JOURNAL OF VIROLOGY, June 1988, p. 2026–2032
0022-538X/88/062026-07$02.00/0

Vol. 62, No. 6

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

MATTHIJS TERSMETTE, RUUD E. Y. DE GOEDE, BERT J. M. AL, IRENE N. WINKEL, ROB A. GRUTERS, H. THEO CUYPERS, HAN G. HUISMAN, AND FRANK MIEDEMA*

Central Laboratory of The Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam, Amsterdam, The Netherlands

Received 30 October 1987/Accepted 1 March 1988

Human immunodeficiency virus isolates were studied with respect to syncytium-inducing capacity, replicative properties, and host range. Five of 10 isolates from patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex were able to induce syncytia in cultures of peripheral blood mononuclear cells (MNC). In contrast, only 2 of 12 isolates from asymptomatic individuals had syncytium-inducing capacity. Syncytium-inducing isolates were reproducibly obtained from the same MNC sample in over 90% of the cases, independent of the donor MNC used for propagation. Syncytium-inducing capacity was shown to be a stable property of an isolate, independent of viral replication rates. Evidence was obtained that the high replication rate of syncytium-inducing isolates observed during primary isolation may be due to higher infectivity of these isolates. The finding that only syncytium-inducing isolates could be transmitted to the H9 cell line is compatible with this higher infectivity. The frequent isolation of syncytium-inducing isolates from individuals with AIDS-related complex or AIDS and the apparent higher in vitro infectivity of these isolates suggest that syncytium-inducing isolates may unfavorably influence the course of human immunodeficiency virus infection.

One of the features of human immunodeficiency virus (HIV) infection is the variability of the latency period between seroconversion and the development of disease. This period may range from a few months to 5 years or more (13, 22), and possibly some HIV-seropositive individuals will never develop acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC). Host-dependent conditions, such as constitutional factors or differences in antigenic load (e.g., allogeneic stimuli, other viral infections), may account for the differences in the clinical course observed. Alternatively, differences in pathogenicity of HIV strains or the development of a more virulent strain in the course of infection may codetermine the outcome of HIV infection.

Recent investigations have demonstrated variability between HIV isolates at the molecular level (1, 3). Variability has been shown to be most pronounced in the *env* gene (10, 26, 30). Also, HIV strains can be distinguished with monoclonal antibodies to *gag* proteins (8; M. Tersmette et al., manuscript in preparation). Differences in in vitro growth characteristics of HIV isolates cultured from peripheral blood mononuclear cells (MNC) of seropositive individuals have been reported (2, 4, 7). In the present study, we investigated differences in syncytium-inducing capacity, replication rate, and host range of HIV isolates obtained from different clinical groups, and we analyzed whether these differences reflected stable viral properties.

MATERIALS AND METHODS

Subjects. HIV isolates were recovered from seropositive individuals visiting the outpatient clinic of the Academic Medical Center, Amsterdam, or participating in a large cohort study (6).

Virus isolation. MNC from seropositive individuals were obtained by isolation on a Percoll density gradient. MNC were used either fresh or after cryopreservation. MNC (5×10^6) from seropositive individuals were cocultivated with 2×10^6 2-day phytohemagglutinin (PHA)-prestimulated MNC from healthy seronegative blood donors. Cells were cultured in 8 ml of Iscove modified Dulbecco medium containing 10% fetal calf serum, 5 μg of Polybrene per ml, partly purified interleukin 2 (20 U/ml), 1 μg of PHA per ml, and antibiotics. Twice a week, starting on day 3, cells were pelleted, and supernatants were polyethylene glycol precipitated (polyethylene glycol 6000, final concentration 7.5%) for testing in reverse transcriptase (RT) and antigen-capture assays. The cells were suspended at a concentration of 10^6/ml in the same medium without PHA. Once a week, fresh 2-day PHA-stimulated MNC of a healthy blood donor were added to the cultures. Cultures were considered positive when supernatants were positive (i.e., over three times negative control) in either antigen-capture or RT assays on at least two occasions. Cultures were kept for at least 40 days, unless the presence of HIV was demonstrated earlier. With this isolation technique, HIV was detected in 100% of MNC cultures from patients with ARC or AIDS, in >90% of MNC cultures from individuals with persistent generalized lymphadopathy, and in ≥70% of MNC cultures from asymptomatic individuals (Tersmette et al., in preparation). The presence of syncytia in coded samples was repeatedly and independently assessed by two observers. The number of syncytia in a culture was scored in a semiquantitative manner when indicated.

In some experiments, HIV was isolated from $CD8^+$ T-cell-depleted MNC. MNC were depleted for $CD8^+$ T cells by a

* Corresponding author.

