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Serum Enhancement of Human Immunodeficiency Virus (HIV) Infection Correlates with Disease in HIV-Infected Individuals

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The sera from 16 individuals infected with the human immunodeficiency virus (HIV) at different clinical stages were evaluated for antibody-dependent neutralization and/or enhancement of infectivity by HIV. The HIV isolate from each individual (homotypic) and established laboratory strains showing broad cellular host range and cytopathicity were used. All sera could neutralize one of the laboratory-passaged isolates, whereas only two could neutralize the corresponding homotypic strain. Seven homotypic isolates were enhanced by serum from the respective individual. This activity was primarily observed in patients with acquired immune deficiency syndrome. Moreover, the tropism for macrophages of four of these seven viral isolates was found to be enhanced by the homotypic sera. Finally, sequential pairs of HIV and sera obtained from five HIV-infected individuals with different clinical progression were studied over time. The enhancing activity of three of the five sera appeared to increase over time, indicating changes in both the host virus population and the type of antibodies produced. These results suggest that enhancing antibodies contribute to the spread and pathogenesis of HIV in vivo. They emphasize the necessity of studying further the association of enhancing antibodies and disease progression in infected individuals.

Infection by the human immunodeficiency virus (HIV), like that of other lentiviruses, is characterized by a slow progression toward disease (5). Despite the formation of both cellular and humoral immune responses to HIV, neither arm of the immune system appears to be able to control definitively the spread of the virus, and eventually most infected individuals develop pathologic symptoms (14). In the acquired immune deficiency syndrome (AIDS), several factors can explain this situation. (i) The primary target cell for HIV infection, the $CD4^+$ helper lymphocyte, is a pivotal cell of the immune response, and abnormalities in its function can have repercussions on other cells of the immune system (11, 12, 18). (ii) HIV may escape immune surveillance by remaining latent for a prolonged period (4, 10, 16), by replicating at low levels (10, 16), and/or by spreading via cell-to-cell contact (15). (iii) HIV variants with different genetic, serologic, and biologic properties may appear over time (1, 2, 6, 21). (iv) Finally, recent evidence has demonstrated the occurrence of antibodies in serum that enhance rather than inhibit the infectivity of HIV in vitro (8, 9, 19, 20).

We sought to examine whether this antibody-dependent enhancement of HIV infectivity could explain the lack of protection conferred by antiviral antibodies and be associated with progression toward disease. Neutralizing antibodies to HIV have usually been measured in infection inhibition assays with viral strains established in the laboratory. However, different HIV strains have varying sensitivity to serum neutralization (1), and some data suggest that sera from HIV-infected individuals are generally less efficient at neutralizing their own isolates than neutralizing these established strains (22). In this study, we tested the ability of the sera of 16 HIV-infected individuals at different clinical stages to neutralize or enhance their own isolate compared with three established laboratory strains. Infection of both peripheral blood lymphocytes and macrophages was evaluated. We also examined the evolution of the antibody response over time in five individuals. The results showed that enhancing antibodies often dominate neutralizing antibodies in their effect on homotypic HIV strains and suggest that this immune response contributes to HIV pathogenesis in vivo.

MATERIALS AND METHODS

Subjects. The HIV-infected individuals used for these studies were randomly selected from homosexual men coming to our laboratory for evaluation of HIV infection. All procedures performed on these volunteers were done according to National Institutes of Health guidelines for human research. Five of the individuals were clinically healthy at the time when sera were collected; four subjects had clinical symptoms diagnosed as the AIDS-related complex (ARC) (lymphadenopathy or oral candidiasis); and seven patients had AIDS as defined by the Centers for Disease Control, including Kaposi's sarcoma and *Pneumocystis carinii* pneumonia. All sera were heat inactivated (30 min at 56°C) before study.

