JOURNAL OF VIROLOGY, July 1991, p. 3938–3942
0022-538X/91/073938-05$02.00/0

Vol. 65, No. 7

Dispensable Role of the Human Immunodeficiency Virus Type 2 Vpx Protein in Viral Replication

LUISA MARCON, FRANK MICHAELS, NAOHIKO HATTORI, KATHLEEN FARGNOLI, ROBERT C. GALLO, AND GENOVEFFA FRANCHINI*

Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20892

Received 11 October 1990/Accepted 9 April 1991

Human immunodeficiency virus type 2 (HIV-2) is similar in genetic organization to HIV-1 but contains a unique gene (*vpx*) that encodes a 16-kDa protein. A replication-competent molecular clone of HIV-2 (HIV-$2_{sbl/isy}$) that infects human primary cells in vitro and rhesus monkeys was used to generate three mutations in the *vpx* gene. In the first mutant, the *vpx* open reading frame was truncated at amino acid 20; the second mutant was tailored to eliminate the proline-rich carboxyl terminus of the protein; and the third mutant was obtained by addition of four amino acids (KDEL) to the carboxyl terminus of the protein to provide a retention signal in the endoplasmic reticulum. The viral infection kinetics of the three mutant viruses and isogeneic HIV-$2_{sbl/isy}$ in the SupT1 cell line were similar. Slight impairment in the early phases of viral replication was observed during infection of primary human peripheral blood mononuclear cells with the *vpx* mutant viruses. All of the *vpx* mutant viruses readily infected macrophages, indicating that *vpx* expression is dispensable for HIV-2 infection and replication in human macrophages.

Two distinct human immunodeficiency viruses (HIVs) are the causative agents of AIDS in humans. HIV type 1 (HIV-1) is associated with AIDS worldwide (1, 9, 10, 24–26). HIV-2 infection is prevalent in West Africa and seems substantially less pathogenic than HIV-1 (2, 15, 18). Retroviruses related to the HIVs have been isolated from several nonhuman primate species and are designated simian immunodeficiency viruses (SIVs) (4, 8, 14, 21, 29). Both the HIV-1 and HIV-2 genomes carry equivalent structural and accessory genes, except that the *vpu* gene is unique to HIV-1 (3, 28) and the *vpx* gene is present only in HIV-2 (7, 13). The *vpx* gene is present in all SIVs except SIV_{mnd} (29) and SIV_{cpz} (14). The x open reading frame (x-ORF) encodes a 16-kDa protein in HIV-2 and a 14-kDa protein in SIV_{mac} (7, 13, 32). The Vpx protein is present in mature virions and is associated with both the cytoplasmic and membrane fractions of infected cells (7).

The HIV-2 used in this study is a biologically active molecular clone of the HIV-2_{6669} West African isolate (5), HIV-$2_{sbl/isy}$. This molecular clone encodes a full-length 16-kDa Vpx protein (7) and infects primary cell cultures of human T lymphocytes and macrophages (12). Unlike a previously reported HIV-2 molecular clone (22), HIV-$2_{sbl/isy}$ also efficiently infects rhesus monkeys (6). We investigated the biological significance of the Vpx protein in HIV-2 replication in vitro with the intention of using the resulting viral mutants to infect rhesus macaques. The HIV-2 proviral DNA from the recombinant DNA was subcloned in two separate plasmids (pEGP and pKF3) because of the instability of the entire provirus in *Escherichia coli*, as previously described for HIV-2 (12) and SIV_{mac} (16). To reconstitute the entire provirus, the two plasmids were linearized at a *Sac*I site and ligated together. Three different mutations in the x-ORF were generated as depicted in Fig. 1. A *Bgl*II-*Sac*I proviral DNA fragment containing the *vpx* ORF was subcloned in the psP72 vector, and the resulting plasmid (pPN) was used to introduce mutations in the *vpx* gene. The *Bgl*II-*Sac*I mutated fragments were then recloned in pEGP to reconstitute the first half of the provirus.

