

The present invention relates more especially to a vaccine designed for the prevention of AIDS.

5 The acquired immune deficiency syndrome (AIDS) is a viral ailment which is now of major importance in North America, Europe and Central Africa.

Recent estimates suggest that approximately 1 million Americans may have been exposed to the AIDS virus. The affected individuals show severe immuno-suppression and the disease is generally fatal.

10 The disease is most commonly transmitted by sexual contact, although people using narcotics intravenously also represent a high-risk group; on the other hand, a large number of individuals have been infected with this virus after receiving contaminated blood or
15 blood products.

The causal agent of this ailment is a retrovirus. Many animal conditions have been attributed to retroviruses, but it is only recently that it has been possible to describe retroviruses affecting man.

20 Whereas human T cell retroviruses (HTLV: human T leukaemia virus) of types I and II have been implicated as the causal agent of certain T cell leukaemias in adults, the retrovirus associated with lymphadenopathies (LAV virus), which is also known as HTLV III or AIDS-related virus (ARV), is now generally accepted as the
25 agent responsible for AIDS.

The genome of the LAV retrovirus has been characterized very completely (Wain-Hobson et al., 1985; Ratner et al., 1985; Muesing et al., 1985; Sanchez
30 Pescador et al., 1985), and data on the sequence indicate a very close relationship with the lentivirus group. Lentiviruses, the prototype of which is ovine Visna virus, are slowly progressing disease agents which

typically show a prolonged incubation period. LAV and Visna virus share many similarities, especially in their tropism for nerve tissue.

As with other well-known retroviruses, the three
5 most important parts of the LAV genome have been designated gag, pol and env. The sequence of the env gene, including the sequence of the gp120 and of the gp41, exhibits characteristics which were expected of a trans membrane envelope glycoprotein, and the identity of
10 the env protein precursor, gp160, consisting of gp120 and gp41, has been confirmed by direct amino acid sequencing.

Hereinafter the gp41 will sometimes be designated gp40 or gp42.

Antibodies produced against the env protein gp160
15 and its cleavage products gp120 and gp41 are commonly detected in the serum of patients who have AIDS, and the env glycoprotein represents the major surface antigen of the AIDS virus.

The env protein is thus the most promising
20 candidate for developing a vaccination strategy, and for this reason attention has been concentrated on this protein and on its coding sequence.

A large number of groups have reported the expression of the env protein in bacteria. However, the
25 absence of glycosylation and post-translational structuring can impair the immunogenic power of the materials synthesized by such microorganisms.

For this reason, the present invention proposes using a viral vector as expression vector for the env
30 protein, this viral vector enabling the protein to be expressed in an environment which will permit its glycosylation and its post-translational restructuring.

Thus, the present invention relates to a viral vector characterised in that it contains all or part of
35 the env gene of the virus responsible for AIDS.

Among the viral vectors which are usable, poxviruses should be mentioned more especially, and vaccinia virus (VV) in particular.

Vaccinia virus is a double-stranded DNA virus

which has been very widely used throughout the world to control and eradicate smallpox. Recent technical developments have enabled this virus to be developed as a cloning vector, and live recombinant viruses have
5 enabled foreign antigens to be expressed and have even enabled immunizations against different viral or parasitic diseases to be obtained.

Thus, several groups have recently demonstrated the use of recombinants of this type to express the
10 influenza antigen, the hepatitis B antigen and the rabies glycoprotein, for immunization against these diseases (Smith et al., 1983; Panicali et al., 1983; Kieny et al 1984).

The expression of a coding sequence for a foreign
15 protein by vaccinia virus (VV) necessarily involves two steps:

- 1) the coding sequence must be aligned with a VV promoter and be inserted in a nonessential segment of the VV DNA, cloned into a suitable
20 bacterial plasmid;
- 2) the VV DNA sequences situated on either side of the coding sequence must permit homologous recombinations in vivo between the plasmid and the viral genome; a double reciprocal
25 recombination leads to a transfer of the DNA insert from the plasmid to the viral genome in which it is propagated and expressed (Panicali and Paoletti, 1982; Mackett et al., 1982; Smith et al., 1983; Panicali et al., 1983).

30 Naturally, the use of this type of vector frequently involves a partial deletion of the genome of the vector virus.

The present invention relates more especially to a viral vector characterized in that it contains at
35 least:

- a part of the genome of a vector virus,
- a gene coding for one of the glycoproteins (gp) of the envelope of the virus responsible for AIDS, and also

- the elements which provide for the expression of this glycoprotein in cells.

The invention also relates to the recombinant DNAs corresponding to the said viral vectors.

5 It is appropriate to point out that 3 glycoproteins (gp) may be counted in the envelope of the virus responsible for AIDS, designated by their mass in kD, namely the gp160, the gp120 and the gp41; the first, gp160, is, in fact, the precursor of the latter two
10 proteins. These designations are not yet firmly established, and the gp41 is sometimes referred to as gp40 or gp42, but these 3 glycoproteins are completely identifiable as a result of the differences in mass, regardless of their designation.

15 Virus responsible for AIDS is understood, in particular, to designate the LAV virus, the HTLV III virus or ARV, and likewise possible point mutants or partial deletions of these viruses, as well as the related viruses.

20 In the part corresponding to the genome of the vector virus (as distinct from the virus responsible for AIDS), the viral vectors can be formed from the genome of a virus of any origin. However, it is preferable to use a part of the genome of a poxvirus, and more especially
25 a part of the genome of vaccinia.

The conditions necessary for the expression of a heterologous protein in the vaccinia virus have been recorded above.

30 In general, to be capable of being expressed, the gene in question, for example the env gene, will have to be under the dependence of a promoter of a vaccinia gene; this promoter will generally be the 7.5 K protein promoter of vaccinia. In addition, the coding sequence will have to be cloned into a nonessential gene of
35 vaccinia, which may possibly serve as a marker gene. In most cases, this will be the TK gene.

Among the glycoproteins of the envelope whose expression it is desired to achieve, the three proteins referred to above, namely the gp160, the gp41 and the

gp120, should be mentioned.

In general, it will be preferable to achieve expression of the complete envelope gene, that is to say the env gene incorporating the signal sequence and the transmembrane sequence of this gene.

As a result of the first tests performed with a viral vector in which the gene coding for the total env protein was cloned, modifications of this gene were proposed in order to improve the immunogenicity of the expression products.

A considerable release of the env protein into the culture supernatants was observed (which release probably occurs in vivo, into the circulating fluids). This may be due to a poor attachment of the protein in the cell membrane; it is known, in addition, that the presentation of the antigens at the cell surface is very important for the induction of an immune response with the vaccinia system. It is hence proposed to modify the env gene so as to improve the anchoring of the glycoprotein in the cell membrane.

To this end, the env gene may be modified at its portion which codes for the transmembrane region, so as to replace the codon corresponding to an arginine by a codon corresponding to an isoleucine.

