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Detection of Plasma Viremia in Human Immunodeficiency Virus-Infected Individuals at All Clinical Stages

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Free virus (virus not present within cells) was detected in the plasma of all human immunodeficiency virus (HIV)-infected individuals studied. Plasma samples from asymptomatic individuals and individuals with HIV disease were tested. The levels of virus varied, but high virus titers correlated directly with HIV-related symptoms and low CD4$^+$ lymphocyte counts. Effective detection of infectious virus depended on the use of an enzyme-linked immunosorbent assay for p24 core antigen and culture conditions in which plasma was added to mitogen-stimulated lymphocytes within 3 h of venipuncture. When there were delays in the time to culturing of plasma, neutralizing antibodies and perhaps other factors present in the plasma were found to reduce the efficiency of virus recovery. Plasma stored at −70°C for several months maintained a stable level of free virus. These results suggest that measurement of HIV present in plasma under optimal conditions could be an efficient way of monitoring the clinical state of an individual and the effects of antiviral therapy.

The human immunodeficiency virus (HIV) was first detected by culturing peripheral blood mononuclear cells (PBMCs) from infected individuals. Initially, virus isolation was limited because of the methods used for HIV recovery in tissue culture (2, 13, 17, 18). More recent approaches that use coculture with mitogen-stimulated PBMCs from HIV-seronegative controls have led to almost 100% isolation of virus from the PBMCs of infected individuals at all clinical stages of HIV infection (3, 12, 16). The efficiency of detection of free virus (virus not present within PBMCs) in the serum or plasma of HIV-infected individuals has also been limited (9, 21, 27). Recently, however, some investigators have presented evidence indicating that free HIV can be found at various concentrations in the blood of most subjects at different stages of HIV infection, including asymptomatic individuals (7, 14). These results have been confirmed in part by other investigators (6, 22, 23, 25), but the prevalence of virus in plasma and the parameters needed for virus detection have not been well defined.

The present study reviews our laboratory's experience in detecting free virus in the serum and plasma of HIV-infected individuals from 1985 to 1991. In the first few years, undiluted plasma or serum was added directly to PBMC cultures, and the procedure gave a moderate success rate for virus isolation (21). More recently, we have used techniques that have led to the detection of virus in all plasma samples, particularly if the p24 (p25) core antigen assay is used. Most importantly, we observed that some plasma specimens left at room temperature for more than 3 h show a substantial loss in the amount of infectious virus. In this report we describe the optimal conditions for detection of viremia and recommend assay of plasma or serum for infectious virus shortly after venipuncture.

MATERIALS AND METHODS

Subjects. For the present studies, 63 blood samples from 38 individuals at various clinical stages of HIV infection, from asymptomatic to AIDS (*Pneumocystis carinii* pneumonia and Kaposi's sarcoma), were studied. Six subjects had received zidovudine (AZT) treatment for at least 3 months. One individual was on dideoxycytidine (ddC) therapy. Four plasma specimens from two individuals were evaluated over time; for five individuals, three specimens were tested, and for nine individuals, two specimens were tested. Fifty-five samples were processed within 1 to 2 h after venipuncture. Fourteen samples were assayed after 3 h after venipuncture; six of these samples were also assayed at <2 h after venipuncture. For comparison, results for blood samples obtained from 91 HIV-infected individuals who were studied from 1985 to 1988 were included. Most of these plasma and serum specimens were assayed for HIV within 8 h of venipuncture. Twelve specimens were evaluated after they had been stored at −70°C for several months. All individuals gave informed consent for participation in the studies described here. The project was approved by the Committee on Human Research, University of California, San Francisco.

Virus. The HIV type 1 (HIV-1) strains HIV-1_{SF2}, HIV-1_{SF33}, and HIV-1_{SF170} were isolated in our laboratory, grown in PBMCs, and stored at −70°C for later use (17, 18). Aliquots of these viruses containing $>10^5$ infectious particles per ml were used for the studies. Infectious virus was detected by a p24 core antigen enzyme-linked immunosorbent assay (ELISA; Coulter Immunology, Hialeah, Fla.) and by a reverse transcriptase (RT) assay (15). Neutralization tests were conducted as described previously (4).