The ISY1 mutant was constructed by linearizing pPN DNA at the *Stu*I site and inserting the *Xba*I linker (TCTAGA), which contains an in-frame termination codon for the Vpx protein. The Vpx protein in this construct is interrupted at amino acid 20, and the mutation introduced a novel *Xba*I restriction site. The ISY12 mutant was generated by linearizing the pPN subclone at the *Nsi*I site, blunting the two ends with T4 polymerase, and ligating them together. Elimination of the *Nsi*I site was associated with a 4-bp deletion and a frameshift in the *vpx* ORF at amino acid 80. In this mutant, the proline-rich tail of the Vpx protein is substituted with a similar-size tail of 28 amino acids.

Since the Vpx protein is present in mature virions, we wanted to investigate whether addition of a signal for the retention in the endoplasmic reticulum to the Vpx protein would change the viral phenotype in infected cells. The amino acid sequence Lys-Asp-Glu-Leu (KDEL) has been shown to anchor proteins to the endoplasmic reticulum (20, 23), and a KDEL receptor has been recently identified (30). The ISY16 mutant was constructed by subcloning the proviral *Bgl*II-*Sac*I fragment of pPN in M13 and then using the M13 mutagenesis kit (Bio-Rad). The mutagenic primer CCA GGT TTA GTC AAG GAT GAA CTA TAA TGA CTG AAG CAC was used to add four amino acids (KDEL) at the carboxyl terminus of the Vpx protein. The mutations introduced in the proviral DNA were verified by DNA sequencing after introduction of the *Bgl*II-*Sac*I fragment into the pEGP DNA before reconstitution of the entire proviral DNA. The HUT78 cell line was transfected with 10 μg of either mutated or wild-type proviral DNA by the DEAE dextran technique to generate viral particles (12). All three mutant proviruses yielded viral protein production and syncytium formation in the transfected cells, indicating that lack of the Vpx protein does not impair viral replication and propagation (data not shown). The supernatants of transfected HUT78 cells were collected and filtered through a 0.22-μm-pore-size filter and used to infect either HUT78 or SupT1 cells to generate viral stocks for infectivity assays.

* Corresponding author.

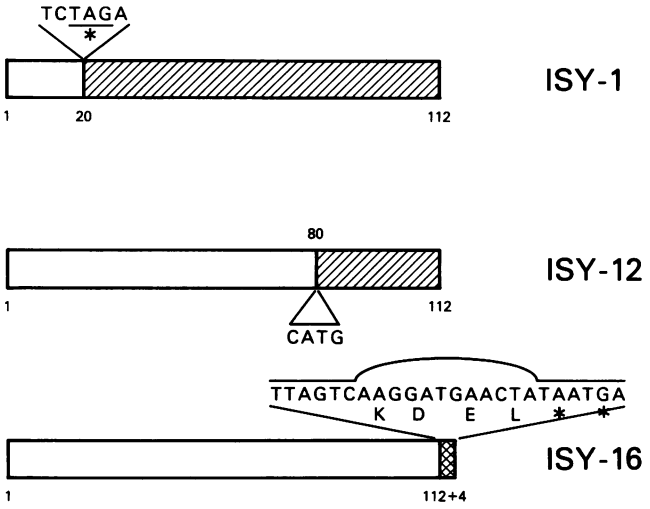

FIG. 1. Graphic representation of *vpx* gene mutant viruses. The bars represent the *vpx* gene. The numbers refer to amino acids of the *vpx* ORF, starting from the first initiating AUG codon (methionine). The letters represent the nucleotides inserted or deleted in the *vpx* ORFs of the mutated viruses. Mutant ISY1 was constructed by insertion of nucleotides TCTAGA, which contain an in-frame stop codon (underlining an asterisk). Mutant ISY12 was generated by deletion of the nucleotides CATG. Mutant ISY16 was constructed by insertion of 12 nucleotides (indicated under the curved line) that encode the four amino acids KDEL before the two naturally existing stop codons of the Vpx protein (asterisks). K, lysine; D, aspartic acid; E, glutamic acid; L, leucine.