There is also a possibility of improving the anchoring by replacing and/or adding the transmembrane region of a heterologous virus, for example the transmembrane region of the gp of rabies virus, to the transmembrane region of the env protein.

Furthermore, there is the possibility that the protein is not satisfactorily assembled following its expression. In effect, the signal peptide is fairly atypical, and might impair the complete exportation of the protein. For this reason, it is proposed to replace and/or add a signal sequence which originates from a heterologous virus, for example the signal sequence of the gp of rabies virus.

Finally, the gp120, rather than the gp160, appears to be the species which is released by the cells.

It may, on the one hand, provide a decoy for the immune system, and on the other hand, in keeping with recent data, become bound to the T4 cells, which might have the effect of inactivating the T4 cells or of making them appear foreign to the other T cells.

It may hence be advantageous to obtain a gp120 env protein which cannot be released. This is accomplished by modifying the env gene between the sequences coding for the gp120 and for the gp41, so as to eliminate the sites for cleavage by proteases situated between the gp120 and the gp41, in particular by elimination of the REKR site.

In the plasmids according to the invention, at least one additional mutation is performed in a site corresponding to a KRR sequence situated 8 amino acids downstream from the REKR sequence. The gp160 thereby obtained is no longer cleaved.

The vectors according to the present invention also contain a sequence coding for the gp41 which is devoid of the sequence corresponding to its hydrophobic N-terminal peptide, which it is thought might be responsible for the syncytial power of the env protein, that is to say the capacity of the virus to cause cell fusion and to produce a giant cell or syncytium.

Finally, the present invention relates to viral vectors in which the extracytoplasmic and intracytoplasmic regions of the gp160 are fused in phase after deletion of the C-terminal hydrophobic peptide, thereby enabling secretion of the glycoprotein to be obtained.

In general, the viral vectors according to the invention contain a sequence coding for one of the following proteins:

J

5 - gp120 ————— gp40 - (tm) -

- S is signal peptide,
- gp120 is the glycoprotein 120,
- J denotes schematically the junction portion between the gp120 and the gp40 which is devoid

of a site for cleavage by proteases,
· gp40 is the glycoprotein 40,
· tm is a transmembrane peptide or alternatively
for a protein of structure:

5 - S - gp40 - tm

or

 - S - gp120 - tm

 The signal peptide and the transmembrane sequence
can be those of the virus responsible for AIDS or can be
10 heterologous, in particular can originate from the rabies
virus or VSV or any virus having an envelope.

 The gp40 can optionally be devoid of its
hydrophobic N-terminal end.

 The first invention relates mainly to the use of
15 viral vectors for obtaining the glycoproteins encoded by
the env gene of LAV virus in cell cultures. The cells in
question are hence initially mammalian cells which have
been infected by a viral vector according to the
invention, or alternatively which may contain the corre-
20 sponding recombinant DNA; among these cells, there should
be mentioned, more especially, human diploid cells, from
primary cultures as well as Vero cells. It is naturally
possible to provide for other types of cells, as will
emerge, moreover, from the examples below.

25 The glycoproteins thereby obtained can be used
after purification for the production of vaccines.

 It is also possible to provide for the direct use
of the viral vectors according to the invention in order
to perform a vaccination, the glycoproteins then being
30 produced in situ and in vivo.

 It is advantageous to provide for the combined
use of several vaccinating agents, administered jointly
or separately, especially the vaccinating agents corre-
sponding to the vectors which separately express the
35 gp120 and the gp40 subjected to the modification
described above. For example, it may be advantageous to
use jointly the vaccinating agents derived from the
vectors 1136 and 1138 which will be described below.

 Finally, the present invention also relates to

the antibodies raised against the above glycoproteins, the antibodies being obtained by infection of a living organism with a viral vector as described above and recovery of the antibodies induced after a specified
5 time.

The techniques employed for obtaining the glycoproteins, the cell cultures and the vaccination techniques are identical to those which are currently performed with known vaccines, and will not be described
10 in detail.

The present invention will be more satisfactorily understood on reading the methods and examples which follow.

Five figures illustrate the examples:

15 Figure 1 shows the action of endo-F on the proteins synthesized by the recombinants VVTGeLAV9-1 and VVTGeLAV1132 and immunoprecipitated by means of an anti-LAV serum. In this figure, the molecular weights are given in kilodaltons and the labelling is as follows:

- 20
- P, the cell pellet
 - S, the supernatant
 - u, the products obtained without treatment
 - e, the products obtained after treatment with endo-F

25 Figure 2 shows the recognition of the proteins of the LAV virus by the sera of mice vaccinated with the recombinant VVTGeLAV9-1. In this figure, T denotes the cases where the serum used is that of a patient suffering from AIDS. The molecular weights are expressed in kilo-
30 daltons.

Figure 3 shows the immunoprecipitation of the proteins synthesized by the recombinant vaccinia viruses bearing the env gene. In this figure, the molecular weights are in kilodaltons.

35 Figure 4 shows an immunoprecipitation of the proteins synthesized by the recombinant viruses VV.TG.eLAV 1135, 1136, 1137 and 1138.

The virus 1135 synthesizes a gp160 which does not appear in the culture supernatant. As regards the viruses

1136 and 1138, they produce proteins gp120 and gp40, respectively, associated with the cell pellet. The virus 1137 produces a slightly smaller protein than the virus 1135, with an MW in keeping with that expected.

5 Figure 5 shows the structure of the env proteins synthesized by the recombinant viruses.

S : signal peptide

H : internal hydrophobic region

TM : transmembrane anchorage region

10 I : gp120/gp40 cleavage site

— : sequence originating from the rabies glycoprotein.

METHODS

Cloning: Maniatis et al., 1982.

15 Enzymes: used according to the supplier's instructions.

Localized mutagenesis: method derived from Zoller and Smith, 1983.

Transfer into vaccinia: Kieny et al., 1984.

20 Only difference: human 143B cells replace the LMTK⁻ cells.

Preparation of the stock virus

25 "Germ-free" chicken primary cells are infected at 0.01 pfu/cell for 4 days at a temperature of 37°C (MEM medium + 5% NCS).

Purification of the virus

30 The above stock virus is centrifuged for 15 minutes at 2500 rpm (Rotor GSA, Sorvall). The supernatant is set aside. The pellet is taken up in an RSB buffer (10 mM Tris-HCl pH 7.4, 10 mM KCl, 1 mM MgCl₂) for 15 minutes at 4°C. The suspension is ground in a Potter and then centrifuged for 15 minutes at 2500 rpm. The supernatant is added to the previous supernatant and a second grinding is then performed in the same manner.

35 All the supernatants are deposited on 10 ml of 36% (w/v) sucrose cushion (10 mM Tris pH 8). The suspension is centrifuged for 2 hours at 14,000 rpm (Rotor SW28, Beckman).

