Neutralization Profiles of Sera from Human Immunodeficiency Virus (HIV)-Infected Individuals: Relationship to HIV Viral Load and CD4 Cell Count

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The relationship of the neutralizing activity (NA) profile of sera from human immunodeficiency virus (HIV)-infected individuals to the HIV viral load and the absolute CD4 count was examined. The NA of 24 serum samples against autologous isolates (AI) and HIV type 1 strain MN was examined. Three NA patterns were recognized. Nine sera neutralized both AI and MN (+/+), six sera neutralized MN but not AI (+/−), and nine sera failed to neutralize both AI and MN (−/−). The identification of the three neutralization patterns (+/+), (−/+), and (−/−) indicated that resistance to neutralization was progressive. A reciprocal relationship between the viral burden of the patients and the NA profiles was observed. The nine subjects with a −/− NA profile had a plasma viral load of ≥5 × 10⁴ copies/ml and a cellular viral burden of ≥1,122 infectious units per million viable cells, which were significantly different from those of the other groups (P < 0.02). These patterns were independent of the phenotypic characteristics of the virus. Longitudinally, subjects with a −/− profile at baseline gained their HIV-specific NA by 24 weeks of antiretroviral therapy when this was associated with a ≥1-log₁₀ decline in the plasma HIV viral load. The sera from week 24 from some patients were able to neutralize both the 24-week and the baseline dominant virus isolates. A change in CD4 cell count of 50 or more in either direction predicted a −/− or +/+ profile. The verification of the autologous NA profile might be important in selecting patients who may benefit from immune-based therapies involving neutralizing monoclonal antibodies.

During primary human immunodeficiency virus (HIV) infection, there is little heterogeneity among HIV strains isolated from an individual patient. This is followed over time by an increase in the genetic diversity of the virus population (3, 12, 20). This increase in genetic diversity is responsible for the emergence of escape mutants that are no longer recognized by autologous neutralizing antibodies or virus-specific T lymphocytes (1, 2, 16, 23, 24, 30). Several studies have indicated that neutralizing antibodies rapidly appear after primary HIV infection and that this is followed by the emergence of virus strains that are resistant to autologous sera (1, 2, 4). This decline in the ability to neutralize autologous strains may be associated with the emergence of more-virulent strains and disease progression. It is important to mention that the patients showing signs of immunological escape retain the ability to make neutralizing antibodies, although these antibodies are not directed against their predominant autologous strains. The sera from these patients fail to neutralize their autologous strains while retaining the ability to neutralize laboratory-adapted HIV type 1 (HIV-1) strains, such as the prototype MN strain (25). This suggests the possibility of halting immune escape, perhaps by effective antiretroviral therapy and therapeutic vaccines, which could lead to delay of the emergence of more-virulent strains.

A syncytium-inducing (SI) phenotype has been reported to be associated with increased virulence and disease progression (6, 26). The relationship of the generation of SI strains to the lack of autologous neutralization and to the sequence of their appearance has not been completely examined. The V3 domain of gp120 is the major neutralization epitope (11, 15, 18) and controls the capability of the virus to form syncytia (9, 10, 14). Thus, factors that may influence the ability to make neutralizing antibodies may potentially impact the cytopathogenicity of the virus and vice versa. However, heterologous antibodies were shown to neutralize infectious molecular clones with V3 loops of both SI and non-SI (NSI) primary and laboratory-adapted viruses (13).

Knowing the neutralization profile might be important in guiding treatment decisions, particularly in immune-based therapy approaches involving neutralizing antibodies. In this study the relationship of escape from autologous viral neutralization and/or neutralization of prototype laboratory strains to markers of disease progression was examined.

MATERIALS AND METHODS

Patient population. The study population consisted of 10 males and 2 females; their absolute CD4 counts at baseline ranged from 116 to 530/mm³, with a mean of 259 ± 98/mm³. They were naive to antiretroviral therapy or had been off therapy for a washout period of 4 weeks at the start of therapy. The patients were on different treatment arms of antiretroviral therapy that were not revealed to the investigators. The patients were treated with two nucleosides or two nucleosides plus a nonnucleoside reverse transcriptase inhibitor. However, none of them were on protease inhibitors.

