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Expression of Envelope Glycoproteins of Human Immunodeficiency Virus by an Insect Virus Vector

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The envelope gene of human immunodeficiency virus was inserted into the genome of an insect virus vector (*Autographa californica* nuclear polyhedrosis virus). Upon infection of tissue culture cells, this recombinant virus produced immunoreactive polypeptides related to the envelope glycoproteins of human immunodeficiency virus. Serological survey indicates such polypeptides would be of value as antigens in diagnostics for acquired immunodeficiency syndrome.

Since the identification of a human retrovirus (human immunodeficiency virus [HIV]) (3, 17, 23) as the etiological agent for acquired immunodeficiency syndrome (AIDS), there has been much interest in using HIV virion proteins for diagnostic tests and possibly for vaccine formulations. Proteins produced by recombinant DNA techniques offer attractive alternatives to virion-derived materials because of their specificity and ease of handling. Several expression systems have been used to produce the envelope (*env*) and the core (*gag*) antigens of HIV. They include *Escherichia coli* (5–8, 28, 32), yeast (13), and mammalian cells (4, 11, 12, 16). In recent years, an insect virus, baculovirus, has also been developed as an expression system (22, 29). Besides the high level of expression obtainable, this system offers the opportunity to study the processing and the biological properties of proteins of mammalian origins in insect cells. The expression and processing of HIV *gag* antigens by baculovirus was recently reported (19). In this communication, we report the expression of the *env* gene of HIV in insect cells by a baculovirus vector and the possible application of such recombinant-produced protein in an AIDS diagnostic test.

The genomes of several isolates of HIV have been cloned (1, 9, 18), and their nucleotide sequences have been determined (21, 25, 27, 33). Besides the typical retroviral genes (*gag*, *pol*, and *env*), the genome of HIV also encodes at least two novel regulatory genes (*tat* and *art*) (30, 31) and two genes of unknown functions (*sor* and 3′ *orf*) (21, 25, 27, 33). We chose to express the *env* gene of HIV, not only because envelope glycoproteins are the most commonly detected antigens by HIV-positive sera (2, 5, 6, 28), but also because these proteins are known to elicit neutralizing (16, 26) and T-cell-mediated immunity in immunized animals (35). The *env* gene encodes a precursor glycoprotein of 150 to 160 kilodaltons (kDa) (gp150/160), which is cleaved to mature virion glycoproteins of 110 to 120 kDa (gp110/120) and 41 kDa (gp41) (21, 25, 27, 33).

To insert the *env* gene of HIV into the genome of the baculovirus *Autographa californica* nuclear polyhedrosis virus (AcNPV), we used a generalized method for the construction of recombinant baculoviruses (20, 22, 29). Briefly, foreign DNA is first inserted into a plasmid vector (pAc610) downstream from the promoter for the baculovirus polyhedrin gene. The chimeric gene in the recombinant plasmid is flanked by additional AcNPV sequences around the polyhedrin gene. This plasmid is then transfected with AcNPV DNA into insect tissue culture cells. Homologous recombination between the plasmid and the viral DNA occurs in the regions flanking the chimeric gene and allows its insertion into the genome of AcNPV. The resultant virus will lack the ability to synthesize polyhedrin and therefore will exhibit an occlusion-negative phenotype in plaque assays. Using this approach, we constructed a recombinant virus, Ac-env5 (Fig. 1), which contains the entire envelope coding sequence of lymphadenopathy-associated virus (LAV) (3), an isolate of HIV. The HIV insert in this recombinant virus also contains 96 and 253 base pairs of 5′ and 3′ untranslated sequences, respectively. The initiation codon of the *env* gene is located 177 base pairs downstream from the usual start site(s) of the polyhedrin transcript (29). Since this codon is most proximal to the 5′ terminus of the transcript, translation initiated at this codon will result in expression of the HIV envelope protein with no additional sequences from the baculovirus polyhedrin gene.