The pellet is taken up, broken up and replaced on

a second identical cushion. The 2nd pellet is taken up in 5 ml of PBS and loaded onto a 20-40% sucrose gradient (10 mM Tris pH 8) (same rotor). The suspension is centrifuged for 45 minutes at 12,000 rpm.

- 5 The virus band is recovered. It is pelleted by centrifugation for 1 hour at 20,000 rpm. The pellet is taken up in 10 mM Tris pH 8.

Immunoprecipitations

- 10 BHK-21 cells are infected (dishes 3 cm in diameter, 10^6 cells per dish, cultured in G-MEM + 10% FCS) at 0.2 pfu/cell for 18 hours. The medium is decanted and replaced by 1 ml of methionine-free medium and 10 μ l of [35 S]methionine (Amersham) per dish.

- 15 An excess of non-radioactive methionine is added after 2 hours.

- 20 After the labelling, the infected cells are scraped off and centrifuged for 1 minute in an Eppendorf centrifuge, the supernatant and pellet fractions are separated, the pellet is washed once in PBS buffer, and then immunoprecipitation is carried out and gel electrophoresis performed (according to Lathe et al., 1980).

Endo-F treatment

- 25 After immunoprecipitation of the labelled proteins with a serum of a patient suffering from AIDS, the protein A-sepharose fraction is taken up in:

- 30 0.2 M Na phosphate, pH 6.1
 0.05% SDS
 0.1% Nonidet P40
 0.1% Beta-mercaptoethanol
 0.1% EDTA pH 8

and boiled for 5 minutes to denature the proteins.

- 35 Incubation is performed for 20 hours at 37°C with 4 units of endo-F per ml, followed by precipitation for 2 minutes in ice with 1/5 volume of 100% TCA. The pellet is washed 3 times with 80% acetone, the sample buffer is added and the mixture is loaded onto an SDS gel.

Antibody assay by ELISA test

LAV

Use of the ELAVIA test (Pasteur-Diagnostic) with a second sheep anti-mouse antibody linked to peroxidase.

5 Vaccinia

Plates having 96 flat-bottomed holes (NUNC) are incubated for 18 hours at 37°C with 10⁷ pfu of wild-type vaccinia virus in carbonate buffer. The plates are then saturated with 0.01% gelatin. The mouse sera are then
10 adsorbed onto the plates and the remainder of the procedure is performed as for the LAV ELISA.

Readings taken at 492 nm.

EXAMPLE 1

Construction of the hybrid plasmids

15 The combined sizes of the different elements required for the transfer of the coding sequence of the env gene into the VV genome, and its subsequent expression are of the order of several kb. It was hence considered necessary to reduce to the minimum the size of
20 the plasmid for replication in E. coli used for the construction work so as to facilitate the necessary manipulations.

The HindIII fragment (Hin-J) of the VV genome contains the complete gene for thymidine kinase (TK);
25 which has already been used previously to permit the exchange and recombination of DNA inserted in the VV genome (Mackett et al., 1982). It is important to note that the transfer of an insert into the TK gene of the VV genome creates a TK-deficient virus which can be
30 selected. It was first necessary to produce a small-sized plasmid carrying a single HindIII site which could be used for the integration of the VV Hin-J fragment. In addition, it was necessary to remove the unnecessary restriction sequences from the plasmid so as to permit
35 the following manipulations.

The construction was primed starting with plasmid pML2 (Lusky and Botchan, 1981), which is a vector derived from plasmid pBR322 by spontaneous deletion in which the segment between nucleotides 1089 and 2491 has been lost.

First, the PstI sequence was removed by insertion of the AhaIII-AhaIII fragment of pUC8 (Vieira and Messing, 1982) between two AhaIII sites of pML2, removing 19 base pairs. The "linker tailing" method (Lathe et al., 1984) was used to insert a HindIII linker between the NruI site and the EcoRI site treated with S1, of this plasmid, the BamHI site being removed. This leads to a plasmid of 2049 base pairs carrying the functional betalactamase gene (which confers resistance to ampicillin) and containing in addition an origin of replication which is active in E. coli and a single HindIII restriction site.

This construction was referred to as pTG1H.

The Hin-J fragment of VV DNA carrying the TK gene has previously been cloned into a vector originating from pBR327 (Drillien and Spehner, 1983). This 4.6-kb fragment was recloned into the HindIII site of pTG1H. A clone was selected in which the TK gene is situated distally with respect to the gene coding for the resistance to ampicillin.

This construction pTG1H-TK was used as a vector in the following experiment.

The following stage was to isolate a VV promoter which could be used to control the expression of the sequence coding for the gene to be expressed. The promoter of an early gene coding for a protein of 7500 daltons (7.5 K) has already been successfully used for an identical purpose (Smith et al., 1983) and the isolation of this segment was hence undertaken.

The 7.5 K gene is situated on one of the smallest SalI fragments (Sal-S fragment) of the VV type WR genome (Venkatesan et al., 1981). Since the small fragments are cloned preferentially, a large proportion of the clones obtained by direct cloning of the DNA of VV type WR cut with SalI in plasmid pBR322 carries the Sal-S fragment. This fragment is transferred to the vector bacteriophage M13mp701 (see Kieny et al., 1983) by SalI digestion and religation, thereby leading to the phage M13TGSal-S.

In this clone, an ScaI site is present in immediate proximity to the initiation ATG of the 7.5 K

gene. Downstream from the 7.5 K gene, there are situated single BamHI and EcoRI sites originating from the vector. The BamHI and ScaI sites are fused by a BglII linker 5' - CAGATCTG-3' after the ends generated by BamHI digestion have been filled in with the Klenow fragment of E. coli. This process removes the ScaI site but reforms the BamHI site and shifts the single EcoRI site downstream. At the same time, the SalI (AccI) site downstream is removed, the SalI site upstream hence becomes unique.

10 This construction is referred to as M13TG 7.5 K.

Within the Hind-J [sic] fragment of VV DNA there are situated ClaI and EcoRI sites which are separated by approximately 30 base pairs (Weir and Moss, 1983). The 7.5 K promoter fragment present in M13TG7.5K is excised with AccI and EcoRI and cloned between the ClaI and EcoRI sites of pTG1H-TK to generate pTG1H-TK-P7.5K.

This construction leads to the transfer of the single BamHI and EcoRI sites from the M13 vector immediately downstream from the 7.5 K promoter sequence. These single BamHI and EcoRI sites are used in the following construction.

The polylinker segment of bacteriophage M13TG131 (Kieny et al., 1983) is excised with EcoRI and BglII and inserted between the EcoRI and BamHI sites of plasmid pTG1H-TK-P7.5K, generating pTG186-poly. In this construction, 10 restriction sites are available for cloning a foreign gene under the control of P7.5K.

EXAMPLE 2

Construction of the plasmid carrying the env sequence.

30 In order to obtain a sequence coding for env, the two proviral segments cloned into plasmids PJ19-6 and PJ19-13 are first assembled.

