Another possibility is to clone small peptides as fusion proteins with glutathione S-transferase of Schistosoma japonicum (Fikrig et al., Science (1990) 250:553).

Furthermore, viruses such as vaccinia, polio or influenza can be used as vectors for immunogens. Rabbits inoculated with recombinant vaccinia virus containing sequences from hepatitis B surface antigen (HBsAg), herpes simplex virus glycoprotein D, and influenza virus hemagglutinin produced antibodies to all three foreign antigens (Perkus et al., Science (1985) 229:981). Furthermore, a chimeric polio virus that expressed an epitope from gp41 of HIV-1 successfully induced neutralizing antibodies against HIV-1 in rabbits (Evans et al., Nature (1989) 339:385).

Since recently, it is also possible to change the genome of influenza virus by in vitro mutagenesis (Enami et al., Proc. Natl. Acad. Sci. USA (1990) 87:3802). By means of this technique it was possible to engineer a stable attenuated influenza A virus (Muster et al., Proc. Natl Acad. Sci. USA (1991) 88:5177).
An advantage of influenza virus in this context is the availability of many variants so that repeated vaccination may be possible. Furthermore, influenza virus induces strong secretory and cellular immune responses, which may be advantageous for an anti-HIV-1 vaccine approach.

It is the object of the instant invention to provide peptide sequences, that are only minimally immunogenic in the context of the whole gp160, and which peptide sequences can be used to elicit antibodies that show neutralizing activity against different strains and/or clinical isolates of HIV-1 and/or that inhibit the fusion of cells caused by HIV-1 in mammals.

It is a further object of the invention to provide strategies to elicit secretory antibodies secreted from mucosal surfaces and directed at HIV-1 virus and virus infected cells. It was presumed according to the invention that eliciting anti-HIV-1 IgA antibodies in mucosal tissues will provide a powerful tool especially in the prophylaxis and prevention of potentially endangered individuals from HIV-1 infection, since many viral infections including HIV-1 are transmitted via mucosal surfaces of the respiratory, gastrointestinal and genital tract.

The objects are achieved by preparing small peptides with seven or eight amino acids obtained either by chemical synthesis or by microbiological methods, the peptides preferably being derived from nucleic acid sequences coding for Glu Leu Asp Lys Trp Ala Ser (= ELDKWAS in the one-letter-code), Leu Glu Leu Asp Lys Trp Ala Ser (LELDKWAS), Glu Leu Asn Lys Trp Ala Ser (ELNKWAS), Leu Glu Leu Asn Lys Trp Ala Ser (LELNKWAS), Glu Leu Asp Asn Trp Ala Ser (ELDNWAS) or Leu Glu Leu Asp Asn Trp Ala Ser (LELDNWAS).

At least one, preferably all, of the above six amino acid sequences (hereinafter referred to as "said six AAS") may then efficiently be presented to the immune system in order to induce formation and release of antibodies that show
neutralizing activity against HIV-1 strains and/or which can inhibit cell fusion caused by these viruses.

It is a major goal of the instant invention to provide a promising anti-HIV-1 vaccine based on a formulation comprising at least one, preferably a mixture of all six, of the above peptides. Special features and advantageous embodiments of the invention are described below.

In a preferred embodiment of the invention, the amino acid sequence Glu Leu Asp Lys Trp Ala (ELDKWA) defining the epitope sequence of the human monoclonal antibody 2F5 (obtained from hybridoma cell line 2F5, PHLS deposit Nr.90091704; deposited 09/17/90) is connected with a Ser or both a Leu and Ser - which are located adjacent to the ELDKWA Sequence on gp41 defining the 2F5-epitope - thereby establishing the peptide sequences Glu Leu Asp Lys Trp Ala Ser (ELDKWAS) and Leu Glu Leu Asp Lys Trp Ala Ser (LELDKWAS) respectively, and inserted into a carrier molecule, namely the antigenic site B of the HA of influenza virus.