PBMCs and culture of plasma. Whole blood was centrifuged at 200 × *g* for 10 min to separate the plasma and cellular fractions. The cellular fractions were isolated by Ficoll-Hypaque gradient centrifugation (17, 18). The PBMCs were then cultured by a standard procedure (3) in which they were mixed with an equal number of normal human PBMCs that were previously stimulated with phytohemagglutinin (PHA; 3 μg/ml) for 3 days. The culture medium was RPMI 1640 with 10% heated (56°C, 30 min) fetal calf serum, 5% interleukin-2 (Electronucleonics, Silver Spring, Md.), 2 mM L-glutamine, 100 μg of streptomycin per ml, and 100 U of penicillin per ml. The supernatants of the PBMC cultures

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were removed twice weekly for up to 28 days and were assayed for virus as described previously (3).

The plasma fraction was further centrifuged at 3,000 × *g* for 15 min to remove all cell debris. For detecting plasma viremia, various quantities of the plasma were added to 2 ml of culture medium containing 4 × 10^6 PHA-stimulated PBMCs obtained from HIV-seronegative individuals. In the earlier studies (1985 to 1988), 0.5 ml of the plasma or serum was added to these PBMCs, and the mixture was cultured overnight before changing the supernatant. For the recent studies, the PBMCs were first pretreated with 2 μg of polybrene per ml for 30 min to increase their sensitivity to virus infection (3). Following a 24-h incubation at 37°C with the plasma sample, the cells were pelleted, washed twice, and refed with fresh medium. For estimating the virus titer in the plasma samples, dilutions were made as described by Ho et al. (14). They ranged from 1,000 μl down to 2 μl, and in some samples, 0.2 μl was used. For all assays, duplicate cultures in six-well plastic plates (Falcon, Lincoln Park, N.J.) were used, and the results reported here are averages.

All cultures, both recent and past, were maintained in 2.5 ml of the complete RPMI 1640 growth medium described above. Twice a week for up to 28 days, half of the supernatant was removed for virus assay and cultures were refed with fresh medium. PBMCs were not added to or removed from the cultures. The presence of virus production in the supernatant was measured by the p24 core antigen and RT assays. A value of at least ≥30 pg (four times the background) was considered positive by the ELISA, and a quantity of 10,000 cpm/ml (background, ~1,000 cpm/ml) was positive by the RT assay. When cultures were positive for HIV on two occasions, they were discarded.

Flow cytometry. The number of $CD4^+$ cells present in a blood sample was determined by flow cytometry by using monoclonal antibodies provided by Becton Dickinson, San Jose, Calif. (19).

IgG separation. Immunoglobulin G (IgG) was separated from serum by binding; this was followed by elution through a protein G affinity column (1). Briefly, protein G-Sepharose beads (4FF; Pharmacia) were washed with phosphate-buffered saline (PBS; pH 7.4) and packed in a C10 column (Pharmacia). Ten milliliters of patient serum diluted 1:2 with PBS was loaded onto the column. IgM and other serum proteins were eluted in the first protein fraction. The column was then washed with PBS (pH 7.4) and then with an eluant of 0.1 M glycine solution (pH 2.7) to release the IgG. The IgG fraction collected was immediately neutralized with 1 M Tris (pH 7.4; 50 μl/ml). The protein concentration of the IgG fraction was quantitated by the Bio-Rad protein assay. Both IgG and non-IgG fractions were then sterilized by filtration through 0.45-μm-pore-size Millipore filters.

RESULTS

Virus detection in plasma and virus recovery from PBMCs. The p24 antigen in the plasma before culture was detected in only 7 of the 63 blood samples examined (11.1%) (data not shown). These seven plasma samples also contained infectious HIV-1 as demonstrated by the RT assay of PBMC culture fluids (see below). In studies that detected HIV in the PBMCs of these individuals, 60 of 63 cultures (95.2%) showed the presence of virus (data not shown); this prevalence resembled that reported by Castro et al. (3). The three negative samples were from asymptomatic individuals whose samples occasionally did not yield virus unless $CD8^+$ cells were first removed from culture (26).

