Is Human Immunodeficiency Virus RNA Load Composed of Neutralized Immune Complexes?

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During acute human immunodeficiency virus (HIV) infection, both virus load (HIV RNA) and infectivity are high (10^3–10^7 RNA copies/mL or TCID_{50}/mL) until antibody is produced, which may reduce the HIV infectivity. In HIV carriers, the HIV RNA load is elevated (10^8–10^10 copies/mL), but infectivity is low (10^0–10^2 TCID_{50}/mL). The low infectivity in carriers could be due to neutralization by antibody in serum, resulting in immune complexes (ICs). We demonstrated that ICs in plasma, prepared with protein A beads, contained HIV RNA (80%–100%) in association with immunoglobulin G (IgG). In comparison, ICs from patients with acute HIV infection and little or no antibody contained virtually no HIV RNA. Moreover, ICs prepared by ultrafiltration contained IgG and specifically and irreversibly neutralized HIV, which indicates that the ICs contained neutralizing antibody. These findings indicate that the HIV RNA in the plasma of carriers is frequently composed of antibody-neutralized HIV as ICs.

The composition of virus load (human immunodeficiency virus [HIV] RNA) in HIV carriers is still an open question. Paradoxically, HIV RNA load in the plasma and secretions of carriers have low infectivity (10^2–10^7 TCID_{50}/mL) but high HIV RNA load (10^3–10^6 copies/mL) [1, 2]. Two possible explanations for the low infectivity are defective HIV and inhibition of infectivity by antibodies. Evidence that neutralizing antibody may be one cause of the low infectivity is the reported decline of the HIV infectivity of plasma after acute infection, when antibody production begins [1] and circulating immune complexes (ICs) appear in the plasma of up to 40% of carriers [3, 4] (J. Denner, personal communication). If neutralization by antibody is an important cause of low infectivity, several predictions can be made: (1) in those carriers who are producing antibody, circulatory HIV would be bound to antibody; (2) in patients with acute infection and less anti-HIV antibody, less HIV would be bound to antibody; (3) the plasma of carriers should contain sufficient antibody to bind to HIV to form neutralized ICs; and (4) in the ICs, bound antibody could neutralize added HIV. These predictions were confirmed experimentally, which indicates that the paradoxically low infectivity of the HIV RNA in carriers often is due to neutralization of infectious HIV by antibody by means of the formation of circulating ICs.

Patients, Materials, and Methods

Patients, plasma, and serum. The first group, comprising 4 patients with chronic HIV infection, was selected for separation of ICs with protein A (figure 1) on the basis of the following criteria: HIV antibody positivity (determined by ELISAs and Western blot assays), presence of HIV RNA in plasma (>200 copies/mL), no treatment, CD4 counts >400 cells/mL, and absence of disease. The criteria for selection of the second group, comprising 2 patients with acute HIV infection, were clinical symptoms of acute HIV infection, high plasma HIV RNA load (>750,000 copies/mL), and ELISAs positive for HIV antibody but indeterminate results of Western blot assays.

The third group, comprising 16 patients with chronic HIV infection, were selected for serum antibody assays (table 1), including separation of ICs by ultrafiltration (10 patients), on the basis of ELISAs and Western blot assays positive for HIV antibody. Serum samples from 5 healthy donors also were studied.

Virus. For in vitro infectivity studies, HIV strain 213 was used. It was propagated in H9 cells and stored at -70°C, as described elsewhere [5]. Infectivity assays (TCID_{50}/100 μL) for HIV were done in quadruplicate on MT-2 cells.

Plasma HIV RNA load. The number of HIV RNA copies per milliliter in plasma was measured by use of the Amplicor HIV Monitor kit (Roche). This assay has a lower detection limit of ~200 copies/mL.

Separation of ICs by use of absorption of plasma protein A beads. To eliminate free antibodies prior to absorption, 300 μL of each serum sample was centrifuged at 23,000 g for 90 min at 4°C. Control
experiments showed that, under these conditions, most of the viral RNA was in the pellet, whereas no RNA was in the supernatant. The pellets that contained HIV RNA were resuspended in 300 μL of PBS and 200 μL containing 10,000–20,000 HIV RNA copies/mL were used to quantify the number of HIV RNA copies.

To absorb the antibody-virion complexes, sequential absorption on protein A–coated beads (Dynal magnetizable polystyrene) was performed. One hundred microliters of Dynabeads was suspended in 80 μL of 0.1 M Na-phosphate buffer, and 20 μL of plasma was added to the washed beads. This suspension was incubated with mixing for 10 min and then placed in a Dynal magnetic particle concentrator to separate the beads. Absorption was repeated 2 more times, and the supernatants were collected in a final volume of 200 μL. Beads obtained from the 3 steps then were washed twice and suspended in 200 μL of PBS. Samples were analyzed with the AmpliCor HIV Monitor kit (Roche), to quantify the number of HIV RNA copies absorbed by the protein A beads.

**Preparation of ICs by ultrafiltration.** Serum samples from 16 patients with chronic HIV infection were studied separately for the presence of neutralizing antibody, and ICs were separated from 10 of these serum samples by use of a 1 × 10³ molecular weight cutoff (MWCO) microconcentration assay (Macrosep; Pall Filtron). The serum samples were sterilized by UV light, and ultrafiltration was done using a 200-μL sample. The retentates were washed 3 times by filtration of 200 mL of medium, to remove free immunoglobulin, and then were resuspended in the initial volume of medium. Control experiments that used HIV and vesicular stomatitis virus showed that antibody unbound to these viruses passed into the filtrate and that most of the free HIV and the ICs were retained by the filter (data not shown).