The modified "chimeric" HA fusion protein, carrying the foreign amino acid sequence ELDKWAS or LELDKWAS, very efficiently elicits antibodies directed at the presented ELDKWAS or LELDKWAS sequence upon application, preferably in the form of an injection, to the immune system of mammals, and humans in particular. The antibodies obtained by this method show neutralizing activity against different strains and/or clinical isolates of HIV-1.

It has, however, turned out that due to the known polymorphism of the HIV-1 virus mutations can occur even within the highly conserved (L)ELDKWAS amino acid sequence of gp41, which corresponds to amino acid 661-668 (LELDKWAS) or 662-668 (ELDKWAS) of HIV-1 isolate BH10. The nucleotide and amino acid numbering used throughout this specification corresponds to that of gp160 of HIV-1 isolate BH10 as used in the Los Alamos data base (Myers et al., Data Base Human
Retrovirus and Aids). The mutations particularly affected the Asp (D) and/or Lys (K) amino acids in the centre of said sequence, thereby reducing the virus' susceptibility to getting neutralized by antibodies carrying the 2F5 epitope. This undesired kind of viral escape mechanism could, however, successfully be overcome by using those peptides according to the instant invention, wherein either Asp (D) or adjacent Lys (K) is replaced with Asparagine (N).

At least one, preferably a mixture of all six, of said six peptides may be used for the manufacture of a pharmaceutical to elicit antibodies that show neutralizing activity against different strains and/or clinical isolates of HIV-1 and/or that inhibit the fusion of cells caused by HIV-1.

In another embodiment, the same peptides may be used either each peptide alone or in a mixture with at least one other of said peptides, to manufacture a pharmaceutical to induce anti-HIV-1 IgA antibodies secreted from mucosal surfaces upon, preferably intranasal, application to mammals.

In a further embodiment of the invention, at least one of said six peptides is linked to a carrier. Such carrier can, for instance, be a virus, preferably in its attenuated and/or recombinant form. Advantageously, influenza virus, baculovirus or vaccinia virus may be used. Linking the small peptides to a carrier such as, for instance, a viral protein or a complete virus enhances the efficacy of antibody induction upon presentation of such chimeric peptide-carrier combinations or chimeric fusion proteins to the immune system.

It is particularly advantageous, to use each of the above peptides as a fusion protein with a viral protein as the carrier, wherein at least one of the amino acid sequences according to the invention substitutes at least one part of the viral carrier protein or is inserted into at least one of the antigenic sites of the viral protein. This feature,
therefore, represents an important embodiment. In a preferred
embodiment, said viral protein is the hemagglutinin (HA) of
influenza virus, the neuraminidase (NA) of influenza virus or
the surface antigen of hepatitis B virus. In another
embodiment, it may be derived from a - preferably recombinant
- baculovirus or vaccinia virus.

The instant invention also relates to the use of any of the
above identified peptides consisting of seven or eight amino
acids and/or peptide-carrier combinations or fusion proteins
for the manufacture of a pharmaceutical to elicit or induce
antibodies that show neutralizing activity against different
strains and/or clinical isolates of HIV-1 and/or that inhibit
the fusion of cells caused by HIV-1. More particularly, it is
intended to provide an effective vaccine based on at least
one, preferably a mixture of all six, of said peptides and/or
said six peptides linked to a carrier, for the prophylactic
treatment of HIV-1 endangered individuals and/or the
therapeutic treatment of HIV-1 infected patients, especially
to prevent the progression of the infection to AIDS.

In a further preferred embodiment, said six peptides and/or
said six peptides linked to a carrier are used to prepare and
manufacture pharmaceutical formulations that - upon
application to the mammalian immune system - lead to the
induction of anti-HIV-1 IgA antibodies secreted from mucosal
surfaces of the animal or human patients. Intranasal
application is preferred in this case.

