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Transmembrane Envelope Glycoproteins of Human Immunodeficiency Virus Type 2 and Simian Immunodeficiency Virus SIV-mac Exist as Homodimers

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An 80-kilodalton glycoprotein (gp80) was produced in human immunodeficiency virus type 2 (HIV-2)-infected cells along with three envelope glycoproteins that we have recently reported: the extracellular glycoprotein (gp125), the envelope glycoprotein precursor (gp140), and the transient dimeric form of the precursor (gp300). gp125 and gp80 were detectable after the synthesis of gp140 and the formation of gp300. Using a specific monoclonal antibody, we showed here that gp80 is a dimeric form of the transmembrane glycoprotein gp36 of HIV-2. Dimerization of the envelope glycoprotein precursor and dimeric forms of the transmembrane glycoproteins were also observed in cells infected with simian immunodeficiency virus (SIV-mac), a virus closely related to HIV-2. Under routine conditions of our experiments (i.e., extraction by 1% Triton X-100 before polyacrylamide gel electrophoresis in sodium dodecyl sulfate [SDS]), monomeric forms of the transmembrane glycoprotein of HIV-2 and SIV-mac were only seldomly observed. Dimeric forms of the envelope precursors and the transmembrane glycoproteins are probably stabilized by extraction in the nonionic detergent Triton X-100 since such dimeric forms resist dissociation during subsequent electrophoresis in the presence of the ionic detergent SDS. However, the dissociation of these dimeric forms might occur when samples are prepared by extraction directly in 1% SDS or by incubation of the purified dimers at acidic pH. Dimerization of the envelope precursor might be required for its processing to give the mature envelope proteins, whereas the transmembrane dimer might be essential for optimal structure of the virion and thus its infectivity.

Two related but distinct human immunodeficiency virus types, 1 and 2 (HIV-1 and HIV-2) have been identified (1, 2, 4, 5, 13, 18, 19, 25). HIV-2 is closely related to the simian immunodeficiency virus (SIV-mac) (3, 6, 9). Alignments of the nucleotide sequences of HIV-1 BRU, HIV-2 ROD, and SIV-mac reveal a considerable homology between HIV-2 and SIV. These two viruses share about 75% overall nucleotide sequence homology, but both of them are only distantly related to HIV-1, with about 40% overall homology (3, 13).

The HIV-1 *env* gene encodes for a 160-kilodalton (kDa) glycoprotein that is proteolytically cleaved to yield the extracellular and transmembrane proteins, gp120 and gp41, respectively (16). Similarly, the HIV-2 *env* gene encodes for a precursor glycoprotein which is then processed into the mature extracellular and transmembrane glycoproteins (21). We have recently suggested that the HIV-2 envelope precursor (gp140) seems to require the formation of a homologous dimer (gp300) during its processing. Interestingly, dimerization of the envelope precursor is also observed in SIV-mac-infected cells (21). In this study, we further investigated the processing of HIV-2 envelope glycoproteins by characterization of the transmembrane glycoprotein. Previously, the detection of the latter had been handicapped by the lack of specific antibodies. For this reason, we prepared a monoclonal antibody (MAb) specific for the transmembrane glycoprotein of HIV-2. With these antibodies, we were then able to identify the transmembrane glycoproteins of HIV-2 and SIV. We showed that the transmembrane proteins exist as a homodimer in the infected cells as well as in the virions. Dimeric forms of the transmembrane glycoproteins of HIV-2 and SIV could be dissociated in an ionic detergent to 36- and 32-kDa proteins, respectively. We speculate that conformational modifications brought about by the dimerization of envelope precursor might be necessary for transport of this glycoprotein to the Golgi apparatus and its processing into the mature glycoprotein products, the extracellular and transmembrane envelope proteins. Furthermore, the transmembrane dimer might be essential for optimal structure of the virion and thus its capacity to fuse with the cellular membrane and be infectious.