Virus reduction neutralization assay. Neutralizing activity (NA) was determined by an infectivity reduction assay as previously described (8, 13). Briefly, virus stocks with 6,000 to 10,000 50% tissue culture infective doses (TCID₅₀)/ml were diluted serially in normal human serum (NHS) using six fivefold dilutions. A fixed concentration (1:20) of autologous serum or NHS was used to neutralize autologous isolates or the laboratory strain MN for 1 h at 37°C. The virus-antiserum mixture was then cocultured with 2 × 10⁶ 48-h phytohemagglutinin (PHA)-stimulated normal donor peripheral blood mononuclear cells (PBMCs) in 2 ml of RPMI 1640 medium with 20% heat-inactivated fetal bovine serum, 5% interleukin-2, penicillin (100 U/ml), streptomycin (100 μg/ml), and l-glutamine in 24-well plates. The plates were incubated at 37°C in a 5% CO₂ humidified atmosphere for 3 days. A fixed serum was used as a positive control and a virus dilution that contained 50 to 100% of the infectivity of the starting stocks was used as a negative control. The verified autologous NA profile was examined.
for each individual well was assayed for HIV-1 p24 antigen (Ag) using the Coulter (Hialeah, Fla.) kit for determination of viral growth. The number of positive and negative wells determined the virus titer, which was calculated using the 50% infective dose computer program developed by J. L. Spouge et al. at the National Center for Biotechnology Information, National Institutes of Health (NIH), Bethesda, Md. (8, 13, 28). The infectivity reduction was calculated as follows: TCID$_{50}$ of NHS-treated virus (NIH), Bethesda, Md. (8, 13, 28). The infectivity reduction was calculated as

\[ \text{IUPM} = \frac{\text{Log ID}_{50} \text{ reduction} \times 100}{10^5} \]

Effect of antiretroviral therapy on neutralization profiles. We next sought to determine whether antiretroviral therapy may affect the pattern of NA. The same group of patients were treated with anti-HIV compounds for 24 weeks and were then examined for NA profiles. The sera and virus isolates at 24 weeks were also from the same venipuncture. The patients were on different therapy regimens that were not revealed to the investigators. Of the five patients with a +/- pattern at entry (Table 2), three became +/- and two continued to be +/- through week 24. Of the four patients with a +/+ profile prior to treatment, one continued to be +/+ and the other three shifted to a +/- profile by week 24. Two patients had a +/- profile (negative for NA against autologous isolates but positive for NA against MN) prior to treatment and held the same pattern over the same time period. Although several outcomes were observed, the data suggest that in some patients primary HIV therapy may halt the deterioration of or even reconstitute the HIV NA.

Relationship of neutralization patterns to phenotypic characteristics of the HIV isolates. Phenotypic characterization of the clinical isolates obtained prior to initiation of treatment identified six strains as SI and six as NSI (Table 2). At 24 weeks of therapy, nine isolates were NSI and three were SI. Although there was a trend for NSI selections, the ability to induce syncytia did not segregate to a particular neutralization pattern.

### RESULTS

**Relationship of HIV neutralization profiles to autologous and MN viral strains in HIV-infected subjects.** Sera from 12 HIV-infected patients were examined for their NA against autologous HIV isolates obtained from the same venipuncture as well as against the laboratory strain MN. The subjects were naive to antiretroviral therapy or had been off therapy for a washout period of 4 weeks at the start of therapy. Their absolute CD4 cell counts ranged between 116 and 530/mm$^3$, with a mean of 271 ± 126/mm$^3$. The patients’ sera in eight instances failed to neutralize the autologous strain (Table 1). Out of those eight, three retained the ability to neutralize MN, indicating competency in producing neutralizing antibodies. Sera from five patients failed to neutralize either strain (double negative [–/–]), and sera from four patients neutralized both strains (double positive [+/+]). The data suggest that HIV patients lose NA against autologous strains before they lose NA against prototype MN.

