JOURNAL OF VIROLOGY, Nov. 1988, p. 4414–4419
0022-538X/88/114414-06$02.00/0

Vol. 62, No. 11

Distinct Replicative and Cytopathic Characteristics of Human Immunodeficiency Virus Isolates

EVA MARIA FENYÖ,[1]* LINDA MORFELDT-MÅNSON,[1,2,3] FRANCESCA CHIODI,[1] BIRGITTA LIND,[1] AGNETA VON GEGERFELT,[1] JAN ALBERT,[1,3] EVA OLAUSSON,[3] AND BIRGITTA ÅSJÖ[1]

Department of Virology,[1] and Department of Infectious Diseases,[2] Karolinska Institutet, and Department of Virology, National Bacteriological Laboratory,[3] S-105 21 Stockholm, Sweden

Received 12 April 1988/Accepted 25 July 1988

According to their capacity to replicate in vitro, human immunodeficiency virus (HIV) isolates can be divided into two major groups, rapid/high and slow/low. Rapid/high viruses can easily be transmitted to a variety of cell lines of T-lymphoid (CEM, H9, and Jurkat) and monocytoid (U937) origin. In contrast, slow/low viruses replicate transiently, if at all, in these cell lines. Except for a few isolates, the great majority of slow/low viruses replicate in peripheral blood mononuclear cells and Jurkat-*tatIII* cells constitutively expressing the *tatIII* gene of HIV-1. The viruses able to replicate efficiently cause syncytium formation and are regularly isolated from immunodeficient patients. Poorly replicating HIV isolates, often obtained from individuals with no or mild disease, show syncytium formation and single-cell killing simultaneously or, with some isolates, cell killing only.

Human immunodeficiency virus (HIV) has been implicated as the etiological agent of the acquired immunodeficiency syndrome (AIDS). Immunodeficiency is at least in part a result of depletion of the CD4 antigen-positive helper subset of T lymphocytes. Since HIV has been shown to preferentially replicate in and kill CD4-positive lymphocytes in vitro (13), it has been suggested that a similar mechanism may be responsible for immunodeficiency in vivo.

Genomic heterogeneity is a prominent characteristic of HIV (4, 22). Restriction enzyme analysis of proviral DNA has been used in most studies to establish the extent of variability. It has been found that the gene encoding the external envelope glycoprotein has the highest rate of genetic change, 10^6-fold greater than that of most genes in other DNA genomes (12). In line with this hypervariability of the *env* gene, antibodies produced in humans to the major envelope glycoprotein appear to be strain specific in virus neutralization assays (21), although cross-reaction can also be detected. The question arises to what extent the observed genomic heterogeneity may lead to alterations in other biological properties of the virus, such as the rate of replication, cytopathic effect, and tissue tropism.

Differences in the in vitro growth properties of different HIV isolates have been described previously (3, 7; E. M. Fenyö, Cancer Proc., in press). We found that the in vitro replication potential of viruses seemed to correlate with the clinical condition of the patient from whom the virus originated. Cultures from peripheral blood mononuclear cells (PBMC) of patients with severe immunodeficiency (mostly AIDS) yielded high reverse transcriptase (RT) activity in culture fluids. In contrast, viruses isolated from HIV-infected individuals with no or milder clinical symptoms, mainly persistent generalized lymphadenopathy (PGL) grew slowly in cultures of PBMC from these individuals. RT activity usually remained low even after prolonged observation (6 weeks). Coculture of RT-positive PBMC derived from AIDS patients with tumor cell lines of T-lymphoid and monocytoid origin regularly yielded lines that continuously produced virus, whereas similar cocultures with lymphocytes from PGL patients only occasionally yielded stable producer lines. In fact, the majority of cocultures of the latter type remained virus negative or showed transient virus replication only. We have therefore suggested that with increasing time of infection, selection may occur in the patient for virus variants that show increased growth potential in T-lymphoid and monocytoid cells (3). In the present work we have characterized HIV isolates from 31 HIV antibody-positive subjects for in vitro replication potential and cytopathic effect after cell-free transmission to PBMC and cell lines.