TABLE 1. Plasma viremia in HIV-1-infected individuals

Clinical stage	No. of individuals positive for virus/total no. tested (%)[a]		
	1985–1988 RT assay	1989–1991	
		p24 antigen ELISA	RT assay
Asymptomatic	5/35 (14.2)	18/18 (100)	16/40 (40.0)[b]
AIDS-related complex	19/41 (46.3)	7/7 (100)[c]	4/7 (57.1)
AIDS	12/15 (80)	8/8 (100)[c]	8/8 (100)
Total	36/91 (39.5)	33/33 (100)	28/55 (50.9)

[a] For 1989 to 1991, only data for plasma assayed within 1 to 2 h of venipuncture are presented. When virus detected by the RT assay was compared for the two time periods, results were statistically different (P = 0.01, χ^2 analysis) for asymptomatic subjects. Other values did not reach statistical significance.

[b] One individual was treated with ddC; two individuals received AZT. One individual treated with ddC and one individual treated with AZT were plasma viremic, as detected by the RT assay.

[c] Two individuals were treated with AZT.

Recovery of virus from serum of HIV-infected individuals. In our initial studies conducted from 1985 to 1988, the overall recovery of virus from the plasma or serum of infected individuals was found to vary according to the clinical stage of HIV infection. In those evaluations, only the RT assay was used. There was a low percentage (14.2%) of virus detected in samples from 35 asymptomatic individuals compared with those samples from symptomatic individuals, particularly those with a documented diagnosis of AIDS (80%) (Table 1). Of the 91 samples evaluated, 36 (39.5%) were found to be positive. The initial studies did not use the culture conditions described below, but the results did reflect the presently appreciated observation that the level of free virus in plasma increases in symptomatic HIV-infected individuals (6, 7, 14).

In subsequent studies conducted from 1989 to 1991, we evaluated our ability to detect HIV in blood by using standard procedures for PBMC culture and methods for the recovery and detection of free virus in plasma that proved to be more sensitive (3) (see Materials and Methods). In these recent approaches, the free virus in plasma was assayed in PBMC cultures by the RT assay and/or the core antigen ELISA. The core antigen ELISA was found to be more sensitive than the RT assay for HIV detection. All 33 of the plasma samples tested by the antigen ELISA, regardless of the clinical state of the subjects, showed the presence of free virus. HIV was even detectable by our assay in an asymptomatic individual with 1,545 $CD4^+$ cells per μl. In contrast, only 28 of 55 samples (50.9%) were found to contain HIV when the culture fluid from PBMCs inoculated with plasma was evaluated by the RT assay (Table 1). Nevertheless, by the latter procedure, plasma viremia was detected in all patients with AIDS and over half of the other symptomatic patients (Table 1). In this regard, the recovery of virus by recent cell culture procedures, as measured by the RT assay, was more efficient (P = 0.01) with specimens from asymptomatic subjects, who generally have less virus in their plasma. The recent results obtained by the RT assay also showed a higher recovery of virus from patients in other clinical groups, but the results did not reach statistical significance.

The RT assay provided similar results regarding the prevalence of viremia in the individuals in relation to their $CD4^+$

cell counts. As noted above, this procedure readily distinguishes between high (positive result) and low (negative result) levels of free virus in plasma. Of the subjects with >500 $CD4^+$ cells per mm^3, 22.7% (5 of 22) were positive for virus, of those with 200 to 500 $CD4^+$ cells per mm^3, 62.5% (10 of 16) were positive for virus, and of those patients with <200 $CD4^+$ cells per mm^3, 91.6% (11 of 12) were positive for virus (data not shown).

Early in the studies described here, the time of plasma culture was found to be important for detecting infectious virus. When plasma cultures were processed more than 3 h after venipuncture, only 7 of the 14 samples tested yielded virus, as detected by the p24 ELISA (data not shown), whereas 33 of 33 samples were found to contain HIV when plasma was cultured 1 to 2 h after venipuncture (Table 1). Moreover, of the 33 samples, six from healthy individuals were also assayed at >3 h, and three of the samples were found to be negative for virus (data not shown).

Relation of virus titer in plasma to clinical state and $CD4^+$ cell counts. The evaluated plasma samples were obtained from individuals with various clinical stages of HIV infection. Seven subjects had received AZT or ddC treatment. These therapies did not affect our ability to detect virus in plasma, as measured in cell culture by the p24 antigen ELISA; however, the quantity of virus in treated patients compared with that in untreated patients was generally lower (see below). In determining the titer of viremia in relation to the patient's clinical state, dilutions of plasma were evaluated and virus was detected by the p24 core antigen ELISA.