To determine whether the *env* gene was expressed by the recombinant virus, we infected insect cells (*Spodoptera frugiperda*) with Ac-env5 and used Western blots (immunoblots) to detect HIV-specific proteins in the infected cell lysates. Three major groups of proteins immunoreactive with HIV-positive human serum were detected in the Ac-env5–infected cells (Fig. 2, lanes 1 and 2) but not in the AcNPV-infected cells (Fig. 2, lanes 10 and 11). The three groups of proteins consisted of a doublet with a molecular size of about 150 kDa, another doublet of about 120 to 130 kDa, and a diffuse band of about 41 kDa. The 150-kDa proteins reacted with HIV-positive human serum as well as with monoclonal sera against LAV gp110 or gp41 (Fig. 2, lanes 1, 7, and 8). These proteins therefore represented the recombinant-made analogs to the precursor envelope glycoprotein gp150 of LAV. The 120- to 130-kDa and the 41-kDa proteins reacted, respectively, to anti-gp110 (Fig. 2, lane 7) and anti-gp41 (Fig. 2, lane 8) monoclonal antibodies and probably represented the recombinant-made analogs of gp110 and gp41, respectively. The time course of appearance of the 150-, 120- to 130-, and 41-kDa proteins was consistent with the notion that the 150-kDa protein was the precursor from which the lower-molecular-size species were derived (Fig. 2, lanes 1 to 4). Minor bands of immunoreactive proteins with molecular sizes of about 92, 65, 43, and 35 kDa were also observed. The identities of these proteins are not

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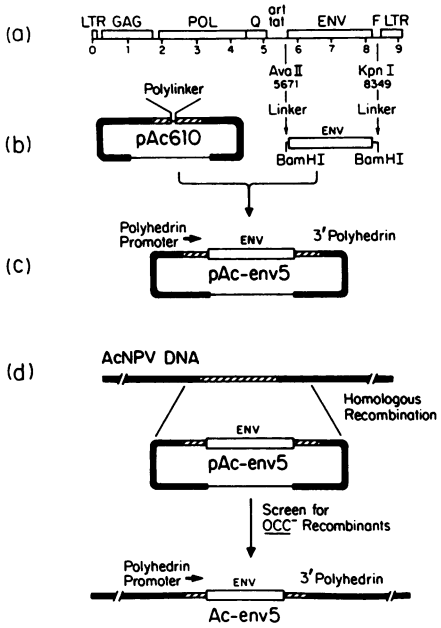

FIG. 1. Construction of recombinant baculoviruses containing the envelope gene of LAV. A portion of the cloned proviral genome of LAV (a), from the *Ava*II site at nucleotide 5671 to the *Kpn*I site at 8349, was isolated (for details, see reference 11). Its termini were converted into *Bam*HI sites by the addition of synthetic linker sequences. This fragment, which contained the entire coding sequence of the *env* gene, was inserted into the *Bam*HI site in the polylinker region of plasmid pAc610 (b). Plasmid pAc610, which is a derivative of pAc461 (J. Luckow and M. Summers, manuscript in preparation), contains AcNPV DNA sequences (▬) which include the polyhedrin gene (▨). The polylinker region is located between the polyhedrin promoter and the 3′ coding sequences of polyhedrin gene. The resulting recombinant plasmid pAc-env5 has the structure shown in panel c. Insertion of the *env* sequence into the AcNPV genome was accomplished by homologous recombination between plasmid pAc-env5 and AcNPV DNA transfected into insect cells. Recombinant virus was recognized as occlusion negative (OCC^-) in plaque assays, and its genome structure is represented in panel d.

clear. They could represent products of partial glycosylation, proteolytic degradation, or incomplete translation. Further analysis is needed to elucidate the processing of the baculovirus-made gp150 analog and to identify the precise cleavage site(s) involved.

