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CD4-Independent, Productive Infection of a Neuronal Cell Line by Human Immunodeficiency Virus Type 1

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One neuronal cell line (SK-N-MC) was found to be susceptible to productive infection by multiple isolates of the human immunodeficiency virus type 1 (HIV-1). Characterization of SK-N-MC cells showed that these cells are neuroectodermal in origin in that they express dopamine hydroxylase, catecholamines, neuron-specific enolase, and neurofilaments. Despite their susceptibility to HIV-1 infection, SK-N-MC cells had no detectable CD4 and this infection was not blocked by anti-CD4 monoclonal antibodies (OKT4A, Leu3A) or recombinant soluble CD4. These experiments demonstrated that certain cells of neuroectodermal origin are susceptible to infection in vitro by HIV-1 via a CD4-independent mechanism.

Subacute encephalitis, also known as acquired immunodeficiency syndrome encephalopathy or dementia complex, is a frequent and devastating complication of infection by the human immunodeficiency virus type 1 (HIV-1) (29). Available evidence strongly supports HIV-1 as the direct cause of this neurologic syndrome (17, 36, 37). Multiple studies on affected brain tissue have identified the monocyte/macrophage as the predominant cell type in the central nervous system infected by HIV-1 (8, 9, 21, 41). However, glial cells have occasionally been found to harbor HIV-1 in vivo (41), a finding consistent with in vitro studies demonstrating the susceptibility of primary glial cells or glioma cell lines to infection (2–5, 12, 23). With the exception of one patient with severe subacute encephalitis (41), neurons in affected brain tissues were found to be free of HIV-1 infection. Taken together, these findings suggest that HIV-1 may induce neurologic dysfunction and degeneration by indirect mechanisms rather than direct infection and lysis of neuronal cells. Nevertheless, to pursue further the possibility of direct infection of neurons by HIV-1, we examined the susceptibility of neuronal cell lines to infection in vitro by multiple strains of HIV-1.

Tumor cell lines of neuronal origin were obtained from the American Type Culture Collection of Rockville, Md. (SK-N-MC, SK-N-SH, IMR-32) and from Robert Seeger of the University of California at Los Angeles (LA-N-1, LA-N-2, LA-N-5). Each cell line was inoculated with a stock of the HTLV-IIIRF isolate of HIV-1 at multiplicities of infection (MOIs) ranging from 0.002 to 1.0 50% tissue culture infective doses ($TCID_{50}$) per cell. The cultures were then monitored twice weekly for core antigen (p24) production in the supernatant fluid by an antigen-capture assay (Abbott Laboratories, North Chicago, Ill.) during the course of the 13-day experiment. As shown in Table 1, productive HIV-1 infection was not observed for SK-N-SH, LA-N-1, LA-N-2, and LA-N-5 cell lines. Transient p24 antigen production was documented only on day 4 in the supernatant fluid of IMR-32 culture inoculated with an MOI of 1.0. Lower MOIs in these cells did not result in HIV-1 replication. In contrast, at all MOIs tested, productive HTLV-IIIRF infection was noted in the SK-N-MC cell line (Table 1), with continuously detectable supernatant p24 antigen starting on day 4. SK-N-MC cells (1), which had the morphology of immature neurons (Fig. 1A), did not exhibit cytopathic effect or evidence of cell-cell fusion associated with this infection. However, the HIV-1-infected cells did appear more granular by light microscopy, with greater numbers of intracellular vacuoles and inclusions (Fig. 1B). The susceptibility of SK-N-MC cells to infection by the HTLV-IIIRF isolate was reproduced in seven similar experiments.

The infection of the SK-N-MC neuronal cell line by HTLV-IIIRF became persistent for over 200 days, with detectable p24 antigen in the supernatant fluid on nearly every determination (Fig. 2). During this time period, multiple attempts to recover infectious virus from culture supernatant fluid were also positive (Fig. 2). For example, on day 104 of culture, the titer of infectious HIV-1 in cell-free culture supernatant fluid was determined by using H9 cells, with a result of 2×10^2 $TCID_{50}$ per ml. The progeny virus was also infectious for SK-N-MC cells but without augmented infectivity.