In relation to the patient's clinical state, the results showed that the mean virus titer was higher (tissue culture infectious dose [TCID] per milliliter) in symptomatic patients than in asymptomatic individuals, although a wide range of HIV levels in plasma was found among HIV-infected patients with similar stages of illness. Asymptomatic individuals (18 subjects) with an average $CD4^+$ count of 612/μl and an HIV titer range of from 1 to 500 TCID/ml showed an average HIV titer of 140 TCID/ml. In contrast, six patients with AIDS who were not on antiviral therapy and who had an average $CD4^+$ count of 30/μl and an HIV titer range of from 100 to 5,000 TCID/ml had a mean HIV titer of 2,683 TCID/ml (data not shown). Three patients with AIDS who were on AZT treatment each had an HIV titer of 25 TCID/ml, supporting the fact that the drug has an effect on virus replication. Peak virus production also occurred earlier in cultures of plasma samples from symptomatic subjects than in cultures of plasma samples from healthy subjects (data not shown). Thus, not only a greater quantity of virus (TCID) but also a more rapid kinetics of virus replication was observed in plasma from HIV-infected patients.

When plasma viremia was evaluated in relation to different $CD4^+$ cell counts, statistically higher HIV titers measured by the p24 antigen ELISA were also found in the patients with the lowest $CD4^+$ cell counts (Table 2). The differences in viremia levels were particularly apparent when very low $CD4^+$ cell counts were present. For example, individuals with ≥500 $CD4^+$ cells per μl had a mean titer of 114 TCID/ml, whereas the four patients with <100 $CD4^+$ cells/μl had a mean titer of 2,750 TCID/ml.

Variations in the levels of virus in plasma over time showed findings similar to those described above (Table 3). In one asymptomatic subject, the free virus titer remained the same; in another, it decreased somewhat. However, in one asymptomatic individual and two patients, the HIV titer increased substantially, and this titer increase was associated with a decline in the $CD4^+$ cell count.

TABLE 2. Titer of HIV-1 recovered from plasma in relation to $CD4^+$ cell count[a]

No. of $CD4^+$ cells/mm^3	No. of individuals	HIV titer range (TCID/ml)	Mean HIV titer (TCID/ml)[b]
≥500	13	1–500	114
300–499	8	1–500	205
100–299	8	25–500	281
<100	4	500–5,000	2,750

[a] Data are for cultures of plasma for viremia detection; plasma was inoculated within 1 to 2 h of venipuncture and was assayed by the p24 antigen ELISA. None of the patients from whom plasma samples were obtained was on antiviral therapy.

[b] The trend of finding an increased virus titer with lower $CD4^+$ cell counts was statistically significant (Kriskal-Wallis test).

Detection of anti-HIV activity in plasma. The initial studies indicated, as noted above, that the time that plasma samples are placed in culture is important for HIV detection. Some plasma samples left in storage for more than 3 h lost a substantial amount of infectious virus, and the result did not reflect the actual presence of HIV in the blood at the time of venipuncture. As further examples, virus recovery from five individuals (four healthy subjects and one patient with Kaposi's sarcoma), was measured 3, 6, or 12 h after phlebotomy. In all subjects except the patient with Kaposi's sarcoma, the titer of virus recovered dropped from up to 500 TCID/ml to 0 (data not shown).

In order to study the effect of the time that plasma samples were placed in culture on the detection of virus, reconstruction experiments were conducted. First, the HIV-1_{SF2} or HIV-1_{SF33} strains at 100 $TCID_{50}$s/ml were added to normal medium or normal human plasma. At various time intervals after storage at different temperatures, these samples were diluted and inoculated onto PHA-stimulated PBMCs from normal HIV-seronegative individuals. The results, as shown in Fig. 1 for HIV-1_{SF2}, indicated that the virus left in medium or normal human plasma for more than 3 h did not lose

TABLE 3. Plasma viremia detected in the same individual over time[a]

Subject no.	Clinical stage[b]	Time of observation (mo)	No. of $CD4^+$ cells/mm^3	TCID/ml
1	Asympt	0	691	100
		5	592	25
2	Asympt	0	1,545	1
		5	1,080	5
3	Asympt	0	344	500
		1	342	500
4	KS	0	144	100
	KS	1	250	500
	KS	2	144	500
	KS	7	47	5,000
5	PCP	0	100	500
		7	9	5,000

[a] Virus was detected in the culture supernatant by p24 antigen ELISA.

