Comparison of Human Immunodeficiency Virus (HIV)-Specific Infection-Enhancing and -Inhibiting Antibodies in AIDS Patients

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Infections with human immunodeficiency virus (HIV) type 1 (HIV-1) induce strong cellular and humoral immune responses in humans. The humoral immune responses comprise the production of virus-specific antibodies directed against all viral proteins. However, the antibodies against viral glycoproteins, i.e., gp120/41, are mainly involved in antiviral responses (for a review, see references 1 and 10). On the basis of their effector functions, these antibodies may be virus-neutralizing or cytotoxic antibodies. Virus-neutralizing antibodies (NAs) bind to specific epitopes on the envelope proteins of virions and render them incapable of infecting target cells by a variety of mechanisms (for a review, see reference 39). These antibodies are very effective in inactivating and eliminating free virions from body fluids; however, they cannot prevent the cell-to-cell spread of HIV infections, which represents a major mechanism of HIV spread in infected humans. The cytotoxic antibodies may be complement-fixing or antibody-dependent cellular cytotoxicity-mediating (ADCC) antibodies. The complement-fixing antibodies activate complement after binding of the virus to the complement epitopes and cause virolysis or death of the infected cells (cytolyis) (39). The ADCC antibodies, on the other hand, effect the interaction between HIV-infected cells expressing envelope glycoproteins and FcyRIII (CD16)-positive natural killer (NK) cells and consequently cause the death of the HIV-infected cells (1, 10). These antibodies are very effective in preventing the cell-to-cell spread of HIV-1 infection. Both NAs and ADCC antibodies have been correlated with better clinical conditions in patients infected with HIV and AIDS patients and therefore may potentially be involved in the in vivo control of HIV infection (2, 6, 24, 28, 31).

Paradoxically, HIV-specific antibodies can also enhance HIV infection and the tropism of the virus (16, 18, 30, 37, 46; for a review, see reference 14). These so-called infection-enhancing antibodies (IEAs) may mediate these effects with and without complement. The complement-dependent IEAs (C-IEAs) enhance infection of complement receptor-bearing cells. All known complement receptors may be used for this process (14, 30, 49, 52). This type of enhancement has been documented with fresh serum or plasma from patients infected with HIV and by adding complement to sera from these patients if the sera had been heat inactivated (15, 37). The complement-independent IEAs enhance HIV infections by mediating binding of virions to Fc receptors (FcRs) on target cells and therefore are FcR-dependent IEAs (FcR-IEAs). The FcRs for immunoglobulin G (IgG) (FcyRI, FcyRII, and FcyRIII) and IgA (FcaR) have all been documented to be involved in this process (19, 45, 46). Human peripheral blood mononuclear cells (PBMCs) have also been reported to be susceptible to the phenomenon of antibody-dependent enhancement of infection by HIV-1 (5, 33). This phenomenon of antibody-dependent infection enhancement occurs in vivo in animal models of AIDS, and IEAs have been demonstrated in HIV-infected and gp160-vaccinated individuals (14, 17, 38, 50, and 52).

Earlier we reported the gp120/41-specific ADCC antibody titers obtained for a cohort of HIV-infected subjects by using our gp120/41-expressing, NK activity-resistant human cell
clones (2). The env gene of a laboratory strain (HXBc2) of HIV-1 was transfected in these cells. The main objective of the present study was to determine and compare the HIV NA and IEA titers in the sera of these patients. To validate our comparisons, we used the same virus (i.e., HXBc2) whose env gene was used for the ADCC antibody studies for determination of the NA and IEA titers. The results of this comparative analysis are reported here.

MATERIALS AND METHODS

Subjects. Sera obtained from 39 individuals infected with HIV in the era before the use of highly active antiretroviral therapy were used in this study after the patients provided informed consent. Details concerning the clinical conditions of the patients and their CD4 counts (range, 0 to 1,200 cells per μl) have been described earlier (2). Eight of these individuals were HIV seropositive but asymptomatic, while the rest had at least one AIDS-defining condition. Only three of the AIDS patients were receiving zidovudine treatment at the time of serum collection. All the sera used were heat inactivated at 56°C for 30 min and kept at −80°C before use.