The HIV-2 ROD isolate (4, 5) was used in this study to infect CEM clone 13 cells (21). Four days after infection with this HIV-2 isolate, about 80 to 90% of the cells produced viral proteins and were recognized by a cytopathic effect corresponding to vacuolization of cells and appearance of small syncitia. Crude extracts from such infected cells were analyzed by the electrophoretic transfer immunoblotting assay (Western blot) with an HIV-1-positive serum and three different HIV-2-positive sera from patients with acquired immunodeficiency syndrome (Fig. 1). The HIV-2-specific sera identified the envelope precursors (gp140 and gp300) and in addition recognized strongly the 80-kDa protein. These sera were specific for HIV-2 proteins since they did not recognize HIV-1 proteins which were detectable with HIV-1-specific serum: the extracellular envelope glycoprotein (gp120) and *gag* precursors (p55 and p40). The relation of the 80-kDa protein to HIV-2 infection was demonstrated by several results in which it was not identified by HIV-

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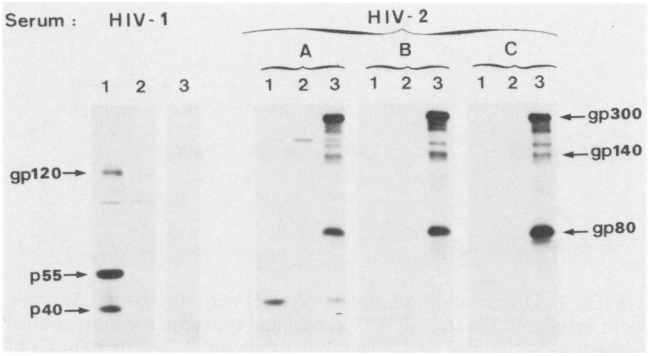

FIG. 1. Identification of a specific 80-kDa protein in HIV-2-infected cells by Western blot analysis with an HIV-1-positive serum and three HIV-2-positive sera (A, B, and C). Extracts (material from 10^6 cells) from uninfected (lanes 2), HIV-1-infected (lanes 1), and HIV-2-infected (lanes 3) CEM cells were analyzed by polyacrylamide gel (7.5%) electrophoresis before the Western blot assay. An autoradiograph is shown. On the left, the arrows indicate the position of HIV-1 extracellular glycoprotein (gp120) and *gag* precursors p55 and p40. On the right are the positions of HIV-2-specific gp300, gp140, and gp80. Conditions for the Western blot assay were as described previously (10). Human polyclonal antibodies in HIV-1 or HIV-2 sera were revealed with a preparation of ^{125}I-labeled protein A (>30 μCi/mg; Amersham Corp., Arlington Heights, Ill.). The labeled bands between gp140 and gp300 probably represent partially degraded products of gp300 since they were not observed routinely. Preparation of extracts from HIV-1 BRU- and HIV-2 ROD-infected CEM cells was as described before (21).

1-positive serum nor was it found in HIV-1-infected cells. Western blot analysis of viral pellets prepared by centrifugation (100,000 × *g* for 30 min) of infected culture medium indicated that the 80-kDa protein was also detectable in HIV-2 particles along with the extracellular glycoprotein, gp125 (data not shown; see below). HIV-2-specific sera could also immunoprecipitate the 80-kDa protein along with the envelope precursor gp140 and its dimeric form gp300 (data not shown; similar to Fig. 1 in reference 21). The glycoprotein nature of the 80-kDa protein (gp80) was demonstrated by its capacity to incorporate radioactive sugars when HIV-2-infected cells were labeled with [^{3}H]glucosamine or [^{3}H]fucose. Tunicamycin blocked such incorporation, indicating that gp80 contains N-linked oligosaccharide chains. In pulse-chase experiments, gp140 was the first envelope product detectable at 15 min after a pulse-labeling, but during the period of chase, gp300 was detectable at 0.5 h, whereas gp 125 and gp80 were detectable at 2 to 3 h (data not shown; similar results were shown previously for the processing of HIV-2 envelope precursor in reference 21). These observations indicated that gp80 is associated with virus particles and suggested that it is a mature product of a precursor which requires processing. The identification of gp80 as the dimeric form of the transmembrane glycoprotein was done with a specific MAb described below.