To verify the genetic stability of the viral mutants, genomic DNA was extracted from chronically infected cells at 1 and 3 months posttransfection, and the polymerase chain reaction (PCR) technique was used to amplify a region of 589 bp containing the *vpx* gene. The two primers (5′ GTC CAA GGT ACC GTC AC3′ and 5′ CCC TAA TGA GCT CTC TGG 3′) used for the amplification procedure overlapped the natural *Kpn*I and *Sac*I restriction sites of HIV-$2_{sbl/isy}$. Since the *Nsi*I site was eliminated in ISY12 and a novel *Xba*I site was introduced in ISY1, the presence or absence of these restriction sites is diagnostic for these mutations. The PCR DNA products were cleaved with the appropriate restriction endonucleases, electrophoresed on a 1.5% agarose gel, and transferred to a nitrocellulose filter (17). The filters were hybridized either with a ^{32}P-labeled HIV-2 probe encompassing the 0.9-kb *Bgl*II-*Sac*I fragment or with labeled oligonucleotides. Figure 2 shows that cleavage of isogeneic HIV-$2_{sbl/isy}$ DNA with *Nsi*I yielded the expected two comigrating bands of 295 and 294 kb upon hybridization, whereas no size reduction of the 589-bp PCR product was observed in ISY12. Conversely, upon *Xba*I cleavage, a reduction in size was seen in amplified ISY1 DNA but not in wild-type DNA. Various internal controls for the cleavage conditions were also performed to ensure that the lack of cleavage was not due to impurities in the DNA (data not shown). To monitor the stability of the ISY16 mutation, the PCR products from the proviral DNAs of the HIV-$2_{sbl/isy}$ and *vpx* mutants were first hybridized with the HIV-2 probe (Fig. 3A), demonstrating the presence of the entire 589-bp fragment in the wild-type and mutant DNAs. The same filter was stripped and rehybridized with an isogeneic oligonucleotide probe corresponding to the 3′ end of the *vpx* gene of HIV-$2_{sbl/isy}$ (5′ CCA GGT TTA GTC AAG GAT GAA CTA TAA TGA CTG AAG CAC 3′). This oligonucleotide failed to anneal to amplified ISY16 DNA (Fig. 3B), although it hybridized to the two DNA fragments from the other viral mutants. However, only the PCR product from ISY16 annealed when the labeled

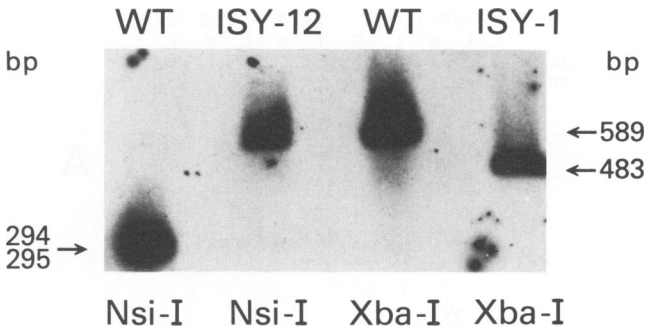

FIG. 2. Southern blot analysis of PCR-amplified DNA fragments obtained from SupT1 cells infected with HIV-$2_{sbl/isy}$ and *vpx* mutant viruses. The virus name is at the top of each lane. WT indicates isogeneic HIV-$2_{sbl/isy}$, and ISY1 and ISY12 indicate the respective mutated viruses. The restriction endonuclease used to cleave the PCR DNA product appears at the bottom of each lane. The size of the uncleaved product is 589 bp. The probe used in this experiment (B8) is a *Bgl*II-*Sac*I fragment containing the DNA sequence from 4970 to 5870 of the HIV-$2_{sbl/isy}$ genome (5).