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TABLE 1. Core antigen (p24) production in culture supernatant fluid of neuronal cell lines inoculated with HTLV-IIIRF[a]

Neuronal cell line	Antigen detection at MOIs with HTLV-IIRF of[b]:					
	1.0	0.2	0.1	0.02	0.01	0.002
SK-N-MC	+	+	+	+	+	+
SK-N-SH	–	–	–	–	–	–
IMR-32	+	–	–	–	–	–
LA-N-1	–	–	–	–	–	–
LA-N-2	–	–	–	–	–	–
LA-N-5	–	–	–	–	–	–

[a] Supernatant fluid was harvested from an HTLV-IIIRF-infected H9 cell culture. Titration of this virus stock on H9 cells yielded an infectious titer of 10^6 $TCID_{50}$ per ml. Each neuronal cell line was grown in six-well plates at a density of 0.5×10^6 cells per well in 6 ml of Dulbecco modified Eagle medium supplemented with 15% fetal calf serum and antibiotics. The neuronal cultures were subsequently inoculated with the virus at the specified MOIs. After 24 h of incubation, the cells were washed six times with medium. The last wash for each culture was free of detectable p24 antigen. Supernatant fluids were harvested on days 4, 7, 10, and 13 for p24 antigen determination (Abbott Laboratories), and cultures were then replenished with fresh medium.

[b] A culture was scored positive for HIV-1 infection if its supernatant p24 antigen concentration was above twice the cutoff value (usually 30 pg/ml) on any determination.

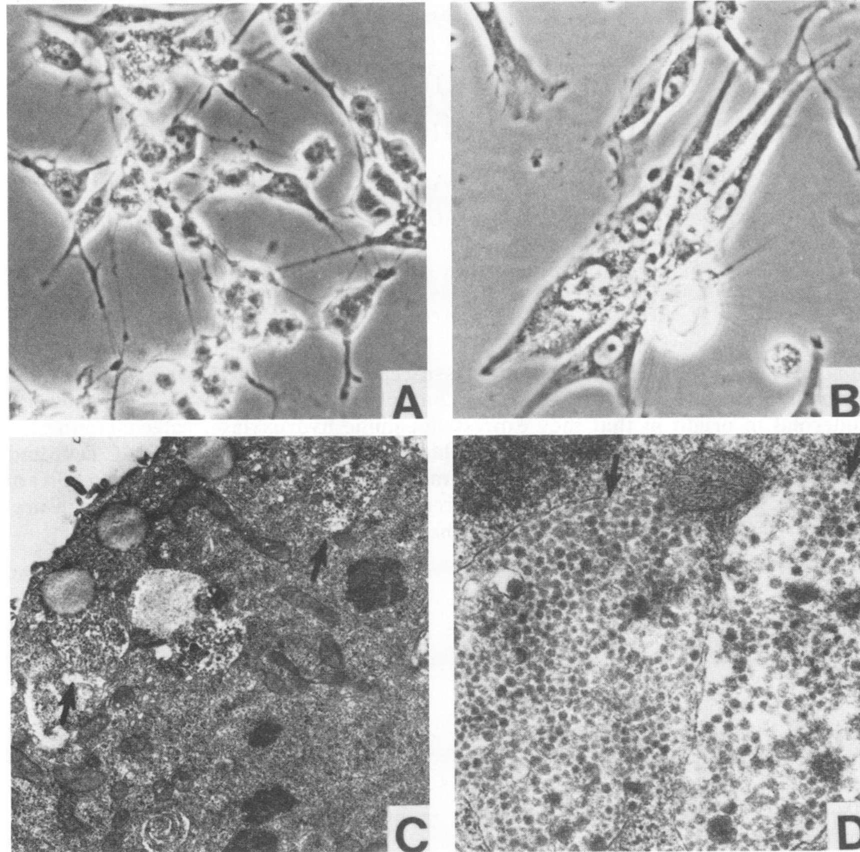

FIG. 1. (A) Uninfected SK-N-MC cells. Magnification, ×1,200. (B) HTLV-IIIRF-infected SK-N-MC cells. Magnification, ×1,200. (C) Electron micrograph of HIV-1-infected SK-N-MC cells, showing several poorly delineated vacuoles (arrows) with particles at various stages of maturation. Magnification, ×7,200. (D) Higher magnification (×29,400) of the subplasmalemmal membrane-bound vacuoles containing incomplete HIV-1 particles.