In order to provide for satisfactory translation of the env mRNA, the sequence of nucleotides around the presumed translation initiation site of the env gene was modified to match the consensus sequence of eukaryotic genes, this being achieved by a directed mutagenesis with an oligonucleotide in proximity to position 5767.

Plasmids pJ19-13 and PJ 19-6 contain HindIII

fragments of the proviral genome of LAV, comprising nucleotides 1258 to 1698 and 1698 to 9173, respectively.

An EcoRI-KpnI fragment of PJ19-13 (containing the env initiation ATG) was inserted in phage M13TG130 and directed mutagenesis was performed with an oligonucleotide (sequence 5'CTCTCATTGTCAGTCTGCTCTTTC), to introduce a PstI site upstream from the env translation initiation codon (position 5767) and in order to substitute the G at the 3-position by an A. The mutated fragment was then introduced between the EcoRI and KpnI sites of plasmid pTG1-POLY (which is a 2.1-kb mini-plasmid similar to pTG1H but which contains a polylinker segment of M13TG131).

The KpnI-HindIII fragment originating from PJ 19-13 was then cloned into the same plasmid (between KpnI and HindIII), followed by a HindIII-XhoI fragment of PJ 19-6 (between HindIII and SalI), to generate a complete env coding sequence flanked by two PstI sites (plasmid pTG1124).

The introduction of these two PstI restriction sites permits easier manipulation of the DNA of the env gene in the subsequent stages of the construction. As stated above, the expression of a heterologous protein in vaccinia virus requires that the coding sequence be aligned with a promoter sequence of vaccinia and be inserted in a nonessential segment of the vaccinia DNA. This DNA situated on each side permits recombination with the vaccinia genome in vivo by a double reciprocal recombination, which transfers the coding sequence and the accompanying promoter into the vaccinia genome.

To this end, the PstI-PstI fragment mentioned above was cloned in the PstI site of pTG186-POLY. A plasmid designated pTG1125 is thereby obtained.

Plasmid pTG186-POLY can be generated from plasmid pTG188 digested with PstI and religated with T4 ligase.

Plasmid pTG188 was deposited on 20th June 1985 at the Collection Nationale de Cultures de Micro-organismes (National Collection of Microorganism

Cultures) of the Institut Pasteur, 28, rue du Docteur Roux, 75015 PARIS under the following number:

E. coli 5KpTG 188 = No. I 458.

The transfer of the coding sequence of the env gene and the accompanying promoter into the vaccinia genome is accomplished as follows.

EXAMPLE 3

Cloning into vaccinia virus to generate VV.TG.e LAV 9-1

The strategy described by Smith et al. (1983) rests on the exchange in vivo between a plasmid carrying an insert in the VV TK gene and the wild-type viral genome so as to inactivate the TK gene carried by the virus. The TK⁻ viruses can be selected by plating on a cell line (TK-negative) in the presence of 5-bromodeoxyuridine (5BUDR) (Mackett et al., 1982). Thymidine kinase phosphorylates 5BUDR to 5'-monophosphate, which is then converted to triphosphate. This compound is an analogue of dTTP and its incorporation in DNA blocks the correct development of the virus. A TK⁻ virus can nevertheless replicate its DNA normally and it leads to visible viral plaques in a cell line which is also TK⁻.

Vaccinia virus is propagated in the cytoplasm of infected cells rather than in their nucleus. For this reason, it is not possible to turn to good account the machinery for replication and transcription of the host DNA, and it is necessary for the virion to possess the components for the expression of its genome. Purified VV DNA is noninfectious.

In order to generate the recombinants, it is necessary to perform simultaneously cellular infection with the VV virion and a transfection with the cloned DNA segment which is of interest. Nevertheless, the generation of the recombinants is limited to the small proportion of cells which are competent for transfection with DNA. For this reason, it was necessary to employ a strategy of indirect "congruence" to reduce the background of non-recombinant parent viruses. This was accomplished using as live infectious virus a temperature-sensitive (ts) mutant of vaccinia which is

incapable of propagation at a nonpermissive temperature of 39.5°C (Drillien and Spehner, 1983). When cells are infected with a ts mutant under nonpermissive conditions and transfected with the DNA of a wild-type virus, viral
5 multiplication will occur only in the cells which are competent for the transfection and in which a recombination between the wild-type viral DNA and the genome of the ts virus has taken place; no virus will multiply in the other cells, despite the fact that they
10 have been infected. If a recombinant plasmid containing a DNA fragment of vaccinia, such as pTG1125, is included in the transfection mixture, at the appropriate concentration, with the wild-type DNA, it is also possible to procure its participation in homologous recombination
15 with the DNA of the vaccinia in the competent cells.

Primary cell monolayers of chick embryo fibroblast (CEF) are infected at 33°C with VV-Copenhagen ts7 (0.1 pfu/cell) and transfected with a calcium phosphate coprecipitate of the DNA of wild-type VV-Copenhagen virus
20 (50 ng/10⁶ cells) and the recombinant plasmid (50 ng/10⁶ cells).

After incubation for 2 hours at a temperature which does not permit the growth of the ts virus (39.5°C), the cells are incubated again for 48 hours at
25 39.5°C. Dilutions of ts⁺ virus are used for reinfecting a monolayer of human 143B cells at 37°C, which are then incubated in the presence of 5BUDR (150 µg/ml). Various plaques of TK⁻ virus are obtained from these cells which have received the recombinant plasmid, while the control
30 cultures without a plasmid do not show visible plaques. The TK⁻ viruses are then subcloned by a second selection in the presence of 5BUDR.

A correct double reciprocal recombination between the hybrid plasmid pTG1125 and the VV genome leads to the
35 exchange of the TK gene carrying the insert with the TK gene of the virus, the recombinants thereby becoming TK⁻.

The DNAs purified from the different TK⁻ recombinant viruses are digested with HindIII and subjected to agarose gel electrophoresis. The DNA

fragments are transferred to a nitrocellulose filter according to the technique described by Southern (1975). The filter is then hybridized with plasmid pTG1125 which has been nick-translated with ^{32}P . After the filter is washed, the latter is fluorographed and 3.85-, 2.9- and 0.8-kb bands are visible on the autoradiograph when the vaccinia virus has incorporated the env gene of LAV. One of these recombinants, VV.TG. eLAV 9-1 was selected for the following studies.

EXAMPLE 4

Env protein synthesized from a recombinant vaccinia-LAV virus

To demonstrate the expression of the env gene of LAV from the hybrid vaccinia virus, rodent cells, BHK21, which are cultured in a G-MEM medium + 10% of foetal calf serum are infected with the same recombinant VV.TG. eLAV 9-1.

A fresh semi-confluent monolayer (10^6 cells) is infected with 0.2 pfu/cell and incubated for 18 hours.