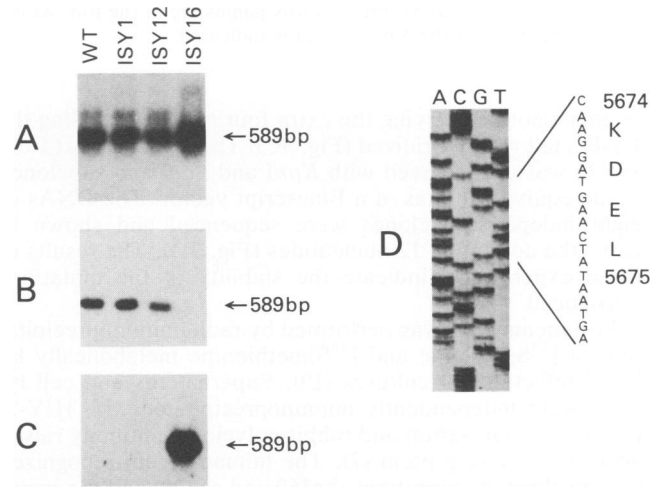

FIG. 3. Southern blot analysis of PCR-amplified DNA fragments obtained from SupT1 cells infected with HIV-$2_{sbl/isy}$ and *vpx* mutant viruses (A, B, and C) and sequence analysis of the PCR product of *vpx* mutant virus ISY16 (D). (A, B, and C) The virus name is at the top of each lane in panel A (WT, wild type). The filter was serially hybridized with the B8 probe (5) (A), and end-labeled oligonucleotide (5′ CCA GGT TTA GTC TAA TGA CTG AAG 3′) corresponding to a DNA sequence overlapping the wild-type 3′ end of the *vpx* gene (B), and the end-labeled oligonucleotide (5′ CCA GGT TA GTC AAG GAT GAA CTA TAA TGA CTG AAG CAC 3′) used to generate mutant ISY16 containing the 12 additional nucleotides before the *vpx* gene termination codons (C); 589 bp is the size of the uncleaved PCR-amplified product. (D) The autoradiograph represents the nucleotide sequence of the 3′ end of *vpx* mutant virus ISY16. The nucleotide designation appears at the top of each lane (A, adenine; C, cytosine; G, guanine; T, thymine). The nucleotide sequence includes the 12 nucleotides inserted between nucleotides 5674 and 5675 which encode lysine (K), aspartic acid (D), glutamic acid (E), and leucine (L).

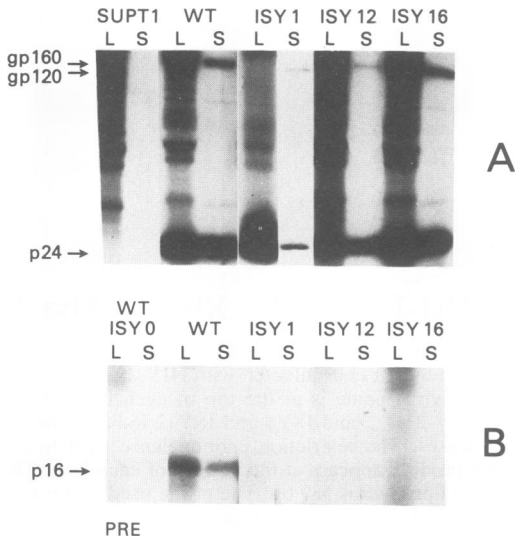

FIG. 4. Radioimmunoprecipitation of viral proteins from SupT1 cells uninfected and infected with wild-type HIV-$2_{sbl/isy}$ and *vpx* mutant viruses. Cell lysates and supernatants (L and S, respectively) of uninfected and infected cell cultures were reacted (19) with human HIV-2 immune serum (A) and rabbit serum raised against the recombinant Vpx protein (7) (B). (A) At the top, SupT1 indicates the uninfected cell culture, and the other notations indicate the viruses (WT, wild type). At the left, the positions of the major viral proteins are indicated. (B) In the first two lanes, the cell lysate and supernatant of a culture infected with the wild-type virus were reacted with preimmune rabbit serum (PRE). Virus names are at the top. At the left, the position of the Vpx protein is indicated.

oligonucleotide carrying the extra four triplets encoding the KDEL tail was hybridized (Fig. 3C). The PCR product from ISY16 was also cleaved with *Kpn*I and *Sac*I and subcloned at the equivalent sites of a Bluescript vector. The DNAs of eight independent clones were sequenced and shown to carry the additional 12 nucleotides (Fig. 3D). The results of these experiments indicate the stability of the mutations introduced.