On the three occasions when the inoculated SK-N-MC cells were examined by indirect immunofluorescence, approximately 10 to 30% of the cells expressed intracellular antigens. In addition, electron microscopy performed on these cells showed the presence of HIV-1-like particles. These particles, which were not seen in the uninfected SK-N-MC cells, were clustered in intracellular vacuoles (Fig. 1C and D), similar to the situation seen with HIV-1 infection of monocyte/macrophages treated with monocyte colony-stimulating factor (11). Most of the virions appeared smaller (50 to 80 nm) than normal HIV-1 (100 to 120 nm) and lacked either an electron-dense core or a surrounding coat, suggesting that many of these particles may be defective.

The susceptibility of SK-N-MC cells to infection by other HIV-1 isolates was also examined. Five additional viruses (HIV-AL and HIV-AC from our laboratory [14, 18], HTLV-IIIB and HTLV-IIIMN from R. Gallo [35], and HIV-Z84 from T. Quinn, Johns Hopkins University, Baltimore, Md.) were used at MOIs of 0.025 or lower. Over the 24-day course of the experiment, productive HIV-1 infection was observed for all cultures inoculated with virus (Fig. 3). These findings suggest that productive infection in this neuronal cell line is not limited to one particular strain of HIV-1. However, the kinetics of viral replication vary from isolate to isolate as shown in Fig. 3.

The SK-N-MC cell line was derived from a lumbar neuroblastoma (1). Upon further morphologic review, the cells more closely resembled those of an immature neuroepithelioma. However, with increasing passage of these cells in culture, they began to develop processes (or neurites), a feature consistent with maturing neurons. This, coupled with the presence of dopamine hydroxylase in the cells and formaldehyde-induced fluorescence indicative of intracellular catecholamines (1), suggested that the SK-N-MC line was indeed neuronal in origin. Further support for this theory derived from our finding that these cells expressed neuron-specific enolase (in nearly 100% of the cells) and neurofilaments (in approximately 5% of the cells) but not glial fibrillary acid protein or S-100 protein within their cytoplasms as determined by indirect immunofluorescence and immunoperoxidase methods previously described (19).

The SK-N-MC cells were then analyzed for the presence of CD4, the known high-affinity receptor for HIV-1. First, the cells were fixed in acetone and subsequently stained with anti-CD4 monoclonal antibodies (Leu3A from Becton-Dickinson and OKT4 and OKT4A from Ortho Diagnostics) in an indirect immunofluorescence assay (19). The results were completely negative, although these antibodies did stain positive controls. The lack of CD4 on SK-N-MC cells was confirmed by radioimmunoprecipitation assay (13) and RNA slot blot hybridization (26). As illustrated in Fig. 4A, OKT4 monoclonal antibody immunoprecipitated CD4 from a T-lymphoblastoid cell line but not from the neuronal cells. Similarly, CD4 mRNA was detected in the T-cell line by slot

