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Isolation of Human Immunodeficiency Virus (HIV) From Plasma During Primary HIV Infection

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Human immunodeficiency virus (HIV) has been isolated from plasma in 6 of 7 patients showing clinical symptoms of a primary HIV infection. Parallel cultures from peripheral blood mononuclear cells (PBMC) yielded virus in 5 patients. In one case, virus could only be isolated from the cerebrospinal fluid but not from peripheral blood. Detectable viremia was transient and preceded the appearance of HIV specific antibodies. After cessation of acute symptoms, the frequency of HIV isolations was similar to that of asymptomatic carriers (23 and 26%, respectively). The role of the immune response in terminating detectable viremia remains to be established.

Key words: HIV, plasma, primary infection

INTRODUCTION

Seroconversion following infection with human immunodeficiency virus (HIV) has been reported to be associated with acute illness in some cases. A mononucleosislike disease was found to coincide with seroconversion in 9 out of 10 patients [Cooper et al, 1985]. By contrast in a study of 15 HIV-infected individuals, others found no signs of clinical illness associated with seroconversion [Weber et al, 1986]. It is not clear to which extent infected persons develop symptoms during the initial phase of the infection. The

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TABLE I. Isolation of HIV From Peripheral Blood Mononuclear Cells of Persons With Different Clinical and HIV Antibody Status

Subjects	HIV antibody status[a]	No. of positive isolations/no. of attempts (% positive)
Patients with diagnosis of		
AIDS	positive	12/12 (100)
AIDS-related complex	positive	13/16 (81)
Lymphadenopathy syndrome	positive	15/22 (68)
Other individuals		
asymptomatic carrier	positive	10/39 (26)
asymptomatic or mild symptoms, at risk for HIV infection	negative	0/77 (0)

[a]All patients were tested by Organon and Abbott ELISA. Positive reactions were confirmed by Western blotting test.

illness, designated primary HIV infection, shows an acute onset and the symptoms most commonly found are fever, malaise, sore throat, lymphadenopathy, rash, and myalgia [Cooper et al, 1985; Sönnerborg et al, 1985]. Aseptic meningitis and encephalitis have been described [Carne et al, 1985]. The acute clinical symptoms usually subside within 1 to 2 weeks, but may in some cases be followed by persistent lymphadenopathy [Sönnerborg et al, 1985].

HIV has been isolated from peripheral blood mononuclear cells (PBMC) of two patients and from cerebrospinal fluid (CSF) of one patient during the acute illness preceding seroconversion [Ho et al, 1985]. Goudsmit et al [1986] showed that HIV antigens were present in serum prior to seroconversion in 5 of 35 patients. The authors suggested that perhaps most HIV-infected individuals have viral antigens circulating in plasma before antibody production. The aim of the present study was to establish whether free virus is present in plasma during primary HIV infection.

SUBJECTS AND METHODS

Subjects

A group of 166 patients from whom we attempted isolation of HIV during September 1985 to September 1986 is listed in Table I. Patients were grouped according to clinical and HIV antibody status. In addition, seven male patients with symptoms suggestive of primary HIV infection were studied. All seven patients had fever, myalgia, and lymphadenopathy; six patients had pharyngitis and exanthema. Transient trombocytopenia and leukopenia were observed in five patients; three patients (nos. 3, 5, and 7) had meningoencephalitis. The acute illness lasted 1–3 weeks as indicated in Figure 1. At follow-up 2 to 6 months after the acute disease, all seven patients still had persisting lymphadenopathy.

Six patients are homosexual or bisexual men. One patient had no identifiable risk factor other than one single heterosexual encounter with a female prostitute during a 4-week stay in East Africa. There was no evidence of infections other than that caused by HIV in any of the patients. Patient no. 7 was treated intravenously with Foscarnet (Astra) (7.5 g every 12 hours) from day 12 to day 22.

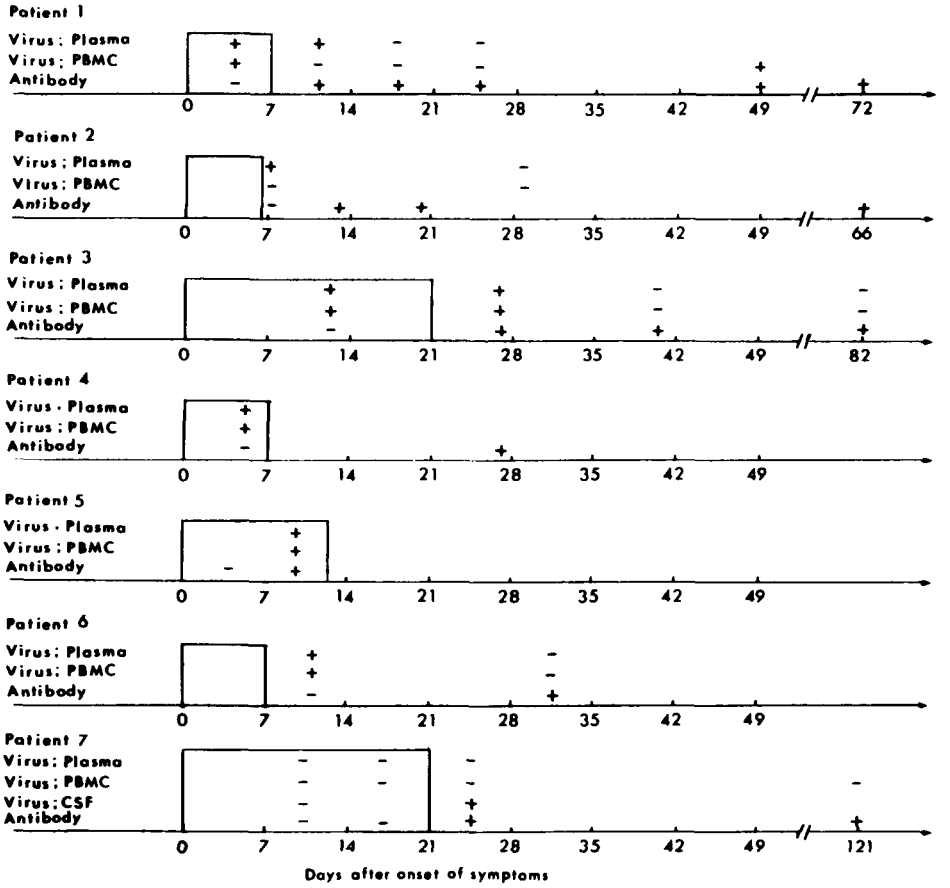

Fig. 1. Isolation of HIV in plasma, peripheral blood mononuclear cells (PBMC), and cerebrospinal fluid (CSF) and demonstration of antibodies by radioimmunoprecipitation assay (RIPA). The time period during which the patients had acute symptoms is indicated.