TABLE 1. Characteristics of 24 HIV isolates in primary cocultures of MNC from seropositive individuals and healthy blood donors

Clinical status[a]	Syncytium-inducing isolates			Non-syncytium-inducing isolates		
	RT assay		Transmission to H9 cells[c]	RT assay		Transmission to H9 cells[d]
	Detection[b] (days)	Peak value[c] (cpm, 10^3)		Detection[b] (days)	Peak value[c] (cpm, 10^3)	
AS	20	106	2/2	22	19	0/10
PGL	11	81	1/1	14	69	0/1
ARC	5	115	2/2	11	81	0/1
AIDS	7	170	3/3	13	54	0/4

[a] AS, Asymptomatic; PGL, persistent generalized lymphadenopathy.
[b] Mean time of culture required for first detection of viral replication by RT assay.
[c] Mean of peak values in RT assay observed for each culture.
[d] Transmission to H9 cells was attempted by cocultivation and cell-free infection.

negative "panning" technique on sheep anti-mouse immunoglobulin G (IgG)-coated petri dishes, as previously described (19). Cell surface marker analysis was performed on a Coulter Epics-C cytofluorometer (Coulter Electronics, Hialeah, Fla.). T-cell subsets were determined with CD4 and CD8 monoclonal antibodies in combination with fluorescein-conjugated goat anti-mouse IgG (produced in our institute).

Transmission to the H9 cell line. For transmission of HIV isolates from MNC cultures to H9 cells (24), both cocultivation and cell-free infection were employed. In vitro HIV-infected MNC (5×10^6) were cocultivated with 5×10^6 H9 cells in a 1-ml volume at 37°C in the presence of Polybrene (2.5 μg/ml) for 1 h. Alternatively, polyethylene glycol 6000-precipitated or ultracentrifuge-pelleted (120,000 × *g*, 2 h) supernatants from HIV-infected MNC cultures were incubated with 10^7 H9 cells under the same conditions. After incubation, cells were cultured at 0.5×10^6/ml in Iscove modified Dulbecco medium containing 10% fetal calf serum, antibiotics, and Polybrene (2.5 μg/ml).

Infection experiments with cell-free virus. Cell-free virus preparations were obtained by ultracentrifuge pelleting (120,000 × *g*, 2 h) of MNC culture supernatants. For virus stock preparation, pellets were carefully suspended, aliquoted, frozen in liquid N_2, and stored at −80°C until used. For cell-free infection, 2-day PHA-stimulated MNC from blood donors were incubated for 2 h at a concentration of 5×10^6 cells per ml in the presence of Polybrene (25 μg/ml) at 37°C. Subsequently, cells were cultured at 10^6/ml in the same medium as used in the virus isolation procedure, without PHA.

$TCID_{50}$ determination. For determination of 50% tissue culture infectious dose ($TCID_{50}$), multiple serial dilutions of virus stocks were titrated onto PHA-stimulated donor MNC (10^6/ml) in microtiter plates. HIV replication was detected by testing of the culture supernatants in a p24 antigen-capture enzyme-linked immunosorbent assay at days 10 and 14. $TCID_{50}$ was defined as the dilution of virus stock resulting in 50% infected cultures under the conditions described above.

RT assay. The RT assay was performed as described before (24) with slight modifications. Briefly, 8-ml samples of culture supernatants were precipitated with polyethylene glycol (7.5% final concentration). The precipitate was suspended in 100 μl of 50 mM Tris hydrochloride (pH 7.5) containing 0.25 M KCl, 20% glycerol, and 0.25% Triton X-100. The assay was performed under conditions as described before (12), with the omission of glutathione.

Antigen-capture assays. HIV p24 in supernatants was detected by two types of antigen-capture assays. In HIV isolation studies, a radioimmunoassay was used, which is described in detail elsewhere (Tersmette et al., in preparation). In this assay, HIV p24 is captured by rabbit IgG anti-HIV coupled to Sepharose beads and detected by a ^{125}I-labeled monoclonal antibody directed to HIV p24. For $TCID_{50}$ determinations, a sandwich enzyme-linked immunosorbent assay for HIV p24 was used. Inactivated culture supernatants were added to microtiter plates coated with purified rabbit IgG. After washing, bound p24 was detected with horseradish peroxidase-labeled monoclonal antibody to p24, followed by substrate.

RESULTS

HIV isolation from MNC from seropositive individuals. In this study, we obtained and analyzed HIV isolates from seropositive individuals, spanning the whole clinical spectrum of HIV infection.