For the preparation of MAbs, mice were immunized with HIV-2 glycoproteins attached to lentil lectin-Sepharose 4B. For this purpose, HIV-2 ROD virions were cultivated in CEM cells and purified from concentrated culture supernatants by banding in sucrose gradients. Purified virus was disrupted in 0.5% Triton X-100–150 mM NaCl–50 mM Tris (pH 8.0)–1% aprotinin (Sigma Chemical Co., St. Louis, Mo.) and clarified by ultracentrifugation. The viral extract was then purified by passing over a lentil lectin-Sepharose 4B affinity column (Pharmacia, Uppsala, Sweden), and the bound glycoproteins eluted with 0.5 M methyl-alpha-D-mannopyranoside (Sigma) were dialyzed overnight against phosphate-buffered saline. BALB/c mice were immunized intraperitoneally with 0.3 ml of purified glycoproteins (2 to 5 μg) reattached to lentil lectin-Sepharose 4B (50 to 100 μl). These mice were boosted every 4 to 6 weeks for 24 weeks with the same immunogen and monitored for the production of HIV-2 glycoprotein antibodies. Three days after the last injection, spleen cells were fused with NS1 myeloma cells by the method of Köhler and Milstein (14). Fusion plates (96-well) were screened for hybridomas secreting anti-HIV-2 antibodies by the Genetic Systems HIV-2 disrupted virion enzyme immunoassay. Methods for the propagation and stabilization of cloned hybridomas and for ascites production have been previously described (12). Hybridoma culture supernatants were screened by radioimmunoprecipitation and Western blot analysis. MAb 1H8, which reacted with the transmembrane glycoprotein, was further mapped to amino acid sequence 579 to 604 within the HIV-2 transmembrane glycoprotein by using a synthetic peptide-based enzyme immunoassay (23). This synthetic peptide, p39′, was synthesized according to the amino acid sequence 579 to 604 deduced from the nucleotide sequence of the HIV-2 ROD envelope. The amino acid sequence of peptide p39′ is VTAIEKYLQDQARLNSWGCAFRQVCH.

In an immunoprecipitation assay with [^{35}S]methionine-labeled extracts from HIV-2-infected cells and from the virus pellet, MAb 1H8 immunoprecipitated gp300, gp140, and gp80 from cell extracts and only gp80 from the virus pellet (Fig. 2A). These results confirmed that gp140 and gp300 are intracellular precursors of the HIV-2 envelope. The weak reactivity of MAb 1H8 with gp36 in both cellular and viral extracts probably reflects low levels of this protein. The MAb did not recognize the extracellular glycoprotein gp125. Furthermore, it did not recognize any proteins in extracts from HIV-1-infected cells or from the HIV-1 virus pellet (data not shown). These results therefore illustrate the specificity of MAb 1H8 for HIV-2 envelope precursors (gp140 and gp300) and the transmembrane glycoprotein.

To show the specificity of MAb 1H8 reactivity with gp80, we did a Western blot assay using extracts from HIV-2 virus pellet. After the transfer of proteins, nitrocellulose sheets were incubated with MAb 1H8 in the absence or presence of peptide p39′ (Fig. 2B). MAb 1H8 gave a strong signal for gp80. In addition, a signal for gp36 was observed, but only after a prolonged exposure of the autoradiogram. Addition of peptide p39′ completely abolished the signals obtained with MAb 1H8 (Fig. 2B). These observations confirm that the reactivity of MAb 1H8 should be within the 26-amino-acid residue corresponding to amino acids 579 to 604 in the transmembrane glycoprotein of HIV-2. Consequently, a sequence corresponding to that of peptide p39′ should be present in gp80. Similar Western blot experiments were done with cell extracts from HIV-2-infected cells (data not shown). MAb 1H8 identified gp80 as well as gp140 and gp300, but such reactivity was abolished in the presence of peptide p39′. The reactivity of a polyclonal antibody specific for HIV-2 envelope glycoproteins (prepared in the laboratory) with gp80, gp140, and gp300 was not modified by peptide p39′ (data not shown). Therefore, these polyclonal antibodies should interact with other epitopes than that corresponding to peptide p39′.