Virus Isolations

The virus isolations from plasma and peripheral blood mononuclear cells (PBMC) were carried out as follows: 5 ml of plasma diluted 1:2 in phosphate-buffered saline (PBS) was centrifuged at 1,200 g for 10 minutes to remove remaining cells and platelets, and the supernatant was centrifuged at 130,000 g for 30 minutes to pellet possible virus. The pellet was resuspended in culture medium and added to 10^7 phytohemagglutinin-P (PHA-P; Difco, 2.5 μg/ml for 3 days) stimulated PBMC obtained from a healthy donor seronegative for HIV; 10^7 PBMC from the healthy donor alone were cultured as control. Our procedure for virus isolation from PBMC has been described [Åsjö et al, 1986]. Cell-free cerebrospinal fluid (CSF) and filtered (0.45 μ Millipore) saliva were centrifuged and cultured in the same way as plasma. Cells from CSF were cocultured with 10^7 PHA-P stimulated PBMC; 3×10^6 PHA-P stimulated PBMC were added to each culture once a week. The culture supernatants were assayed for reverse transcriptase (RT) activity two times per week for 4–6 weeks as described [Åsjö et al, 1986]. RT tests resulting in

radioactivity counts above 8,000 cpm/ml of culture medium were considered positive. Background radioactivity counts ranged from 400 to 1,000 cpm/ml. The cultures of patients nos. 3–7 were also assayed by fixed-cell direct immunofluorescence using the fluorescein isothiocyanate conjugated Ig fraction of a serum with high antibody titers against HIV. RT positive cultures were cocultivated with H9 or CEM cells as described [Åsjö et al, 1986].

Detection of HIV-Specific Antibodies

Antibodies to HIV proteins in patients' sera were detected by radioimmunoprecipitation assay (RIPA), Western blot (WB) test, and ELISA. The RIPA antigen was prepared from cell lysates of ^{35}S cystein-labeled cells of the monocytic cell line U937 clone 16 [Sundström et al, 1976]. The details of immunoprecipitation and gel electrophoresis have been described previously [Essex et al, 1983]. Two major modifications were made; protein A sepharose was added after the formation of immune complexes and a 9–16% gradient polyacrylamide gel was used. WB test (Dupont) and ELISA (Organon and Abbott) were performed according to manufacturers' recommendations.

RESULTS

Isolation of HIV From Patients With Varying Severity of HIV Infection

According to our experience, the frequency of positive HIV isolations from peripheral blood mononuclear cells (PBMC) of patients with HIV-specific antibodies varies depending on the severity of clinical conditions. Hence lymphocyte cultures from patients with advanced AIDS yielded virus regularly in contrast to only 26% of cultures from asymptomatic carriers (Table I). Among 77 seronegative individuals "at risk" for HIV infection, most of them being sex partners to seropositive individuals, none yielded virus upon isolation. At variance with these data are the positive virus isolations obtained from the 7 patients with clinical signs of primary HIV infection.

Isolation of HIV From Patients Undergoing a Primary HIV Infection

Figure 1 shows the results of virus isolations and antibody tests for all 7 patients. HIV has been isolated from plasma in 6 patients, in some on two separate occasions (patients nos. 1 and 3). All successful isolations from plasma were from samples taken during the acute illness or within one week after the disappearance of acute symptoms. Further attempts to isolate virus from plasma of four patients were unsuccessful. PBMC did not always yield virus, even if virus could be recovered from plasma. None of the cocultivations with H9 and CEM cells yielded virus.

Patient no. 7 did not have detectable virus in plasma or PBMC in samples taken 11, 17, and 25 days after onset of symptoms. The cerebrospinal fluid (CSF), although initially negative (11 days), yielded HIV on day 25. CSF samples of patients nos. 1 and 3, as well as saliva samples of all patients were virus negative (data not shown).

In summary, infectious HIV can be isolated from patients showing clinical signs of a primary HIV infection. After the cessation of acute disease symptoms, virus isolation becomes more difficult and is comparable to the frequency of positive HIV isolations from asymptomatic carriers, 23% and 26%, respectively.

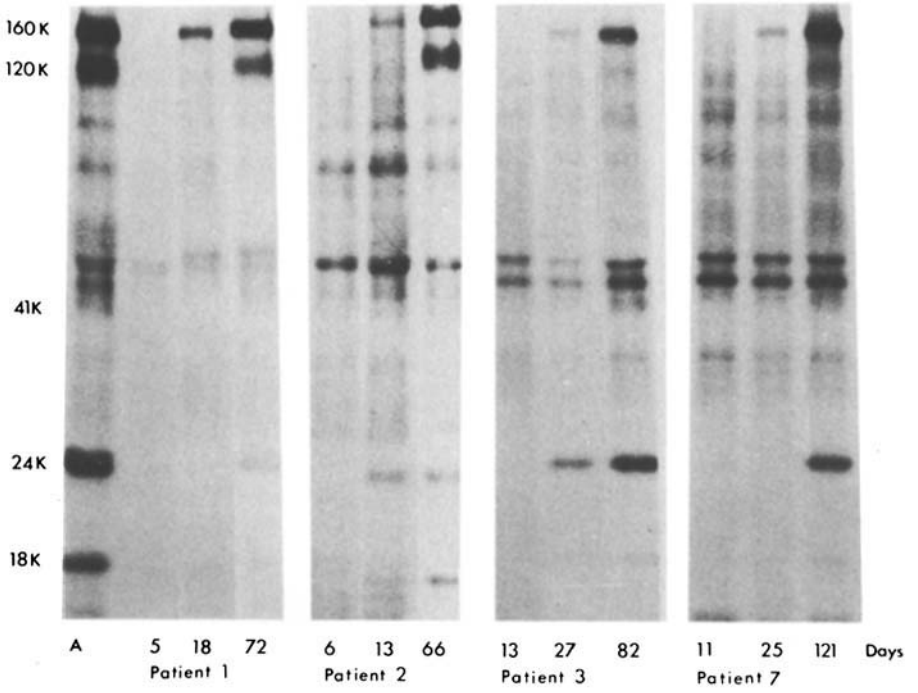

Fig. 2. Reactivity of serum samples to HIV (HTLV-IIIB strain) proteins as determined by radioimmunoprecipitation assay. Lane A: Serum from a patient with persistent generalized lymphadenopathy known to react with several HIV proteins (positive control). Serum samples from patients 1, 2, 3, and 7 were drawn at indicated number of days after onset of acute symptoms.