The main features of virus isolates consecutively obtained from 24 individuals belonging to different clinical groups are shown in Table 1. The mean time of culture required for detection of HIV in the supernatant by RT assay decreased with progression of disease. In about 30% of the cultures, syncytium formation was observed (Fig. 1). Syncytium-inducing isolates were seen in MNC cultures from 5 of 10 patients with ARC or AIDS, but only in 2 of 12 MNC cultures from asymptomatic individuals (Table 1). In these cultures, RT activity was detected earlier than in MNC cultures from individuals of the same clinical group, and RT peak activity was consistently higher (Table 1). Transmission to H9 cells was attempted by cocultivation and infection with cell-free virus. Successful transmission, resulting in continuous replication, was obtained with all syncytium-inducing but none of the non-syncytium-inducing isolates.

HIV was repeatedly isolated from cryopreserved MNC from the same sample used for the initial isolation from 13 of 24 subjects described in Table 1. In most cases, all isolates obtained from one person either did or did not induce syncytia, the only exception being an asymptomatic individual (no. 33) from whom a syncytium-inducing HIV isolate, transmissible to H9, was obtained in only one of five isolations (Table 2). Moreover, preliminary studies on sequential HIV isolates from six individuals obtained over a period of 18 months showed remarkably constant properties with respect to replication rate, syncytium induction, and transmissibility to the H9 cell line (Tersmette et al., in preparation).

MNC of three seropositive asymptomatic individuals, who previously had yielded non-syncytium-inducing isolates, were cultured in parallel with and without depletion for $CD8^+$ cells. Depletion for $CD8^+$ cells did result in earlier