Virus has been isolated from 5×10^6 Ficoll-Isopaque-separated PBMC or lymph node cells as described previously (3). The clinical status of subjects and the level of T-helper cells ($CD4^+$ cells) at the time of virus isolation are presented in Table 1. Figure 1 shows the RT activity detectable in lymphocyte cultures from 14 HIV antibody-positive subjects, randomly selected from the 31 samples. RT activity in 0.3% Triton X-100-disrupted high-speed pellets of supernatant culture fluids was measured as described previously (2). The six cultures shown in Fig. 1A exhibited high RT activity within 2 weeks, whereas the eight cultures shown in Fig. 1B either showed low activity for the entire observation period or displayed higher RT activity only after 3 weeks. Viruses were therefore designated rapid/high and slow/low, respectively. To test whether these viral replication patterns were characteristic of the particular virus isolate rather than variations in culture conditions, we performed repeated isolations from one patient in each category at 3-month intervals. Within the limits of experimental variation, each replication pattern, rapid/high or slow/low, was reproducible for a particular patient (Fig. 2). Of the 31 HIV antibody-positive subjects, patients 1 to 23 yielded slow/low virus, whereas patients 24 to 31 yielded rapid/high virus (Table 1).

Cell-free supernatants of cultures of PBMC from patients and containing various amounts of RT activity were transferred to phytohemagglutinin-stimulated PBMC from normal blood donors (Fig. 3). All six rapid/high HIV isolates could easily be transmitted, whereas not all the slow/low viruses were able to replicate in PBMC. Successful transmission

* Corresponding author.

TABLE 1. Clinical status of the patients according to the Centers for Disease Control classification system for HIV infection (1986)[a]

Virus type	Patient no. with following status:						
	II	III-a	IV B	III-b	IVC-2	IVC-1	IV D
Slow/low	1–3	5–8, 10–17, 19, 20	4, 21	9, 18	22		23
Rapid/high				24, 26	25, 27	28, 29, 31	30

[a] Symbols: II, asymptomatic infection; III, PGL (III-a, PGL and CD4-positive cells at ≥250 × 10^6/liter; III-b, PGL and CD4-positive cells at <250 10^6/liter); IV B, neurologic disease; IV C-1, secondary infectious diseases (opportunistic infection); IV C-2, other secondary infectious diseases (oral candidiasis); IV D, secondary cancers (Kaposi sarcoma, lymphoma). Groups II, IIIa, and IV B are associated with CD4-positive cells at ≥250 × 10^6/liter; groups III-b, IV C-2, IV C-1, and IV D are associated with CD4-positive cells at <250 × 10^6/liter. Patients in groups IVC-1, IVC-2, and IV D suffered from AIDS.

was not simply dependent on the amount of virus, as quantitated by RT activity. Some isolates replicated readily with small inocula, whereas others did not, even if the RT activity of the inoculum was increased 50-fold.

Two weeks after the infection of PBMC described above, cocultures were initiated with CEM, H9, U937-16, Jurkat, and Jurkat-*tatIII* cells (Table 2). CEM and H9 (15) are T leukemia cell lines. Jurkat-*tatIII* was originally derived from the Jurkat T cell line by transfection with the transactivator gene, *tatIII*, of the human T-cell lymphotropic virus type IIIB (HTLV-IIIB) isolate of HIV and, as a consequence, shows constitutive expression of the transactivator protein (16). U-937 is a monocytoid cell line originally obtained from a histiocytic lymphoma (18). The clone 16 subline used in the present study (U937-16) contains 95% CD4-positive cells and can easily be infected with HTLV-IIIB (2). The Jurkat-*tatIII* cell line was selected for these experiments because, according to our experience, it supports the replication of most of the poorly replicating HIV isolates (1). On the basis of their capacity to replicate in cell lines, slow/low viruses could be subdivided into three groups. Group 1 viruses did not replicate in PBMC or in any of the cell lines used. HIV isolates belonging to group 2 could replicate in PBMC but not in cell lines such as CEM, H9, Jurkat, or U937-16. However, all nine isolates replicated in Jurkat-*tatIII* cells. Slow/low viruses in group 3 replicated in PBMC as well as in cell lines. However, only 1 to 2% of CEM and U937-16 cells expressed viral antigens. In contrast, cultures infected with rapid/high viruses showed an efficient replication and 10 to 70% viral antigen-positive cells. Such viruses replicated equally well in Jurkat-*tatIII* cells. The *tat* product is known to play a crucial role in HIV replication (9). Successful