Appearance of HIV-Specific Antibodies in Patients Undergoing a Primary HIV Infection

HIV-specific antibodies appeared in all patients and were demonstrated by radioimmunoprecipitation assay (RIPA), WB tests, and ELISA. Detailed comparison of the different serological methods will be presented elsewhere. The results of RIPA are shown in Figure 1 and representative gels in Figure 2. HIV-specific antibodies were first detected 10 to 33 days after the onset of symptoms. The sera of all patients precipitated the precursor of viral envelope glycoproteins, gp160, at the time of seroconversion. In fact, this was the only viral protein precipitated by patients nos. 1 and 7 at day 18 and 25, respectively. Sera from other patients precipitated a number of viral proteins, such as p24 (the major core protein), as illustrated by patients nos. 2 and 3 in Figure 2. HIV was isolated from plasma on three occasions (patients nos. 1, 3, and 5) in the presence of antibodies.

DISCUSSION

Our results indicate that patients with symptomatic primary HIV infection may transiently carry infectious virus in plasma. In a recent report, 5 of 35 patients were shown

to have HIV antigen in serum prior to seroconversion [Goudsmit et al, 1986]. As judged by our results, it seems probable that the demonstration of HIV antigens in serum reflects true viremia at this early stage. The fact that we were able to demonstrate viremia in the majority (6/7) of cases, whereas Goudsmit et al [1986] found antigenemia in only 5/35 cases, suggests that virus isolation is a more sensitive method for virus detection than detection of viral antigens. It cannot be excluded, however, that differences in time of blood sampling and selection of patients are responsible for the apparent differences.

The isolation of infectious virus from plasma in 6 out of 7 patients in the present study indicates that individuals with primary HIV infection may be contagious during the early events of the disease, before the appearance of serum antibodies. In accordance with our observation, it has recently been reported that two blood recipients became infected by blood given by one seronegative donor who probably had a subclinical primary HIV infection at the time of the donation [Anonymous, 1986].

In two cases we recovered virus from plasma when no virus could be isolated from PBMC. This might be a result of technical difficulties in recovering HIV from infected PBMC, but it is also possible that cells other than PBMC are infected during the early stages of primary HIV infection. The possibility that monocytes are the initially infected cells cannot be ruled out since the presence of infected monocytes may not be detected in PBMC cultures due to unfavorable culture conditions [Gartner et al, 1986].

The absence of detectable infectious virus in plasma and, except on one occasion, in PBMC after the cessation of the acute symptoms is interesting. The immune response of the infected individual can be presumed to play a role in terminating the acute disease and detectable viremia. In the present study there was a certain correlation in time between the appearance of antibodies and the disappearance of detectable viremia. However, this correlation should not be overinterpreted since the neutralizing capacity of these sera has not been tested. Other mechanisms, such as the recently described suppressing action of OKT 8 positive T-cells [Walker et al, 1986], may prove to be more important in terminating detectable viremia.

In summary, we have shown that HIV was present in plasma of 6 of 7 patients during the early stages of primary HIV infection. This suggests that most patients with symptomatic primary HIV infection are viremic prior to seroconversion. Probably the immune response of the infected person terminates viremia and allows clinical recovery. Since HIV is a retrovirus that integrates into the genome of infected cells, the virus will remain in the infected individuals with the potential of causing chronic disease many years later. However, the initial steps of infection seem to follow the classical pattern of acute viral disease.

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Isolation Frequency of Human Immunodeficiency Virus from Cerebrospinal Fluid and Blood of Patients with Varying Severity of HIV Infection

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ABSTRACT

Isolation of the human immunodeficiency virus (HIV) has been attempted from the cerebrospinal fluid (CSF) of 63 subjects at different stages of HIV infection, including asymptomatic carriers and patients with or without neurologic or psychiatric complications. In addition blood was collected from 40 of these subjects for virus isolation. HIV could be isolated from the CSF at all clinical stages with an overall frequency of 40%. In contrast, the frequency of HIV isolation from the blood was lower (32%) at the early stages of infection than in patients with severe disease (77%).

HIV isolation from the CSF was more frequently positive in patients with neurologic or psychiatric complications than in patients showing no such disturbances (48 and 32%, respectively).

INTRODUCTION

INFECTION WITH THE HUMAN immunodeficiency virus (HIV) results in a broad variety of clinical symptoms among which immunodeficiency and neurologic complications are the principal disorders. The acquired immunodeficiency syndrome (AIDS) dementia complex,[1,2] also named AIDS encephalopathy or subacute encephalitis, is the most common neurologic manifestation in AIDS; other focal neurologic symptoms include vacuolar myelopathy,[3] aseptic meningitis,[4] peripheral neuropathy,[5] opportunistic infections,[4,6,7] secondary Kaposi's sarcoma,[8] and cerebral lymphoma.[9]

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HIV infects the brain and may be directly involved in the pathogenesis of the AIDS dementia complex. The HIV genome has been detected in the brain of patients with AIDS,[10] and chimpanzees can be infected with HIV by intracerebral or intravenous inoculation of human AIDS brain material.[11] Specific intrathecally synthesized antibodies have been detected in the cerebrospinal fluid (CSF),[12-14] and the virus has been isolated not only from the CSF and neural tissue of patients with AIDS-related neurologic syndromes[15,16] but also from asymptomatic carriers[17,18] and from patients with primary HIV infection.[19]

Extensive data are available on virus isolation from the peripheral blood of HIV-infected patients, but the frequency of HIV isolation from the CSF is unknown. According to our experience,[19] it is more difficult to isolate HIV from the peripheral blood mononuclear cells (PBMC) of asymptomatic carriers (26% positive) than from the PBMC of patients with the AIDS-related complex (ARC) and AIDS (81–100% positive). In the present study we asked the question whether the ease of virus isolation from the CSF similarly corresponds to the clinical stage of HIV infection and/or to the presence of neurologic symptoms. Consequently, HIV isolation was attempted from the CSF of 63 individuals with varying severities of HIV infection, including 29 patients with neurologic symptoms. Virus isolation from the blood of 40 of these patients was also attempted.