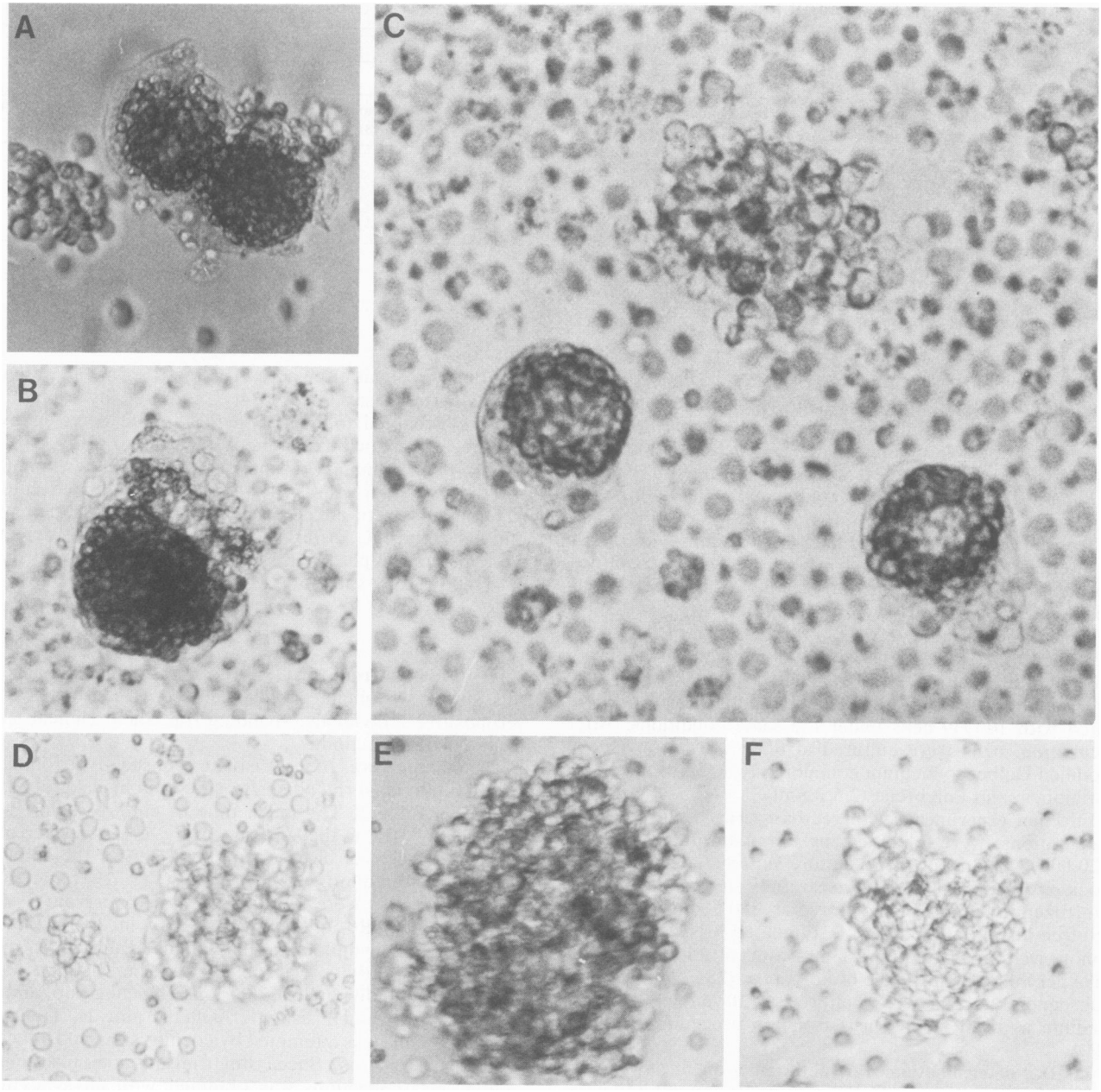

FIG. 1. Representative examples of MNC cultures infected with syncytium-inducing (A, $HIV_{AMS}16$; B, $HIV_{AMS}42$; C, $HIV_{AMS}42$) or non-syncytium-inducing (D, $HIV_{AMS}24$; E, $HIV_{AMS}35$) HIV isolates. The large, multinucleated syncytial cells with irregularly shaped cytoplasm can be easily distinguished from normal MNC clusters (for instance, note the difference between the two syncytia and the normal cell cluster in panel C). (F) Uninfected MNC control.

detection of HIV replication, as has been described before (29). However, no effect was observed with respect to the occurrence of syncytia (Table 3).

Induction of syncytia is independent of donor leukocytes. To study whether nonviral factors, such as the number of infected patient cells or the level of CD4 expression of the donor cells, are major determinants in the process of syncytium induction, MNC of five different blood donors were infected with fresh, cell-free inocula of three syncytium-inducing isolates ($HIV_{AMS}37$, $HIV_{AMS}42$, and human T-cell lymphotropic virus type IIIB) and one non-syncytium-inducing isolate ($HIV_{AMS}24$). As has been reported before (7, 9), some differences in HIV replication were observed between MNC cultures from different donors (Fig. 2). With all five MNC, the ranking order of HIV isolates with respect to replication rate was roughly the same (Fig. 2). Within 8 days, all three syncytium-inducing isolates produced numerous (over five syncytia per 100× field) large syncytia in cultures from all five donor MNC. Only minor variations in size and number of syncytia could be observed among cultures of different donor MNC. HIV replication in $HIV_{AMS}24$-infected cultures was higher than or equal to the replication observed in cultures infected with $HIV_{AMS}42$ and -37, respectively (Fig. 2). Nevertheless, in cultures infected with $HIV_{AMS}24$, only a few, small (<10 nuclei), transiently present syncytia could be observed.

TABLE 2. Reproducibility of isolation of syncytium-inducing HIV strains

Subject no.	Clinical status	No. of isolations	No. of syncytium-inducing isolates
33	AS[a]	5	1
35	AS	4	0
49	AS	3	0
52	AS	3	0
53	AS	3	0
16	ARC	6	6
42	ARC	4	4
24	AIDS	3	0
32	AIDS	3	3
37	AIDS	5	5
38	AIDS	5	0
54	AIDS	2	0
55	AIDS	2	2

[a] AS, Asymptomatic.

Syncytium induction is a replication-independent property of HIV isolates. To address the relation between HIV replication and syncytium formation more fully, MNC of two different healthy donors were infected with high equal doses of five different HIV isolates (three syncytium inducing, two non-syncytium inducing). Inocula were carefully adjusted by titration in the RT assay. Also, the $TCID_{50}$s of the inocula were determined. After a 2-h incubation, cells were washed twice to remove free virus. All three isolates which induced syncytia in cocultivation experiments produced high numbers of large syncytia. In contrast, in cultures infected with $HIV_{AMS}53$ and $HIV_{AMS}35$, only few small syncytia or no syncytia at all, respectively, were observed (Table 4). At day 5, 10 to 15% of the cells of cultures infected with either type of virus expressed HIV antigen, as detected with a biotin-avidin staining technique. Virus replication, as determined by RT activity measured at three time points, did not significantly differ between syncytium- and non-syncytium-inducing isolates. This experiment demonstrates that occurrence of syncytia is not just a result of high virus replication and that between HIV isolates there are obvious differences in syncytium-inducing capacities.

Rapid spread of syncytium-inducing isolates in MNC. In the previous experiment, differences in replication were not found when identical MNC were infected with high equal doses of syncytium- and non-syncytium-inducing isolates. Nevertheless, in primary isolations, starting with low numbers of infected cells, syncytium-inducing isolates grew more rapidly to high titers (Table 1). To study the latter phenomenon, the following experimental setup was designed. PHA-prestimulated MNC (0.5×10^6) were infected with high equal inocula of the same strains used in the previous experiment (Table 5). After a 2-h incubation, the cells were thoroughly washed and added to cultures of 4.5×10^6 PHA-prestimulated MNC of the same donor. Thus, on the basis of the data presented in Table 4, these cultures may be expected to have been inoculated with comparable numbers of infected autologous cells. In agreement with observations in primary isolations, the syncytium-inducing isolates were found to replicate much more rapidly compared to non-syncytium-inducing isolates (Table 5), indicating a more rapid spread of the former.