In all our experiments, we prepared extracts from infected cells or from viral pellets using a lysis buffer containing a nonionic detergent, Nonidet P-40 or Triton X-100. Under

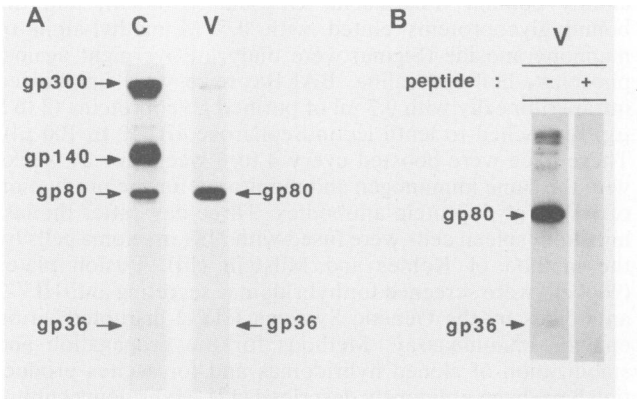

FIG. 2. Characterization of HIV-2 envelope glycoproteins by the MAb 1H8. (A) HIV-2-infected CEM cells were labeled for 16 h with [^{3}H]glucosamine (200 μCi/ml; 4 × 10^6 cells per ml). Extracts from infected cells (lanes C) and from the virus pellet (lanes V) were purified by immunoprecipitation with MAb 1H8, and the labeled proteins were analyzed by polyacrylamide gel (12.5%) electrophoresis. A fluorograph is shown. The arrows indicate the positions of the envelope precursor (gp140), the dimeric form of the precursor (gp300), and the monomeric (gp36) and dimeric (gp80) forms of the transmembrane glycoprotein. (B) Peptide p39′ blocks the binding of MAb 1H8 to gp80. Extracts from HIV-2 virus pellets were analyzed by Western blot assay with MAb 1H8 (at 1:100 dilution). Incubation with the MAb was done in the absence (−) or presence (+) of 1 μg of peptide p39′ per ml. Conditions for the Western blot assay were as described before (10). The MAb was revealed by ^{125}I-labeled goat anti-mouse immunoglobulins (7.5 μCi/μg; Dupont, NEN Research Products, Boston, Mass.). The results of the autoradiography are shown. HIV-1 ROD-infected human CEM clone 13 cells (21) were cultured in suspension in RPMI 1640 medium containing 10% fetal calf serum and 2 μg of Polybrene (Sigma) per ml. On day 4, infected cells were labeled with [^{3}H]glucosamine (20 to 40 μCi/mmol; Amersham). Cell and viral extracts were prepared in lysis buffer containing 10 mM Tris hydrochloride (pH 7.6), 150 mM NaCl, 1 mM EDTA, 1% (vol/vol) Triton X-100, 0.2 mM phenylmethylsulfonyl fluoride, 5 mM β-mercaptoethanol, and 100 U of aprotinin (Iniprol) per ml. Immunoprecipitation in the presence of protein A-Sepharose was done in a modified radioimmunoprecipitation buffer containing 20 mM Tris hydrochloride (pH 7.6), 50 mM KCl, 150 mM NaCl, 1 mM EDTA, 1% (vol/vol) Triton X-100, 20% (vol/vol) glycerol, 7 mM β-mercaptoethanol, 0.2 mM phenylmethylsulfonyl fluoride, and 100 U of aprotinin per ml (21).