SUBJECTS AND METHODS

Patients

CSF, serum, and blood samples obtained on the same day were collected during the period September 1985 to September 1987. The study comprises samples from 63 patients (Table 1), including a total of 73 CSF specimens: 5 patients showed signs of primary HIV infection, 18 were asymptomatic carriers, 9 patients had lymphadenopathy syndrome (LAS), 11 were classified as AIDS-related complex, and 16 as AIDS. An additional 4 patients had no other somatic complications but neurologic or psychiatric disturbances of different types; they were included in the asymptomatic group and we refer here to this group as "only neurologic or psychiatric symptoms."

CD4 cell counts were available for 50 of the patients included in the study at the time of isolation. All subjects but 1 in the asymptomatic group and 4 of 7 LAS patients had CD4 counts in the normal range ($>0.34 \times 10^9$ liter^{-1}). Low CD4 counts were found in 2 patients with primary HIV infection and 19 of the patients with ARC and AIDS. All 4 patients with only neurologic or psychiatric symptoms had normal CD4 counts.

Neurologic or psychiatric symptoms were present in 29 patients (Table 2). This group included meningoencephalitis accompanying seroconversion,[20] polyneuropathy, psychosis (listed under psychiatric disturbances), focal central nervous system (CNS) complications (including CNS malignancies, opportunistic infections, and cerebral ataxia), and AIDS dementia complex.[1] Of the 63 subjects (57 males and 6 females), 44 were homosexuals and 2 bisexual men, 7 were drug abusers, 4 were infected through blood transfusion, and 6 acquired infection heterosexually.

TABLE 1. SUMMARY OF HIV ISOLATIONS FROM CEREBROSPINAL FLUID (CSF) AND PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC)

	Isolations/no. of patients	
Stages of HIV infection[a]	*CSF*	*PBMC*
Primary	1/5	4/5
Asymptomatic	10/22	5/17
LAS	4/9	2/5
ARC-AIDS	10/27	10/13
Total	25/63	21/40

[a]Excluding neurologic symptoms.

TABLE 2. FREQUENCY OF HIV ISOLATION FROM CEREBROSPINAL FLUID (CSF) AND PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) IN SPECIMENS OBTAINED FROM PATIENTS WITH AND WITHOUT NEUROLOGIC OR PSYCHIATRIC COMPLICATIONS

	Neurologic & psychiatric complications											
	Meningoencephalitis accompanying seroconversion		*None*		*Polyneuropathy*		*Psychiatric disturbances*		*Focal CNS complications*[b]		*AIDS dementia complex*	
Stages of HIV infection[a]	*CSF*	*PBMC*	*CSF*	*PBMC*	*CSF*	*PBMC*	*CSF*	*PBMC*	*CSF*	*PBMC*	*CSF*	*PBMC*
Primary	1/3 (2)[c]	1/3 (2)[c]	0/3 (3)	3/3 (3)								
Asymptomatic			7/21 (18)[d]	4/15 (14)	1/2 (1)	1/1 (1)	2/2 (2)	0/2 (2)				
LAS			4/8 (7)	2/5 (5)	0/2 (2)							
ARC-AIDS			0/6 (6)	4/4 (4)	1/3 (1)	2/2 (1)			2/5 (5)	2/2 (2)	7/18 (16)	5/8 (6)
Total	1/3 (2)	1/3 (2)	11/38 (34)	13/27 (26)	2/7 (4)	3/3 (2)	2/2 (2)	0/2 (2)	2/5 (5)	2/2 (2)	7/18 (16)	5/8 (6)

[a]Excluding neurologic symptoms.

[b]These include CNS malignancies, opportunistic infections, and cerebral ataxia.

[c]Isolations/number of specimens (number of patients).

[d]One patient with myotonia, probably not HIV related.

HIV serologic tests

Sera and CSF samples were tested by ELISA using commercially available kits. Positive specimens were confirmed by radioimmunoprecipitation assay or Western blot technique.[21]

Virus isolation

Cells obtained from 5–10 ml of CSF were collected by low-speed centrifugation (1000 rpm for 10 minutes).The cell-free supernatant of CSF was then centrifugated at 45,000 rpm for 30 minutes to pellet any free virus. Cells from the CSF and the pelleted material were then added to separate bottles with 5×10^6 PBMC each. PBMC had been obtained from healthy seronegative blood donors and pretreated for 3 days with phytohemagglutinin (PHA; Difco, 2.5 μg/ml). The cultures were grown in the RPMI medium supplemented with 10% fetal calf serum, 10% T cell growth factor (TCGF; Cellular Products, Buffalo NY), 45 IU of sheep anti-human-α-interferon serum, 2 μg/ml polybrene, and antibiotics. The cultures were monitored twice a week for reverse transcriptase (RT) activity[22] and cytopathic changes (CPE). Positive cultures were confirmed by indirect immunofluorescence using monoclonal antibodies to the HIV core proteins p24 and p15 (gift of Dr. M. Popovic, NIH, Bethesda).

Isolation of HIV from PBMC was attempted in 40 patients (45 specimens) and was carried out as previously described.[22]

RESULTS

The results of virus isolations from the CSF and blood are shown in Table 1. HIV was isolated from the CSF (CSF cells and/or cell-free supernatant) of 25 of the 63 patients (40%) included in the study. Positive CSF cultures were obtained at all stages of HIV infection, notably from 1 of 5 patients undergoing seroconversion, 10 of 22 (45%) asymptomatic HIV carriers, 4 of 9 patients with LAS, and 10 of 27 (37%) with ARC and AIDS. Table 2 shows the distribution of isolation frequencies in the presence or absence of neurologic or psychiatric complications. From a total of 38 specimens obtained from patients without neurologic symptoms, 11 cultures (29%) yielded virus compared with the 7 (39%) positive cultures from the 18 specimens from patients presenting with AIDS dementia complex. When considering the total number of patients, isolation frequency was higher (48%) in the group with neurologic or psychiatric symptoms than in the group showing no such disturbances (32%). It has to be pointed out that HIV could be isolated from the CSF of all 4 patients with only neurologic or psychiatric disturbances, including Guillain-Barré syndrome, myotonia, and psychosis. Conversely, the CSF of all 6 patients with severe immunodeficiency but without neurologic or psychiatric symptoms did not yield virus.