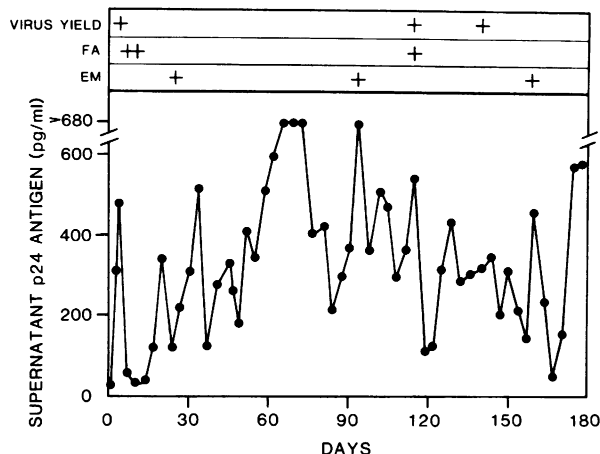

FIG. 2. Persistent HIV-1 infection of SK-N-MC cells. HTLV-IIIRF (5×10^5 $TCID_{50}$) was incubated with 10^6 SK-N-MC cells for 24 h before the culture was washed six times. Thereafter, twice weekly, the cells were split 1:3 and refed with fresh medium. In addition, sequentially harvested supernatant fluids were assayed for viral antigen production. Levels of p24 antigen above 30 pg/ml are considered positive. Virus yield assays were performed by inoculating 0.5 ml of SK-N-MC culture supernatant onto 10^6 H9 cells. Recovery of infectious HIV-1 was demonstrated by the development of syncytia and by p24 antigen expression in the H9 cells. Immunofluorescence assays (FA) were done by using the virus-inoculated SK-N-MC cells (0.2×10^6 per well) grown in 8-well culture-chamber slides as previously described (16). After fixation in acetone, an indirect immunofluorescence assay was performed by using goat anti-gp120 and mouse anti-p24 followed by the appropriate rabbit anti-goat or anti-mouse antibody conjugated to fluorescein isothiocyanate (19). EM, Electron microscopy.

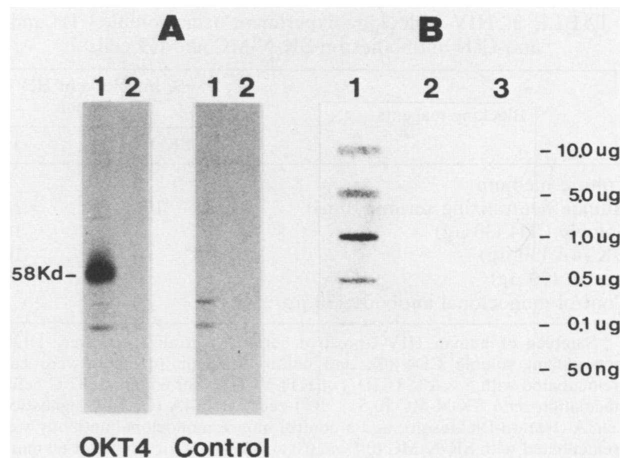

FIG. 4. (A) Lack of CD4 on SK-N-MC cells as analyzed by an immunoprecipitation assay. This procedure was based on a protocol for immunoprecipitation previously described (13). Briefly, 6×10^6 SK-N-MC (lanes 2) and 5×10^6 HPB-ALL (a known CD4+ T-cell line; lanes 1) cells were labeled with 100 μCi of [^{35}S]cysteine and [^{35}S]methionine per ml for 16 h and then lysed. These cell lysates were subsequently preclarified and subjected to immunoprecipitation by OKT4 (Ortho Diagnostics) and by a control mouse monoclonal antibody. Both were linked to Sepharose beads through a rabbit anti-mouse antibody and *Staphylococcus aureus* protein A. The precipitated proteins were then solubilized and resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by autoradiography. (B) Lack of CD4 mRNA in SK-N-MC cells by slot blot hybridization. RNA preparations extracted from 2×10^6 HPB-ALL human T (lane 1), B-35 rat neuroblastoma (lane 2), and SK-N-MC (lane 3) cells were each spotted onto a nitrocellulose membrane, using the Minifold II slot blot system (Schleicher & Schuell, Inc., Keene, N.H.). Hybridization was performed by using a 1.8-kilobase fragment of the *XbaI-XhoI* digestion product of the CDM7:CD4 plasmid (gift of D. Camerini and B. Seed, Massachusetts General Hospital, Boston, Mass.) and was done by the method of Maniatis et al. (26).

blot hybridization but, again, not in the SK-N-MC cells (Fig. 4B). Therefore, it appears that the SK-N-MC cells do not express detectable CD4 despite their susceptibility to HIV-1 infection.