Radioisotope labeling with [^{3}H]mannose indicated that the recombinant-made 150-kDa proteins were glycosylated (Fig. 3). It has been shown that insect cells recognize the same glycosylation signals on polypeptides as do mammalian cells, although the pattern of glycosylation appears to be different (10). It remains to be shown whether this is the case for the HIV-*env* proteins made by recombinant baculovirus. Very little, if any, of the 120- to 130-kDa and 41-kDa *env*-related proteins was detected by radioimmunoprecipitation of metabolically labeled cell lysates (Fig. 3). This result could be due to the slow processing of the 150-kDa precursor, since the smaller-molecular-size species did not appear until more than 24 h after the 150-kDa precursor was detected (Fig. 2, lanes 2 and 4). This result is in contrast to the efficient processing observed for the HIV *env* protein expressed by the vaccinia virus vector in mammalian cells (Fig. 3, lane 13). It is interesting that a similarly slow rate was observed for the processing of the baculovirus-expressed hemagglutinin of influenza virus (14). The processing of hemagglutinin also involves the proteolytic cleavage of arginine- or lysine-rich sequences similar to that proposed for the processing of HIV envelope proteins (21, 25, 27, 33). It remains to be tested whether these results reflect the inefficiency of insect cells to process proteins of mammalian origins at this cleavage site(s).

Using an immunoassay specific for HIV envelope glycoproteins gp150 and gp110, we estimated that the amount of immunoreactive protein produced by recombinant infected cells was 1 to 2 mg/liter (data not shown). Although this level is not as high as that achieved for some other proteins expressed by the same vector system (22), it represents a significant increase over the level obtainable with purified HIV virions (23).

Since the recombinant baculovirus produced immunoreactive proteins related to the envelope glycoproteins of HIV, these proteins could be useful as antigens in diagnostic tests for AIDS. To demonstrate this potential, we used infected cell lysate as antigens in a solid-phase immunoassay to screen a panel of 48 serum samples. This panel included sera from patients with AIDS, AIDS-related complex, or lymphadenopathy syndrome, and sera from other high-risk individuals, as well as sera from healthy normal donors. This immunoassay correctly identified all 35 positive and 13 negative samples previously classified by Western blot and enzyme-linked immunoassays on the basis of disrupted virions (Fig. 4). The serum sample that gave the lowest positive reading in this immunoassay reacted weakly to gp110 but not to gp41 in Western blots, indicating the comparable sensitivity of these two assays in detecting antibodies against envelope glycoproteins.

Antibodies against envelope antigens appeared to be one of the best markers for detecting HIV-positive sera (2). Several envelope-related polypeptides have been used as antigens in immunoassays, with a high degree of specificity

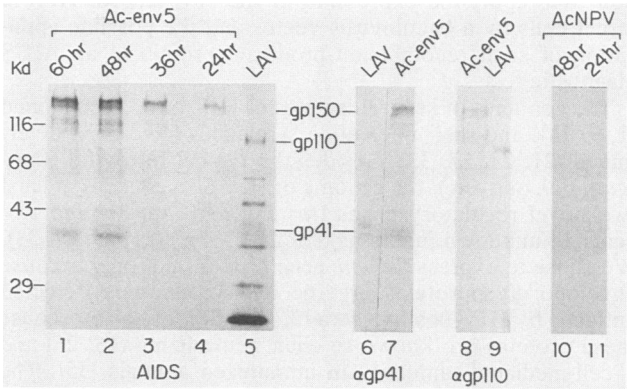

FIG. 2. Western blot analysis of *env*-related proteins expressed by a recombinant baculovirus. Insect cells were infected with recombinant virus Ac-env5 (lanes 1 through 4, 7, and 8) or its parental strain AcNPV (lanes 10 and 11), both at a multiplicity of infection of 5. At the indicated times after infection, cells were harvested and lysed by being boiled in Laemmli sample buffer (15). Total cellular proteins were resolved on a 10% sodium dodecyl sulfate-polyacrylamide gel and electrotransferred onto nitrocellulose filters. Proteins immobilized on filters were reacted with HIV-positive human sera (lanes 1 through 5, 10, and 11) or with monoclonal antibodies against gp41 (lanes 6 and 7) or gp110 (lanes 8 and 9). Immunoreactive proteins were detected by goat anti-immunoglobulin antibodies conjugated with alkaline phosphatase (Zymed). Purified LAV virion proteins were used as markers in lanes 5, 6, and 8. kd, Kilodaltons.