HIV isolation has been attempted from both cells and cell-free supernatants of CSF. Virus could be isolated from the cell-free supernatant of CSF from six asymptomatic carriers, three LAS patients, and eight ARC-AIDS patients. Reverse transcriptase (RT) activity was detected within 1–2 weeks in cultures infected with virus from ARC and AIDS patients, whereas in cultures infected with virus from the CSF of patients in the early stages of HIV infection (asymptomatic carriers with or without neurologic symptoms and LAS patients) RT activity was first detected between 2 and 5 weeks. The maximum values of RT activity were higher in the cultures infected with virus from patients with severe disease ($6–232 \times 10^3$ cpm/ml in the ARC-AIDS patients) than in cultures infected with virus from subjects at the early stages of HIV infection ($4–36 \times 10^3$ cpm/ml) (Fig. 1). There was no difference in the appearance and peak of RT activity in cultures established from the cells of the CSF of patients at different stages of HIV infection.

HIV was isolated from the PBMC of 21 of the 40 patients (53%) tested (Table 1). A total of eight patients (one primary, four asymptomatic, one LAS, and two with only neurologic or psychiatric symptoms) were positive in the CSF and negative in the PBMC cultures. Paired CSF and PBMC isolates were obtained from nine patients: two asymptomatic carriers, one LAS, one ARC, three AIDS, and two patients presenting with Guillain-Barré syndrome and myotonia, respectively, but no other somatic symptoms. In some cases the time of appearance and peak of RT (Fig. 2a) in cultures obtained from the

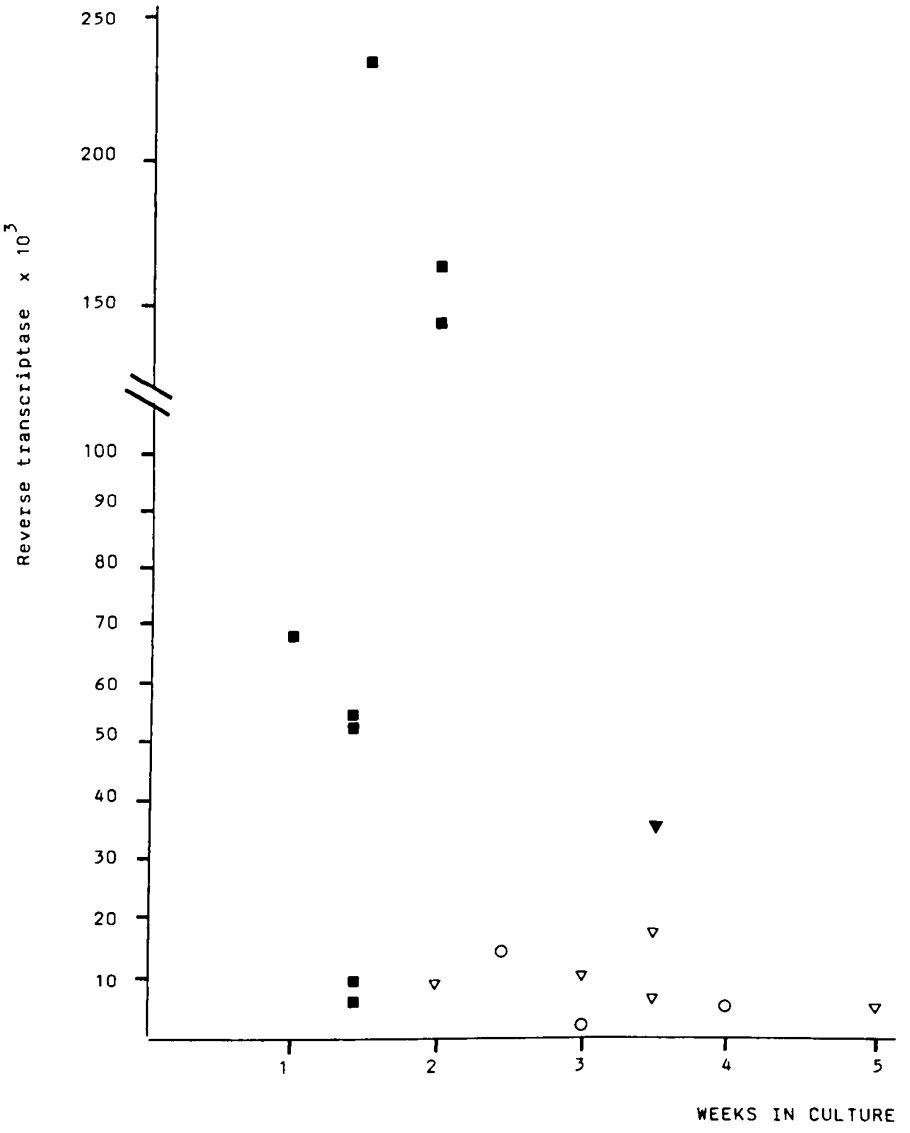

FIG. 1. Isolation of virus from the fluid of CSF of HIV-infected patients. RT = maximum value for RT activity detected in single cultures; ▽ = asymptomatic; ▼ = only neurologic or psychiatric symptoms; ○ = LAS; ■ = ARC-AIDS.

CSF and PBMC were nearly overlapping; in other paired cultures the kinetics of RT activity showed minor (Fig. 2b) or substantial (Fig. 2c) differences.

Except for the 5 patients undergoing seroconversion at the time of sampling,[23] the remaining 58 patients included in the study were HIV antibody positive in serum. Of these, the CSF of 51 patients were assayed for HIV-specific antibodies and all were found to be positive.