[b] Asympt, asymptomatic; KS, Kaposi's sarcoma; PCP, *P. carinii* pneumonia.

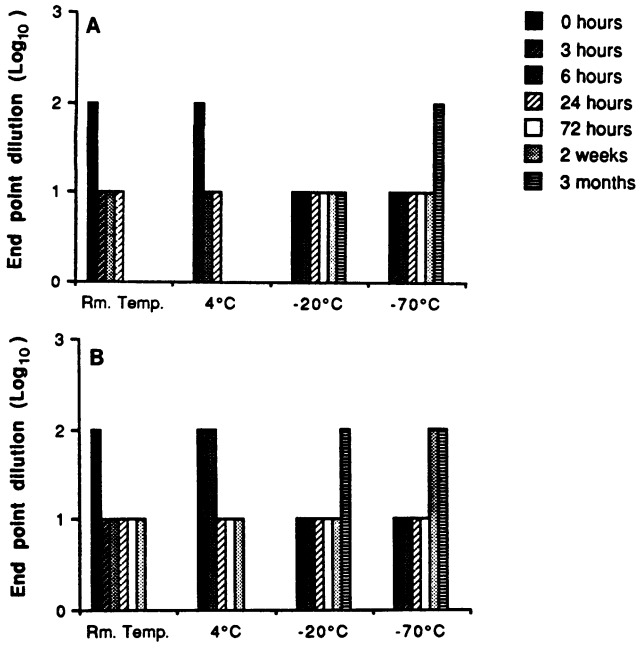

FIG. 1. HIV-1_{SF2} was added to medium (A) or normal human plasma (B) and left for various time periods at different temperatures. The relative level of HIV detected in inoculated PHA-stimulated normal PBMCs is indicated by the bar graph. A representative result of at least two separate studies is shown. Similar results were obtained with HIV-1_{SF33}.

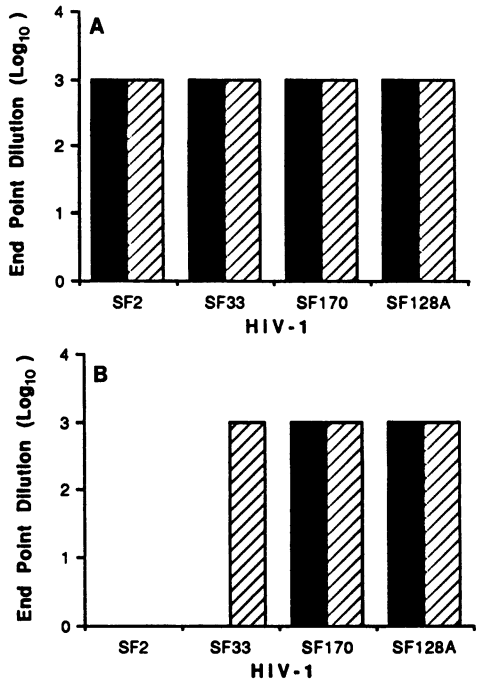

FIG. 2. HIV-1 strains (SF2, 33, 128A, 170) were mixed with 1 ml of plasma from an uninfected (A) or infected (B) individual. After 24 h at room temperature (■) or after storage at −70°C for 24 h (▨), the mixtures were diluted 10-fold and added to PHA-stimulated normal PBMCs. The relative level of HIV-1 detected is indicated by the bar graph.

substantial amounts of infectivity. The results also suggested that storage of plasma at 4, 20, or −70°C helped to maintain the virus infectivity. Nevertheless, the data demonstrated that the virus in normal human plasma was more stable than virus in medium after long periods of time at a high temperature; normal plasma appeared to protect the virus from inactivation. Similar results were observed with HIV-1_{SF33} (data not shown).

These findings were in contrast to our subsequent observations on HIV-1 added to the plasma of infected individuals; virus infectivity was markedly reduced after a few hours. In those studies, HIV-1_{SF2} or HIV-1_{SF33} was placed into plasma obtained from HIV-1-infected, healthy individuals; the plasma was kept at room temperature for several hours to eliminate any endogenous virus. The results showed decreased levels of virus after the plasma was held at room temperature for 6 to 24 hours (Fig. 2 shows the results obtained after 24 h). In one reconstituted plasma sample, virus (HIV-1_{SF33}) was detected only when the plasma was stored at −70°C for 24 h.