Cell lines. The MT-2 and U937 cell lines were used in this study. The MT-2 cell line was derived from in vitro transformation of cord blood lymphocytes by coculture with human T-cell leukemia virus type 1-producing human leukemic cells. The MT-2 cell line is CD4 positive and abundantly expresses CR2 (26). It has been used for demonstration of C-IEAs in the sera of patients infected with HIV. U937 is a promyelomonocytic cell line that expresses CD4, FcγR, and FcγRIII or any of the complement receptors and has been used for the demonstration of FeR-mediated IEAs in the sera of patients infected with HIV (22, 45, 46). Both of these lines were cultured in RPMI 1640 containing 10% heat-inactivated fetal bovine serum and antibiotics (50 μg of streptomycin per ml, 50 U of penicillin per ml, 30 μg of gentamicin per ml); this medium is hereafter referred to as the culture medium.

ADCC antibody assay. A standard 51Cr-release assay was used to determine ADCC antibody activity. This assay was described in detail (2). The target cells used in this assay were our env gene-transfected, gp120/41 expressing, NK activity-resistant cloned human Raji cells. The env gene was derived from the HXBc2 molecular clone of HIV-1. Control vector-transfected cells (which did not express gp120/41) were used as negative controls. PBMCs obtained from HIV-seronegative donors served as effector cells in these assays. Sera from HIV-seronegative donors were also used in the ADCC antibody assays for comparison with the sera from patients infected with HIV. Briefly, 10,000 3H-chromium-labeled target cells were incubated in triplicate with PBMCs at a 1:20 ratio in the presence of 25 μl of a dilution of serum in the wells of 96-well microculture plates. Tenfold dilutions of serum starting with a dilution of 1:10 were used for this assay. After 16 h of incubation at 37°C in a humidified 5% CO₂ atmosphere, the amount of radioactivity released in the culture supernatants was measured in a gamma counter (Cobra II; Labs安全, Turku, Finland) and was compared to the maximum amount of radioactivity released (100% cytotoxicity) and the spontaneous amount of radioactivity released (0% cytotoxicity), as described earlier (2). The ADCC antibody titer of a serum sample was defined as the reciprocal of the highest dilution that resulted in a significantly (P ≤0.05) higher level of lysis of the target cells compared to the level of lysis achieved with the corresponding dilution of normal human serum.

HIV neutralization assay. The titers of the HIV NAs in the sera were determined by a previously published protocol (37), with some modifications. Briefly, 100 syncytium-forming units of the virus (in 50 μl of culture medium) were mixed with an equal volume of twofold serial dilutions of the sera (starting with a dilution of 1:10) in triplicate in 96-well microculture plates, and the mixture was incubated at room temperature for 1 h. After this incubation, 5 × 10⁴ MT-2 cells were added (100 μl volume) and the plates were incubated at 37°C in a humidified 5% CO₂ atmosphere. The microcultures were examined daily under an inverted microscope for the appearance of syncytia. All the microcultures were scored for the presence or absence of syncytia when all wells containing virus and no serum showed syncytia. The neutralization titer of the serum was defined as the reciprocal of the highest dilution at which at least two replicate wells were free of syncytia.

Assay for C-IEA titers. The assay for C-IEA titers used was essentially the same as that used for assay of the NA titers described above, except that the serum dilutions were made in culture medium containing 10% (vol/vol) fresh human serum from an HIV-seronegative donor. The fresh serum served as a source of complement in these assays. For determination of C-IEA titers, 100 μl of the cell suspension was transferred to the wells of a 96-well flat-bottom microculture plate that had been precoated with polyl-l-lysine. The syncytia were counted under an inverted microscope after the cells had settled and adhered to the plate. The C-IEA titer was defined as the serum dilution that resulted in a 50% or more increase in the number of syncytia, whereas the virus neutralization titer (the C-NA titer) in this assay was that which resulted in a 50% or more decrease in the number of syncytia compared to the number of syncytia for the positive control (virus-infected cells).