DISCUSSION

In the present report, we studied differences in in vitro properties of HIV isolates. In the initial isolation experiments, two observations attract attention (Table 1). First, the time of culture required for first detection of viral replication by RT activity was decreased in MNC cocultures from individuals at later stages of disease. This is in agreement with the results of Åsjö et al. (2). Second, in cultures from about 30% of the individuals, syncytia could be detected. Although the number of subjects tested is too small for definite conclusions, it seems that syncytium-inducing isolates are found more often in cultures from patients with ARC and AIDS.

TABLE 3. Effects of $CD8^+$ cell depletion on the isolation of slow-replicating, non-syncytium-inducing HIV isolates

Subject no.[a]	CD8 depletion	% $CD8^+$ cells	Antigen-capture assay detection[b] (days)	RT assay	
				Detection[b] (days)	Peak value[c] (cpm, 10^3)
46	−	43	15	>30	<5
	+	13	8	13	18
52	−	34	13	>30	6
	+	7	13	19	16
53	−	31	30	>30	<5
	+	6	19	>30	<5

[a] All subjects were asymptomatic.
[b] Mean time of culture required for detection of HIV replication in the antigen-capture assay or RT assay (cultures in duplicate).
[c] Mean peak value in the RT assay observed at 30 days of culture.

Remarkably, transmission to the H9 cell line, resulting in stable infection, could be achieved with all syncytium-inducing HIV isolates, but with none of the non-syncytium-inducing isolates. This is in agreement with previous reports that only about 30% of the HIV isolates can be transmitted to HIV-permissive cell lines (2, 7). The failure of HIV isolates to induce syncytium formation was not due to suppressor activity mediated by $CD8^+$ cells (29).

From cell-free transmission experiments, it can be concluded that the capacity to induce syncytia in culture is a stable property exhibited by particular HIV strains.

Several groups have shown that the binding of the viral gp120 to the CD4 molecule is the initial step in both syncytium formation (16, 25) and cell-free infection (17, 18). Since syncytium formation can be blocked by certain mono-

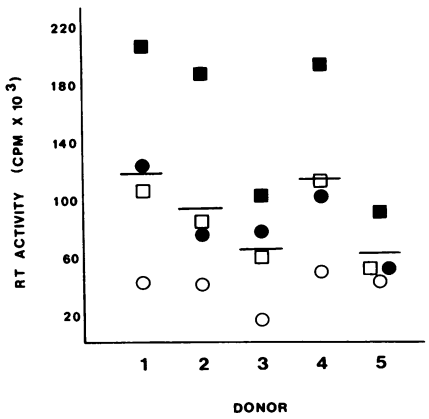

FIG. 2. RT activity at day 8 in supernatants of cultures of five different donor MNC, infected with four different HIV isolates. Symbols: □, $HIV_{AMS}24$; ●, $HIV_{AMS}37$; ○, $HIV_{AMS}42$; ■, human T-cell lymphotropic virus type IIIB. $HIV_{AMS}24$ was non-syncytium inducing; $HIV_{AMS}37$ and -42 were syncytium inducing in primary isolation. Horizontal bars represent means.

TABLE 4. Differences in syncytium formation in MNC cultures infected with high, equal infectious doses of different HIV isolates[a]

HIV isolate (condition[b])	Presence of syncytia in original isolation	Inoculum		MNC cultures							
		RT activity (cpm, 10^5)	$TCID_{50}$	Syncytia[c] at day:					RT activity[d] (cpm, 10^3) at day:		
				3	5	7	10	12	5	10	13
$HIV_{AMS}16$ (ARC)	+	34	$10^{4.0}$	+	++	++	++	++	115	20	6
$HIV_{AMS}35$ (AS)	−	39	$10^{5.2}$	−	−	−	−	−	139	22	7
$HIV_{AMS}42$ (ARC)	+	28	$10^{5.0}$	+	++	++	++	++	114	5	5
$HIV_{AMS}53$ (AS)	−	38	$10^{4.0}$	−	±	−	±	±	113	15	5
$HIV_{AMS}55$ (AIDS)	+	33	$10^{4.5}$	+	+	++	++	++	165	11	4

[a] MNC were incubated with viral inocula for 2 h. Cells were then washed thoroughly and put into culture at 10^6 cells per ml.
[b] Clinical condition of the individual from whom the isolate was obtained; AS, asymptomatic.
[c] −, No syncytia present; ±, only occasionally small syncytia present; +, one to five syncytia per 100× field; ++, over five syncytia per 100× field.
[d] Mean of duplicate cultures.

clonal antibodies to CD4 at a lower concentration than cell-free infection, it is thought that for syncytium formation higher numbers of interacting CD4 and gp120 molecules are required (5). Therefore, differences in syncytium-inducing capacity of HIV isolates may be due to differences in regulation of viral expression, as a consequence of which some HIV isolates induce higher densities of gp120 molecules at the cell surface. Second, the gp120 of some HIV variants could have higher affinity for the CD4 molecules than others. Third, differences in other HIV components thought to be active in the process of virus entry, particularly gp41 (14) and the 23-kilodalton SOR protein (27), may contribute to differences in syncytium induction by HIV isolates.