Protein analysis was performed by radioimmunoprecipitation of [^{35}S]cysteine and [^{35}S]methionine metabolically labeled infected cell cultures (19). Supernatants and cell lysates were independently immunoprecipitated with HIV-2-positive human serum and rabbit polyclonal antibody raised against the Vpx protein (7). The human serum recognized the envelope glycoproteins (gp160 and gp120) and the major *gag*-encoded antigen, p24, in all of the cells infected with the viral mutants (Fig. 4A), indicating that the mutations in the *vpx* gene did not interfere with viral protein expression in the SupT1 cell line. The polyclonal rabbit serum raised against a recombinant Vpx protein recognized the 16-kDa protein only in the supernatant and the lysate of cells infected with the wild-type virus and not in cells infected with the *vpx* mutant viruses (Fig. 4B). The results obtained with the ISY1 mutant were predictable, since the introduced mutation eliminated 80% of the Vpx protein, but those obtained with ISY12 and ISY16 were not. In ISY12, the last 31 amino acids of the Vpx protein were substituted with 28 amino acids encoded by another reading frame, and detection of a protein similar in size to Vpx in both the lysates and supernatants of infected cells was expected. In ISY16, because of addition of the KDEL sequence retention signal, we expected the presence of the Vpx protein in the cell lysate but not in the infected cell supernatant. The failure to immunoprecipitate the Vpx protein from both the cell lysates and supernatants of ISY12- and ISY16-infected cells was surprising. It is unlikely that the rabbit immune serum recognizes only epitopes resident within the last 28 amino acids of the Vpx protein, since the rabbits were immunized with the entire Vpx protein and most of the immunogenic hydrophilic domains are located in the first portion of the Vpx protein. It is possible that introduction of extraneous amino acids at the carboxyl terminus in both ISY12 and ISY16 induced conformational changes which could interfere with antibody recognition. Alternatively, the modifications introduced in both the ISY12 and ISY16 mutants could render the Vpx protein particularly unstable and therefore subject it to rapid intracellular degradation.

To investigate the role of the Vpx protein in viral replication, we infected primary peripheral blood mononuclear cells (PBMCs). A total of 1.5×10^6 PBMCs obtained from two different human donors were phytohemagglutinin stimulated for 3 days and infected with 2×10^5 cpm of reverse transcriptase (RT) activity per ml for each virus. Each culture was monitored for viral expression by measuring the RT activity in the supernatant and by radioimmunoprecipitation of metabolically labeled viral proteins. The results of experiment 1 (Fig. 5) indicated a delay in the appearance of the peak RT activity in all cultures infected with the *vpx*-negative mutants compared with those infected with HIV-$2_{sbl/isy}$ (15 versus 11 days). To ascertain whether a delay in virus release from infected cells or a delay in the synthesis of viral proteins occurred, we metabolically labeled viral proteins after infection. In experiment 2, radioimmunoprecipitation of total cellular proteins with HIV-2 immune serum showed a lower amount of intracellular p24 present at days 9 through 14 in cultures infected with the *vpx* mutant viruses than in cultures infected with isogeneic HIV-$2_{isy/isy}$ (Fig. 5). These results parallel the RT activity data from experiment 1 and indicate that mutations that interfere with the production (ISY1), or perhaps the metabolism (ISY12 and ISY16), of the Vpx protein decrease the viral replication rate in the early days of infection. However, in another experiment, performed with PBMCs from a third blood donor, such a clear delay in the *vpx* mutant was not observed.