The medium is then removed and a medium having a low concentration of methionine (1 ml for 10^6 cells), supplemented with 10 $\mu\text{l/ml}$ of [^{35}S]methionine, is added. The cells are incubated at 37°C and the labelled proteins are collected by centrifugation. After separation into pellet and supernatant, the proteins are incubated with a serum belonging to a patient suffering from AIDS. The proteins which react with the serum are recovered by adsorption on a protein A-Sepharose resin, and spread by electrophoresis on an SDS polyacrylamide gel and autoradiographed according to a technique described by Lathe et al., 1980. The autoradiographs show that the serum of the patient suffering from AIDS specifically binds three proteins in the infected cell extracts (the result is identical or similar to that obtained with other sera of patients). The apparent molecular weights of 160, 120 and 41 kD suggest equivalence with the gp160, gp120 and gp 41 bands identified by means of sera of patients suffering from AIDS in an authentic env glycoprotein preparation and in extracts of cells infected with the LAV virus.

This observation, that three proteins are expressed from the recombinant vector which carries only the coding sequence of the env gene of LAV, supports the hypothesis that the gp120 and gp41 are generated by proteolytic cleavage of the primary translation product, gp160.

The sequence coding for env leads to a primary translation product of approximately 90 kD, whereas the env precursor obtained by the above method possesses an apparent molecular weight of approximately 160 kD. This difference is attributed to the presence of a very considerable amount of glycosylation. By digestion with endoglycosidase F, which removes the glycosyl groups, a good correlation could be demonstrated between the products obtained by the present invention and the predicted products (Figure 1).

EXAMPLE 5

Demonstration of anti-env antibodies in mice vaccinated with the VV.TG.e LAV 9-1 virus

5-week-old male Balb/c mice are vaccinated by subcutaneous injection of 5×10^7 pfu of VV.TV.e LAV 9-1 virus per animal. They receive a booster injection with the same dose after 2 weeks, and blood samples are drawn 1, 2 and 4 weeks after the booster. The presence of antibodies directed against determinants of LAV virus and of vaccinia virus in their sera is sought.

All the vaccinated animals give sera capable of reacting with vaccinia virus in an ELISA test. In contrast, the response in the ELISA test against LAV virus is weak and of low reproducibility. To improve the sensitivity of the tests, a "Western blot" technique was used. This method enables antibodies capable of reacting with the proteins of LAV virus to be demonstrated after these proteins have been denatured with SDS in an electrophoresis gel and transferred to a nitrocellulose membrane. In this experiment, the nitrocellulose membranes employed are those of the LAV-BLOT kit sold by Diagnostic-Pasteur and to which the proteins of LAV virus are already bound. These membranes are cut into strips and each strip is incubated with the serum of the

vaccinated mice (1/20 dilution). A second antibody (sheep anti-mouse) linked to peroxylase enables the proteins of the LAV virus which have bound mouse antibodies to be visualized.

5 Several sera (12/27) give a specific reaction with a protein of molecular weight about 160 kD, corresponding to the gp160 of env (Figure 2). In a certain number of sera, a reaction is also observed with the gp41 protein. It should be noted that the sera of a
10 few mice produce signals in Western blot corresponding to unidentified proteins of the LAV virus preparation bound to the membranes.

EXAMPLE 6

Construction of pTG1128

15 This plasmid pTG1128 is identical to plasmid 1125 with the exception that the sequence coding for the trans-membrane region has been mutated to replace the arginine by an isoleucine, this being in order to improve the attachment of the protein in the cell membrane.

20 The HindIII-BamHI fragment of pTG1124 containing the transmembrane region of env described in Example 2 is inserted into phage M13 TG131 after a HindIII-BamHI digestion. A phage M13 TG154 is thereby obtained.

25 A localized mutagenesis designed to replace the codon coding for arginine by a codon coding for isoleucine is then performed on this phage M13 TG154. For this purpose, the following oligonucleotide is used:

5' GGT TTA ATA TAG TTTT 3'.

30 Phage M13 TG155 is thereby obtained, the sequences having been modified as follows:

	Gly	Leu	Arg	Ile	Val
Original sequence:	GGT	TTA	AGA	ATA	GTT
Mutated sequence:	GGT	TTA	<u>ATA</u>	ATA	GTT
			Ile		

35 The BamHI-HindIII fragment thereby mutated is transferred from M13 TG155 into plasmid pTG1124 in equivalent sites, to give plasmid pTG1127 which re-forms the env gene as above except that the arginine codon has

been replaced by an isoleucine codon.

As described in Example 1, the PstI-PstI fragment of pTG1127 is cloned into the PstI site of plasmid pTG186-POLY to give plasmid pTG1128.

5

EXAMPLE 7

Construction of plasmid pTG1130

In this plasmid, the sequence coding for the trans-membrane region of the rabies glycoprotein is fused with the beginning of the sequence coding for the hydrophobic portion of the env glycoprotein.

10

The transmembrane region of the rabies glycoprotein originates from a BamHI-PstI fragment of phage M13 TGRG151.

This fragment is cloned in phage M13 TG154 between the BamHI and PstI sites (see above example). The phage M13 TG156 is thereby obtained.

15

A localized mutagenesis is then performed on M13 TG156 in order to fuse in phase the env and rabies sequences with an oligonucleotide by forming a 5' GCTGTGGTATATAAAATATGTATTACTGAGTG 3' loop

20

Tyr	Leu	Lys	Ile	Phe		Gly	Lys	Tyr	Val
TAT	ATA	AAA	ATA	TTC	-----	GGG	AAG	TAT	GTA
			env tm					rabies tm	

The phage M13 TG157 is thereby obtained.

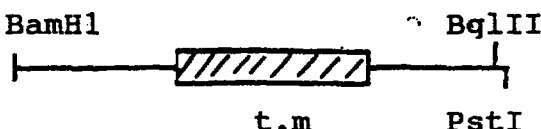
25

The transmembrane (tm) region of the rabies glycoprotein which has just been fused with the env gene is then transferred into plasmid pTG1124.

For this purpose, the HindIII-BglII fragment of M13 TG157 is cloned into pTG1124 on which a HindIII-BamHI restriction has been performed (this destroys the BamHI and BglII sites).

30

The BglII site of M13 TG157 originates from the rabies gp fragment:



35

Plasmid pTG1126 is thereby obtained.

As above, PstI-PstI fragment of pTG1126 is cloned into the PstI site of pTG 186-POLY to give plasmid pTG1130.

EXAMPLE 8

Construction of pTG1131

The objective of the construction of this plasmid is to fuse the signal sequence of the env gene and the signal sequence of the rabies glycoprotein.