When MNC cultures were infected with high, equal inocula of different HIV isolates, no differences in viral replication rate were observed between syncytium- and non-syncytium-inducing isolates. Since in this type of experiment it may be expected that, due to high multiplicity of infection, most susceptible cells become simultaneously infected, this finding indicates that there is no difference in efficiency of virion production per cell between syncytium- and non-syncytium-inducing isolates. On the other hand, experiments with cellular inocula, containing comparable numbers of infected cells, revealed clear differences in virus replication rates in MNC culture between the two types of isolates.

TABLE 5. Rapid replication of syncytium-inducing HIV isolates in MNC cultures infected with cellular inocula

HIV isolate (condition[a])	Presence of syncytia in original isolation	RT activity of HIV prepn[b] (cpm, 10^4)	MNC cultures					
			Syncytia[c] at day:			RT activity[d] (cpm, 10^3) at day:		
			4	7	10	4	7	11
$HIV_{AMS}16$ (ARC)	+	78	++	++	+	36	34	12
$HIV_{AMS}35$ (AS)	−	102	−	−	−	3	3	11
$HIV_{AMS}42$ (ARC)	+	78	++	++	+	35	74	7
$HIV_{AMS}53$ (AS)	−	96	−	−	−	3	3	31
$HIV_{AMS}55$ (AIDS)	+	66	+	++	+	28	76	8

[a] Clinical condition of the individual from whom the isolate was obtained; AS, asymptomatic.
[b] HIV preparation used to prepare the cellular inoculum. Cells (0.5×10^6) were incubated with the virus preparation for 2 h, washed thoroughly, and added to cultures of 4.5×10^6 autologous MNC.
[c] −, No syncytia present; +, one to five syncytia per 100× field; ++, over five syncytia per 100× field.
[d] Mean of duplicate cultures.

Apparently, syncytium-inducing isolates have a comparatively high infectivity, resulting in a more rapid spread, leading to higher replication rates as detected by RT activity in the culture supernatant. The early and high RT activity produced by syncytium-inducing isolates in this experiment is in agreement with the early and high RT activity observed during primary isolation of syncytium-inducing isolates (Table 1).

Both syncytium-inducing capacity and high infectivity may well be a consequence of the same viral denominator. Moreover, the ability of syncytium-inducing isolates to persistently infect the H9 cell line may also be related to this same property. Since in experiments with cell-free virus inocula no difference in replication rate was observed, it seems most likely that this property is structural rather than regulatory. Thus, the biological differences between syncytium- and non-syncytium-inducing isolates are probably caused by structural differences in gp120, gp41, or possibly SOR molecules.

One of the central, unresolved problems in the pathogenesis of AIDS is the exact mechanism by which $CD4^+$ T-cell depletion occurs, since at no point in time in the course of infection could more than 1 in about 10^4 MNC be shown to be infected with HIV (11). A possible explanation for this apparent paradox is the hypothesis according to which $CD4^+$ T-cell depletion occurs by syncytium formation of long-living, low CD4-expressing, infected cells (e.g., monocytes [15], dendritic cells [21], or Epstein-Barr virus-transformed B cells [20, 28]) with uninfected high $CD4^+$-expressing T cells (24). If this mechanism were to account for the $CD4^+$ T-cell depletion, it may be expected that syncytium-inducing capacity of HIV isolates would correlate with later stages of disease. The results of our experiments seem partially to favor this hypothesis. Syncytium-inducing isolates were observed more frequently in MNC cocultures from patients with ARC and AIDS than in cultures from asymptomatic individuals (Table 1). The apparent higher infectivity of these isolates may lead to a more rapid spread of the virus. Moreover, the ability of syncytium-inducing HIV isolates to persistently infect H9 cells may reflect the fact that these isolates have acquired a broader host range, enabling them to infect more subpopulations of $CD4^+$ T cells. Recent observations in a large cohort of homosexual men identified sexual contact with a person in whom AIDS developed as an independent risk factor for progression to AIDS (23). This also may indicate the presence of more virulent HIV strains in such persons. Nevertheless, in our study also non-syncytium-inducing HIV isolates were ob-