Virus production. HIV strain HXBc2 was produced in the COS-7 cell line, an African green monkey kidney cell line transformed by an origin-defective mutant of simian virus 40 and maintained in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum and antibiotics. The cells were transfected with 20 μg of the HXBc2 provirus plasmid by the calcium phosphate method, as described previously (42). The transfected cells were grown at 37°C and the culture supernatant was collected 48 h later. The supernatant was passed through a 0.45-μm-pore-size filter and concentrated 100-fold by centrifugation (42). The titers of the virus preparation were measured for determination of the 50% tissue culture infective doses and the number of syncytium-forming units per milliliter by using CEM-SS cell monolayers, as described previously (32).

Statistical analysis. The relationships between the titers of different types of antibodies were determined by multiple stepwise regression analysis with statistical software (SPSS Inc., Chicago, Ill.). Fisher's exact probability test was used to compare the percentages of sera positive for antibodies between different patient groups. The nonparametric Mann-Whitney U test and the Kruskal-Wallis analysis of variance test were used to compare titers between different patient groups. In the case of significant results (P < 0.05) by the Kruskal-Wallis analysis of variance test, group comparisons were made by Dunn's multiple comparison test. Results were deemed statistically significant when the α value was <0.05.

RESULTS

Relative prevalence of antibodies in sera. The percentages of sera positive for each of the different gp120/41-specific antibodies and the geometric mean antibody titers are shown in Table 1. While ≥60% of the serum samples tested were positive for NAs or ADCC antibodies, only 38% of the serum samples neutralized virus in the presence of human complement. An overwhelming majority of the serum samples (72%) were positive for antibodies that enhanced HIV infection in
the presence of complement. The percentages of serum samples positive for the different types of antibodies are shown in Table 2. Higher percentages of serum samples positive for NAs or ADCC antibodies were also positive for IEAs compared with the percentages of serum samples negative for NAs or ADCC antibodies but positive for IEAs. However, smaller percentages of serum samples positive for C-NAs were also positive for IEAs. These data suggest that the presence of NAs or ADCC antibodies in sera is more likely to accompany the presence of IEAs, while the converse is true for the presence of C-NAs. It may be noted in Table 2 that 11 (28%) of the serum samples neutralized virus in the presence of complement as C-NAs. It may be noted in Table 2 that 11 (28%) of the serum samples neutralized virus in the presence of complement as C-NAs. It may be noted in Table 2 that 11 (28%) of the serum samples neutralized virus in the presence of complement as C-NAs. It may be noted in Table 2 that 11 (28%) of the serum samples neutralized virus in the presence of complement as C-NAs. It may be noted in Table 2 that 11 (28%) of the serum samples neutralized virus in the presence of complement as C-NAs. It may be noted in Table 2 that 11 (28%) of the serum samples neutralized virus in the presence of complement as C-NAs. It may be noted in Table 2 that 11 (28%) of the serum samples neutralized virus in the presence of complement as C-NAs. It may be noted in Table 2 that 11 (28%) of the serum samples neutralized virus in the presence of complement as C-NAs. It may be noted in Table 2 that 11 (28%) of the serum samples neutralized virus in the presence of complement as C-NAs.