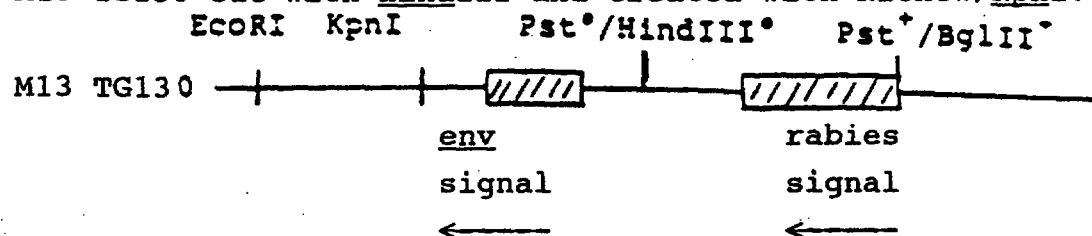
The signal sequence of the rabies glycoprotein is removed from plasmid pTG155 PRO in the form of a BglII-HindIII fragment, which is cloned into the PstI-HindIII sites of M13 TG130 by means of a single-stranded adaptor having the following sequence:

5' GATCTGCA 3'

The phage M13 TG158 is thereby obtained.

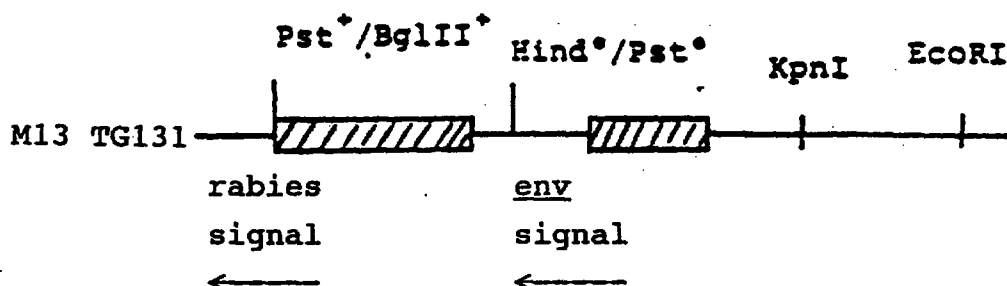
The transfer of the env signal peptide into M13 TG158 is then carried out in order to fuse the latter with the gene coding for the signal peptide of the rabies glycoprotein.

For this purpose, the PstI fragment treated with S1 nuclease and then with Klenow and KpnI is cloned into M13 TG158 cut with HindIII and treated with Klenow/KpnI:



The phage M13 TG159 is thereby obtained.

The KpnI-PstI block of M13 TG159 is transferred into M13 TG131 to obtain plasmid M13 TG160.



A localized mutagenesis on M13 TG160 enables the env and rabies glycoprotein sequences to be fused in phase (by forming a loop). This is achieved by means of the oligonucleotide

5' GACCCACAATTTTCTGTAATAGGGAATTTCCCAA 3'

	rabies signal									<u>env</u>			
	<u>Cys</u>	<u>Phe</u>	Gly	Lys	Phe	Pro	Ile	Tyr		Thr	Gln	Lys	Leu
5'	TGT	TTT	GGG	AAA	TTC	CCT	ATT	TAC	---	ACA	GAA	AAA	TTG

Phage M13 TG161 is thereby obtained.

5 The PvuII-KpnI fragment of M13 TG161 is then cloned into pTG1126 cut with EcoRI and treated with Klenow/KpnI (the PvuII site of M13 TG161 originates from M13 in the region situated upstream from the polylinker). This leads to plasmid pTG1129.

10 By cloning the PstI-PstI fragment of pTG1129 into
plasmid pTG186-POLY cut with PstI, plasmid pTG1131 is
obtained.

EXAMPLE 9

Preparation of plasmid pTG1132

15 By cloning the PstI-PstI fragment of pTG1128 into the PstI site of M13 TG131, plasmid M13 TG162 is obtained.

A localized mutagenesis is then performed by means of the oligonucleotide

20 5' ATTCCCACTGCTTAGTATTCATTCTGCACCACTC 3'

This enables a stop codon to be placed at the end of the gp120. The sequences obtained are as follows:

Original sequence: CAG AGA GAA AAA AGA GCA GTG

25 presumed cleavage site
of gp120

		Q	E	Y	xxx		
Mutated sequence:	CAB	AAT	GAA	TAC	TAA	GCA	GTG

The phage M13 TG168 is thereby obtained.

30 By recloning the PstI fragment of M13 TG168 into the PstI site of pTG186-POLY, plasmid pTG1132 is obtained.

EXAMPLE 10

Construction of plasmid pTG1133

35 By localized mutagenesis on M13 TG162 by means of
the following oligonucleotide:

5' ATTCCCACTGCTTGGTGTTCATTCTGCACCACTC 3'

a bacteriophage is obtained in which a potential cleavage site separating qp 120 and qp 40 has been destroyed.

The modified sequences are as follows:

5 Original sequence: : CAG AGA GAA AAA AGA ↑ GCA GTG
R E K R A V
cleavage site

Mutated sequence: : CAG AAT GAA CAC CAA GCA
 Q E H N

10. The phage M13 TG165 is thereby obtained.

By recloning the PstI-PstI fragment of M13 TG165 into pTG186-POLY at the PstI site, plasmid pTG1133 is obtained.

EXAMPLE 11

15 Construction of plasmid pTG1134

By cloning the PstI-PstI fragment of pTG1131 into the PstI site of M13 TG131, the phage M13 TG163 is obtained.

20 A localized mutagenesis is performed on M13 TG163 in order to destroy the same cleavage site of the gp120 as above. For this purpose, the following oligonucleotide is used:

5' ATCCCACTGCTTGATGTTCACTCTGCACCACTC 3'

25 This enables the sequences to be modified in the following manner:

		R	E	K	R		
Original sequence:	CAG	AGA	GAA	AAA	AGA	↑	GCA GTG
Mutated sequence:	GAG	AAT	GAA	CAT	CAA		GCA
		N	E	H	Q		

30 Under these conditions, the phage M13 TG166 is obtained.

By recloning the PstI-PstI fragment of this phage M13 TG166 into the PstI site of pTG186-POLY, plasmid pTG-1134 is obtained.

EXAMPLE 12

Immunoprecipitation of the proteins synthesized by the
VV.TG. eLAV recombinant viruses.

5 By working as described above for plasmid
pTG1125, the hybrid vaccinia vectors corresponding to the
different plasmids prepared above are obtained.

These viral vectors will be referred to,
respectively, as

10 VV.TG. eLAV 1128
VV.TG. eLAV 1130
VV.TG. eLAV 1131
VV.TG. eLAV 1132
VV.TG. eLAV 1133
VV.TG. eLAV 1134.

15 The proteins obtained as described above are
tested by immunoprecipitation (Figure 3).

For the virus 9-1, the group of immunoprecipitates reveals an immunoprecipitation corresponding to the
gp160, the gp120 and the gp41.

20 The same applies for the virus 1128.

The virus 1130 also shows a gp160 and a gp120.

The protein corresponding to the gp41 has a
slightly lower weight, due to the modification of its C-
terminal end.

25 The virus 1131 shows a spectrum which is substan-
tially identical to that obtained for the virus 1130.

The virus 1132 naturally does not show the
protein corresponding to the gp41. The 105-kD protein
present in the pellets is an isoform of the gp120
30 (different glycosylation).