Consequently, said six peptides and/or peptide-carrier
combinations of the instant invention may also be used for
the manufacture of a pharmaceutical for the prophylaxis and
prevention of endangered individuals from HIV-1 infection.

It appears, that this special feature of the instant
invention may give rise to the hope that by way of eliciting
anti-HIV-1 specific secretory IgA antibodies in mucosal
surfaces according to the instant invention a medical tool is
provided that effectively attacks the viral invaders already at the very first stage of their entrance to the mammalian body. It is believed that the prior art methods to treat HIV-1 infected patients at least partly fail to defend the disease because the virus is chased primarily in the bloodstream and at a rather late stage, i.e. after widespread distribution in the humoral system.

The instant invention further relates to an antibody showing HIV-1 neutralizing activity and being capable of preventing fusion of cells caused by HIV-1, characterized in that it is elicited by one of the said six peptides.

In a preferred embodiment, the said antibody is a secretory antibody, preferably an anti-HIV-1 IgA antibody, and advantageously being secreted by mucosal surfaces.

Furthermore, the instant invention also refers to a process for the manufacture of anyone of said six peptides by either microbiological or chemical methods.

In one embodiment said six peptides are chemically synthesized following standard biochemical procedures, preferably using an Applied Biosystems 431A Peptide Synthesizer.

In another embodiment, said amino acid sequences are obtained by a microbiological process comprising the steps of inserting a nucleotide sequence selected from the group consisting of

a) a nucleotide sequence corresponding to one of said six peptides;

b) a nucleotide sequence hybridizing to one of the nucleotide sequences under a);

c) a nucleotide sequence deduced from one of the nucleotide sequences under a) by degeneration;
into the genome of a host organism, carrying out expression of the genome using standard microbiological cultivation techniques, and isolating at least one, preferably a multitude, of said peptides.

Chimeric fusion proteins or peptide-carrier combinations may be manufactured according to an embodiment comprising linking an amino acid sequence selected from the group consisting of said six peptides, to a carrier, preferably a virus or viral protein, by chemical or microbiological methods.

In a preferred embodiment, the process for the manufacture of the said six peptides linked to a carrier, preferably a virus or viral protein, is microbiological and comprises the steps of linking a nucleotide sequence selected from the group consisting of

a) a nucleotide sequence corresponding to one of the amino acid sequences of the said six peptides;

b) a nucleotide sequence hybridizing to one of the nucleotide sequences under a);

c) a nucleotide sequence deduced from one of the nucleotide sequences under a) by degeneration;

with a nucleotide sequence corresponding to an amino acid sequence of the carrier, transferring the linked nucleotide sequences to a host organism, carrying out expression of the linked sequence using standard microbiological techniques, and isolating at least one, preferably a multitude, of said peptides linked to a carrier.

In a further embodiment, the process for the manufacture of said six peptides linked to a carrier, preferably a virus or viral protein, is characterized in that at least one of the nucleotide sequences selected from the group consisting of

a) a nucleotide sequence corresponding to one of the amino acid sequences of said six peptides;
b) a nucleotide sequence hybridizing to one of the nucleotide sequences under a); 

c) a nucleotide sequence deduced from one of the nucleotide sequences under a) by degeneration;

is linked with a nucleotide sequence corresponding to an amino acid sequence of a viral protein as the carrier, thereby substituting at least part of the nucleotide sequence corresponding to the amino acid sequence of the viral protein or being inserted into at least one site of said sequence corresponding to an antigenic site of the viral protein.

In a specific embodiment, the applied viral protein is selected from the group consisting of the hemagglutinin of influenza virus, neuraminidase of influenza virus and the surface antigen of hepatitis B virus, and in another characteristic embodiment, an influenza virus, baculovirus or vaccinia virus - each of them preferably in a recombinant form - is used as the host organism.