When SupT1 cells were used as targets in infectivity assays, no differences in the rate of protein synthesis or virus release in the medium were detected. Similarly, productive infection of human macrophages was obtained readily with the *vpx*-negative viruses, as shown by radioimmunoprecipitation of de novo-synthesized viral proteins from cell lysates and supernatants of infected cultures (Fig. 5C). In summary, we confirmed and extended the observation that introduction of stable mutations in the HIV-2 *vpx* gene does not alter viral infection and replication in human PBMCs or in enriched macrophage cultures (27, 31). However, we and others (11) observed a delay in viral production in human PBMCs. The results presented in the cited study were obtained with a *nef*-defective molecular clone. The quantitative differences between the results of the two studies could be due to an intrinsic difference in the replicative abilities of the two clones. Modifications introduced at the carboxyl terminus of the Vpx protein confer the same viral phenotype displayed when almost the entire *vpx* ORF is eliminated in HIV-$2_{sbl/isy}$, suggesting that the protein carboxyl terminus is important for its function.

The delay in viral replication observed in human PBMC infection was not observed when a neoplastic cell line was used as the target, indicating that the target cell background

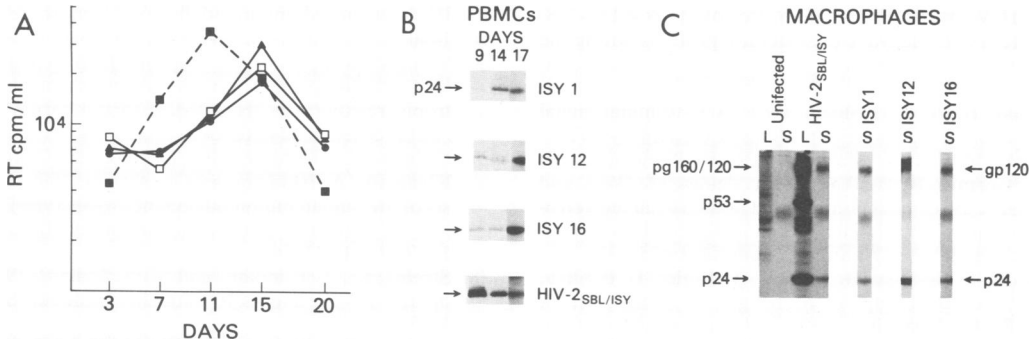

FIG. 5. Kinetics of viral infection in human PBMCs (A) and radioimmunoprecipitation in human PBMCs (B), and macrophages (C) infected with wild-type HIV-$2_{sbl/isy}$ and *vpx* mutant viruses. (A) RT activity detected in the supernatant of infected PBMCs at different days during infection. Symbols: ■, wild-type virus; ▲, ISY1; ●, ISY12; □, ISY16. (B) Radioimmunoprecipitation of total cellular proteins with human HIV-2 immune serum from infected PBMCs on different days during infection. The virus name is on the right of each panel, and the position of the major Gag protein is indicated on the left. The arrows point at the protein bands. (C) Total cellular proteins from uninfected and infected macrophages were metabolically labeled on day 25 postinfection and immunoprecipitated (19) with human HIV-2 immune serum. The first two lanes contained uninfected macrophages, and the rest contained the viruses indicated (L, lysate; S, supernatant). The positions of viral proteins are indicated at the right and left. The arrows point at the protein bands.

can influence viral expression. The significance of the *vpx* gene of HIV-2 in infection of rhesus monkeys is being investigated, and preliminary results indicate that lack of the *vpx* gene does not interfere with viral infectivity in vivo (4a). Whether ablation of the *vpx* gene from the HIV-$2_{sbl/isy}$ virus, which is nonpathogenic in rhesus monkeys, can modulate its pathogenicity is a subject of active investigation.

We are grateful to Randy Zicht for technical assistance and Oliviero Varnier for the gift of HIV-2 immune serum.