If CD4 is present on the surface of SK-N-MC cells at a subdetectable level, then the infection of these cells by HIV-1 via the CD4 pathway should be exquisitely sensitive to blockade by monoclonal antibodies (Leu3A, OKT4A) directed against the gp120-binding site on the CD4 molecule. Results from three blocking experiments are summarized in Table 2. As previously described (28), both OKT4A and

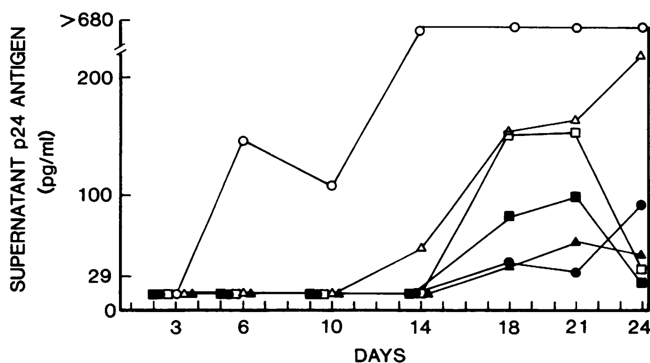

FIG. 3. Infection of SK-N-MC cells by multiple strains of HIV-1. Symbols: ○, HIV-AL; △, HTLV-IIIRF; □, HIV-AC; ●, HTLV-IIIB; ▲, HIV-Z84; and ■, HTLV-IIIMN. In this experiment, p24 antigen concentrations above 29 pg/ml were considered significant.

Leu3A blocked (95 to 100%) HIV-1 infection in H9 cells. However, they failed (0 to 6%) to prevent infection in SK-N-MC cells. In addition, recombinant soluble CD4 (Biogen Research, Inc., Cambridge, Mass.) (6) also did not block infection in these neuronal cells, although it completely inhibited infection in T cells (Table 2). Taken together, the above results strongly suggest that HIV-1 infection of the SK-N-MC neurons is mediated by an interaction between the virus and a cell-surface protein other than CD4.

We have shown for the first time that a neuronal cell line can be productively and persistently infected by HIV-1 in vitro. Prior studies have found that neuronal cell lines can transiently replicate HIV-1 when transfected with infectious full-length viral DNA (38). Obviously, our in vitro findings with tumor cell lines cannot be extrapolated to in vivo situations with normal neurons. Nevertheless, they do suggest that similar in vitro studies on primary neuronal cultures should be conducted, as should more extensive in situ analyses of affected brain tissues for evidence of direct neuronal involvement by HIV-1. The inability to find evidence of HIV-1 in these cells to date may simply reflect the limitations of the technical methods employed. If neurons are indeed infected, they may have shortened survival or loss of luxury functions, thus resulting in neurologic dysfunction. Such a finding may also redirect some of the current research effort on HIV-1 neuropathogenesis, which

TABLE 2. HIV-1 blocking experiment using soluble CD4 and anti-CD4 antibodies on SK-N-MC and H9 cells[a]

Blocking reagents	% Inhibition of HIV-1 infection	
	SK-N-MC	H9
Culture medium	0	0
Human neutralizing serum (10 μl)	100	100
Soluble CD4 (30 μg)	6	100
OKT4A (30 μg)	0	100
Leu3A (30 μg)	0	95
Control monoclonal antibody (30 μg)	0	0

[a] Samples of human HIV-1-positive serum (neutralization titer, 1:128), recombinant soluble CD4 (6), and culture medium (control) were each preincubated with 5×10^4 $TCID_{50}$ of HTLV-IIIRF for 60 min at 37°C before inoculation onto SK-N-MC (0.5×10^6) cells. OKT4A (Ortho Diagnostics), Leu3A (Becton-Dickinson), and a control mouse monoclonal antibody were preincubated with SK-N-MC (0.5×10^6) and H9 (1×10^6) cells for 60 min at 37°C before the cells were challenged with HTLV-IIIRF (5×10^4 $TCID_{50}$). After 24 h, all cultures were washed six times and then replenished with fresh medium. Each sample of the last wash was negative for detectable p24 antigen. On day 7 of the experiment, supernatant fluids were examined for HIV-1 expression by using a p24 antigen assay (Abbott Laboratories). This experiment was repeated twice with nearly identical results.

is largely focused on indirect mechanisms mediated by the infected monocyte/macrophage (8, 9, 15, 21, 41).