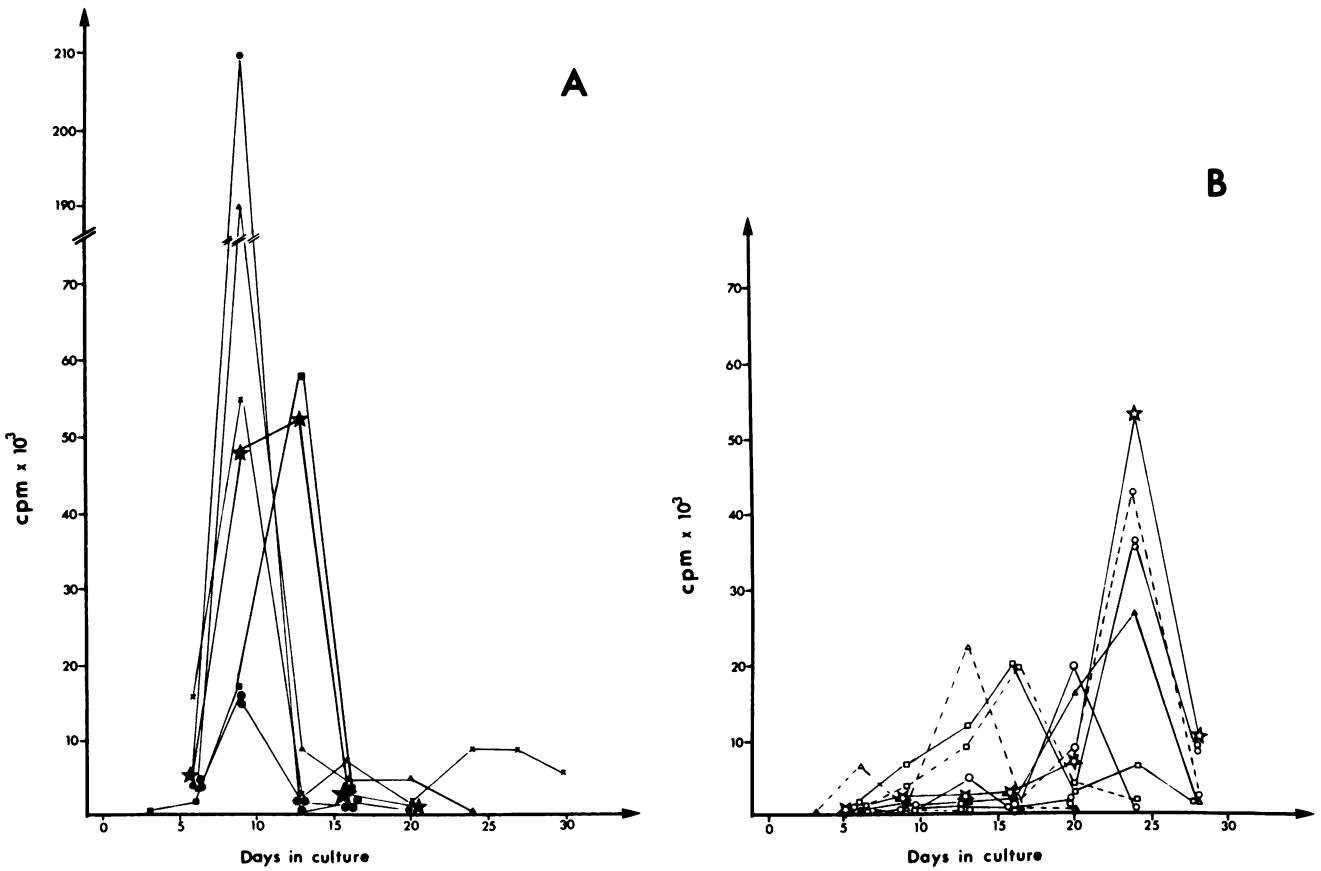

FIG. 1. RT activity in patient PBMC cultures showing (A) the rapid/high pattern (patients 24, 26, 28, 29, 30, and 31) and (B) the slow/low pattern (patients 6, 7, 8, 10, 12, 13, 15, and 17).