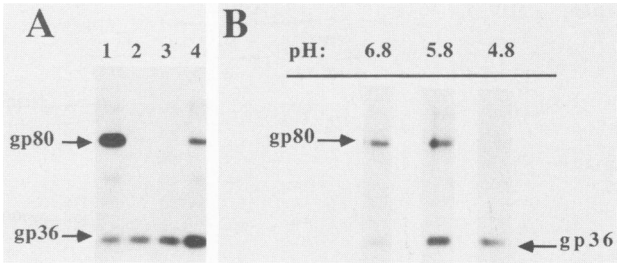

FIG. 3. Dissociation of gp80. (A) HIV-2-infected CEM cells were incubated for 16 h at 37°C in minimal essential medium culture without L-methionine and serum but supplemented with 200 μCi of [^{35}S]methionine (1,000 μCi/mmol; Amersham) per ml. HIV-2 virus pellets from [^{35}S]methionine-labeled cells (each corresponding to material from 10^7 cells) were suspended in different buffers: lane 1, lysis buffer containing Triton (10 mM Tris hydrochloride [pH 7.6], 150 mM NaCl, 1 mM EDTA, 1% [vol/vol] Triton X-100, and 100 U of aprotinin per ml); lane 2, lysis buffer containing SDS (as in buffer 1 but containing 1% [vol/vol] SDS instead of Triton X-100); lane 3, lysis buffer containing SDS and then heated (95°C, 5 min); lane 4, lysis buffer but also containing 0.1% (vol/vol) SDS and 0.2% (vol/vol) deoxycholate. All these samples were then diluted 10-fold in lysis buffer without Triton before immunoprecipitation with MAb 1H8. (B) Dissociation of the purified gp80 into gp36. Extracts of HIV-2 virus pellet from [^{35}S]methionine-labeled cells (2 × 10^7 cells) were immunoprecipitated with MAb 1H8, and gp80 was purified by preparative gel electrophoresis (21). Aliquots of the purified gp80 preparation were lyophilized and suspended in 100 mM acetate at pH 6.8, 5.8, and 4.8 containing 1% (vol/vol) SDS, 100 U of aprotinin per ml, and 5 mM EGTA (to inhibit calcium-dependent proteolysis). All the samples were incubated at 30°C for 60 min before dilution in twofold-concentrated electrophoresis sample buffer. Samples were analyzed by polyacrylamide gel (12.5%) electrophoresis. Fluorographs are shown.

these conditions, gp80 is stable even after addition of the ionic detergent sodium dodecyl sulfate (SDS). We investigated the effect of SDS when it substituted for Triton for the preparation of extracts from [^{35}S]methionine-labeled HIV-2 pellets. Labeled proteins were solubilized in lysis buffer containing 1% Triton or 1% SDS or in radioimmunoprecipitation buffer containing 0.1% SDS and 1% deoxycholate. Extracts in 1% SDS lysis buffer were also heated at 95°C. These extracts were then diluted 10-fold in buffer without detergent and immunoprecipitated with MAb 1H8 (Fig. 3A). In the immune complex prepared from Triton extracts, gp80 and gp36 were the major proteins immunoprecipitated by MAb 1H8. On the other hand, gp80 became undetectable and the level of gp36 was slightly increased in SDS extracts. A somewhat better result was observed with the radioimmunoprecipitation buffer, in which most of gp80 was dissociated and about 30% of the label was recovered in gp36 (Fig. 3A, lane 4). To show that gp80 is composed of only gp36, dissociation experiments were done with [^{35}S]methionine-labeled gp80 purified by MAb 1H8 immunoprecipitation and by preparative gel electrophoresis (21). Lyophilized gp80 preparations were then suspended directly in an acetate buffer with SDS at pH 6.8, 5.8, and 4.8. At pH 5.8, there was a partial dissociation, whereas at pH 4.8 all gp80 was converted to gp36 (Fig. 3B). These results therefore illustrate that the dissociation of gp80 into gp36 might occur when samples are prepared by extraction directly in the ionic detergent SDS or by incubation of the purified gp80 at acidic pH, as we have reported to be the case for the dissociation of gp300 into gp140 (21). Dissociation of gp300 and gp80 does not occur in the presence of reducing agents, in high concentrations of salt, or by the metal chelators EDTA or EGTA (data not shown). A significant degradation of proteins occurred during dissociation reactions since not all the label in gp80 was recovered as gp36 (Fig. 3). The presence of 200 U of aprotinin per ml and 0.2 mM phenylmethylsulfonyl fluoride did not prevent such degradation. A similar degradation also occurs during dissociation of the dimeric form of the precursor gp300 to gp140 (21). It might be that the dimeric forms of these proteins have a conformation which can resist proteolysis. Dissociation of gp300 and gp80 might then lead to conformational modifications, making the proteolytic sites accessible.