HIV-1 is known to infect T-helper lymphocytes [20] and monocyte/macrophages (10, 16, 31), both of which express CD4 on the cell surface. It is now well documented that CD4 serves as a receptor for HIV-1 (25, 27, 28), and specific domains on both gp120 and CD4 involved in this envelope-receptor interaction have been carefully defined (22, 24, 32, 40). However, there is a growing awareness that HIV-1 tropism may be quite broad, including infection of tissues or cells which were not known to be CD4 positive, such as the colon (30), retina (34), and cervix (33) in vivo and glioma (2–5, 12, 23), rhabdomyosarcoma (4), hepatocellular carcinoma (our unpublished data), fibroblast (39), and bone marrow-derived CD34+ precursor (7) cells in vitro. Our current findings clearly document that HIV-1 can infect neuronal cells which have no detectable CD4. Moreover, this infection cannot be blocked by recombinant soluble CD4 or anti-CD4 monoclonal antibodies which are capable of preventing HIV-1 infection of T-helper lymphocytes. Similar results have been obtained by Clapham and associates (4) with HIV-1 susceptible glioma and rhabdomyosarcoma cells. Therefore, we believe there is an alternate pathway by which HIV-1 can enter and replicate in CD4-negative cells. The search for the putative second receptor for HIV-1 may be facilitated by the use of these susceptible neuronal cells.

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LITERATURE CITED

1. **Biedler, J. L., L. Helson, and B. A. Spengler.** 1973. Morphology and growth, tumorigenicity, and cytogenetics of human neuroblastoma cells in continuous culture. Cancer Res. **33**:2643–2652.
2. **Cheng-Mayer, C., J. T. Rutka, M. L. Rosenblum, T. McHugh, D. P. Stites, and J. A. Levy.** 1987. Human immunodeficiency virus can productively infect cultured human glial cells. Proc. Natl. Acad. Sci. USA **84**:3526–3530.
3. **Chiodi, F., S. Fuerstenberg, M. Gidlund, B. Asjo, and E. M. Fenyo.** 1987. Infection of brain-derived cells with the human immunodeficiency virus. J. Virol. **61**:1244–1247.
4. **Clapham, P., J. N. Weber, D. Whitby, K. McKintosh, A. G. Dalgleish, P. J. Maddon, K. C. Deen, R. W. Sweet, and R. A. Weiss.** 1989. Soluble CD4 blocks the infectivity of diverse strains of HIV and SIV for T cells and monocytes but not for brain and muscle cells. Nature (London) **337**:368–370.
5. **Dewhurst, S., K. Sakai, J. Bresser, M. Stevenson, M. J. Evinger-Hodges, and D. J. Volsky.** 1987. Persistent productive infection of human glial cells by human immunodeficiency virus (HIV) and by infectious molecular clones of HIV. J. Virol. **61**:3774–3782.
6. **Fisher, R. A., J. M. Bertonis, W. Meier, V. A. Johnson, D. S. Costopoulos, T. Liu, R. Tizard, B. D. Walker, M. S. Hirsch, R. T. Schooley, and R. A. Flavell.** 1988. HIV infection is blocked in vitro by recombinant soluble CD4. Nature (London) **331**: 76–78.