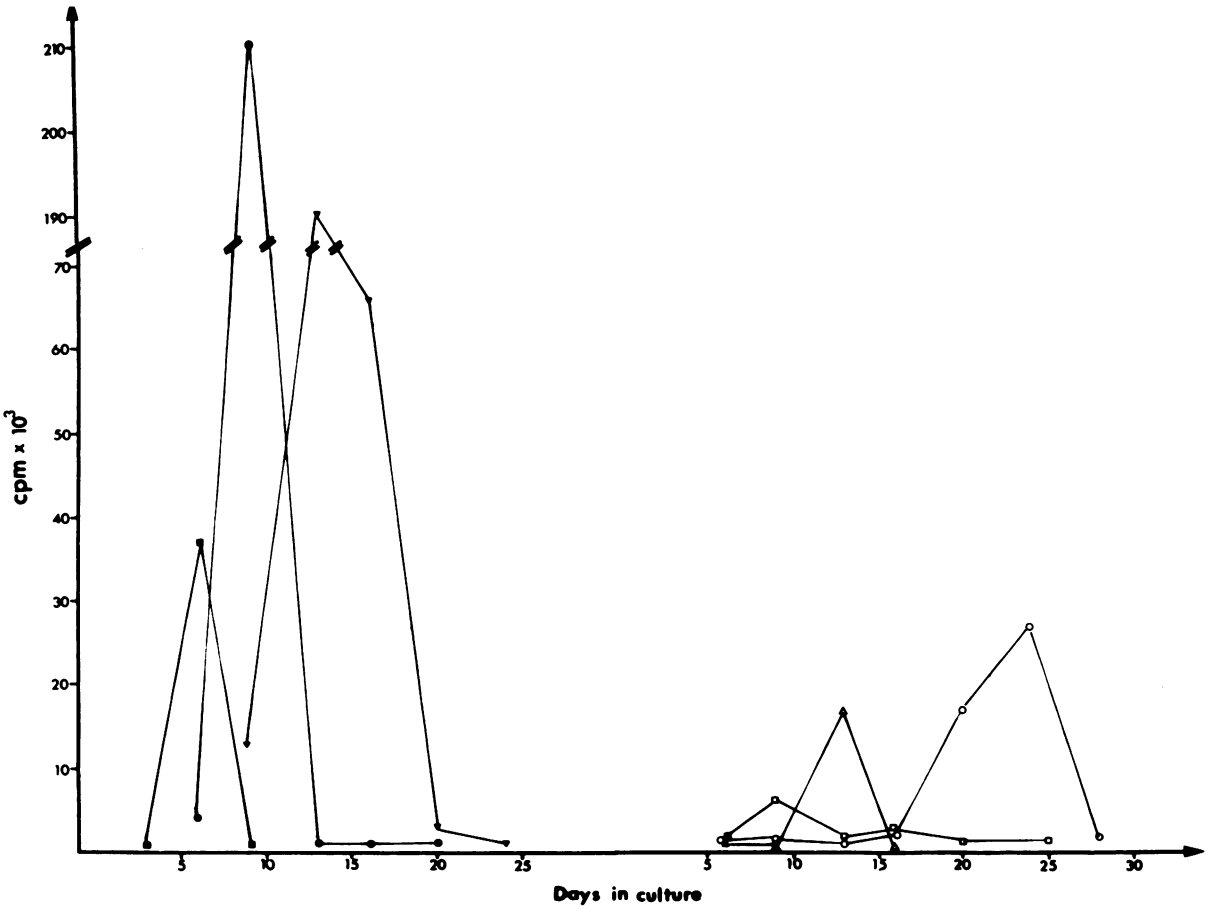

FIG. 2. RT activity in PBMC cultures derived from patients 29 and 7.

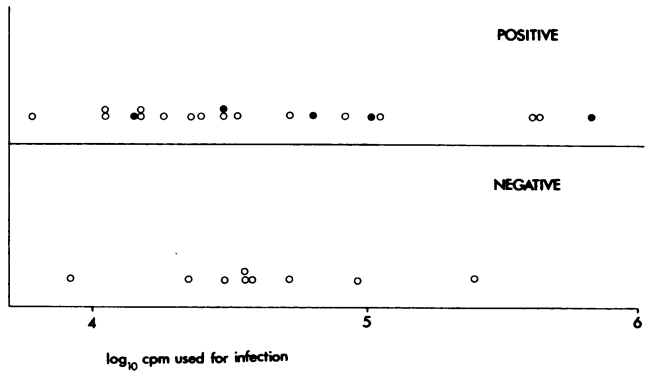

FIG. 3. Infection of 10^6 phytohemagglutinin-stimulated (PHA-P [Difco Laboratories], 2.5 μg/ml for 3 days) PBMC from normal blood donors with cell-free virus. The inoculum dose was quantitated by RT activity. The figure shows the minimum amount of virus used for successful infection (positive) or the maximum amount of virus used that did not replicate (negative) during the 4-week observation period. Each virus was tested two to six times. Viruses were tested in a series of 10 to 22 isolates to minimize the experimental variation due to differences in sensitivity to HIV infection of PBMCs from different individuals (7, 10). At least two rapid/high isolates were included in each series. Symbols: ●, rapid/high virus; ○, slow/low virus.