DISCUSSION

Our findings show that HIV can be isolated from the CSF of patients with varying severities of HIV infection. The overall frequency of HIV isolation from the CSF was 40% and from the blood, 53%. The frequency of isolation from CSF is roughly the same in the early (asymptomatic + LAS, 45%) and late

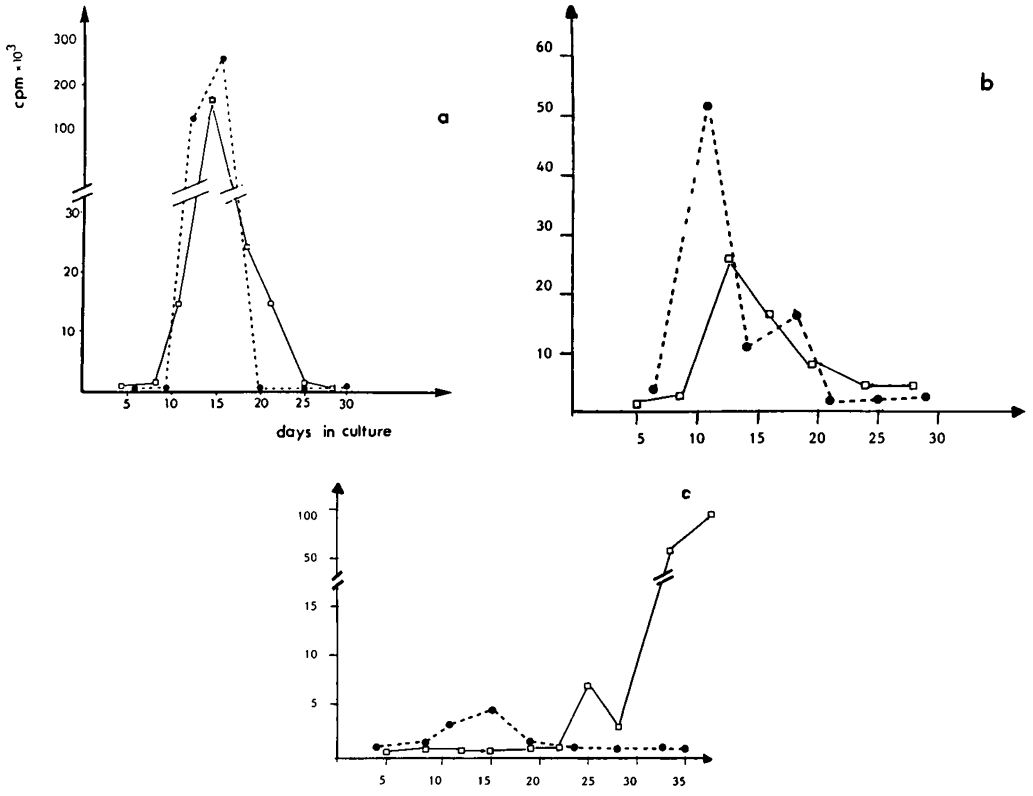

FIG. 2. RT activity in paired cultures from blood and CSF; □ = PBMC; ● = cell-free supernatant of CSF in b and CSF cells in a and c.

stages of HIV infection (ARC and AIDS, 37%). In contrast, the frequency of HIV isolation from the blood was 32% at the early stages and 77% in the late stages. This suggests that whatever the mechanism for the increase in frequency of virus isolation from the blood, it does not operate beyond the blood-brain barrier. A possibility is that a large number of HIV-infected cells are present in the CSF compared with blood and this may contribute to successful virus isolation. Antibodies do not seem to influence the outcome of virus isolation since all CSF samples contained HIV-specific antibodies.[18]

The frequency of virus isolation from the CSF was higher in patients with neurologic or psychiatric symptoms than in patients without symptoms (48 and 32%, respectively). Interestingly, HIV was isolated from the CSF in all four patients with solely neurologic or psychiatric symptoms.

In the majority of patients with positive virus isolation from the cells of the CSF, free virus was also recovered. Viruses isolated from asymptomatic carriers and LAS patients replicated slowly and gave lower maximum RT values than viruses obtained from the CSF of ARC and AIDS patients. This suggests that in the early stages of HIV infection a smaller amount of free virus is present in the CSF than in the late stages. That virus expression is low or perhaps intermittent in these patients is also suggested by the fact that HIV isolation is successful only in one or two of three CSF samples obtained from the same patient (four patients). Our results are in accordance with the report of Goudsmit et al.[24] showing that HIV antigen is undetectable in the CSF of asymptomatic carriers.

In the group of patients in the late stages of HIV infection large amounts of free virus in the CSF were not uniformly present. In fact, of the eight patients with positive CSF cultures only six yielded high RT activity (>50 × 10^3 cpm/ml). That virus production may not be massive in the brain of all AIDS patients with encephalopathy is suggested by histopathologic studies: Pumarola-Sune and coworkers[25] detected HIV antigen in the brain of only 8 of 20 demented AIDS patients, and Gyorkey and collaborators[26] found HIV

virions in five of the seven brain biopsies obtained from AIDS patients. In our experiment virus could not be isolated from the six patients showing no clinical signs of neurologic or psychiatric disturbances.

We obtained paired CSF and PBMC isolates from nine patients and have shown that the isolation patterns can be different in the paired viruses: preliminary results suggest that these differences in the replication pattern in vitro correlate to differences in the size of the virus-associated proteins and proviral DNA (not shown). We are now attempting biologic and genomic characterization of the paired viruses to establish the degree of similarity between CSF and blood isolates.

In conclusion there is no strict correlation between HIV isolation from the CSF and the stage of HIV infection or the presence of neurologic complications. Our findings underscore the possibility that virus invades the CNS at a very early stage of the disease and additional mechanisms may account for the overt appearance of neurologic symptoms.

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SIMULTANEOUS ISOLATION OF HIV-1 AND HIV-2 FROM AN AIDS PATIENT

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Summary Two distinct human immunodeficiency viruses, HIV-1_{SF480} and HIV-2_{UC2} were isolated simultaneously from the blood of an Ivory Coast patient with AIDS. The HIV subtypes were segregated by their differential ability to infect established human cell lines and by the cell surface expression of type-specific viral antigens. The viruses could be distinguished by both immunoblot and Southern blot analyses. The results indicate that an individual can be infected by both HIV subtypes.

Introduction

THE two subtypes of the human immunodeficiency virus, HIV-1 and HIV-2, have approximately 45% homology for aminoacid sequence and genome.[1] Techniques to distinguish between HIV-1 and HIV-2 have been based on the close relation of HIV-2 to simian immunodeficiency virus (SIV),[2-4] and type-specific immunodominant peptide sequences in the virus transmembrane envelope proteins.[5] Serological studies of West African individuals[4,6] have indicated simultaneous infection by both subtypes of HIV. However, confirmation of dual infection requires the isolation of both virus subtypes from the same individual because of the possibility of cross-reactivity of HIV-1 and HIV-2 antigens.[7,8] We describe simultaneous recovery of distinct HIV-1 and HIV-2 viruses from an AIDS patient.