7. **Folks, T. M., S. W. Kessler, J. M. Orenstein, J. S. Justement, E. S. Jaffe, and A. S. Fauci.** 1988. Infection and replication of HIV-1 in purified progenitor cells of normal human bone marrow. Science **242**:919–922.
8. **Gabuzda, D. H., D. D. Ho, S. M. de la Monte, M. S. Hirsch, T. R. Rota, and R. A. Sobel.** 1986. Immunohistochemical identification of HTLV-III antigen in brains of patients with AIDS. Ann. Neurol. **20**:289–295.
9. **Gartner, S., P. Markovits, D. M. Markovitz, R. F. Betts, and M. Popovic.** 1986. Virus isolation from and identification of HTLV-III/LAV-producing cells in brain tissue from a patient with AIDS. J. Am. Med. Assoc. **256**:2365–2371.
10. **Gartner, S., P. Markovits, D. M. Markovitz, M. H. Kaplan, R. C. Gallo, and M. Popovic.** 1986. The role of mononuclear phagocytes in HTLV-III/LAV infection. Science **233**:215–219.
11. **Gendelman, H. E., J. M. Orenstein, M. A. Martin, C. Ferrua, R. Mitra, T. Phipps, L. A. Wahl, H. C. Lane, A. S. Fauci, D. E. Burke, D. Skillman, and M. S. Meltzer.** 1988. Efficient isolation and propagation of human immunodeficiency virus on rCSF-1 treated monocytes. J. Exp. Med. **167**:1428–1441.
12. **Harouse, J. M., C. Kunsch, H. T. Hartle, M. A. Laughlin, J. A. Hoxie, B. Wigdahl, and F. Gonzalez-Scarano.** 1989. CD4-independent infection of human neural cells by human immunodeficiency virus type 1. J. Virol. **63**:2527–2533.
13. **Ho, D. D., J. C. Kaplan, I. E. Rackauskas, and M. E. Gurney.** 1988. Importance of the second conserved domain of gp120 for HIV infectivity and antibody neutralization. Science **239**:1021–1023.
14. **Ho, D. D., T. Moudgil, H. S. Robin, M. Alam, B. J. Wallace, and Y. Mizrachi.** 1989. HIV-1 in a seronegative patient with visceral Kaposi's sarcoma and hypogammaglobulinemia. Am. J. Med. **86**:349–351.
15. **Ho, D. D., R. J. Pomerantz, and J. C. Kaplan.** 1987. Pathogenesis of infection with the human immunodeficiency virus. N. Engl. J. Med. **317**:278–286.
16. **Ho, D. D., T. R. Rota, and M. S. Hirsch.** 1986. Infection of monocyte-macrophages by human T-lymphotropic virus type III. J. Clin. Invest. **77**:1712–1715.
17. **Ho, D. D., T. R. Rota, R. T. Schooley, J. C. Kaplan, J. D. Allan, J. E. Groopman, L. Resnick, D. Felsenstein, C. A. Andrews, and M. S. Hirsch.** 1985. Isolation of HTLV-III from cerebrospinal fluid and neural tissues of patients with neurologic syndromes related to the acquired immunodeficiency syndrome. N. Engl. J. Med. **313**:1493–1497.
18. **Ho, D. D., M. G. Sarngadharan, M. S. Hirsch, R. T. Schooley, T. R. Rota, R. C. Kennedy, T. C. Chanh, and V. Sato.** 1987. Human immunodeficiency virus neutralizing antibodies recognize several conserved domains on the envelope glycoproteins. J. Virol. **61**:2024–2028.
19. **Ho, D. D., R. T. Schooley, T. R. Rota, J. C. Kaplan, S. Z. Salahuddin, M. A. Gonda, and M. S. Hirsch.** 1984. HTLV-III in semen and blood of a healthy homosexual man. Science **226**: 451–453.
20. **Klatzmann, D., F. Barré-Sinoussi, M. T. Nugeyre, C. Dauguet,**