passage of many of the slow/low isolates in Jurkat-*tatIII* cells suggests that if the transactivator protein is provided, the efficiency of replication can be increased. Alternatively, selection for transfected cells following the introduction of the *tatIII* gene into the Jurkat cell line has also selected for a cell clone particularly susceptible to HIV infection.

PBMC and Jurkat-*tatIII* cells were infected with cell-free virus classified as rapid/high or slow/low according to their replication patterns, and the cultures were observed for cytopathic changes (Table 3). Two types of changes could be observed: one involved predominantly syncytium formation (Figure 4A), and the other involved cell death (Fig. 4B). It is interesting that HIV isolates grouped according to replication potential also, in some cases, differed in their cytopathic characteristics. Rapid/high viruses all formed syncytia. With some of the isolates, syncytium formation was very pronounced, and these cultures died unless fresh cells were added. Cell lines that produced virus continuously could regularly be established from these cultures. This type of cytopathic effect could clearly be distinguished from cell killing without syncytium formation, which was a characteristic of some slow/low viruses. Cultures infected with these viruses died in the absence (three of four isolates in group 1) or presence (one of three isolates in group 3) of virus replication. With this type of cell death, addition of fresh cells could not save the cultures, as was the case with syncytium formation. Seven slow/low virus isolates showed a mixed type of cytopathic effect, inasmuch as syncytium

TABLE 2. Cell-free transmission and replication patterns of HIV isolates in PBMC and different cell lines

Virus type[a]	No. tested	Successful transmission of free virus to PBMC	Cocultivation of PBMC with:		
			CEM, H9, Jurkat, and/or U937 clone 16		Jurkat-*tat III* (no. RT positive)
			No. RT positive	IF (% positive cells)[b]	
Slow/low					
Group 1	4	0	0	0	0
Group 2	9	9	0	0	9
Group 3	4	4	4	4 (1–2%)	4
Rapid/high	6	6	6	6 (10–70%)	6

[a] Slow/low, group 1: patients 7, 9, 10, and 11; group 2: patients 6, 8, 12, 13, 17, 18, 19, 22, and 23; group 3: patients 5, 15, 16, and 21; rapid/high: patients 24, 25, 26, 29, 30, and 31.

[b] Indirect immunofluorescence (IF) assay was carried out on fixed cells with mouse monoclonal antibodies against the viral core proteins p24 and p15 (kindly provided by M. Popovic) and fluorescein isothiocyanate-labeled rabbit anti-mouse immunoglobulin G (Dakopatts).

formation and degradation of individual cells could be observed simultaneously in infected cultures. Heterogeneity among HIV isolates with regard to cytopathic effect has been reported from several laboratories (6, 7, 11, 14, 20). Dahl et al. (6) have used a lymphoblastoid cell line immortalized by Epstein-Barr virus to distinguish the capacity of various HIV isolates to induce cytolysis or persistent infection. These authors noted that the type of cytopathic effect—syncytium formation alone or syncytium formation combined with degradation of individual cells—varied with the virus isolate used. Similarly, Tersmette et al. (19) have observed differential syncytium-inducing capacity of HIV isolates propagated in PBMC. Syncytium-inducing isolates were frequently derived from patients with AIDS or with other signs of severe immunodeficiency, had a higher infectivity, and replicated in the H9 cell line. In this communication, such viruses are designated rapid/high. The non-syncytium-inducing variants found by Tersmette et al. (19) may correspond to some of our slow/low viruses. It must be noted, however, that the Jurkat-*tatIII* cells (a cell line not used by Tersmette et al. [19]) seem to be more sensitive to the cytopathic effect of HIV than PBMC are (Table 3). Several of our slow/low viruses form syncytia in Jurkat-*tatIII* cells but not in PBMC.