As regards the virus 1133, this clearly shows the
proteins 160, 120 and 41, but the bands corresponding to
the proteins 120 and 41 are weaker than in the other
spectra.

35 The same applies for the virus 1134, with which
the gp41 also shows a lower molecular weight but for
which it is clear that the cleavage took place with
slower kinetics than those of the viruses VV.TG.1125 (9-
1) to 1131.

EXAMPLE 13

Construction of plasmid pTG1135

The kinetics of release performed on the VV.TG. eLAV1133 and 1134 viruses show that, although the kinetics of cutting between the gp120 and the gp40 are slower, the cleavage still takes place. The examination of the DNA sequence of the env gene reveals another potential cleavage site (KRR) 8 amino acids downstream from the first cleavage site. It may hence be important to mutate this second site so as to obtain a recombinant vaccinia virus which expresses only the gp160.

By localized mutagenesis on M13TG166 by means of the following nucleotide:

5' ATTCTGCACCACGTGATTCTGTGCCTTGGTGGGT 3'

a phage is obtained in which the second cleavage site is modified. The modified sequences are as follows:

Original sequence:

GCA	AAG	AGA	AGA	GTG
A	K	R	R	V

↑
potential cleavage site

Mutated sequence: GGA CAG AAT CAG GTG
 A Q N H V

The PstI-PstI fragment of the phage obtained (M13TG181) is cloned into pTG186-POLY at the PstI site to generate plasmid pTG1135.

EXAMPLE 14

Construction of plasmid pTG1139

The C-terminal portion of the env gene synthesized by the recombinant vaccinia vector VV.TG.eLAV1135 is a sequence derived from the rabies glycoprotein. It may hence appear to be useful also to have another recombinant in which this C-terminal portion was replaced by the C-terminal portion of the env gene of the LAV virus.

For this purpose, the same mutagenesis is performed on the phage M13TG165 as that performed (see

Example 13) on the phage M13TG166, to generate the phage M13TG184.

The PstI-PstI fragment of M13TG184 is then recloned into plasmid pTG186-POLY to generate plasmid pTG1139.

EXAMPLE 15

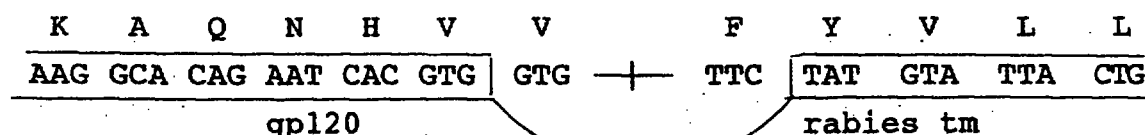
Construction of plasmid pTG1136

It would also be desirable to have a recombinant vaccinia virus which expressed only the gp120. This gp120 will, in distinction to the case obtained with the VV.TG.eLAV1132 virus, be equipped with a C-terminal anchorage region.

For this purpose, the sequences corresponding to the gp40 in the phage M13TG181 are removed by localized mutagenesis using the following nucleotide:

5' TGCACTCAGTAATACATACACGTGATTCTGTGCCTT 3'

This oligonucleotide enables the gp120 sequences (with the 2 modified cleavage sites) and the sequences of the transmembrane region of the rabies glycoprotein to be fused in phase.



Phage M13TG182 is thereby obtained. PstI-PstI fragment of M13TG182 is then inserted at the PstI site of pTG186-POLY to generate plasmid pTG1136.

EXAMPLE 16

Construction of plasmid pTG1137

The role of the hydrophobic region situated at the N-terminal portion of the gp40 is little known. This region of the env protein may be responsible for the power for inducing formation of syncytia.

It hence appears to be advantageous to produce a gp160 which does not contain this sequence.

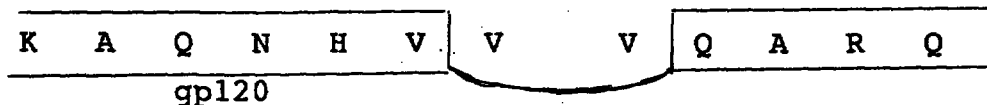
For this purpose, the sequences upstream and downstream from the portion coding for this hydrophobic peptide in the phage M13TG181 are fused in phase by means

of the following oligonucleotide:

5' CAATAATTGTCTGGCCTGCACGTGATTCTGTGCCTT 3'

This enables the phage M13TG183 to be obtained by carrying out the fusion:

5 AAG GCA CAG AAT CAC GTG GTG +— GTA CAG GCC AGA CAA



10 The PstI-PstI fragment of M13TG183 is recloned into the PstI site of pTG186-POLY to generate plasmid pTG1137.

EXAMPLE 17

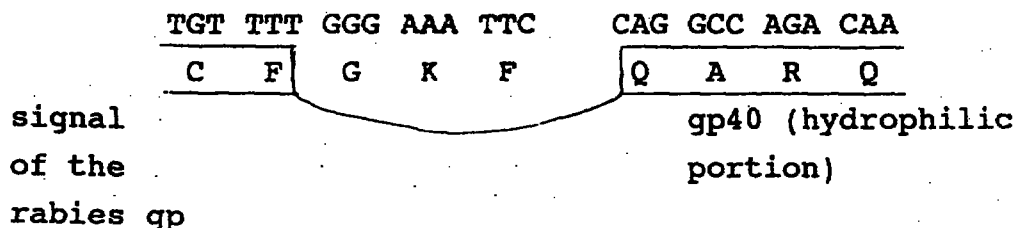
Construction of plasmid pTG1138

15 In addition to recombinant viruses which express the gp160 or the gp120, it may be useful to generate a recombinant vaccinia virus which expresses the gp40 alone.

20 For this purpose, the sequences coding for the signal peptide are fused with the sequences coding for the gp40 on the phage M13TG163, by means of the following nucleotide:

5' CAATAATTGTCTGGCCTGAATAGGGAATTTCCCAAA 3'

25 This enables the phage M13TG180 to be generated, which contains the fusion:



30 The PstI-PstI fragment of M13TG180 is inserted at the PstI site of pTG186-POLY to give plasmid pTG1138.

EXAMPLE 18

Construction of plasmid pTG1162

35 As in the case of the gp160 (plasmid pTG1139), it may also be important to have a recombinant virus which expresses a gp40 in which the anchorage region and the intracytoplasmic region are the sequences of the env gene

of the LAV virus, rather than the corresponding sequences of the rabies glycoprotein.

To obtain this, the HindIII-BglI fragment of M13TG180 is replaced by the HindIII-BglI fragment of M13TG-165, generating phage M13TG190.

Plasmid pTG1162 is obtained by cloning the PstI-PstI fragment of the phage M13TG190 into the PstI site of plasmid pTG186-POLY.

EXAMPLE 19

Construction of plasmid pTG1163.