Viruses. The HIV type 1 (HIV-1) strains used for these studies were those previously obtained in the laboratory from infected individuals. These included HIV-1_{SF33}, a highly cytopathic strain obtained from an individual with thrombocytopenia (13). HIV-1_{SF128A} was isolated from the brain of an individual who died with neurologic symptoms resulting from HIV infection of the central nervous system (1). HIV-1_{SF170} came from patient with AIDS in Rwanda and has a limited host range as defined by infection of established T cells and macrophages (1). In previous studies, HIV-1_{SF33} was found to be highly susceptible to serum neutralization (1) (subgroup A); the other laboratory strains were relatively resistant to neutralization by human sera (1) (subgroups C and D).

The homotypic HIV-1 strains were recovered from phytohemagglutinin (3 μg/ml)-stimulated peripheral blood mononuclear cells (PMC) of the individuals being studied at

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approximately the same time as the serum was obtained. For the sequential studies, the HIV isolates were selected at exactly the same time as the serum tested. All viruses were grown in PMC from seronegative individuals until they reached titers of more than 10^6 cpm/ml of culture supernatant as measured in reverse transcriptase (RT) assays (7). Subsequently, the infectious titer was determined in PMC as described previously (17).

Cells. For these studies, phytohemagglutinin (3 μg/ml)-stimulated PMC from seronegative individuals were used as target cells for measuring HIV infection. They were cultured in the presence of 5% interleukin-2 (Electronucleonics) in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum, 2 mM glutamine, and 1% antibiotics (100 U of penicillin per ml, 100 μg of streptomycin per ml) (13).

Monocytes-macrophages were isolated from normal PMC by adherence onto the bottom of polystyrene flasks. After 3 days in culture in the RPMI 1640 medium containing in addition 5% heat-inactivated blood group-matched human serum, the nonadherent cells were removed by aspiration and the remaining cells were trypsinized and washed twice to remove any additional nonadherent cells. Adherent cells were allowed to mature into macrophages for 7 to 8 days (3) and were then used for infection studies as described previously (8). This procedure was performed to focus specifically on differentiated macrophages as the targets for HIV infection and enhancement.

Neutralization-enhancement assays. For the neutralization-enhancement assays, viruses at 100 50% tissue culture infective doses per ml were mixed with individual sera initially screened at 10-fold dilutions and subsequently retested at 2-fold dilutions when necessary (1, 8). The mixtures were kept at room temperature for 60 min and then added onto PMC. The PMC were pretreated with Polybrene (10 μg/ml) only in the neutralization assays (1). Virus production was measured by a standard RT assay (7). Neutralization was considered present when more than two-thirds (67%) of RT activity was eliminated from the culture supernatants compared with control cultures receiving virus incubated with normal serum. Enhancement was considered present if more than twice (200%) the level of RT activity was produced in the cell culture fluid compared with the control cultures. All experiments were repeated at least twice.

RESULTS

Neutralization versus enhancement. In the initial evaluation of sera from individuals at different clinical states, the ability to neutralize or enhance laboratory strains of HIV-1 was examined. All 16 sera tested readily neutralized the highly cytopathic HIV-1_{SF33} strain, and 6 sera neutralized HIV-1_{SF128A}, a strain relatively insensitive to serum neutralization (1) (Table 1). Only one serum showed limited activity against HIV-1_{SF170}. This latter result was not unexpected since this strain has been shown to be neutralized only by sera from Africa (1; unpublished observations). In contrast to the results with HIV-1_{SF33}, only two sera from an asymptomatic subject (no. 1) and a patient with AIDS (11) showed neutralization of the homotypic isolate (Table 1). Furthermore, five sera from patients with AIDS, one serum sample from a patient with ARC, and one serum sample from an asymptomatic individual enhanced infection of their own HIV-1 isolate (Table 1). The two sera with neutralizing antibody against the homotypic isolates showed no enhancement against these HIV even when diluted 100-fold past the neutralization titers (data not shown).