TABLE 3. Cytopathic characteristics of the different isolates

Virus isolate	CPE[a] at following day after infection:				Virus replication in:	
	7		14		PBMC	J-*tatIII*
	PBMC	J-*tatIII*	PBMC	J-*tatIII*		
Slow/low group 1						
7	S	S/D	S	S/D	–	–
9	–	–	–	D	–	–
10	–	–	D	D	–	–
11	–	–	D	D	–	–
Slow/low group 2						
6	–	S	S/D	D	+	+
8	S	S	S	D	+	+
12	S	S	–	S	+	+
13	–	S	D	D	+	–[b]
17	–	–	–	D	+	+
18	–	–	S	S	+	–[b]
19	–	S	–	S	+	+
22	–	S	–	S	+	+
23	S	S	S/D	D	+	+
Slow/low group 3						
5	S	S	S	D	+	+
15	D	D	D	D	+	+
16	S	S	D	D	+	+
21	–	S	–	S	+	+
Rapid/high						
24	S	S	S	S	+	+
25	S	Small S	S	Small S	+	+
26	S	S	S	S	+	+
29	S/D	S/D	S/D	S/D	+	+
30	S	S	S	S	+	+
31	S	S	S	S	+	+

[a] S, Syncytia; D, cell death, consisting of pyknosis and degradation of individual cells; –, no effect. CPE, Cytopathic effect.

[b] Viruses could be transmitted to Jurkat-tat_{III} (J-*tatIII*) cells by cocultivation with RT-positive PBMC.

The difference between slow/low and rapid/high viruses may reside in their capacity to generate infectious virus particles. Accordingly, slow/low viruses may contain 10- to 1,000-fold less infectious virus on the basis of equal particle numbers. Poorly infectious virus particles are generated by deletion mutations in the *sor* gene (8, 17). It is therefore conceivable that some of our HIV isolates represent variants in *sor*. Molecular cloning in conjunction with infectivity assays may enable us to localize the determinants for efficient viral replication.

It is also possible that slow/low and rapid/high viruses differ in target cell tropism. For HIV isolates derived from brain tissue, a monocyte tropism has been demonstrated (11, 14), whereas isolates derived from PBMC cultures regularly showed T-lymphocyte tropism. Accordingly, rapid/high viruses in our material may be lymphotropic, whereas slow/low viruses may preferentially replicate in monocytes. The presence of monocytes in PBMC cultures would allow these viruses to replicate to some extent.

Rapid/high viruses were all obtained from patients showing a severe immunodeficiency, reflected by low CD4 cell counts ($<250 \times 10^6$/liter) (Table 1). None of the subjects with CD4 cell counts above this value yielded rapid/high virus. Recently, Cheng-Mayer et al. (5) found that a few individuals harbored HIV variants that had higher replication potential and were more cytopathic in vitro as the disease progressed. Since HIV isolates with increased replicative capacity in T cells are regularly isolated from patients with severe immunodeficiency, it is likely that variant viruses will be selected in HIV-infected patients over time. Whether these variant viruses are indeed more virulent and