It also appears to be important to obtain a recombinant vaccinia virus which synthesizes a gp160 which is not cleaved and which is secreted into the medium. In effect, this protein might be used as a killed vaccine, in combination with adjuvants, or included in liposomes or ISCOMS [Morein et al., Nature (1984) 308, 5958 p.457-60].

For this purpose, the bacteriophage M13TG194, in which the sequences coding for the extracytoplasmic and intracytoplasmic regions are fused in phase in the bacteriophage M13 TG184, is constructed by means of the following oligonucleotide:

5' TCCCTGCCTAACTCTATTTTTTATATACACAGCCA 3'

The PstI-PstI fragment of M13TG194 is then cloned at the PstI site of pTG186-POLY to give pTG1163.

The recombinant proteins thereby obtained, and especially the non-cleavable gp160, can be used in diagnostic kits for detecting potential antibodies present in the blood of patients who have been in contact with the virus. These tests can be carried out according to processes known to those skilled in the art, for example by ELISA, RIPA or "Western [sic] blot" (immunoprinting).

These proteins can also be used for the production of hybridomas and monoclonal antibodies designed to detect the presence of virus in samples.

The different plasmids and M13 phages are described, in particular, in the following patent applications:

M13 TG131 : Kieny et al., 1983

M13 TGRG151 : WO 83/04052

pTG155 PRO : FR 84 06499

M13 TG130 : Kieny et al., 1983.

5 The following plasmids were deposited on 16th November 1984 at the Collection Nationale de Cultures de Microorganismes [National Collection of Microorganism Cultures] of the Institut Pasteur and are described in Patent GB-A-84 29099:

10 PJ 19-6 : CNCM No. 366-I

PJ 19-13 : CNCM No. 367-I.

Plasmid pTG1125 was deposited on 6th June 1986 in the same collection, in the form of a transformed bacterium, E. coli 1106/pTG1125, under no. I-557.

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- 25

CLAIMS

(except for Spain)

1. Viral vector, characterized in that it contains at least:
 - 5 - a part of the genome of a virus,
 - a sequence coding for the signal sequence of the precursor of the gp160 glycoprotein of the envelope of the virus responsible for AIDS, or a signal sequence originating from a heterologous virus,
 - 10 - the sequence of the gene coding for the gp160 glycoprotein of the envelope of the virus responsible for AIDS, the gene coding for the said gp160 not containing the site for cleavage by proteases of sequence REKR,
 - and also the elements providing for the expression of
15 this glycoprotein in cells.
2. Viral vector according to Claim 1, characterized in that the gene for the gp160 codes for a gp160 not containing the sites for cleavage by proteases of sequence REKR and KRR.
- 20 3. Viral vector according to Claim 1, characterized in that the REKR sequence is replaced by the sequence NEHQ.
4. Viral vector according to Claim 2, characterized in that the REKR and KRR sequences are replaced respectively by the sequences NEHQ and QNH.
25
5. Viral vector according to one of Claims 1 to 4, characterized in that the part of the genome of a virus is a part of the genome of a poxvirus.
6. Viral vector according to Claim 5, characterized in that the poxvirus is vaccinia virus.
30
7. Viral vector according to Claims 1 to 6, characterized in that it does not contain the sequence coding for the transmembrane region of the natural gp160.
8. Viral vector according to Claims 1 to 7, characterized in that the sequence coding for the C-terminal hydrophobic peptide corresponding to the transmembrane (tm) region of the natural gp160 has been mutated to replace the Arg codon by an Ile codon.
35
9. Viral vector according to Claim 7, characterized





in that it contains a sequence coding for a transmembrane region originating from a heterologous virus, in particular from the rabies virus.

10. Viral vector according to Claim 7, characterized
5 in that it does not contain a sequence coding for a transmembrane anchorage region.

11. Viral vector according to one of Claims 1 to 10,
characterized in that the DNA sequence coding for the
gpl60 is under the dependence of a promoter of a pox-
10 virus gene.

12. Viral vector according to Claim 10, characterized
in that the promoter is a promoter of a gene of vaccinia
virus.

13. Viral vector according to Claim 12, characterized
15 in that the DNA sequence coding for the gpl60 is under
the control of the promoter of the gene for the 7.5 K
protein of vaccinia virus.

14. Viral vector according to one of Claims 11 to 13,
characterized in that the sequence coding for the gpl60
20 is cloned into the TK gene of vaccinia virus.

15. Viral vector according to one of Claims 1 to 7
and 9 to 14, characterized in that the C-terminal
hydrophobic peptide of the gpl60 is deleted.

16. Recombinant DNA corresponding to a viral vector
25 according to one of Claims 1 to 15.

17. Culture of mammalian cells infected by a viral
vector according to one of Claims 1 to 15 or containing
a DNA according to Claim 16.

18. Method for preparing envelope glycoproteins of
30 the virus responsible for AIDS, characterized in that
cells according to Claim 17 are cultured and in that the
glycoprotein produced is recovered.

19. Non-cleavable gpl60 envelope glycoprotein of the
virus responsible for AIDS which can be obtained, in
35 particular, by employing the process according to Claim
18.

20. Non-cleavable gpl60 glycoprotein of the envelope
of the virus responsible for AIDS, characterized in that
it does not contain the site for cleavage by proteases of



sequence REKR of the natural gp160 glycoprotein.

21. Non-cleavable gp160 glycoprotein according to Claim 20, characterized in that it does not contain the sites for cleavage by proteases of sequences REKR and KRR
5 of the natural gp160 glycoprotein.

22. Non-cleavable gp160 glycoprotein according to Claim 20 or 21, characterized in that the REKR sequence is replaced by the sequence NEHQ.

23. Non-cleavable gp160 glycoprotein according to
10 Claim 20 or 21, characterized in that the REKR and KRR sequences of the natural gp160 are replaced by the sequences NEHQ and QNH respectively.

24. Glycoprotein according to one of Claims 20 to 23, characterized in that it does not contain the trans-
15 membrane anchorage region of the natural gp160.

25. Glycoprotein according to Claim 24, characterized in that it contains the transmembrane region of the rabies virus.

26. Glycoprotein according to Claim 24, characterized
20 in that the C-terminal hydrophobic peptide corresponding to the transmembrane region of the gp160 is modified, the arginine being replaced by an isoleucine.

27. Glycoprotein according to Claim 24, characterized in that it does not contain a transmembrane anchorage
25 region.

28. Glycoprotein according to one of Claims 22 to 25 or 27, characterized in that the C-terminal hydrophobic peptide of the natural gp160 is deleted.

29. Vaccine, characterized in that it consists of a
30 viral vector according to one of Claims 1 to 13 and/or a glycoprotein according to one of Claims 19 or 28.

30. Antibodies raised against the envelope glycoproteins of the virus responsible for AIDS, according to Claims 19 to 28, other than the antibodies
35 raised against the natural gp160 glycoproteins.