TABLE 1. Effect of serum antibodies on infection by heterotypic versus homotypic HIV[a]

Subject	Diagnosis	HIV_{SF33} (N)	HIV_{SF128} (N)	HIV_{SF170} (N)	Homotypic HIV	
					N	E
1	A	1,000	10	–	200	–
2	A	200	–	–	–	≥2,000
3	A	800	–	–	–	–
4	A	800	10	–	–	–
5	A	10	–	–	–	–
6	ARC	400	–	–	–	–
7	ARC	100	–	–	–	–
8	ARC	100	–	10	–	20
9	ARC	20	–	–	–	–
10	AIDS	100	–	–	–	200
11	AIDS	400	10	–	10	–
12	AIDS	100	100	–	–	200
13	AIDS	100	–	–	–	≥2,000
14	AIDS	100	10	–	–	–
15	AIDS	800	10	–	–	2,000
16	AIDS	20	ND[b]	ND	–	20

[a] Sera were obtained from 16 HIV-infected individuals who were asymptomatic (A) or had ARC or AIDS. Control sera came from seronegative donors. Results represent the reciprocal of serum dilutions at which virus production was neutralized (N) or enhanced (E) as defined in Materials and Methods. Negative results (–) indicate no neutralization or enhancement at a serum dilution of 1:10 or 1:20, respectively.

[b] ND, Not done.

Infection of macrophages. Five of the isolates that were enhanced by the homotypic serum were further tested for homotypic neutralization or enhancement in peripheral blood macrophages (Table 2). Three of these isolates from subjects 8, 10, and 15 grew readily in macrophages, while the other two from subjects 2 and 12 did not. However, in the presence of the homologous serum, the two isolates from subjects 2 and 12 infected and replicated substantially in macrophages. Moreover, the growth of two of the macrophage-tropic HIV-1 strains from subjects 10 and 15 was enhanced when they were mixed with the homotypic serum (Table 2).

Presence of antiviral activity over time in infected individuals. To investigate whether progression toward disease is associated with the appearance or an increased titer of enhancing antibodies, we tested the sequential sera and corresponding isolates from five HIV-1-infected individuals over a 1- to 3-year period (subjects 1, 5, 9, 10, 11; Table 1).

TABLE 2. Effect of homotypic versus normal serum on HIV infection of macrophages[a]

Patient	Diagnosis	(RT activity (10^3 cpm/ml)	
		Normal serum	Homotypic serum
2	A	2.0[b]	31.6
8	ARC	25.4	13.8
10	AIDS	133.4	700.1
12	AIDS	3.0[b]	30.2
15	AIDS	72.5	224.1

[a] The ability of virus isolates from five HIV-infected individuals to grow in peripheral blood macrophages in the presence of normal human serum or homotypic serum is indicated by the peak level of particle-associated RT activity in culture supernatants measured at days 12 to 15 postinfection (7). Sera were used at 1:20 and 1:200 dilutions and gave comparable results. A, Asymptomatic.

[b] RT levels under 5×10^3 cpm/ml indicate no virus production measured over 35 days of culture.