E. Vilmer, C. Griscelli, F. Brun-Vezinet, C. Rouzioux, J. C. Gluckman, J. C. Chermann, and L. Montagnier. 1984. Selective tropism of lymphadenopathy associated virus (LAV) for helper-inducer T lymphocytes. Science **225**:59–64.

21. **Koenig, S., H. E. Gendelman, J. M. Orenstein, M. C. Dal Canto, G. H. Pezeshkpour, M. Yungbluth, F. Janotta, A. Aksamit, M. A. Martin, and A. S. Fauci.** 1986. Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. Science **233**:1089–1093.
22. **Kowalski, M., J. Potz, L. Basiripour, T. Dorfman, W. C. Goh, E. Terwilliger, A. Dayton, C. Rosen, W. Haseltine, and J. Sodroski.** 1987. Functional regions of the envelope glycoprotein of human immunodeficiency virus type 1. Science **237**:1351–1355.
23. **Koyanagi, Y., S. Miles, R. T. Mitsuyasu, J. E. Merrill, H. V. Vinters, and I. S. Y. Chen.** 1987. Dual infection of the central nervous system by AIDS viruses with distinct cellular tropisms. Science **236**:819–822.
24. **Lasky, L., G. Nakamura, D. H. Smith, C. Fennie, C. Shimasaki, E. Patzer, P. Berman, T. Gregory, and D. J. Capon.** 1987. Delineation of a region of the human immunodeficiency virus type 1 gp120 glycoprotein critical for interaction with the CD4 receptor. Cell **50**:975–985.
25. **Maddon, P. J., A. G. Dalgleish, J. S. McDougal, P. R. Clapham, R. A. Weiss, and R. Axel.** 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. Cell **47**:333–348.
26. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
27. **McDougal, J. S., M. S. Kennedy, J. M. Sligh, S. P. Cort, A. Mawle, and J. K. A. Nicholson.** 1986. Binding of HTLV-III/LAV to T4+ T cells by a complex of the 110K viral protein and the T4 molecule. Science **231**:382–385.
28. **McDougal, J. S., A. Mawle, S. Cort, J. Nicholson, G. Gross, J. Shepple-Campbell, D. Hicks, and J. Sligh.** 1986. Cellular tropism of the human retrovirus HTLV-III/LAV. 1. Role of T-cell activation and expression of the T4 antigen. J. Immunol. **135**: 3151–3162.
29. **Navia, B. A., B. D. Jordan, and R. W. Price.** 1986. The AIDS dementia complex. I. Clinical features. Ann. Neurol. **19**:517–524.
30. **Nelson, J. A., C. A. Wiley, C. Reynolds-Kohler, C. E. Reese, W. Margaretten, and J. A. Levy.** 1988. Human immunodeficiency virus detected in bowel epithelium from patients with gastrointestinal symptoms. Lancet **i**:259–262.
31. **Nicholson, J. K. A., G. D. Gross, C. S. Callaway, and J. S. McDougal.** 1986. In vitro infection of human monocytes with human T-lymphotropic virus type III/lymphadenopathy-associated viruses (HTLV-III/LAV). J. Immunol. **137**:323–329.
32. **Peterson, A., and B. Seed.** 1988. Genetic analysis of monoclonal antibody and HIV binding sites on the human lymphocyte antigen CD4. Cell **54**:65–72.
33. **Pomerantz, R. J., S. M. de la Monte, S. P. Donegan, T. R. Rota, M. W. Vogt, D. E. Craven, and M. S. Hirsch.** 1988. Human immunodeficiency virus (HIV) infection of the uterine cervix. Ann. Intern. Med. **108**:321–327.
34. **Pomerantz, R. J., D. R. Kuritzkes, S. M. de la Monte, T. R. Rota, A. S. Baker, D. Albert, D. H. Bor, E. L. Feldman, R. T. Schooley, and M. S. Hirsch.** 1987. Infection of the retina by human immunodeficiency virus type 1. N. Engl J. Med. **317**: 1643–1647.
35. **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.
36. **Resnick, L., F. diMarzo-Veronese, J. Shupbach, W. W. Tourtellotte, D. D. Ho, F. Muller, P. Shapshack, M. Vogt, J. E. Groopman, P. D. Markham, and R. C. Gallo.** 1985. Intra-blood-brain barrier synthesis of HTLV-III specific IgG in patients with neurologic symptoms associated with AIDS or AIDS-related complex. N. Engl. J. Med. **313**:1498–1504.
37. **Shaw, G. M., M. E. Harper, B. H. Hahn, L. G. Epstein, D. C. Gajdusek, R. W. Price, B. A. Navia, C. K. Petito, C. J. O'Hara, J. E. Groopman, E.-S. Cho, J. M. Oleske, F. Wong-Staal, and R. C. Gallo.** 1985. HTLV-III infection in brains of children and adults with AIDS encephalopathy. Science **227**:177–182.
38. **Srinivasen, A., D. Dorsett, D. York, C. Bohan, and R. Anand.** 1988. Human immunodeficiency virus replication in human brain cells. Arch. Virol. **98**:135–141.
39. **Tateno, M., F. Gonzalez-Scarano, and J. A. Levy.** 1989. Human immunodeficiency virus can infect CD4-negative human fibroblastoid cells. Proc. Natl. Acad. Sci. USA **86**;4287–4290.
40. **Traunecker, A., W. Luke, and K. Karjalainen.** 1988. Soluble CD4 molecules neutralize human immunodeficiency virus type 1. Nature (London) **331**:84–86.
41. **Wiley, C. A., R. D. Schrier, J. A. Nelson, P. W. Lampert, and M. B. A. Oldstone.** 1986. Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. Proc. Natl. Acad. Sci. USA **83**:7089–7093.