and sensitivity (5, 6, 28, 34; W. L. Cosand, U.S. Patent 4,629,783, Dec., 1986). Results from the present study indicated the same potential for the envelope-related glycoproteins produced by the recombinant baculovirus. It is important that a relatively low cutoff value (0.18) and a high signal-to-background ratio (average, >9) were obtained with crude infected cell lysate without any purification. This result is probably related to the fact that the baculovirus is only known to infect cells of invertebrate origins. In this regard, baculovirus-produced proteins may have certain advantages over *E. coli*-produced proteins, which require purification before being used as antigens in assays.

Another important feature of the baculovirus-produced proteins is that they contain the entire amino acid sequence of the envelope protein of HIV. This sequence might allow a more complete representation of both linear and conformational epitopes than do *E. coli*-produced or chemically synthesized peptides. On the other hand, it is known that glycosylation in insect cells follows different patterns than those in mammalian cells. It is not clear how this difference in glycosylation will affect the antigenic property of the baculovirus-made proteins. A more thorough study is required to determine whether any of these factors could contribute to the relative merits of the baculovirus-produced proteins compared with HIV virion proteins or other recombinant-made analogs as antigens in immunoassays.

The purified envelope glycoprotein (gp120) of HIV (25) and its recombinant-made analogs (16, 24) have also been used to immunize animals which subsequently developed neutralizing antibodies. These findings raised the possibility that gp120 or its recombinant analogs would be useful as immunogens in the formation of a vaccine against AIDS.

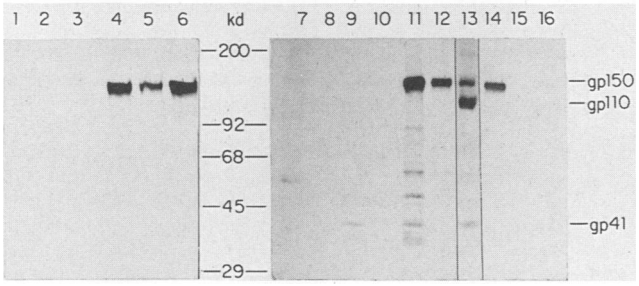

FIG. 3. Radioimmunoprecipitation analysis of *env*-related proteins expressed by recombinant baculovirus. Insect cells were mock infected (lanes 7, 8, and 16), infected with recombinant baculovirus Ac-env5 (lanes 4 to 6, 11, 12, and 14), or infected with parental wild-type virus (lanes 1 to 3, 9, 10, and 15) at a multiplicity of infection of 5. At 40 h postinfection, cultures were either labeled for 16 h with 160 μCi of [^{3}H]mannose per ml (54 Ci/mmol; Amersham; lanes 1 through 6) or for 2 h with 100 μCi of [^{35}S]methionine per ml (>800 Ci/mmol; Amersham; lanes 7 through 12 and 14 through 16). At the end of the labeling period, cells were harvested and lysed in 0.01 M Tris (pH 7.4)–0.1 M NaCl–1% Nonidet P-40–0.5% deoxycholate–1 mM EDTA. Cell lysates were reacted with HIV-positive human sera (lanes 3, 6, and 14 through 16) and with monoclonal antibody against gp110 (lanes 1, 4, 7, 9, and 11) or against gp41 (lanes 2, 5, 8, 10, and 12). Immunoprecipitated proteins were resolved on a 10% sodium dodecyl sulfate-polyacrylamide gel and detected by fluorography. Lane 13 contained proteins from African green monkey kidney cells (BSC-1) infected by recombinant vaccinia virus v-env5 (11), at a multiplicity of infection of 10. Cells were labeled with [^{35}S]methionine for 2 h, starting at 10 h postinfection. At the end of the labeling period, cells were harvested and lysed and the lysate was reacted with HIV-positive human sera. kd, Kilodaltons.