Insertion of the sequences of said six peptides into the antigenic site B of the HA of influenza strains other than WSN, which strain is mainly referred to in the following examples, will equally lead to fusion proteins and/or chimeric viruses that elicit neutralizing anti-HIV-1 antibodies and prevent HIV-1-driven cell fusion.

Also, introduction of these sequences into other sites of the HA of influenza virus or into the neuraminidase (NA) of influenza virus leads to peptide-carrier fusion proteins and/or chimeric viruses that elicit neutralizing anti-HIV antibodies and prevent HIV-driven cell fusion.

The present invention will be further explained and demonstrated in the following examples, which do not limit in any respect the scope of the present invention.
Example 1: Epitope mapping

The epitope of the monoclonal antibody 2F5 was identified by immunoblotting using a method described by Towbin et al., Proc. Natl. Acad. Sci. USA (1979) 76:4350. Peptides of overlapping fragments of gp41 of HIV-1 isolate BH10 were cloned as fusion peptides with glutathiontransferase. The different fusion peptides were obtained through hybridization of gp41 corresponding oligonucleotides which were cloned between the Bam HI and the Eco RI site of the plasmid pGEX-2T (Pharma-
cia). The recombinant plasmids were transformed into the E. coli strain DH5 and expression of the fusion proteins was induced with isopropylthiogalactoside (IPTG). The E. coli extract was then purified with glutathion-sepharose 4B columns, loaded on sodium dodecylsulfate-polyacrylamide gels, separated by electrophoresis, and protein expression was analyzed by silver staining and immunoblotting (Fig.1). The data from the immunoblotting show that the epitope of human monoclonal antibody 2F5 comprises the amino acid sequence ELDKWA corresponding to amino acids 662-667 of gp160.

Fig. 1 illustrates the specificity of human monoclonal anti-
body 2F5 (see Example 1).

Fig. 2 illustrates the construction of chimeric hemagglu-
tinins that carry the 2F5-epitope sequence (see Example 2)

Fig. 3 illustrates ELISA titers of antisera of mice immunized
with the chimeric influenza viruses (see Example 3)

Fig. 4 illustrates ELISA titers of mice immunized with cells
infected with the recombinant baculoviruses (see Example 5).

Fig. 1 shows immuno blots of fusion peptides with overlapping
fragments of gp160 of HIV-1 (isolate BH10). In contrast to
constructs that comprise amino acids 597 to 677 (lane 2), 634
to 677 (lane 3) and 648 to 677 (lane 4) which were reactive
with the antibody 2F5, a fusion peptide comprising amino
acids 667 to 677 (lane 5) did not show a positive reaction.
This was the first indication that the epitope of the monoclonal antibody 2F5 is formed by amino acids within the sequence from position 648 to 667 of gp160. Based on these results, overlapping 6-mer peptides of this region were fused with the glutathion S-transferase.

As shown in Fig.1b, the peptide containing the aminoacid sequence GLU LEU ASP LYS TRP ALA (amino acids 662-667, lane 2) was highly reactive with the antibody 2F5 whereas for peptides containing the aminoacid sequence LEU ASP LYS TRP ALA SER (LDKWAS, amino acids 663-668, lane 3) or ASP LYS TRP ALA SER LEU (DKWASL, amino acids 664-669, lane 4) reactivity with the monoclonal antibody was reduced. A peptide containing aminoacid sequence LEU GLU LEU ASP LYS TRP (LELDKW, amino acids 661-666, lane 1) showed no significant reactivity. These data show that the epitope of the monoclonal antibody comprises the amino acid sequence GLU LEU ASP LYS TRP ALA (ELDKW) that corresponds to amino acids 662-667 on gp160 of the HIV-1 BH10 isolate. In this context, both a synthetic peptide corresponding to this epitope sequence and a fusion protein containing this sequence were able to inhibit neutralization mediated by the 2F5 antibody.