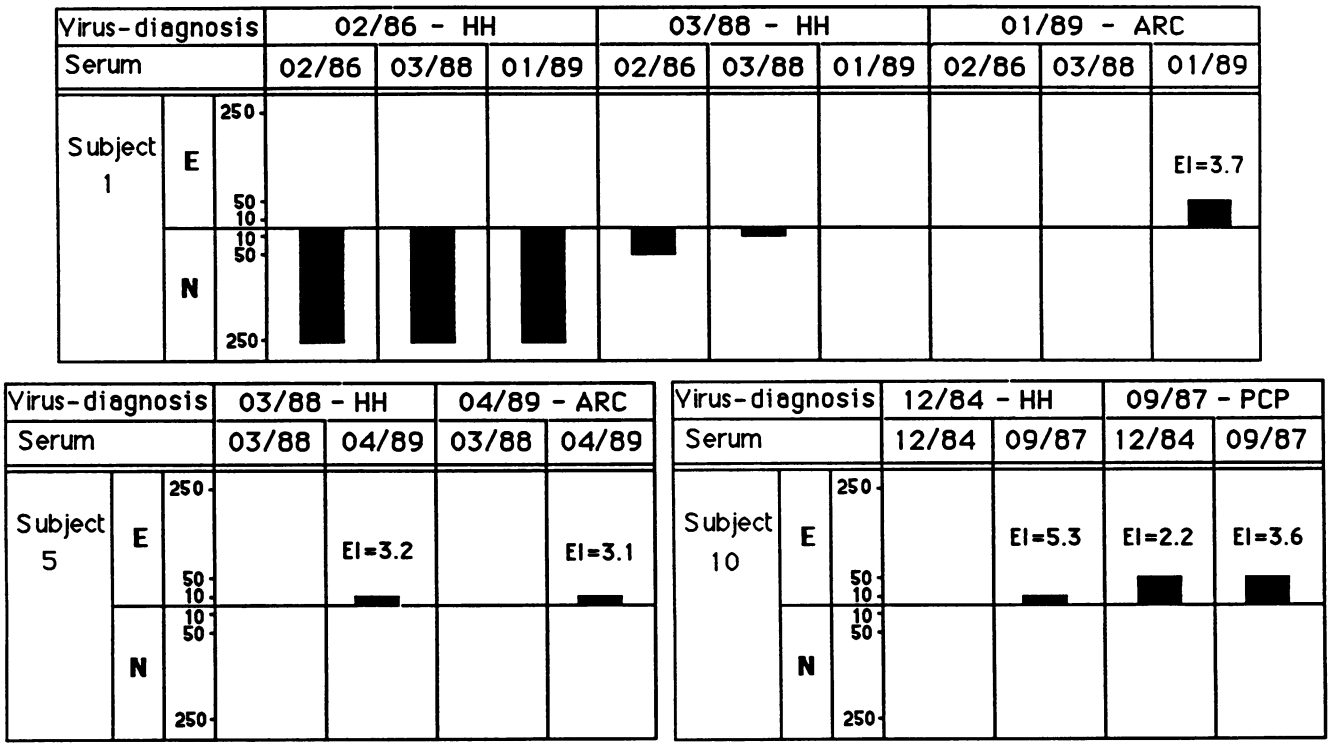

FIG. 1. Neutralization or enhancement of sequential homotypic HIV-1 isolates. Early and late HIV-1 isolates from each individual (1, 5, and 10) were tested for neutralization (N) or enhancement (E) by the corresponding early or late sera. The sera were evaluated at five fivefold dilutions (1:10 to 1:1,250). Normal control serum was used at corresponding dilutions. The clinical status of each individual at the different time points is indicated as HH, healthy homosexual man; ARC; and PCP, *P. carinii* pneumonia. Enhancement indexes (EI) are shown above bars and were defined as the ratio of the RT activity of supernatants of cultures receiving virus preincubated with homotypic sera versus virus preincubated with control sera.

During this time, all the subjects advanced in disease state. Early and late HIV-1 isolates from each individual and the corresponding serum specimens were used in this experiment (Fig. 1). The results indicate that the late isolates of three of the five individuals (1, 5, and 10) were increasingly enhanced by the homotypic sera, while the earlier isolates were either not affected or neutralized by the early sera. The late sera also enhanced the early isolates from subjects 5 and 10, who progressed to ARC and AIDS, respectively. This result suggests that enhancing antibodies were selected over time. However, this finding was not observed with the late serum from subject 1, who remained asymptomatic throughout the follow-up period but in January 1989 had a sudden drop in $CD4^+$ cell count and developed symptoms of ARC. Moreover, no change in antibody activity was found in the two other subjects (9 and 11) who progressed to ARC. Their sera did not show any enhancement or neutralization with either one of their virus isolates (data not shown). Finally, it is noteworthy that only with subject 10 did the early serum enhance the late isolate. This finding suggests the occurrence over time of new viral epitopes sensitive to antibody enhancement.