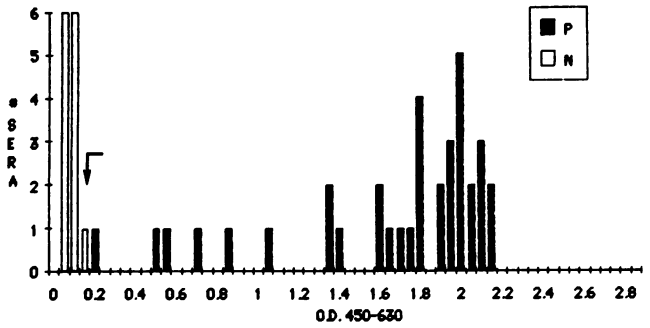

FIG. 4. Immunoassay with lysates from recombinant baculovirus-infected cells as antigens. Insect cells were infected with recombinant baculovirus Ac-env5 at a multiplicity of infection of 5. Two days later, infected cells were harvested, washed in phosphate-buffered saline, and lysed in phosphate-buffered saline–0.5% Nonidet P-40. Crude lysate was frozen and thawed twice and cleared by low-speed centrifugation. Cleared lysate was diluted 400-fold with carbonate buffer (15 mM Na_2CO_3–35 mM $NaHCO_3$, pH 9.6), and samples were used to coat the wells of microtiter plates (~0.3 μg of total protein per well). Antigen immobilized on these wells was reacted with a panel of human sera previously characterized as HIV-positive (P) or HIV-negative (N) by whole-virus enzyme-linked immunosorbent assays and Western blots. HIV antibodies were detected by goat anti-immunoglobulin antibodies conjugated with horseradish peroxidase. Reactivity was expressed as optical density (O.D.) values after a standard chromogen reaction. To control for background variations of individual wells, optical density values are expressed as the difference of the absorbances measured at 450 and 630 μm. The arrow indicates the cutoff value (0.18), which is defined as the mean optical density value of negative sera plus 3 standard deviations.

Studies are in progress to determine whether the envelope glycoprotein analogs produced by the recombinant baculovirus will have similar immunogenic properties.

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LITERATURE CITED

1. **Alizon, M., P. Sonigo, F. Barre-Sinoussi, J. C. Chermann, P. Tiollais, L. Montagnier, and S. Wain-Hobson.** 1984. Molecular cloning of lymphadenopathy-associated virus. Nature (London) **312**:757–760.
2. **Barin, F., M. F. McLane, J. S. Allan, T. H. Lee, J. E. Groopman, and M. Essex.** 1985. Virus envelope protein of HTLV-III represents major target antigen for antibodies in AIDS patients. Science **228**:1094–1096.
3. **Barre-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Brun-Vezinet, 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.
4. **Chakrabarti, S., M. Robert-Guroff, F. Wong-Staal, R. C. Gallo, and B. Moss.** 1986. Expression of the HTLV-III envelope gene by a recombinant vaccinia-virus. Nature (London) **320**:535–537.
5. **Chang, T. W., I. Kato, S. McKinney, P. Chanda, A. D. Barone, F. Wong-Staal, R. C. Gallo, and N. Chang.** 1985. Detection of antibodies to human T-cell lymphotropic virus III (HTLV-III) with an immunoassay employing a recombinant *Escherichia coli* derived viral antigenic peptide. Bio/Technology **3**:905–909.
6. **Crowl, R., K. Ganguly, M. Gordon, R. Conroy, M. Schaber, R. Kramer, G. Shaw, F. Wong-Staal, and E. P. Reddy.** 1985. HTLV-III *env* gene products synthesized in *E. coli* are recog-

nized by antibodies present in the sera of AIDS patients. Cell **41**:979–986.