Example 2: Construction of plasmids and the chimeric influenza viruses

All genetic manipulations used were done according to standard procedures described by Sambrook et al., (1989) in "Molecular Cloning", A Laboratory Manual, Cold Spring Harbour Laboratory Press, New York. As illustrated in Fig.2, the gp41-sequences Leu Glu Leu Asp Lys Trp Ala Ser (LELDKWAS) or Glu Leu Asp Lys Trp Ala Ser (ELDKWAS) were inserted into the antigenic site B of the HA of influenza WSN virus. The plasmids pHA-ELDKWAS and pHA-LELDKWAS were constructed by replacing the Pst I-Hind III fragment of plasmid pT3/WSN-HAm1 which is described by Li et al. (Proc. Natl. Acad. Sci. USA 90, 5214-5218; 1993) with a PCR product which was obtained by using pT3/WSN-HAm1 as a template and sense and antisense
primers derived from influenza virus WSN HAm1, whereby the
antisense primer further contained a 21 or 24 nucleotide
insertion corresponding to gp160 positions 1981 or 1984 to
2004. The plasmids pHA-ELNKWAS, pHA-LELNKWAS, pHA-ELDNWAS and
pHA-LELDNWAS were prepared in the same way.

The sequence of the WSN HA is provided by Hiti et al.
(J.Virol.41,730-734,1982) and the sequence of gp160 by the
Swissprot database entry ENV$HIV10. Transfection of RNA de-
derived from this plasmid into MDBK cells and selection and
preparation of chimeric viruses was done according to Enami
et al. (Proc.Natl.Acad. Sci. 87,3802-3805,1990) with modi-
fications described by Enami and Palese (J.Virol.65, 2711-

Example 3: Immunization and antibody response of mice
immunized with the chimeric influenza viruses

OF-1 mice were immunized with the chimeric influenza-ELDKWAS
virus (mice M1, M2, M3, M4) or influenza-L ELDKWAS virus
(mouse M5). Mice were first immunized with 10² TCID₅₀
intranasally (i.n.) followed by an i.n. booster immunization
with 5x10⁵ TCID₅₀ after 6 weeks, an intraperitoneal (i.p.)
immunization with 20µg sucrose-purified live virus after 3
weeks and a final boost with 20µg of SDS-denatured virus in
incomplete Freunds adjuvant after 3 more weeks intervals. For
intranasal immunization, mice were under ether anesthesia.
For the wild-type WSN control virus (wt 1, wt 2), the same
protocol was used. Mice were bled 12 days after the final
boost, antisera were inactivated 1h at 56°C and ELISA titers
and neutralizing activity of antisera was determined.

Fig.3A shows the binding of influenza-ELDKWAS antisera to a
glutathion S-transferase (GST) fusion peptide containing the
sequence ELDKWA on its C-terminus. The sequence was deter-
mined using ELISA. 96-well microtitre plates were coated with
the GST-ELDKWA fusion peptide at 4µg/ml (100µl/well) in car-
bonate buffer, pH 9.6 for 4h at room temperature. Plates were
then washed with PBS/0.05% Tween. Antiserum diluted in PBS/
1% BSA/0.05% Tween was added and incubated for 1h at RT.
After washing, antibodies were detected by incubation with a
goat anti-mouse IgG γ-chain antibody, conjugated with horse
radish peroxidase. The plates were stained using o-phenylene-
diamine-dihydrochloride as a substrate. The reaction was
stopped with 2.5 M H2SO4 and the plates were measured
(measure wavelength 492 nm, reference wavelength 620 nm).

Fig 3B: The same procedure as described in Fig.3A was fol-
lowed except that for detection of antibodies a goat anti-
mouse IgA antibody, conjugated with horse-radish peroxidase
was used. The neutralizing activity of the antisera was
determined by syncytia inhibition assays. The reciprocal
serum dilutions that inhibit syncytia formation by 50% (EC50)
are shown in Table 1. Antisera from mice M1 – M3 neutralized
the entire test panel at various serum dilutions. Antisera M4
and M5 neutralized HIV-1 isolates MN and RF but not IIIB. The
antisera induced by the WSN wild-type virus did not neutral-
ize any of the HIV-1 isolates tested at the lowest serum
dilution (1:20).