### Prevalence of antibodies in patient groups

We classified the patient sera on the basis of the number of CD4⁺ T cells per microliter, as described earlier (2). CD4⁺ T-cell counts have been widely used as surrogate markers for HIV disease progression (for a review, see reference 51). As shown in Table 3, NA, ADCC antibody, and C-NA titers were the lowest in the group of patients with <200 CD4⁺ T cells/μl. However, there were no statistically significant (P < 0.05) differences in the ADCC antibody and NA titers between the patient groups. Only the titers of the C-NAs differed significantly (P < 0.05) between the patient groups (Table 3). In contrast to the ADCC antibody, NA, and C-NA titers, the titers of both types of IEA (i.e., C-IEA and FcR-IEA) were the lowest in the group of patients with the highest CD4⁺ T-cell counts (>400 T cells/μl), although the differences between the patient groups were not significant (P > 0.05) (Table 3). Since patients with <200 CD4⁺ T cells per μl have relatively advanced HIV disease, we also compared their antibody profiles with those of the rest of the patients (Table 3). The percentages of serum samples that were antibody positive and the ADCC antibody, NA, and C-NA titers were higher for patients with CD4⁺ T-cell counts of >200 cells/μl (although the values were significantly [P < 0.05] higher only for C-NAs), while the converse was true for samples positive for IEA and IEA titers. Taken together, these data suggest that the components of the anti-HIV humoral immune response with respect to these different antibody types may vary during different stages of HIV disease.

### Regression analysis of antibody titers

We performed a stepwise multiple regression analysis between the titers of different antibodies, as described in Materials and Methods. The relationships between these antibody types for all serum samples are depicted in Table 4. Only ADCC antibody titers showed a significant (P < 0.05) positive correlation with the virus NA titers determined in the presence of complement (C-NA titers). The regression equation between these two antibody types is depicted in Fig. 1. It is evident from Fig. 1 that sera from HIV-infected individuals may be positive for ADCC antibody even though they are negative for C-NAs. The titers of all other types of antibodies varied independently of each other, suggesting that the immunogenicities of their epitopes may vary in different individuals.

### DISCUSSION

This comparative study demonstrates the prevalence of various types of antibodies with different effector functions against the viral envelope proteins in HIV-infected individuals. The titers of these antibodies against the same viral strain or its antigens were simultaneously determined and compared in the sera of the same cohort of patients infected with HIV. This study demonstrates that HIV-1 induces not only antibodies that mediate virus neutralization and kill HIV-infected gp120/41-

### Table 2. Relative prevalence of different antibody types in sera

<table>
<thead>
<tr>
<th>Antibody and result</th>
<th>C-IEA Positive (%)</th>
<th>C-IEA Negative (%)</th>
<th>NA Positive (%)</th>
<th>NA Negative (%)</th>
<th>ADCC antibody Positive (%)</th>
<th>ADCC antibody Negative (%)</th>
<th>C-NA Positive (%)</th>
<th>C-NA Negative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-IEA</td>
<td>FcR-IEA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>18 (46)</td>
<td>12 (32)</td>
<td>17 (44)</td>
<td>11 (28)</td>
<td>11 (28)</td>
<td>17 (44)</td>
<td>11 (28)</td>
<td>14 (38)</td>
</tr>
<tr>
<td>Negative</td>
<td>9 (23)</td>
<td>6 (19)</td>
<td>6 (15)</td>
<td>5 (13)</td>
<td>7 (18)</td>
<td>8 (22)</td>
<td>8 (22)</td>
<td>14 (38)</td>
</tr>
<tr>
<td>Total</td>
<td>28 (72)</td>
<td>18 (49)</td>
<td>25 (68)</td>
<td>21 (57)</td>
<td>22 (56)</td>
<td>25 (65)</td>
<td>21 (57)</td>
<td>28 (72)</td>
</tr>
</tbody>
</table>