For those studies, the African strain HIV-1_{SF170} and the brain-derived virus HIV-1_{128A} were also used. These viruses are not usually neutralized by sera from infected individuals (17). As shown in Fig. 2, they were not inactivated by plasma from HIV-infected subjects. In a further investigation of HIV inactivation by plasma or serum from HIV-1-infected individuals, we heated three serum specimens at 56°C for 30 min. No effect on the inactivation of HIV by these sera was observed (data not shown). These findings indicate that in infected individuals, the blood contains a heat-stable factor that can inactivate HIV-1, but only those strains that are neutralizable.

In related studies, plasma samples obtained within 2 h following venipuncture were stored at −70°C for several months; the detected virus titer was not diminished. For example, plasma from five individuals that was stored for up to 13 months showed no decrease in virus titer (virus titer range, 5 to 5,000 TCID/ml, depending on the subject). These observations suggest that plasma kept at −70°C for several months can maintain infectious virus. Our early observations (1985 to 1988) with some sera stored at −70°C support this conclusion (Table 1).

Identification of a plasma factor that inactivates HIV. Our studies with HIV-1_{SF170} and HIV-1_{SF128}, noted above, strongly suggested an involvement of antibodies in the inactivation of HIV. This possibility was substantiated by showing that the plasma samples obtained from HIV-infected individuals neutralized strains HIV-1_{SF2} and HIV-1_{SF33} at titers of 1:100 dilution or greater but did not neutralize the other two HIV-1 strains (data not shown).

In a further evaluation of the involvement of antibodies in the inactivation of HIV-1, we purified IgG from the plasma of four HIV-1-infected healthy individuals and tested this material and the remaining IgG-depleted plasma sample for anti-HIV activity. All plasma samples neutralized HIV-1_{SF2}. In the experiment for which representative results are presented in Table 4, the subject's plasma used neutralized HIV-1_{SF2} but not the autologous virus (HIV-1_{SF1002}) (data not shown). In the related studies, viruses mixed with the plasma or plasma fractions were left for 1 and 6 h at room temperature and were then inoculated onto PHA-stimulated PBMCs. The normal plasma and its fractions had no substantial effect on HIV-1_{SF2} or the primary isolate HIV-1_{SF1002}, as expected. With the plasma and the IgG fractions from the HIV-infected individual, HIV-1_{SF2} infection was

TABLE 4. Plasma inactivation factor associated with antibodies in serum[a]

Virus strain and sample	HIV replication as RT activity (10^3 cpm/ml) at:	
	1 h	6 h
HIV-1_{SF2}		
Normal plasma	295	168
IgG fraction	126	117
IgG-depleted fraction	202	111
Seropositive plasma	5.5	0.8
IgG fraction	1.7	1.5
IgG-depleted fraction	217	164
HIV-1_{SF1002}		
Normal plasma	352	145
IgG fraction	265	158
IgG-depleted fraction	333	183
Seropositive plasma	261	89
IgG fraction	212	114
IgG-depleted fraction	300	188

[a] Virus and plasma or its fractions were mixed and left for 1 or 6 h at room temperature and were then added to PBMCs. HIV replication is shown as RT activity in the culture supernatant at the first peak of RT activity (~10 to 15 days). Normal plasma and IgG fractions were used at an ~1:3 dilution. Representative results from at least four independent experiments are presented. HIV-1_{SF1002} was obtained from an asymptomatic individual.

substantially reduced, particularly after 6 h (Table 4); the IgG-depleted plasma fraction showed no effect on HIV-1_{SF2}. In contrast, when the HIV-1_{SF1002} primary isolate was used with the subject's own plasma sample, which did not neutralize HIV-1_{SF1002}, no effect of the plasma or of its fractions was observed (Table 4). Similar results were observed in three other experiments; the IgG fraction was associated with inhibition of HIV. The results, therefore, further support the fact that IgG plays a role in inactivating HIV-1.