tained from patients with ARC and AIDS. Some HIV isolates, $HIV_{AMS}24$ and -53, that were not able to produce syncytia in cocultivation assays, temporarily induced some small syncytia when MNC were infected at a high multiplicity of infection, presumably due to synchronization of the replication cycle occurring in this type of experiment. This may indicate that the differences in syncytium-inducing capacity between HIV isolates are gradual rather than absolute. In our preliminary, longitudinal study, a switch from one type of HIV to the other was not observed. However, the isolation of a syncytium-inducing HIV variant from one asymptomatic individual in one out of five experiments (Table 2) may indicate that these changes do occur, albeit at a low frequency. Closer analysis of the putative variability of *env* or *sor* gene products between syncytium-inducing and non-syncytium-inducing HIV variants may contribute to a better understanding of the pathogenesis of HIV infection.

ACKNOWLEDGMENTS

We are grateful to the following physicians for assistance in obtaining blood samples from seropositive individuals and providing clinical information: F. de Wolf, J. K. Eeftinck-Schattenkerk, and S. Danner. We thank J. Goudsmit for helpful discussions and Joline Claassen for technical assistance.

This study was supported in part by grants 28-1079 and 28-1379 from The Netherlands Foundation for Preventive Medicine. F.M. is a senior fellow of the Royal Netherlands Academy of Arts and Sciences.