### Table 3. Antibody profiles of AIDS patients with different CD4⁺ T-cell counts

<table>
<thead>
<tr>
<th>Antibody</th>
<th>GMT ± SE (median GMT; no. of positive sera) for sera with the following CD4⁺ T-cell counts (no. of cells/μl):</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;200 (n = 23) (group A)</td>
<td>200–400 (n = 7) (group B)</td>
</tr>
<tr>
<td>ADCC antibody</td>
<td>13.49 ± 1.92 (10; 56.2)</td>
<td>193.06 ± 5.18 (100; 71.42)</td>
</tr>
<tr>
<td>NA</td>
<td>17.95 ± 1.65 (40; 65.22)</td>
<td>41.40 ± 3.03 (40; 71.42)</td>
</tr>
<tr>
<td>C-NA</td>
<td>1.89 ± 1.36 (0; 17.39)</td>
<td>22.86 ± 2.34 (40; 71.42)</td>
</tr>
<tr>
<td>C-IEA</td>
<td>55.85 ± 1.73 (160; 73.91)</td>
<td>26.07 ± 1.84 (40; 85.71)</td>
</tr>
<tr>
<td>FcR-IEA</td>
<td>168.65 ± 3.36 (10; 50.00)</td>
<td>46.42 ± 5.76 (6; 50.00)</td>
</tr>
</tbody>
</table>

a GMT, geometric mean titer. n, the number of patients in each group.

b Values are percentages.

c P values are given only for comparison of means between groups A and D. Group D represents both group B and group C.
d The group with the lowest CD4 counts had significantly (P < 0.05) lower titers than the other three groups.

e The geometric mean titers of these antibodies tend to increase as CD4 counts decrease.
expressing target cells via ADCC but also antibodies that can enhance HIV infection with or without the involvement of complement. Both NAs and ADCC antibodies are generally considered protective for the host, as the former may neutralize free virions and effect their clearance from plasma, whereas the latter may prevent the cell-to-cell spread of infection by destroying virus-infected cells (10, 39). Both kinds of antibodies have been shown to mediate various degrees of protection destroying virus-infected cells (10, 39). Both NAs and ADCC antibodies are generally considered protective for the host, as the former may neutralize free virions and effect their clearance from plasma, whereas the latter may prevent the cell-to-cell spread of infection by destroying virus-infected cells (10, 39). Both kinds of antibodies have been shown to mediate various degrees of protection

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variable</th>
<th>Coefficient</th>
<th>t value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADCC antibody</td>
<td>NA</td>
<td>-0.078</td>
<td>-0.582</td>
<td>0.5650</td>
</tr>
<tr>
<td></td>
<td>C-NA</td>
<td>0.661</td>
<td>5.033</td>
<td>0.0001*</td>
</tr>
<tr>
<td></td>
<td>C-IEA</td>
<td>-0.117</td>
<td>-0.877</td>
<td>0.3870</td>
</tr>
<tr>
<td></td>
<td>FeR-IEA</td>
<td>-0.081</td>
<td>-0.627</td>
<td>0.5350</td>
</tr>
<tr>
<td>NA</td>
<td>C-NA</td>
<td>0.185</td>
<td>1.140</td>
<td>0.262</td>
</tr>
<tr>
<td></td>
<td>C-IEA</td>
<td>-0.213</td>
<td>-1.312</td>
<td>0.198</td>
</tr>
<tr>
<td></td>
<td>FeR-IEA</td>
<td>-0.078</td>
<td>-0.482</td>
<td>0.633</td>
</tr>
<tr>
<td>C-NA</td>
<td>C-IEA</td>
<td>-0.099</td>
<td>-0.594</td>
<td>0.556</td>
</tr>
<tr>
<td></td>
<td>FeR-IEA</td>
<td>-0.040</td>
<td>-0.243</td>
<td>0.810</td>
</tr>
<tr>
<td>C-IEA</td>
<td>FeR-IEA</td>
<td>-0.078</td>
<td>-0.475</td>
<td>0.638</td>
</tr>
</tbody>
</table>

* Significant correlation.