REFERENCES

1. **Barré-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vexinet-Brun, C. Rouzioux, W. Rozenbaum, 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. **Clavel, F., D. Guetard, F. Brun-Vezinet, 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 human retrovirus from West African patients with AIDS. Science **233**:343–346.
3. **Cohen, E. A., E. F. Terwilliger, J. K. Sodroski, and W. A. Haseltine.** 1988. Identification of a protein encoded by the vpu gene of HIV-1. Nature (London) **334**:532–534.
4. **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 T-cell tropic HTLV-III-like retrovirus from macaques. Science **228**:1201–1204.

4a. **Franchini, G., et al.** Unpublished data.

5. **Franchini, G., K. A. Fargnoli, F. Giombini, L. Jagodzinski, A. De Rossi, M. Bosch, G. Biberfeld, E. M. Fenyo, J. Albert, R. C. Gallo, and F. Wong-Staal.** 1989. Molecular and biological characterization of a replication competent human immunodeficiency type 2 (HIV-2) proviral clone. Proc. Natl. Acad. Sci. USA **86**:2433–2437.
6. **Franchini, G., P. Markham, E. Gard, K. Fargnoli, S. Kubaruwa, L. Jagozinsky, M. Robert-Guroff, P. Lusso, G. Ford, F. Wong-Staal, and R. C. Gallo.** 1990. Persistent infection of rhesus macaques with a molecular clone of HIV-2: evidence of minimal genetic drift and low pathogenetic effects. J. Virol. **64**:4462–4467.
7. **Franchini, G., J. R. Rusche, T. J. O'Keeffe, and F. Wong-Staal.** 1988. The human immunodeficiency virus type 2 (HIV-2) contains a novel gene encoding a 16 kD protein associated with mature virions. AIDS Res. Hum. Retroviruses **4**:243–250.
8. **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 (Cercocebus arys). Proc. Natl. Acad. Sci. USA **83**:5286–5290.
9. **Gallo, R. C., S. Z. Salahuddin, M. Popovic, G. M. Shearer, M. Kaplan, B. F. Haynes, T. J. Palker, R. Redfield, J. Oleske, B. Safai, G. White, P. Foster, and P. D. Markham.** 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science **224**:500–503.
10. **Gottlieb, M. S., R. Schroff, H. M. Schanler, J. D. Weismere, P. T. Fan, R. A. Weld, and A. Saxon.** 1981. Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men. Evidence of a new acquired cellular immunodeficiency. N. Engl. J. Med. **305**:1426–1431.
11. **Guyader, M., M. Emerman, L. Montagnier, and K. Peden.** 1989. VPX mutants of HIV-2 are infectious in established cell lines but display a severe defect in peripheral blood lymphocytes. EMBO J. **8**:1169–1175.
12. **Hattori, N., F. Michaels, K. Fargnoli, L. Marcon, R. C. Gallo, and G. Franchini.** 1990. The HIV-2 vpr gene is essential for productive infection of human macrophages. Proc. Natl. Acad. Sci. USA **87**:8080–8084.
13. **Henderson, L. E., R. C. Sowder, T. D. Copeland, R. E. Benveniste, and S. Oroszlan.** 1988. Isolation and characterization of a novel protein (x-ORF product) from SIV and HIV-2. Science **241**:199–201.
14. **Huet, T., R. Cheynier, A. Meyerhans, G. Roelants, and S. Wain-Hobson.** 1990. Genetic organization of a chimpanzee lentivirus related to HIV-1. Nature (London) **345**:356–359.
15. **Kanki, P. J., F. Barin, S. M'Boup , J. S. Allan, J. L. Romet-Lemonne, R. Marlink, M. F. McLane, T.-H. Lee, B. Arbeille, F. Denis, and M. Essex.** 1986. New human T-lymphotrophic retrovirus related to simian T-lymphotrophic virus III (STLV-III_{agm}). Science **232**:238–243.
16. **Kestler, H., T. Kodama, D. Ringler, M. Marthas, N. Pedersen, A. Lackner, D. Regier, P. Sehgal, M. Daniel, N. King, and R. Desrosiers.** 1990. Induction of AIDS in rhesus monkeys by molecularly cloned simian immunodeficiency virus. Science **248**:1109–1112.
17. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning; a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Marlink, R., D. Ricard, S. M'Boup, P. Kanki, J. L. Romet-Lemonne, I. N'Doye, M. A. Simpson, F. Greco, and M. J. Chou.** 1988. Clinical, hematologic, and immunologic cross-sectional evaluation of individuals exposed to human immunodeficiency