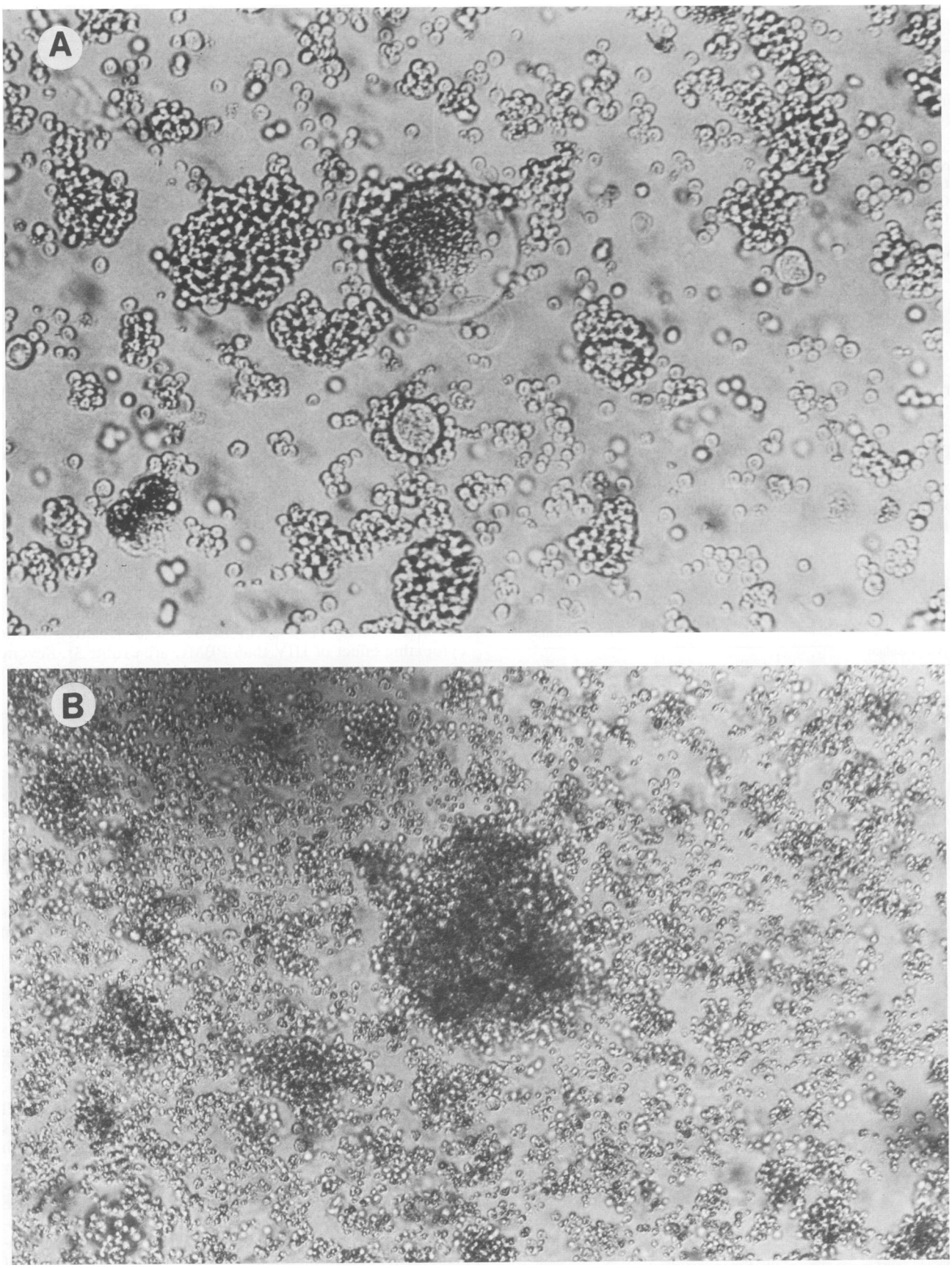

FIG. 4. Cytopathic effect in unstained Jurkat-*tatIII* cells. (A) Cells infected with virus isolate 24 show syncytium formation. (B) Cells infected with virus isolate 15 show pyknosis and degradation of individual cells.

cause disease progression or whether the severe immunodeficiency in the host allows the replication of such viruses, is presently unclear. To better understand the pathogenesis of HIV, we are carrying out longitudinal studies with patients progressing from the asymptomatic stage to severe immunodeficiency.

We thank E. Norrby for critical reading of the manuscript.

This investigation was supported by grants from the Swedish Cancer Society, from the Swedish Medical Research Council (grants B87-16H-7737-02B, K87-16P-07749-02, and K87-16P-07784-02B), and from the Swedish National Board for Technical Development.