**HIV neutralization by patients’ serum and ICs.** To determine the neutralization titer of antibody, 2-fold serial dilutions of each serum sample (100 μL) were mixed with 100 TCID₅₀ of HIV strain 213 and were incubated for 1 h at 37°C. Residual virus infectivity was determined by titrating 0.5 log₁₀ dilutions of the mixtures in 4 replicate cultures of MT-2 cells [5, 6].

To determine the neutralization index (the quantity of virus that can be neutralized by the serum), 25 μL of the serum sample was mixed with 25 μL of 100 TCID₅₀ of HIV and was incubated for 1 h at 37°C before the assay for residual HIV infectivity (TCID₅₀/100 μL) to determine the log₁₀ inhibition. To determine specificity of neutralization by ICs, neutralization assays were done using the heterologous Sindbis and vesicular stomatitis viruses. No inhibition occurred.

**Statistical analysis.** In addition to the basic descriptive statistics given in the legend for figure 1, regression analysis and nonparametric statistical methods were used. In each instance, the pertinent trends and differences observed were large, and all related significance levels were small (P < .05), even with the sample sizes used.

**Results**

**HIV-immunoglobulin ICs.** To determine whether immunoglobulin in the plasma of untreated carriers was bound to HIV, protein A beads were used to absorb immunoglobulin, and the
amount of coabsorbed HIV RNA was measured. The absorption was repeated sequentially 3 times. As shown in figure 1A, most of the HIV RNA in the plasma samples from 4 carriers was removed from the plasma when the immunoglobulin was absorbed by protein A beads. After absorption, the residual HIV RNA in the sample ranged from 3% to 15% of the HIV RNA input. In contrast, plasma samples from 2 untreated patients with primary HIV infection and with low levels of antibody to HIV (ELISA positive but Western blot indeterminate), showed no HIV RNA bound to protein A–coated beads, and most of the HIV RNA was in the plasma (figure 1B). This indicates that during the acute stage of infection, unlike during the chronic stage, most of the virus is not bound to immunoglobulin. Control experiments demonstrated that free protein A blocked HIV RNA binding to the protein A beads, thus confirming that the protein A on the beads is the molecule that binds the HIV-immunoglobulin ICs.

Discussion

Plasma samples from persons with acute HIV infection have both high HIV infectivity (10^3–10^7 TCID_{50/mL}) and high HIV RNA load (10^3–10^7 copies/mL) [1, 7]. This correspondence of high infectivity and high HIV RNA load does not occur in carriers with chronic HIV infection: plasma samples from these persons have low infectivity (10^0–10^2 TCID_{50/mL}) but, paradoxically, higher HIV RNA load (10^3–10^4 copies/mL) [1, 7]. The mechanism that causes the disproportionately low infectivity of the HIV RNA load in plasma from carriers is as yet unexplained. Possible mechanisms for low infectivity include circu-
lating defective HIV and neutralization of the HIV by the patient’s antibody, resulting in circulating ICs, which have been found in patients and which may be noninfectious [3, 4] (J. Denner, personal communication). Similar ICs occur during other chronic viral infections, such as equine infectious anemia [8], Aleutian mink disease [9], lactic dehydrogenase infection [10], lymphocytic choriomeningitis [11], and herpesvirus infection [12], and some of these ICs have diminished infectivity. Our findings support the mechanism of neutralization of HIV in ICs, by demonstrating that (1) in most patients with chronic, but not acute, infection the HIV RNA in plasma is bound to immunoglobulin in an IC (figure 1); (2) the plasma of carriers contains HIV-specific neutralizing antibody (table 1), which is available to bind to circulating HIV; (3) the circulating ICs from the plasma of carriers contain neutralizing antibody; and (4) the neutralization activity often is sufficiently potent to account for the low infectivity of the ICs. Future use of different strains of HIV for neutralization tests may increase their sensitivity [13]. Similarly, it seems possible that the low infectivity of cell-free HIV in mucosal secretions, such as seminal fluid and milk [6], may be due in part to neutralization by the antibody in those fluids. The role of subtypes of IgG in ICs will be of interest, because IgG3 does not bind to protein A.

The infectious titer of cell-associated virus, which is not accessible to antibody, is ~10-fold higher than that of cell-free HIV in both blood [1] and seminal fluid [14]. The present findings suggest that the higher infectivity of leukocytes, compared with that of cell-free body fluids, may be due to exclusion of neutralizing antibody from cells.

The nature of the HIV RNA in the ICs may be surmised. Unprotected RNA is unlikely to occur in plasma, because of its sensitivity to plasma RNases. The HIV RNA may exist in ICs as the same HIV virions that initially circulate as high-titered infectious HIV during acute infection and then are neutralized when antibody is produced.

The HIV in the plasma of carriers retains low infectivity (10^2–10^3 TCID_{50}/mL) [1, 2] in the presence of neutralizing antibody. A possible explanation for the low residual infectivity is a small nonneutralizable fraction that has been found with other viruses in the presence of antibody [15]. Possible mechanisms of a nonneutralizable fraction include steric hindrance of antibody neutralization [15] and escape variants [13].

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References