DISCUSSION

These results with sera from HIV-infected individuals indicate that neutralizing activity of serum against established laboratory strains does not reflect the ability of these individuals to control the infectivity of their own isolates. This observation supports those of others showing that high levels of neutralizing antibodies against standard HIV-1 strains can be found even in severely ill patients (1, 22). In the present study, we determined that not only do the sera from many HIV-infected individuals lack neutralizing activity against homotypic isolates, but they often enhance infection by their own HIV-1 strain (Tables 1 and 2). This observation was particularly true for patients with AIDS.

Previous studies had indicated that the enhancement of HIV infectivity is mediated by antibodies (8, 9, 19, 20). The present findings indicate that the HIV-infected individual probably produces a mixed population of antibodies, the net effect of which may more often facilitate rather than inhibit HIV infection. The observation that enhancing antibodies were found primarily in individuals with disease (Table 1) suggests that this immune response is involved in the increased spread and replication of HIV in vivo and is an important factor in disease progression. In the presence of enhancing antibodies, infection of T lymphocytes can be increased, with subsequent cell death and immunologic disorders (8, 12). Likewise, these types of antibodies to HIV can permit some HIV strains to spread to macrophages (Table 2), regarded as important reservoirs of the virus. In both instances, the overall immune capabilities of the infected individual can be compromised by this increased infection of immune cells.

The study of sera and HIV isolates obtained from five infected individuals over time, although limited in number, also suggests that enhancing antibody production plays a role in progression toward disease. Serum samples from three HIV-infected individuals (1, 5, and 10) who had in their serum either no or in one case (1) limited neutralizing

activity against their own virus in the relatively early time of their infection were found to enhance the infectivity of their related virus isolated up to 3 years later. The data suggest both that enhancing antibodies develop over time and that HIV variants with higher susceptibility to enhancing antibodies are selected over time and eventually may predominate in the individual. These results coupled with those showing the emergence over time of more pathogenic variants of an HIV-1 strain in an infected individual (2) emphasize further the important role of the virus in disease progression.

In all, the results of this study indicate that enhancing antibodies may contribute to the severity of HIV infection and therefore represent an important issue to be considered in both the treatment and prevention of AIDS. A block in their production may help delay disease progression, and the identification of the viral epitope(s) eliciting their production seems critical for vaccine development.