7. **Dowbenko, D. J., J. R. Bell, C. V. Benton, J. E. Groopman, H. Nguyen, D. Vetterlein, D. J. Capon, and L. A. Lasky.** 1985. Bacterial expression of the acquired immunodeficiency syndrome retrovirus p24 *gag* protein and its use as a diagnostic reagent. Proc. Natl. Acad. Sci. USA **82**:7748–7752.
8. **Ghrayeb, J., I. Kato, S. McKinney, J. J. Huang, P. K. Chanda, D. D. Ho, M. G. Sarangadharan, T. W. Chang, and N. T. Chang.** 1986. Human T-cell lymphotropic virus type III (HTLV-III) core antigens: synthesis in *Escherichia coli* and immunoreactivity with human sera. DNA **5**:93–99.
9. **Hahn, B. H., G. M. Shaw, S. K. Arya, M. Popovic, R. C. Gallo, and F. Wong-Staal.** 1984. Molecular cloning and characterization of the HTLV-III virus associated with AIDS. Nature (London) **312**:166–169.
10. **Hsieh, P., and P. W. Robbins.** 1984. Regulation of asparagine-linked oligosaccharide processing. J. Biol. Chem. **259**:2375–2382.
11. **Hu, S.-L., S. G. Kosowski, and J. Dalrymple.** 1986. Expression of AIDS virus envelope gene in recombinant vaccinia viruses. Nature (London) **320**:537–540.
12. **Kieny, M. P., G. Rautmann, D. Schmitt, K. Dott, S. Wain-Hobson, M. Alizon, M. Girard, S. Chamaret, A. Laurent, L. Montagnier, and J.-P. Lecocq.** 1986. AIDS virus *env* protein expressed from a recombinant vaccinia virus. Bio/Technology **4**:790–795.
13. **Kramer, R. A., M. D. Schaber, A. M. Skalka, K. Ganguly, F. Wong-Staal, and E. P. Reddy.** 1986. HTLV-III *gag* protein is processed in yeast cells by the virus *pol*-protease. Science **231**:1580–1584.
14. **Kuroda, K., C. Hauser, R. Rott, H. D. Klenk, and W. Doerfler.** 1986. Expression of the influenza virus haemagglutin in insect cells by a baculovirus vector. EMBO J. **5**:1359–1365.
15. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
16. **Lasky, L. A., J. E. Groopman, C. W. Fennie, P. M. Benz, D. J. Capon, D. J. Dowbenko, G. R. Nakamura, W. M. Nunes, M. E. Renz, and P. W. Berman.** 1986. Neutralization of the AIDS retrovirus by antibodies to a recombinant envelope glycoprotein. Science **233**:209–212.
17. **Levy, J. A., A. D. Hoffman, S. M. Kramer, J. A. Landis, J. M. Shimabukuro, and L. S. Oshiro.** 1984. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. Science **225**:840–842.
18. **Luciw, P. A., S. J. Potter, K. Steimer, D. Dina, and J. A. Levy.** 1984. Molecular cloning of AIDS-associated retroviruses. Nature (London) **312**:760–763.
19. **Madisen, L., B. Travis, S.-L. Hu, and A. F. Purchio.** 1987. Expression of the human immunodeficiency virus *gag* gene in insect cells. Virology **158**:248–250.
20. **Miyamoto, C., G. E. Smith, J. Farrell-Towt, R. Chizzonite, M. D. Summers, and G. Ju.** 1985. Production of human c-*myc* protein in insect cells infected with a baculovirus expression vector. Mol. Cell. Biol. **5**:2860–2865.
21. **Muesing, M. A., D. H. Smith, C. D. Cabradilla, C. V. Benton, L. A. Lasky, and D. J. Capon.** 1985. Nucleic acid structure and expression of the human AIDS/lymphadenopathy retroviruses. Nature (London) **313**:250–258.
22. **Pennock, G. D., C. Shoemaker, and L. K. Miller.** 1984. Strong and regulated expression of *Escherichia coli* β-galactosidase in insect cells with a baculovirus vector. Mol. Cell. Biol. **4**: 399–406.
23. **Popovic, M., M. G. Sarangadharan, 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.