Table 1
NEUTRALIZATION OF HIV-1 BY SERA RAISED AGAINST CHIMERIC
INFLUENZA VIRUS

Neutralization titer (EC50)

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<td>95</td>
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<tr>
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<td>not tested</td>
</tr>
<tr>
<td>M5</td>
<td>20</td>
<td>34</td>
<td>24</td>
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For syncytia inhibition assay the indicator cell line AA-2
which is described by Chaffee et al., J. Exp. Med (1988)
168:605, and as virus inoculum frozen stocks of HIV-1 strains
MN, RF and IIIB were used. All virus stocks were diluted to
101.9-102.5 TCID50 per ml. Mouse-antisera were diluted 2-fold in medium and distributed in 96-well microtiter plates (4 replicas of each dilution). 50 µl of virus was added to 50 µl of diluted antisera and the virus/antibody mixture was preincubated for 2h at 4°C. For infection, 100µl of AA-2cells (5x10^6 cells/ml) were added to each well; presence of syncytia was recorded after 5 days as an indication of HIV-1 infection. Estimation of 50% inhibiting dose (EC50) was done according to Reed and Muench as described in Am. J. Hyg. (1938) 27:493. The reciprocal serum dilutions that inhibit syncytia formation by 50% (EC50) are shown.

Example 4: Expression of chimeric hemagglutinins carrying the extended 2F5-epitopes by recombinant baculoviruses

The chimeric hemagglutinins containing the said six peptides were prepared as described in Example 1. The coding sequence of these chimeric hemagglutinins was flanked by restriction enzyme site BamHI and inserted into the BamHI site of plasmid (Bluebac III, Invitrogen, San Diego CA). Transfection experiments in order to obtain recombinant baculoviruses that contain the chimeric hemagglutinins were done according to methods described by Groebe et al., Nucleic. Acids Res. (1990) 18:4033, and Felgner et al., Proc. Natl. Acad. Sci. USA (1987) 84:7413. The resulting recombinant chimeric baculoviruses can be used to elicit HIV-1 neutralizing antibodies.

Example 5: Immunization and antibody response of mice immunized with cells infected by the recombinant baculoviruses

Sf9 cells that were used for immunizations, were infected by recombinant baculoviruses at a multiplicity of infection (MOI) of 1-5, and harvested 3 days post infection. The cells were washed twice with PBS and resuspended in sterile PBS at a concentration of 5x 10^6 cells/ml. These cells specifically reacted with monoclonal antibody 2F5 in Western blots, indicating that these cells contained the hemagglutinin carrying
the ELDKWAS or LELDWAS sequence. Balb/cA mice were immunized with four intraperitoneal injections of 1x10^6 infected Sf9 cells at 2 weeks intervals (Van Wyyke Coelingh et al., Virology (1987) 160: 4465). Seven days after the fourth immunization, the mice were bled and ELISA titers of the antisera were determined. Fig.4 shows the binding of the recombinant baculovirus derived antisera to a synthetic peptide comprising the sequence Gly Gly Gly Glu Leu Asp Lys Trp Ala (GGGELDKWA) in an ELISA that was performed as described in Example 2.

**Induction of secretory antibodies**

Immunizations carried out by application of at least one, preferably a mixture, of said six peptides resulted in significantly improved IgG ELDKWAS-specific ELISA titers. Furthermore, in contrast to immunizations using the ELDKWAS sequence, immunizations with the said six peptides resulted also in a significant IgA response. Surprisingly, this IgA immune response was also triggered at the mucosal level.