A untoward consequence of the HIV-induced humoral immune response is the induction of gp120/41-specific antibodies, which enhance HIV infection. This infection enhancement can occur through FcRs for IgG and IgA antibodies present on target cells, as well as through complement receptors (19, 45, 46). All four complement receptors have been demonstrated to bind to HIV-1 and mediate infection in the presence of complement in different human cell types (30, 47, 49, 52). Several researchers have demonstrated that the ability of sera to enhance HIV infection in the presence of complement correlated with the progression toward AIDS (15, 18, 43, 48). This may be the reason why CR2-positive CD4+ T cells are preferentially depleted in HIV-infected patients (20). However, some researchers could not find a correlation between the presence of IEAs and disease progression (27, 28). Nevertheless, the same group (29) has reported on the in vivo relevance of these antibodies to increased viral burden and viral antigenemia in the simian immunodeficiency virus-infected macaque model of AIDS. The probable reasons for these discrepancies have also been described (14, 43, 48). Our results suggest that HIV IEAs are at least as prevalent in the sera of HIV-infected individuals as NAs or ADCC antibodies. These findings are in agreement with those reported by others (for a review, see reference 14). In fact, the in vitro assays used to determine infection enhancement may be underestimating the real prevalence of these antibodies in sera since any given assay measures enhancement by only one type of FcR or complement receptor, whereas in vivo all these receptors may be contributing to enhancement. This has also been demonstrated in vivo in animal models of enhancement (35). Based upon the neutralization indices derived from the plots generated from in vitro neutralization assays, pairwise serum vector distances, and cluster analyses, Kostrikis et al. (21) have shown that the phenomenon of

![Figure 1](http://jcm.asm.org/)

**FIG. 1.** Regression equation for ADCC antibodies and NAs. The equation between the geometric mean titers of these two antibody types was built by using Spearson’s regression formula. ADCC antibody titers were calculated from this equation for three arbitrary values for C-NA and are presented here.
fection enhancement is widespread in patients infected with HIV. Unfortunately, the practical implications of these findings have not been fully realized in HIV immunization and vaccination efforts and the IEA titers have rarely been determined in such studies.

There was a statistically significant (P < 0.05) positive correlation only between the titers of ADCC antibodies and C-NAs. There was a lack of a correlation between the titers of all other antibody pairs (Table 4). This suggests the presence of distinct epitopes for these different types of antibodies and the differential immunogenicities of these epitopes in HIV-infected individuals. A lack of correlation between ADCC anti-body and NA titers has been reported by others (7). This is supported by reports that, in general, ADCC antibody-mediating monoclonal antibodies (MAbs) do not mediate virus neutralization and vice versa (8, 9, 11; for a review, see references 1 and 10). However, there may be exceptions: some HIV-neutralizing MAbs may mediate ADCC antibodies, provided that they bind to their epitopes with a high affinity and these epitopes become accessible to the FcRs of ADCC effector cells (4). Similarly, neutralizing MAbs do not mediate infection enhancement and vice versa (44). Complement alone or in the presence of antibodies, however, may cause increased levels of adhesion of the virions to target cells via complement receptors and may thus abrogate the neutralizing effect of sera, as reported here and by McDougal et al. (25). It is noteworthy that epitopes for the IEAs have been mapped to the N-termini two-thirds of the extracellular part of gp41 (36), whereas the NAs recognize epitopes mainly in the variable and CD4 binding domains of the viral envelope proteins of HIV-1 (13, 33a, 34, 35, 39). These findings may be exploited to the advantage of the host for the design of vaccines that do not carry epitopes for IEAs. However, a corollary of the presence of distinct epitopes for these antibody types is their independent induction, which necessitates monitoring of each of these antibody types in individuals infected with HIV and individuals vaccinated against HIV.

In conclusion, our study underscores the fact that the humoral immune response of the human host directed against HIV envelope glycoproteins consists of a mixture of IEAs and infection-inhibiting antibodies. A net balance between these two types of antibodies may vary at different stages of the infection and may determine the usefulness of the humoral immune response for the infected host.

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