24. **Putney, S. D., T. J. Matthews, W. G. Robey, D. L. Lynn, M. Robert-Guroff, W. T. Mueller, A. J. Langlois, J. Ghrayeb, S. R. Petteway, K. J. Weinhold, P. J. Fischinger, F. Wong-Staal, R. C. Gallo, and D. P. Bolognesi.** 1986. HTLV-III/LAV-neutralizing antibodies to an *E. coli*-produced fragment of the virus envelope. Science **234**:1392–1395.
25. **Ratner, L., W. Haseltine, R. Patarca, K. Livak, B. Stavcich, S. F. Josephs, E. R. Doran, J. A. Ratalski, E. A. Whitehorn, K. Baumeister, L. Ivanoff, S. R. Petteway, M. L. Pearson, J. A. Lautenberger, T. S. Papas, J. Ghrayeb, N. T. Chang, R. C. Gallo, and F. Wong-Staal.** 1985. Nucleotide sequence of the AIDS virus, HTLV-III. Nature (London) **313**:277–284.
26. **Robey, W. G., L. O. Arthur, T. J. Matthews, A. Langlois, T. D. Copeland, N. W. Lerche, S. Oroszlan, D. P. Bolognesi, R. V. Gilden, and P. J. Fischinger.** 1986. Prospect for prevention of human immunodeficiency virus infection: purified 120 KDa envelope glycoprotein induces neutralizing antibody. Proc. Natl. Acad. Sci. USA **83**:7023–7027.
27. **Sanchez-Pescador, R., M. D. Power, P. J. Barr, K. S. Steimer, M. M. Stempien, S. L. Brown-Shimer, W. Gee, A. Renard, A. Randolph, J. A. Levy, D. Dina, and P. Luciw.** 1985. Nucleotide sequence and expression of an AIDS-associated retrovirus (ARV-2). Science **227**:484–492.
28. **Schulz, T. F., J. M. Aschauer, P. Hengster, C. Larcher, H. Wachter, B. Fleckenstein, and M. P. Dierich.** 1986. Envelope gene-derived recombinant peptide in the serodiagnosis of human immunodeficiency virus infection. Lancet **ii**:111–112.
29. **Smith, G. E., M. D. Summers, and M. J. Fraser.** 1983. Production of human beta interferon in insect cells infected with a baculovirus expression vector. Mol. Cell. Biol. **3**:2156–2165.
30. **Sodroski, J., W. C. Goh, C. Rosen, A. Dayton, E. Terwilliger, and W. A. Haseltine.** 1986. A second post-transcriptional *trans*-activator gene required for HTLV-III replication. Nature (London) **321**:412–417.
31. **Sodroski, J., C. Rosen, F. Wong-Staal, S. Z. Salahuddin, M. Popovic, S. Arya, R. C. Gallo, and W. A. Haseltine.** 1985. *Trans*-acting transcriptional regulation of human T-cell leukemia virus type III long terminal repeat. Science **227**:171–173.
32. **Steimer, K. S., J. P. Puma, M. D. Power, M. A. Powers, C. George-Nascimento, J. C. Stephens, J. A. Levy, R. Sanchez-Pescudor, P. A. Luciw, P. J. Barr, and R. A. Hallewell.** 1986. Differentiated antibody responses of individuals infected with AIDS-associated retroviruses surveyed using the viral core antigen $p25^{gag}$ expressed in bacteria. Virology **150**:283–290.
33. **Wain-Hobson, S., P. Sonigo, O. Danus, S. Cole, and M. Alizon.** 1985. Nucleotide sequence of the AIDS virus, LAV. Cell **40**:9–17.
34. **Wang, J. J. G., S. Steel, R. Risniewolski, and C. Y. Wang.** 1986. Detection of antibodies to human T-lymphotropic virus type III by using a synthetic peptide of 21 amino acid residues corresponding to a highly antigenic segment of gp41 envelope protein. Proc. Natl. Acad. Sci. USA **83**:6159–6163.
35. **Zarling, J. M., W. Morton, P. A. Moran, J. McClure, S. G. Kosowski, and S.-L. Hu.** 1986. T-cell responses to human AIDS virus in macaques immunized with recombinant vaccinia viruses. Nature (London) **323**:344–346.