**Example 5:** IgA antibodies in respiratory secretions of Balb/c mice immunized with influenza-ELDKWAS virus.

Mice were immunized with 10^2 PFU intranasally and boosted after 4 weeks with 10^5 PFU by the same route. The third immunization was given intranasally (IN) or intraperitoneally (IP) with 10^7 PFU after 4 more weeks. Nasal washings were collected and pooled 8 weeks after the third immunization and reactivity of these samples with the peptide ELDKWAS was determined by ELISA. As a control (WT pool) nasal washings of mice immunized with the wild-type (WT) influenza virus were pooled and analyzed. The same immunization scheme as for the IN group was used (ref. Fig.5). The elicited antibodies have primarily been produced by the nasal mucosa and it can be recognized that the antibody titers are unusually high. It can further be seen in Fig.5 that intranasal application of the influenza-ELDKWAS-virus leads to a higher concentration
of HIV-1 neutralizing antibodies than intraperitoneal application.

Example 7: IgA antibodies in intestinal secretions of Balb/c mice immunized with influenza-ELDKWAS virus

Mice were immunized with $10^2$ PFU intranasally and boosted after 4 weeks with $10^5$ PFU by the same route. The third immunization was given intranasally (rf. Fig. 6a) or intraperitoneally (Fig. 6b) with $10^7$ PFU after 4 more weeks. Faecal pellets containing the antibodies released from the intestinal mucosa were collected and extracted 8 weeks after the third immunization, and reactivity of these samples with the peptide ELDKWA was determined by ELISA. IN O, IN R, IN B and IN S are samples from mice that received the third immunization intranasally, and the samples IP O, IP R, IP RS and IP S were collected from mice that received the third
CLAIMS

What is claimed is

5 1. A peptide eliciting antibodies that show neutralizing activity against different strains and/or clinical isolates of HIV-1 and/or that inhibit the fusion of cells caused by HIV-1, characterized in that the peptide is composed of an amino acid sequence selected from the group consisting of ELDKWAS, LELEDKWAS, ELDWNAS, ELNKWAS, LELEDNWAS and LELENKWAS.

2. A peptide according to claim 1, characterized in that it is linked to a carrier.

15 3. A peptide according to claim 2, characterized in that it is part of a - preferably recombinant - virus, advantageously selected from the group consisting of an influenza virus, a baculovirus and a vaccinia virus.

20 4. A peptide according to claim 2 or 3, characterized in that at least one of said amino acid sequences substitutes at least part of the amino acid sequence of a viral protein, or is inserted into at least one antigenic site of a viral protein.

25 5. A peptide according to claim 4, characterized in that the viral protein is selected from the group consisting of hemagglutinin (HA) of influenza virus, neuraminidase (NA) of influenza virus and the surface antigen of hepatitis B virus.

30 6. A peptide according to claim 4, characterized in that the viral protein is derived from a - preferably recombinant - baculovirus or vaccinia virus.

35 7. Use of at least one, preferably all six, of peptides being composed of a sequence of seven or eight amino acids, the amino acid sequences being selected from the group consisting of ELDKWAS, LELEDKWAS, ELDWNAS, ELNKWAS, LELEDNWAS
and LELNKWAS, for the manufacture of a pharmaceutical to elicit antibodies that show neutralizing activity against different strains and/or clinical isolates of HIV-1 and/or that inhibit the fusion of cells caused by HIV-1.

8. Use of a peptide according to claim 1 for the manufacture of a pharmaceutical to induce anti-HIV-1 IgA antibodies secreted from mucosal surfaces upon, preferably intranasal, application to mammals.

9. Use of a peptide according to any one of claims 2 to 6 for the manufacture of a pharmaceutical to elicit antibodies that show neutralizing activity against different strains and clinical isolates of HIV-1 and that inhibit the fusion of cells caused by HIV-1.

10. Use of a peptide according to any one of claims 2 to 6 for the manufacture of a pharmaceutical to induce anti-HIV-1 IgA antibodies secreted from mucosal surfaces upon, preferably intranasal, application to mammals.