LITERATURE CITED

1. **Åsjö, B., J. Albert, F. Chiodi, and E. M. Fenyö.** 1988. Improved tissue culture technique for production of poorly replicating human immunodeficiency virus isolates. J. Virol. Methods **19:** 191–196.
2. **Åsjö, B., I. Ivhed, M. Gidlund, S. Fuerstenberg, E. M. Fenyö, K. Nilsson, and H. Wigzell.** 1987. Susceptibility to infection by the human immunodeficiency virus (HIV) correlates with T4 expression in a parental monocytoid cell line and its subclones. Virology **157:**359–365.
3. **Åsjö, B., L. Morfeldt-Månson, J. Albert, G. Biberfeld, A. Karlsson, K. Lidman, and E. M. Fenyö.** 1986. Replicative capacity of human immunodeficiency virus from patients with varying severity of HIV infection. Lancet **ii:**660–662.
4. **Benn, S., R. Rutledge, T. Folks, J. Gold, L. Baker, J. McCormick, P. Feorino, P. Piot, T. Quinn, and M. Martin.** 1985. Genomic heterogeneity of AIDS retroviral isolates from North America and Zaire. Science **230:**949–951.
5. **Cheng-Mayer, C., D. Seto, M. Tateno, and J. A. Levy.** 1988. Biologic features of HIV-1 that correlate with virulence in the host. Science **240:**80–82.
6. **Dahl, K., K. Martin, and G. Miller.** 1987. Differences among human immunodeficiency virus strains in their capacities to induce cytolysis or persistent infection of a lymphoblastoid cell line immortalized by Epstein-Barr virus. J. Virol. **61:**1602–1608.
7. **Evans, L. A., T. M. McHugh, D. P. Stites, and J. A. Levy.** 1987. Differential ability of human immunodeficiency virus isolates to productively infect human cells. J. Immunol. **138:**3415–3418.
8. **Fisher, A. G., B. Ensoli, L. Ivanoff, M. Chamberlain, S. Petteway, L. Ratner, R. C. Gallo, and F. Wong-Staal.** 1987. The sor gene of HIV-1 is required for efficient virus transmission in vitro. Science **237:**888–893.
9. **Fisher, A. G., M. B. Feinberg, S. F. Josephs, M. E. Harper, L. M. Marselle, G. Reyes, M. A. Gonda, A. Aldovini, C. Debouk, R. C. Gallo, and F. Wong-Staal.** 1986. The trans-activator gene of HTLV-III is essential for virus replication. Nature (London) **320:**367–371.
10. **Folks, T., J. Kelly, S. Benn, A. Kinter, J. Justement, J. Gold, R. Redfield, K. W. Sell, and A. S. Fauci.** 1986. Susceptibility of normal human lymphocytes to infection with HTLV-III/LAV. J. Immunol. **136:**4049–4053.
11. **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.
12. **Hahn, B. H., G. M. Shaw, M. E. Taylor, R. R. Redfield, P. D. Markham, S. Z. Salahuddin, F. Wong-Staal, R. C. Gallo, E. S. Parks, and W. P. Parks.** 1986. Genetic variation in HTLV-III/LAV over time in patients with AIDS or at risk for AIDS. Science **232:**1548–1553.
13. **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:**59–63.
14. **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.
15. **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.
16. **Rosen, C. A., J. G. Sodroski, K. Campbell, and W. A. Haseltine.** 1986. Construction of recombinant murine retroviruses that express the human T-cell leukemia virus type II and human T-cell lymphotropic virus type III *trans*-activator genes. J. Virol. **57:**379–384.
17. **Strebel, K., D. Daugherty, K. Clouse, D. Cohen, T. Folks, and M. A. Martin.** 1987. The HIV "A" (sor) gene product is essential for virus infectivity. Nature (London) **328:**728–730.
18. **Sundström, C., and K. Nilsson.** 1976. Establishment and characterization of a human histiocytic lymphoma cell line (U-937). Int. J. Cancer **17:**565–577.
19. **Tersmette, M., R. E. Y. de Goede, B. J. M. Al, I. N. Winkel, R. A. Gruters, H. T. Cuypers, H. G. Huisman, and F. Miedema.** 1988. Differential syncytium-inducing capacity of human immunodeficiency virus isolates: frequent detection of syncytium-inducing isolates in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. J. Virol. **62:**2026–2032.
20. **von Briesen, H., W. B. Becker, K. Henco, E. B. Helm, H. R. Gelderblom, H. D. Brede, and H. Rübsamen-Waigmann.** 1987. Isolation frequency and growth properties of HIV-variants: multiple simultaneous variants in a patient demonstrated by molecular cloning. J. Med. Virol. **23:**51–66.
21. **Weiss, R. A., P. R. Clapham, J. N. Weber, A. G. Dalgleish, L. A. Lasky, and P. W. Berman.** 1986. Variable and conserved neutralization antigens of human immunodeficiency virus. Nature (London) **324:**572–575.
22. **Wong-Staal, F., G. M. Shaw, B. H. Hahn, S. Z. Salahuddin, M. Popovic, P. Markham, R. Redfield, and R. C. Gallo.** 1985. Genomic diversity of human T-lymphotropic virus type III (HTLV-III). Science **229:**759–762.