DISCUSSION

The studies described here were conducted to determine the prevalence and extent of plasma viremia by procedures that would be optimal for virus detection. The results confirm the findings of Ho et al. (14) that free virus can be detected in the plasma of all individuals infected with HIV. Even those asymptomatic individuals with high $CD4^+$ cell counts (e.g., 1,545/μl) had detectable levels of HIV in their plasma or serum. Other studies have also reported high virus recovery rates from plasma, but not from all infected individuals (6–8, 22). The detection of free virus in our studies did not appear to result from the cells that remained in the centrifuged plasma samples, since filtration of plasma from three of the asymptomatic individuals studied did not change the results (data not shown). Moreover, other investigators have found that filtration of plasma did not affect their observations on plasma viremia (22). The results suggest that HIV can successfully be detected by cell culture methods involving plasma inoculation onto polybrene-treated, PHA-stimulated PBMCs and a core antigen ELISA. This ELISA procedure has been shown by other investigators (10) to be more sensitive than the RT assay at detecting low levels of virus. Our observations also support the conclusion that for infectious virus detection, culture of plasma is much more sensitive than the p24 core antigen ELISA on the plasma itself.

We did find with some specimens, however, that a delay in culturing of the plasma can inhibit virus recovery. In previous studies in our laboratory, a lower level of virus was detected (Table 1) (21). These results reflect the lower sensitivity of the RT assay used and perhaps the length of time between venipuncture and plasma culture. Finally, our data suggest that if plasma is stored at −70°C, the virus can remain stable for several months.

As reported by other investigators (6–8, 11, 14, 22, 23, 25), our previous (1985 to 1988) and recent results also indicate that the level of viremia increases as individuals' symptoms progress and they lose $CD4^+$ cells (Tables 2 and 3). However, only virus titers determined when the $CD4^+$ cell counts were low (<100 TCID/ml) differed substantially from those of others to be predictive of a highly symptomatic course of infection. It is not known whether the viremia was responsible for the loss of $CD4^+$ cells or reflects the reduced $CD4^+$ lymphocyte number. Previous work in our laboratory and the work of other investigators has suggested that the virus changes over time to become more cytopathic (5, 24) and, thus, might cause a reduction in $CD4^+$ cell numbers. Studies of the virus recovered from the plasma of these individuals over time should elucidate this possibility.

We investigated the reason that plasma (or serum) tested 3 h after venipuncture gave a lower frequency of infectious virus detection. Whereas the plasma of normal individuals stabilized the virus (Fig. 1), that of infected individuals appeared to inactivate HIV (Fig. 2). Further studies on this virus inactivation indicated that the activity is heat stable and is associated with the IgG fraction of the blood (Table 4). This conclusion is supported by the inactivation of only those strains (e.g., HIV-1_{SF2} and HIV-1_{SF33}) that are susceptible to neutralization by serum and not ones that are resistant to serum neutralization (e.g., HIV-1_{SF170}, HIV-1_{SF128A}, and HIV-1_{SF1002}). Moreover, the observation explains why the plasma from some infected individuals (e.g., subject 1002) did not inactivate the free virus that was present. The phenomenon most likely represents the presence of escape mutants (20). In this regard, preliminary data obtained in our laboratory suggest that virus inactivation by plasma is less common in symptomatic patients. Nevertheless, the reason that the effect of virus inactivation by plasma sometimes takes up to 3 h was not answered by the experiments described here; it is possible that other factors in plasma are also involved. The relevance of these findings to the in vivo situation is not evident. Most likely, in infected individuals, fresh HIV particles are constantly released into the plasma from PBMCs and lymph nodes, and so the effect of an inactivating factor would not be immediately apparent.

In summary, results of the present study further indicate the wide prevalence of viremia in individuals at many different clinical stages of HIV infection, including those who are asymptomatic. In our studies, all seropositive individuals, even those with high $CD4^+$ counts, had plasma viremia. Thus, culture of cell-free plasma or serum could be a feasible method of measuring virus production in infected individuals and monitoring the effects of antiviral therapy, particularly in symptomatic patients. Of the assays described here, the p24 core antigen ELISA is the preferred virus detection procedure. However, the variability in virus titers in plasma among the subjects at different clinical stages of HIV-1 infection suggests that only by following plasma viremia in a particular individual over time can the results help in establishing a prognosis or evaluating the results of

therapy. Nevertheless, titers of >1,000 TCID/ml would be highly suggestive of a low $CD4^+$ count and progression of the pathogenic course of infection. Our observations also indicate that the time of culture after venipuncture can be important for infectious virus detection. The time of culture after venipuncture should be short in order to avoid the inactivating effects of neutralizing antibodies and, perhaps, other factors in plasma. Finally, the experiments described here provide a practical means of monitoring plasma viremia; storage of plasma at −70°C appears to stabilize the virus in plasma for several months.

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