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LITERATURE CITED

1. **Cheng-Mayer, C., J. Homsy, L. A. Evans, and J. A. Levy.** 1988. Identification of human immunodeficiency virus subtypes with distinct patterns of sensitivity to serum neutralization. Proc. Natl. Acad. Sci. USA **85**:2815–2819.
2. **Cheng-Mayer, C., D. Seto, M. Tateno, and J. A. Levy.** 1988. Biologic features of HIV that correlate with virulence in the host. Science **240**:80–82.
3. **Clarkson, S. B., and P. A. Ory.** 1988. CD16: developmentally regulated IgG Fc receptors on cultured human monocytes. J. Exp. Med. **167**:408–417.
4. **Folks, T., D. M. Powell, M. M. Lightfoote, S. Benn, M. A. Martin, and A. S. Fauci.** 1986. Induction of HTLV-III/LAV from a nonvirus-producing T-cell line: implications for latency. Science **231**:600–602.
5. **Haase, A. T.** 1986. Pathogenesis of lentivirus infections. Nature (London) **322**:130–136.
6. **Hahn, B. H., M. A. Gonda, G. M. Shaw, M. Popovic, J. A. Hoxie, R. C. Gallo, and F. Wong-Staal.** 1985. Genomic diversity of the acquired immune deficiency syndrome virus HTLV-III: different viruses exhibit greatest divergence in their envelope genes. Proc. Natl. Acad. Sci. USA **82**:4813–4817.
7. **Hoffman, A. D., B. Banapour, and J. A. Levy.** 1985. Characterization of the AIDS-associated retrovirus reverse transcriptase and optimal conditions for its detection in virions. Virology **147**:326–335.
8. **Homsy, J., M. Meyer, M. Tateno, S. Clarkson, and J. A. Levy.** 1989. The Fc and not CD4 receptor mediates antibody enhancement of HIV infection in human cells. Science **244**:1357–1360.
9. **Homsy, J., M. Tateno, and J. A. Levy.** 1988. Antibody-dependent enhancement of HIV infection. Lancet **i**:1285–1286.
10. **Hoxie, J. A., B. S. Haggarty, J. L. Rackowsky, N. Pilsbury, and J. A. Levy.** 1985. Persistent non-cytopathic infection of human lymphocytes with AIDS-associated retrovirus (ARV). Science **229**:1400–1402.
11. **Klatzmann, D., F. Barré-Sinoussi, M. T. Nugeyre, C. Dauguet, E. Vilmer, C. G. F. Brun-Vezinet, C. Rouzioux, J. C. Gluckman, J.-C. Chermann, and L. Montagnier.** 1984. Selective tropism of lymphadenopathy associated virus (LAV) for helper-inducer T lymphocytes. Science **225**:59–63.
12. **Fauci, A. S.** 1988. The human immunodeficiency virus: infectivity and mechanism of pathogenesis. Science **239**:617–622.
13. **Levy, J. A., and J. Shimabukuro.** 1985. Recovery of AIDS-associated retrovirus from patients with AIDS or AIDS-related conditions, and from clinically healthy individuals. J. Infect. Dis. **152**:734–738.
14. **Lifson, A. R., G. W. Rutherford, and H. W. Jaffe.** 1988. The natural history of human immunodeficiency virus infection. J. Infect. Dis. **158**:1360–1367.
15. **Lifson, J. D., G. R. Reyes, M. S. McGrath, B. S. Stein, and E. G. Engleman.** 1986. AIDS retrovirus induced cytopathology: giant cell formation and involvement of CD4 antigen. Science **232**: 1123–1127.
16. **Luciw, P. A., C. Cheng-Mayer, and J. A. Levy.** 1987. Mutational analysis of the human immunodeficiency virus (HIV): the orf-B region down-regulates virus replication. Proc. Natl. Acad. Sci. USA **84**:1434–1438.
17. **McDougal, J. S., S. P. Cort, M. S. Kennedy, C. D. Cabridilla, P. M. Feorino, D. P. Francis, D. Hicks, V. S. Kalyanaraman, and J. S. Martin.** 1985. Immunoassay for the detection and quantitation of infectious human retrovirus, lymphadenopathy-associated virus (LAV). J. Immunol. Methods **76**:171–183.
18. **McDougal, J. S., M. S. Kennedy, J. M. Sligh, S. P. Cort, A. Mawle, and J. K. A. Nicholson.** 1985. Binding of HTLV-III/LAV to $T4^+$ T cells by a complex of the 110K viral protein and the T4 molecule. Science **231**:382–385.
19. **Robinson, W. E., Jr., D. C. Montefiori, and W. M. Mitchell.** 1988. Antibody-dependent enhancement of human immunodeficiency virus type 1 infection. Lancet **i**:790–794.
20. **Takeda, A., C. U. Tuazon, and F. A. Ennis.** 1988. Antibody-enhanced infection by HIV-1 via Fc receptor-mediated entry. Science **242**:580–583.
21. **Tersmette, M., R. A. Gruters, F. De Wolf, R. E. Y. De Goede, J. M. A. Lange, P. T. A. Schellekens, J. Goudsmit, H. G. Huisman, and F. Miedema.** 1989. Evidence for a role of virulent immunodeficiency virus (HIV) variants in the pathogenesis of acquired immunodeficiency syndrome: studies on sequential HIV isolates. J. Virol. **63**:2118–2125.
22. **Weiss, R. A., P. R. Clapham, J. N. Weber, A. G. Dalgleish, L. A. Lasky, and P. W. Berman.** 1986. Variable and conserved neutralization antigens of HIV. Nature (London) **324**:572–575.