Previously, we have shown that the glycoprotein precursor (gp140) of SIV forms a dimer during its processing (21). For this reason, it was important to investigate whether the transmembrane glycoprotein was also detectable as a dimer. SIV-mac-infected HUT-78 cells were labeled with [^{3}H]glucosamine, and extracts prepared in lysis buffer containing Triton were immunoprecipitated with MAb 1H8. The MAb precipitated three glycosylated proteins: the envelope precursor gp140, the dimer precursor gp300, and a 65-kDa

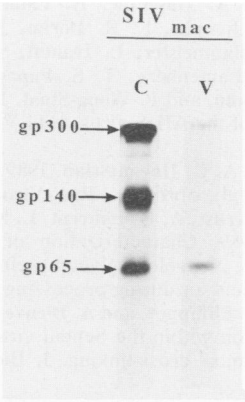

FIG. 4. Transmembrane glycoprotein of SIV exists as a dimer. SIV-mac-infected HUT-78 cells (21) were labeled for 16 h with [^{3}H]glucosamine (200 μCi/ml; 4 × 10^6 cells per ml). Extracts (prepared in lysis buffer as described in the legend to Fig. 2) from infected cells (lane C) and from virus pellets (lane V) were purified by immunoprecipitation with MAb 1H8, and the labeled proteins were analyzed by polyacrylamide gel (12.5%) electrophoresis. A fluorograph is shown.

protein (gp65) which is probably the counterpart of HIV-2 gp80. gp65 was found to be associated with SIV particles (Fig. 4). In these experiments, monomeric forms of the transmembrane glycoprotein of SIV-mac were not detectable. The HIV-2 ROD amino acid sequence 579 to 604 corresponds to SIV-mac sequence 595 to 620 (3, 13). Since these two sequences are highly homologous, then MAb 1H8 cross-reacts with envelope proteins of both HIV-2 ROD and SIV-mac.

By the use of an MAb, Veronese et al. (24) have recently reported that the transmembrane glycoprotein of SIV-mac is a 32-kDa protein (gp32). However, in their immunoprecipitation assays, they reported the presence of unidentified 75- and 300-kDa proteins at high levels along with the envelope precursor gp140. In analogy with our data, the 75-kDa protein is probably the dimeric form of the transmembrane glycoprotein gp32, whereas the 300-kDa protein should be the dimeric form of the envelope precursor as we have reported previously (21). Under our experimental conditions, we did not detect dimeric forms of the HIV-1 transmembrane glycoprotein, gp41. On the other hand, Pinter et al. (17) have recently reported that the native form of gp41 in HIV-1 virions exists as noncovalently associated tetramers and trimers. Such oligomeric forms of gp41, however, are less stable than the dimeric forms of HIV-2 or SIV transmembrane glycoproteins. For HIV-2 and SIV, the nonionic detergent Triton conserves the native forms of the envelope dimers, the precursor, and the transmembrane glycoproteins.

To confirm the molecular weights of HIV-2 envelope glycoproteins under native conditions, we did gel filtration experiments using a Sephacryl S-300 column and [^{35}S]methionine-labeled extracts from HIV-2-infected cells prepared in lysis buffer containing Triton. Under these experimental conditions, gp300 eluted as the second peak after the peak of aggregated proteins. The transmembrane glycoprotein dimer eluted as a 75- to 80-kDa protein after the peak of gp125 and before the peak of the bovine serum albumin (68 kDa) marker (data not shown). These observations indicated that the molecular weight estimations of the native and denatured dimers gp300 and gp80 are comparable. Previously, the formation of oligomeric complexes of some viral structural glycoproteins has been reported, for example, the hemagglutinin of influenza virus (11, 26, 27), the G protein of vesicular stomatitis virus (7, 15), the F protein of Sendai virus (22), and the envelope glycoprotein of Rous sarcoma virus (8). In Rous sarcoma virus, the oligomeric structure of the transmembrane glycoproteins seems to be the functional form found in virions. All these observations enforce the suggestion that efficient processing of some glycoproteins requires the formation of oligomeric structures, and in some cases, oligomeric forms of the mature glycoprotein might be essential for infectivity. Accordingly, antiviral agents designed to block the formation of oligomeric precursors or cause dissociation of oligomeric complexes of the mature protein might be employed to prevent virus replication and spreading.