virus type 2 (HIV-2). AIDS Res. Hum. Retroviruses **4**:137–148.
19. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
20. **Munro, S., and H. R. B. Pelham.** 1987. A C-terminal signal prevents secretion of luminal ER proteins. Cell **48**:899–907.
21. **Murphey-Corb, M., L. N. Martin, S. R. S. Rangan, G. B. Baskin, B. J. Gormus, R. H. Wolf, W. A. Andes, M. West, and R. C. Montelaro.** 1986. Isolation of an HTLV-III-related retrovirus from macaques with simian AIDS and its possible origin in asymptomatic mangabeys. Nature (London) **321**:435–437.
22. **Naidu, Y. M., H. W. Kestler III, Y. Li, C. V. Butler, D. P. Silva, D. K. Schmidt, C. D. Troup, P. K. Sehgal, P. Sonigo, M. D. Daniel, and R. C. Desrosiers.** 1988. Characterization of infectious molecular clones of simian immunodeficiency virus (SIV_{mac}) and human immunodeficiency virus type 2: persistent infection of rhesus monkeys with molecularly cloned SIV_{mac}. J. Virol. **62**:4691–4696.
23. **Paabo, S., B. M. Bhat, W. S. M. Wold, and P. A. Peterson.** 1987. A short sequence in the coho-terminus makes an adenovirus membrane glycoprotein a resident of the endoplasmic reticulum. Cell **50**:311–317.
24. **Popovic, M., M. G. Sarngadharan, E. Read, and R. C. Gallo.** 1984. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science **224**:497–500.
25. **Safai, B., M. G. Sarngadharan, J. E. Groopman, K. Arnett, M. Popovic, A. Sliski, J. Schupbach, and R. C. Gallo.** 1984. Seroepidemiological studies of human T-lymphotropic retrovirus type III in acquired immunodeficiency syndrome. Lancet **i**:1438–1440.
26. **Sarngadharan, M. G., M. Popovic, L. Bruch, J. Schupbach, and R. C. Gallo.** 1984. Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS. Science **224**:506–508.
27. **Shibata, R., T. Miura, M. Hayami, K. Ogawa, H. Sakai, T. Kiyomasu, A. Ishimoto, and A. Adachi.** 1990. Mutational analysis of the human immunodeficiency virus type 2 (HIV-2) genome in relation to HIV-1 and simian immunodeficiency virus SIV_{agm}. J. Virol. **64**:742–747.
28. **Strebel, K., T. Klimkait, and M. A. Martin.** 1988. A novel gene of HIV-1, vpu, and its 16-kilodalton product. Science **241**:1221–1223.
29. **Tsujimoto, H., A. Hasegawa, N. Maki, M. Fukasawa, T. Miura, S. Speidel, R. W. Cooper, E. Moriyama, T. Gojobori, and M. Hayami.** 1989. Sequence of a novel simian immunodeficiency virus from a wild-caught African mandrill. Nature (London) **341**:539–541.
30. **Vauz, D., J. Tooze, and S. Fuller.** 1990. Identification by anti-idiotype antibodies of an intracellular membrane protein that recognizes a mammalian endoplasmic reticulum retention signal. Nature (London) **345**:495–502.
31. **Wen, H., N. Vander Heyden, and L. Ratner.** 1989. Analysis of the function of viral protein x(vpx) of HIV-2. Virology **173**:624–630.
32. **Yu, X.-F., S. Ito, M. Essex, and T.-H. Lee.** 1988. A naturally immunogenic virion-associated protein specific for HIV-2 and SIV. Nature (London) **335**:262–265.