Materials and Methods

Cells

Mitogen-stimulated human, chimpanzee, and rhesus monkey peripheral blood mononuclear cells (PMC) and unstimulated human peripheral blood macrophages were prepared and maintained as previously described.[4,9] The human T cell lines HUT-78, CEM, and *Sup*T1; the U-937 monocytic cell line; and 5 different Epstein-Barr virus-transformed human B-cell lines were cultured in RPMI-1640 that contained 10% fetal calf serum. (Cell lines were obtained from the American Tissue Culture Collection or derived in this laboratory.[4])

Viral Studies

Serum samples from individuals who attended the Treichville Hospital in Abidjan, Ivory Coast, were analysed for type-specific antibodies to HIV by enzyme-linked immunosorbent assay (ELISA) and immunoblot.[5,10] Of 67 HIV antibody-positive samples tested to date, 23 (34%) had reactivity by both procedures to HIV-1 and HIV-2.[4] PMC from 13 of these 23 individuals with dual reactivity were co-cultivated with PMC from normal

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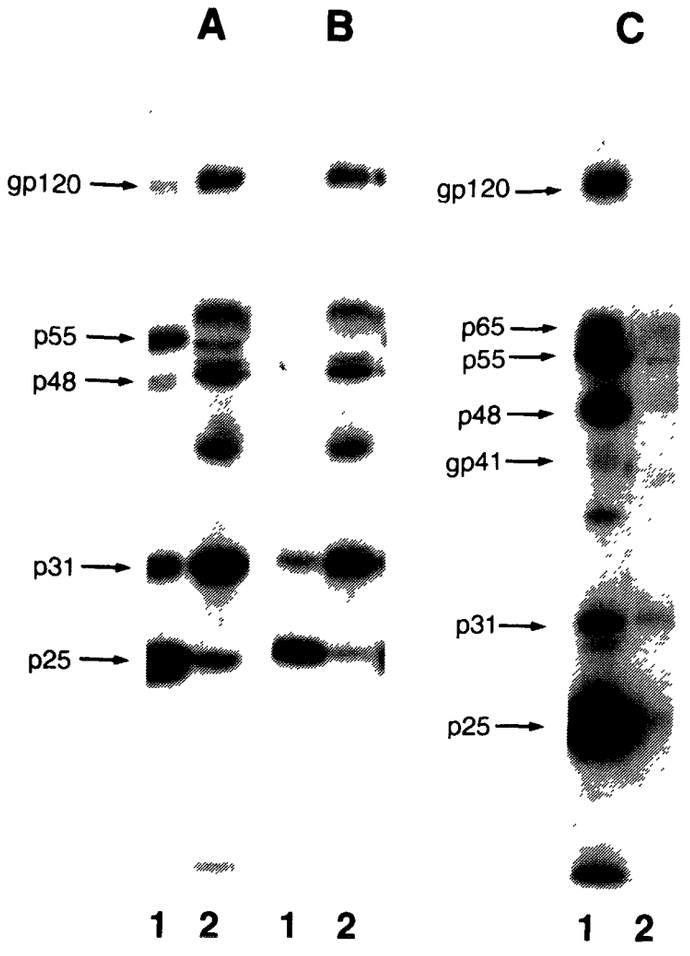

Fig 1—Immunoblot analyses of viral antigens in infected cell culture supernatants.

Viral antigens detected by serum with antibodies to HIV-1 only (lanes 1) or to HIV-2 only (lanes 2).[4]

A. Patient's peripheral blood mononuclear cells (PMC) co-cultivated with mitogen-stimulated PMC from seronegative donors.

B. After passage through the CEM T-cell line.

C. After panning for surface expression of HIV-1 gp41.

seronegative donors.[4] Culture supernatants with high levels of reverse transcriptase activity[11] were concentrated by centrifugation, and viral antigens were analysed by immunoblot with either HIV-1 or HIV-2 type-specific sera.[4,5] Virus replication in different cell lines was measured by reverse transcriptase activity and viral antigen production.[12]

Clinical Details

The isolates HIV-1_{SF480} and HIV-2_{UC2} were obtained from the PMC of a 37-year-old woman with dual antibody reactivity. This woman was originally from Burkina Faso. She had had tuberculosis in 1986, but her admission to Treichville Hospital at the time of this study was due to recurrent vomiting and weight loss of 39 kg. She had prolonged fever, a white cell count of 12 300/ml (8% lymphocytes) but no lymphadenopathy. *Necator americanus* infection was diagnosed before she died. (15 of the other 22 individuals with dual antibody reactivity presented with parasitic bowel infections, chronic diarrhoea and extreme weight loss; the remainder had pulmonary disease.)

Cell Enrichment

Cells with surface expression of gp41 of HIV-1 were selected by panning.[13] Immunoglobulin G specific for gp41 (0·5 μg) was diluted in 2 ml of buffered 'tris' hydrochloride (pH 9·5) and added to each 60 mm^2 bacterial grade petri plate and incubated at 37°C for 45 min. This immunoglobulin had been affinity purified from a pool of HIV-1 antibody-positive sera and did not detect the envelope protein of either HIV-2 or SIV by immunoblot analyses. The plates were then blocked for 40 min at 37°C with phosphate-buffered saline (PBS) containing 1% fetal calf serum before HIV infected PMC were added for 2 h at 4°C. Non-adherent cells were removed by washing six times, then adherent cells were removed by a high pressure stream of PBS/1% fetal calf serum. These cells were co-cultured with uninfected mitogen-stimulated human PMC; the culture supernatant was then concentrated and viral antigens analysed.[4]

Southern Blot Analyses

High molecular weight whole-cell DNA was extracted from PMC cultures 7 days after inoculation and 15 μg of each DNA sample was digested with *Hind*III or *Pvu*II restriction enzymes. Restriction fragments were separated by electrophoresis on 0·8% agarose gels, transferred to nylon membranes (Biodyne, Pall Laboratories), and detected with ^{32}P-labelled probes (Nick Translation System, Bethesda Research Laboratories) that represented the entire genome of either HIV-1_{SF2}[14] or SIV_{mac}.[15]

Results

Both HIV-1-specific (lane 1) and HIV-2-specific (lane 2) sera recognised viral antigens, including external envelope glycoproteins, in the PMC culture of an individual with dual antibody reactivity (see fig 1A). This result indicates that both types of virus were present in the infected culture supernatant, and was observed for cultures from 3 of 13 individuals with dual antibody reactivity. Identical results were also obtained when virus pools were passed through peripheral blood human macrophages, and chimpanzee and rhesus monkey PMC. However, when mononuclear cells from the other 10 individuals with dual reactivity were cultured, only antigens characteristic of HIV-1 were detected (data not shown).