11. Use of a peptide according to any one of claims 1 to 6 for the manufacture of a pharmaceutical for prophylaxis and prevention of HIV-1 infection in endangered individuals.

12. An antibody showing HIV-1 neutralizing activity and being capable of preventing fusion of cells caused by HIV-1, characterized in that it is elicited by one of the peptides of claim 1.

13. An antibody according to claim 12, characterized in that it is a secretory antibody, preferably an anti-HIV-1 IgA antibody, and advantageously being secreted by mucosal surfaces.

14. A process for the manufacture of peptides according to claim 1, wherein at least one of said amino acid sequences is produced by chemical or microbiological methods.
15. A process according to claim 14 wherein the method is microbiological and comprises inserting a nucleotide sequence selected from the group consisting of

5  a) a nucleotide sequence corresponding to one of said amino acid sequences;
   b) a nucleotide sequence hybridizing to one of the nucleotide sequences under a);
   c) a nucleotide sequence deduced from one of the nucleotide sequences under a) by degeneration;

into the genome of a host organism, carrying out expression of the genome using standard microbiological cultivation techniques, and isolating at least one, preferably a multitude, of said peptides.

16. A process for the manufacture of peptides according to anyone of claims 2 to 6 comprising linking an amino acid sequence of claim 1 to a carrier, preferably a virus or viral protein, by chemical or microbiological methods.

17. A process according to claim 16 wherein said method is microbiological and comprises the steps of linking a nucleotide sequence selected from the group consisting of

25  a) a nucleotide sequence corresponding to one of said amino acid sequences;
   b) a nucleotide sequence hybridizing to one of the nucleotide sequences under a);
   c) a nucleotide sequence deduced from one of the nucleotide sequences under a) by degeneration;

5 to a nucleotide sequence corresponding to an amino acid sequence of the carrier, transferring the linked nucleotide sequences to a host organism, carrying out expression of the linked sequence using standard microbiological techniques, and isolating at least one, preferably a multitude, of peptides linked to a carrier.
18. A process according to claim 17, wherein at least one of the nucleotide sequences under a) through c) is linked to a nucleotide sequence corresponding to an amino acid sequence of a viral protein as the carrier, thereby substituting at least part of the nucleotide sequence corresponding to the amino acid sequence of the viral protein or being inserted into at least one site of said sequence corresponding to an antigenic site of the viral protein.

19. A process according to claim 18, wherein a nucleotide sequence corresponding to a viral protein selected from the group consisting of the hemagglutinin of influenza virus, neuraminidase of influenza virus and the surface antigen of hepatitis B virus, is used.

20. A process according to anyone of claims 15 and 17 to 19, wherein a virus of the group consisting of a - preferably recombinant - influenza virus, baculovirus and vaccinia virus is used as the host organism.
Fig. 1

a

b

106-
80-
49-
32-
27-

1 2 3 4 5

106-
80-
49-
32-
27-

1 2 3 4 5 6

Fig. 2

HA (wt):

ACG AAG AAG GGG GAT TCA TAC CCA AAG
T K K G D S Y P K

1984 2004

HA-ELDKWAS:

AAG AAG GGG GAG CTC GAT AAA TGG GCT AGC GAT TCA TAC
K K G E L D K W A S D S Y

1981 2004

HA-LELDKWAS:

AAG AAG GGG TTG GAG CTC GAT AAA TGG GCT AGC GAT TCA TAC
K K G L E L D K W A S D S Y

SUBSTITUTE SHEET (RULE 26)
A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/48 C07K14/16 A61K39/21 A61K39/395 C07K16/10

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 C12N C07K A61K

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 database and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<th>Category</th>
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<td>ALLAWAY ET AL. 'Synergistic inhibition of HIV-1 envelope-mediated cell fusion by CD4-based molecules in combination with antibodies to gp120 or gp41' see table 1</td>
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