Several observations emphasize that dimerization is a natural step in the processing pathway of the envelope precursor gp140, i.e., dimerization is not due to accumulation of unprocessed gp140 nor is it an artifact of the experimental procedure (20). The mechanism of dimerization of the envelope glycoprotein precursor is not yet clear. This is an intrinsic property of the polypeptide moiety of the envelope precursor, and it occurs in a compartment of the endoplasmic reticulum with an environment favoring the process of dimerization (21). The fact that the transmembrane glycoproteins exist in dimeric forms (gp80) suggests that dimerization of gp140 occurs through interactions between the transmembrane regions of the envelope precursor.

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LITERATURE CITED

1. **Barré-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Brun-Vézinet, C. Rouzioux, W. Rozembaum, and L. Montagnier.** 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science **220**:868–871.
2. **Brun-Vézinet, F., M. A. Rey, C. Katlama, P. H. Girard, D. Roulot, P. Yeni, L. Lenoble, F. Clavel, M. Alizon, S. Gadelle, J. J. Madjar, and M. Harzic.** 1987. Lymphadenopathy associated virus type 2 in AIDS-related complex. Lancet **i**:128–132.
3. **Chakrabarti, L., M. Guyader, M. Alizon, M. D. Daniel, R. C. Desrosiers, P. Tiollais, and P. Sonigo.** 1987. Sequence of simian immunodeficiency virus from macaque and its relationship to other human and simian retroviruses. Nature (London) **328**: 543–547.
4. **Clavel, F., D. Guétard, F. Brun-Vézinet, S. Chamaret, M. A. Rey, M. O. Santos-Ferreira, A. G. Laurent, C. Dauguet, C. Katlama, C. Rouzioux, D. Klatzmann, J. L. Champalimaud, and L. Montagnier.** 1986. Isolation of a new retrovirus from West African patients with AIDS. Science **233**:343–346.
5. **Clavel, F., M. Guyader, D. Guétard, M. Sallé, L. Montagnier, and M. Alizon.** 1986. Molecular cloning and polymorphism of the immune deficiency virus type 2. Nature **324**:691–694.
6. **Daniel, M. D., N. L. Letvin, N. W. King, M. Kannagi, P. K. Sehgal, R. D. Hunt, P. J. Kanki, M. Essex, and R. C. Desrosiers.** 1985. Isolation of a T-cell tropic HTLV-III-like retrovirus from macaques. Science **228**:1201–1204.
7. **Doms, R. W., A. H. Helenius, and J. M. White.** 1985. Membrane fusion activity of influenza hemagglutinin. J. Biol. Chem. **260**: 2973–2981.
8. **Einfeld, D., and E. Hunter.** 1988. Oligomeric structure of a prototype retrovirus glycoprotein. Proc. Natl. Acad. Sci. USA **85**:8688–8692.
9. **Fultz, P. N., H. M. McClure, D. C. Anderson, R. B. Swenson, R. Anand, and A. Srinivasan.** 1986. Isolation of a T-lymphotropic