HIV-1 and HIV-2 differ in their ability to replicate in various cell types.[4] To determine if both HIV subtypes were

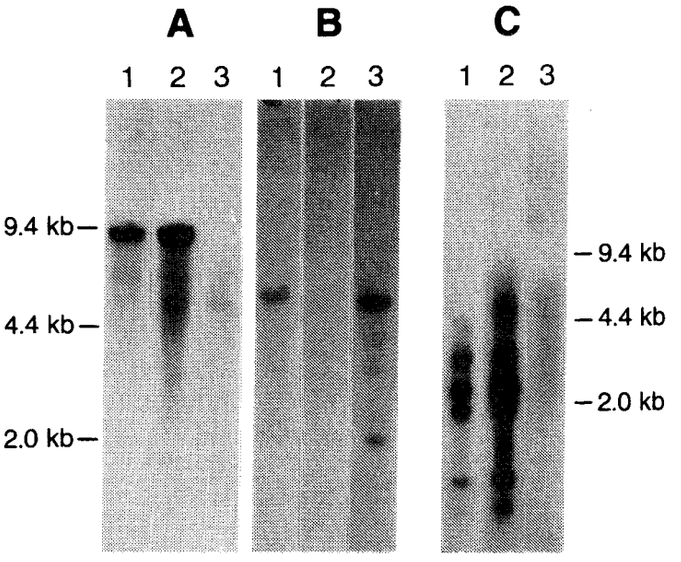

Fig 2—Southern blot of HIV isolates from dually seropositive individual.

DNA from PMC cultures with viral antigens typical of both HIV-1 and HIV-2 (lanes 1); HIV-1 only (after panning, lanes 2); or HIV-2 only (after passage through CEM cells, lanes 3) were digested with either *Hind*III (A and B) or *Pvu*II (C). DNA analysed for homology to either HIV-1 (A and C) or SIV_{mac} (B).

present in the same individual, PMC from different primate species, human peripheral blood macrophages, and established human cell lines were inoculated with supernatant from one of the cultures that contained both HIV-1 and HIV-2 viral antigens. Antigens of both subtypes were detected in all the peripheral blood mononuclear cell cultures and the human macrophages, but only proteins characteristic of the HIV-2 isolate (now designated as HIV-2_{UC2}) were observed after passage through the CEM established T-cell line (fig 1B). To enrich for the suspected HIV-1 isolate, the panning technique of Wysocki and Sato[16] was used. Affinity-purified human immunoglobulin, which recognises the transmembrane envelope protein of many HIV-1 isolates from both Africa and the United States, was used as the capture antibody. Fig 1C shows that only antigens characteristic of HIV-1 were detected in the culture supernatant when the adherent cells obtained by panning were co-cultivated with normal human PMC. This isolate was designated HIV-1_{SF480}. (The external envelope glycoprotein of HIV-2_{UC2} has a slightly higher molecular weight than that of HIV-1_{SF480}.)

Southern blot analyses were done to confirm dual infection with HIV-1 and HIV-2 and to demonstrate the genomic identity of these viruses. PMC were inoculated with supernatants from cultures for which immunoblot showed viral antigens specific for both HIV-1 and HIV-2, only HIV-1 (after panning), or only HIV-2 (after passage through CEM cells). Fig 2 shows sequences reactive with both HIV-1 and SIV genomic probes were present in the unseparated cultures (fig 2A and B, lanes 1). In contrast only HIV-1-like sequences were present in the panned mononuclear cell cultures (fig 2A, lane 2) and only HIV-2-like sequences in the CEM culture (fig 2B, lane 3). Digestion with *Pvu*II was done to identify conclusively the genome of HIV-1 (fig 2C). The results confirmed the presence of both HIV subtypes in this infected individual.

Both HIV subtypes replicated in human, rhesus monkey, and chimpanzee PMC, human peripheral blood macrophages, and the HUT-78 T cell line. HIV-2_{UC2} replicated in the CEM and U937 human cell lines, but no replication of HIV-1_{SF480} in these cells could be detected. Ballooning and mononuclear giant cells were observed in both HIV-1_{SF480}-infected and HIV-2_{UC2}-infected mononuclear cells and purified human CD4-positive cells. When these infected cells were mixed with the *Sup*T1 T-cell line (99% CD4-positive), extensive syncytial formation and cell death occurred. CD4 surface antigen expression on the inoculated panned T helper cells was also assessed by flow cytometry using anti-leu3a monoclonal antibody (Becton Dickinson). At the peak of viral antigen expression (15–20% viral antigen-positive by immunofluorescence assay), both HIV-1_{SF480}-infected and HIV-2_{UC2}-infected cultures showed reduced expression of the CD4 protein (81% and 64% reduction, respectively) and visible cytopathic effects.

Discussion

Both HIV-1 (HIV-1_{SF480}) and HIV-2 (HIV-2_{UC2}) viruses were identified and separated from the PMC of an infected individual. Although evidence of both subtypes was only detected for 3 of 13 individuals with dual antibody reactivity, the other individuals with dual reactivity may also be infected by both subtypes. However, in view of the rate of sequence and antigenic change which has been reported for HIV, the possibility of cross-reactive antibodies in some of these individuals cannot be excluded.

The highly cytopathic HIV-2_{UC2} strain, which reduces surface CD4 expression, differs from the recently described HIV-2_{UC1} strain, which does not induce cytopathic effects or modulate CD4 antigen expression in infected cells.[4] These results emphasise the heterogeneity among different isolates of both HIV-1 and HIV-2. Superinfection of HIV-1-infected chimpanzees with a different HIV-1 strain has been described,[16] and so human beings may become infected by two distinct HIV-1 strains. We have not been able to infect the same cell with both HIV subtypes isolated from this patient, nor did we recover HIV-2_{UC2} from the panned HIV-1-producing mononuclear cell cultures: we suggest that the two subtypes had infected different cells in this patient.

We observed no differences in the clinical manifestations of individuals with dually reactive sera and of patients with antibodies specific to either HIV-1 or HIV-2 alone. However, it is not possible to assess the role of dual infection in the exacerbation of HIV-associated illness as all patients in this study were selected on the basis of their clinical manifestations.

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