LITERATURE CITED

1. **Alizon, M., S. Wain-Hobson, L. Montagnier, and P. Sonigo.** 1986. Genetic variability of the AIDS virus. Cell **46**:63–74.
2. **Åsjö, B., J. Albert, A. Karlsson, L. Morfeldt-Månson, G. Biberfeld, K. Lidman, and E. M. Fenyö.** 1986. Replicative properties of human immunodeficiency virus from patients with varying severity of HIV infection. Lancet **ii**:660–662.
3. **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**:1548–1553.
4. **Dahl, K., K. Martin, and G. Miller.** 1987. Differences among HIV strains in their capacity to induce cytolysis or persistent infection of a lymphoblastoid cell line immortalized by Epstein-Barr virus. J. Virol. **61**:1602–1608.
5. **Dalgleish, A. G., P. C. L. Beverley, P. R. Clapham, D. H. Crawford, M. F. Greaves, and R. A. Weiss.** 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature (London) **312**:763–767.
6. **De Wolf, F., J. Goudsmit, D. A. Paul, J. M. A. Lange, C. Hooijkaas, P. T. A. Schellekens, R. A. Coutinho, and J. van der Noordaa.** 1987. Persistent HIV antigenemia is associated with rapid clinical and immunological deterioration. Br. Med. J. **295**:569–572.
7. **Evans, L. A., T. M. McHugh, D. P. Stites, and J. A. Levy.** 1987. Differential ability of HIV isolates to productively infect human cells. J. Immunol. **138**:3415–3418.
8. **Ferns, R. B., R. S. Tedder, and R. A. Weiss.** 1987. Characterisation of monoclonal antibodies against the HIV gag products and their use in monitoring HIV isolate variation. J. Gen. Virol. **68**:1543–1551.
9. **Folks, T., J. Kelly, S. Bean, A. Kinker, J. Juntenant, 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.
10. **Hahn, B. H., M. A. Gonda, G. M. Shaw, M. Popovic, J. A. Hoxie, R. C. Gallo, and F. Wong-Staal.** 1985. Genomic diversity of the acquired immunodeficiency virus HTLV III: different viruses exhibit greatest divergence in their envelope genes. Proc. Natl. Acad. Sci. USA **82**:4813–4817.
11. **Harper, M. E., L. M. Marselle, R. C. Gallo, and F. Wong-Staal.** 1986. Detection of lymphocytes expressing HTLV-III in lymph nodes and peripheral blood from infected persons by in situ hybridization. Proc. Natl. Acad. Sci. USA **83**:772–776.
12. **Hoffman, A. D., B. Banapour, and J. A. Levy.** 1985. Characterization of the AIDS-associated retrovirus reverse transcriptase and optimal conditions for its detection in virions. Virology **147**:326–335.
13. **Jaffe, H. W., W. W. Darrow, D. F. Echenberg, P. M. O'Malley, J. P. Getchell, V. S. Kalyanaraman, R. H. Byers, D. P. Drennan, E. H. Braff, J. W. Curran, and D. P. Francis.** 1985. The acquired immunodeficiency syndrome in a cohort of homosexual men. A six year follow-up study. Ann. Intern. Med. **103**: 210–214.
14. **Kowalski, M., J. Potz, L. Basiripour, T. Dorfman, W. C. Goh, E. Terwilliger, A. Dayton, G. Rosen, W. Haseltine, and J. Sodroski.** 1987. Functional regions of the envelope glycoprotein of human immunodeficiency virus type I. Science **237**:1351–1355.
15. **Levy, J. A., J. Shimabukuro, T. McHugh, C. Casavant, D. Stites, and L. Oshiro.** 1985. AIDS-associated retrovirus (ARV) can productively infect other cells besides human T helper cells. Virology **147**:441–448.
16. **Lifson, J. D., M. B. Feinberg, G. R. Reyes, L. Rabin, B. Banapour, S. Chakrabarti, B. Moss, F. Wong-Staal, K. S. Steiner, and E. G. Engelman.** 1986. Induction of CD4-dependent cell fusion by the HTLV-III/LAV envelope glycoprotein. Nature (London) **323**:725–728.
17. **McDougal, J. S., M. S. Kennedy, J. M. Sligh, S. P. Cort, A. Marole, and J. K. A. Nicholson.** 1986. Binding of HTLV-III/LAV to $T4^+$ T cells by a complex of the 110 K viral protein and the T4 molecule. Science **231**:382–385.
18. **McDougal, J. S., J. K. A. Nicholson, G. D. Cross, S. P. Cort, M. S. Kennedy, and A. C. Mawle.** 1986. Binding of the human retrovirus HTLV-III/LAV/ARV/HIV to the CD4 (T4) molecule: conformation dependence, epitope mapping, antibody inhibition, and potential for idiotypic mimicry. J. Immunol. **137**: 2937–2944.
19. **Miedema, F., J. W. Van Oostveen, R. W. Sauerwein, F. Terpstra, L. A. Aarden, and C. J. M. Melief.** 1985. Induction of immunoglobulin synthesis by I12 is $T4^+T8^-$-cell-dependent. A role for I12 in the pokeweed-mitogen-driven system. Eur. J. Immunol. **15**:107–112.
20. **Montagnier, L., J. Gruest, S. Chamaret, C. Dauguet, C. Axler, C. Guetard, M. T. Nugeyre, F. Barré-Sinoussi, J. C. Chermann, J. B. Brunt, D. Klatzmann, and J. C. Gluckman.** 1984. Adaption of lymphadenopathy-associated virus (LAV) to replicate in EBV-transformed B-lymphoblastoid cell lines. Science **225**: 63–65.
21. **Patterson, S., and S. C. Knight.** 1987. Susceptibility of human peripheral blood dendritic cells to infection by HIV. J. Gen. Virol. **68**:1177–1181.
22. **Peterman, T. A., H. W. Jaffe, P. M. Feorino, J. P. Getchell, D. T. Warfield, H. W. Haverkos, R. L. Stoneburner, and J. W. Curran.** 1985. Transfusion-associated AIDS in the United States. J. Am. Med. Assoc. **254**:2913–2917.
23. **Polk, B. F., R. Fox, R. Brookmeyer, S. Kanchanaraska, R. Kaslow, B. Visscher, C. Rinaldo, and J. Phair.** 1987. Predictors of the acquired immunodeficiency syndrome developing in a cohort of homosexual men. N. Engl. J. Med. **316**:61–66.
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. **Sodroski, J., W. C. Goh, C. Rosen, K. Campbell, and W. A. Haseltine.** 1986. Role of the HTLV-III/LAV envelope in syncytium formation and cytopathicity. Nature (London) **322**:470–474.
26. **Starcich, B. R., B. H. Hahn, G. M. Shaw, P. D. McNeely, S. Modrow, H. Wolf, E. S. Parks, W. Parks, S. F. Josephs, R. C. Gallo, and F. Wong-Staal.** 1986. Identification and characterisation of conserved and variable regions in the envelope gene of

HTLV-III/LAV, the retrovirus in AIDS. Cell **45**:637–648.
27. **Stiebel, K., D. Daugherty, K. Clouse, D. Cohen, T. Folks, and M. A. Martin.** 1987. The HIV "A" (SOR) gene product is essential for viral infectivity. Nature (London) **328**:728–730.
28. **Tersmette, M., F. Miedema, J. G. Huisman, J. Goudsmit, and C. J. M. Melief.** 1985. Productive HTLV-III infection of human B-cell lines. Lancet **i**:815–816.
29. **Walker, C. M., D. J. Moody, D. P. Stites, and J. A. Levy.** 1986. $CD8^+$ lymphocytes can control HIV infection *in vitro* by suppressing virus replication. Science **234**:1563–1566.
30. **Willey, R. L., R. A. Rutledge, S. Dias, T. Folks, T. Theodore, C. E. Buckler, and M. A. Martin.** 1986. Identification of conserved and divergent domains within the envelope gene of the AIDS retrovirus. Proc. Natl. Acad. Sci. USA **83**:5038–5042.