retrovirus from naturally infected sooty mangabey monkeys (Cercocebusatys). Proc. Natl. Acad. Sci. USA **83**:5286–5290.
10. **Galabru, J., and A. Hovanessian.** 1987. Autophosphorylation of the protein kinase dependent on double-stranded RNA. J. Biol. Chem. **262**:15538–15544.
11. **Gething, M. J., K. McCammnon, and J. Sambrook.** 1986. Expression of wild-type and mutant forms of influenza hemagglutinin: the role of folding in intracellular transport. Cell **46**:939–950.
12. **Gosting, L., J. McClure, E. Dickenson, S. Watanabe, K. Shriver, and L. Goldstein.** 1987. Monoclonal antibodies to gp110 and gp41 of human immunodeficiency virus. J. Clin. Microbiol. **25**:845–848.
13. **Guyader, M., M. Emerman, P. Sonigo, F. Clavel, L. Montagnier, and M. Alizon.** 1987. Genome organization and transactivation of the human immunodeficiency virus type 2. Nature (London) **326**:662–669.
14. **Köhler, G., and C. Milstein.** 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature (London) **256**:495–497.
15. **Kreis, T. E., and H. F. Lodish.** 1986. Oligomerization is essential for transport of vesicular stomatitis viral glycoprotein to the cell surface. Cell **46**:929–937.
16. **Montagnier, L., F. Clavel, B. Krust, S. Chamaret, F. Rey, F. Barré-Sinoussi, and J. C. Chermann.** 1985. Identification and antigenicity of the major envelope glycoprotein of lymphadenopathy associated virus. Virology **144**:283–289.
17. **Pinter, A., W. J. Honnen, S. A. Tilley, C. Bona, H. Zaghouani, M. K. Gorny, and S. Zolla-Pazner.** 1989. Oligomeric structure of gp41, the transmembrane protein of human immunodeficiency virus type 1. J. Virol. **63**:2674–2679.
18. **Popovic, M., M. G. Sarngadharan, E. Read, and R. C. Gallo.** 1984. Detection, isolation and continuous production of cytopathic retrovirus (HTLV-III) from patients with AIDS and pre-AIDS. Science **224**:497–500.
19. **Ratner, L., W. A. Haseltine, R. Patarca, K. J. Livak, B. Starcich, S. F. Josephs, E. R. Doran, J. A. Rafalski, E. A. Whitehorn, K. Baumeister, L. Ivanoff, S. R. Petteway, J. A. Pearson, J. A. Lantenberg, T. S. Papas, J. Ghrayeb, N. T. Chang, R. C. Gallo, and F. Wong-Staal.** 1985. Complete nucleotide sequence of the AIDS virus, HTLV-III. Nature (London) **313**:277–284.
20. **Rey, M. A., and A. G. Hovanessian.** 1989. On the processing of HIV-2 envelope glycoproteins. Res. Virol. **140**:271–274.
21. **Rey, M. A., B. Krust, A. G. Laurent, L. Montagnier, and A. G. Hovanessian.** 1989. Characterization of human immunodeficiency virus type 2 envelope glycoproteins: dimerization of the glycoprotein precursor during processing. J. Virol. **63**:647–658.
22. **Sechoy, O., J. R. Philippot, and A. Bienvenue.** 1987. F protein-F protein interaction within the Sendai virus identified by native bonding or chemical cross-linking. J. Biol. Chem. **262**:11519–11523.
23. **Shriver, K., A. Watson, A. Flesher, J. McClure, D. Wierzbicki, R. Houghton, W. Cosand, L. Goldstein, and M. Tam.** 1988. Advances in the diagnosis of blood virus infections, p. 209–224. *In* L. M. de la Maza and E. M. Peterson (ed.), Medical virology VII. Elsevier Science Publishing, Inc., New York.
24. **Veronese, F. D. M., B. Joseph, T. D. Copeland, S. Oroszlan, R. C. Gallo, and M. G. Sargadharan.** 1989. Identification of simian immunodeficiency virus SIV_{mac} *env* gene products. J. Virol. **63**:1416–1419.
25. **Wain-Hobson, S., P. Sonigo, O. Danos, S. Cole, and M. Alizon.** 1985. Nucleotide sequence of the AIDS virus LAV. Cell **40**: 9–17.
26. **Wiley, D. C., J. Skehel, and M. Waterfield.** 1977. Evidence from studies with a cross-linking reagent that the hemagglutinin of influenza virus is a trimer. Virology **79**:446–448.
27. **Wilson, I. A., J. Skehel, and D. C. Wiley.** 1981. Structure of the hemagglutinin membrane glycoprotein of influenza virus at 3Å resolution. Nature (London) **289**:366–373.