**Effect of antiretroviral therapy on neutralization profiles.**

#### TABLE 2. Relationship of neutralization profiles of HIV patient sera to CD4 cell count and HIV viral load

<table>
<thead>
<tr>
<th>Patient</th>
<th>Entry Week 24</th>
<th>HIV RNA (copies/ml)</th>
<th>IUPM</th>
<th>CD4 cell count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>+/-</td>
<td>SI NSI</td>
<td>3.2 x 10$^4$</td>
<td>206</td>
</tr>
<tr>
<td>2</td>
<td>+/-</td>
<td>SI SI</td>
<td>4.9 x 10$^4$</td>
<td>206</td>
</tr>
<tr>
<td>3</td>
<td>-/-</td>
<td>SI NSI</td>
<td>6.8 x 10$^5$</td>
<td>2503</td>
</tr>
<tr>
<td>4</td>
<td>+/-</td>
<td>SI NSI</td>
<td>3.6 x 10$^4$</td>
<td>363</td>
</tr>
<tr>
<td>5</td>
<td>-/-</td>
<td>SI NSI</td>
<td>1.1 x 10$^5$</td>
<td>112</td>
</tr>
<tr>
<td>6</td>
<td>-/-</td>
<td>SI NSI</td>
<td>2.1 x 10$^5$</td>
<td>112</td>
</tr>
<tr>
<td>7</td>
<td>-/-</td>
<td>SI NSI</td>
<td>9.9 x 10$^4$</td>
<td>421</td>
</tr>
<tr>
<td>8</td>
<td>-/-</td>
<td>SI NSI</td>
<td>3.2 x 10$^4$</td>
<td>81</td>
</tr>
<tr>
<td>9</td>
<td>+/-</td>
<td>SI NSI</td>
<td>1.2 x 10$^4$</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>+/-</td>
<td>SI SI</td>
<td>7.4 x 10$^3$</td>
<td>206</td>
</tr>
<tr>
<td>11</td>
<td>+/-</td>
<td>SI NSI</td>
<td>1.6 x 10$^4$</td>
<td>8</td>
</tr>
<tr>
<td>12</td>
<td>+/-</td>
<td>SI NSI</td>
<td>4.9 x 10$^4$</td>
<td>82</td>
</tr>
</tbody>
</table>

**a** Determined by using sera and autologous virus isolates obtained from the same venipuncture.

**b** W24, week 24 of antiretroviral therapy.
therapy are shown in Table 2. Patient 1, who was +/- at both entry and week 24, as well patients 5, 7, 11, and 12, who regained their NA by week 24, had a significant increase in their CD4 cell counts ($P < 0.05$). Patients 2, 4, and 10 had a +/+ profile at entry that deteriorated to -/- by week 24. The CD4 cell counts of patients 2 and 10 substantially decreased; however, patient 4 had a 32-cell increase. Patients 8 and 9 had a -/+ profile which was maintained through 24 weeks, and their CD4 cell counts did not change significantly. These data suggest that a change in CD4 cell count of 50 or more in either direction may predict a -/- or +/+ profile ($x^2 = 10.37, P = 0.005$). A lack of a change in CD4 cell count would imply a lack of autologous NA. Although patient 3 had maintained a -/- profile through week 24, his CD4 cell count significantly increased. This may have been due to sequestration of the neutralizing antibody by inactivated HIV particles in the serum as a consequence of a high level of viremia ($1.9 \times 10^5$ copies/ml).

**Relationship of NA to plasma HIV RNA and cellular viremia.** The relationship of the NA profile to cellular viremia was investigated. The cellular viremia was expressed as the number of IUPM. The cumulative number of sera with a -/- profile was associated with a mean level of cellular viremia of 2,324 ± 666 IUPM in the corresponding patients and was significantly different ($P < 0.02$) from the numbers of +/+ (289 ± 126 IUPM) and +/+ (161 ± 72 IUPM) sera (Fig. 1). Nine of the 10 subjects (90%) belonging to this -/- group had cellular viremia of 1,100 IUPM or above, suggesting an association between the two events (Table 2). The tendency of patients to regain NA by week 24 was coupled with a decrease of cellular viremia (e.g., patients 5, 7, 11, and 12). In two of three patients losing their NA by week 24 (patients 2 and 10), a substantial increase in cellular viremia was observed.

Plasma HIV RNA in all patients was measured at entry and at week 24. The sera with a -/- profile were associated with a high level of circulating plasma HIV RNA. In all these instances, the HIV RNA copy number was more than $5 \times 10^5$/ml (Fig. 1 and Table 2). The HIV RNA viral load of the -/- group (201,638 ± 62,266 copies/ml) was significantly different ($P < 0.02$) from those of the +/+ (18,490 ± 6,454 copies/ml) and +/+ (18,566 ± 5,507 copies/ml) groups. The pattern of change in the viral load of individual patients reflected their NA profile. Patients who showed a shift from -/- to +/+ (patients 5, 11, and 12) had a significant drop (to an undetectable level or a $>1$-log$_{10}$ reduction) in their viral load. Patient 5 had no detectable levels of RNA, and patients 11 and 12 had an approximately 1-log$_{10}$ drop in their RNA copy numbers by week 24. Four of five patients who maintained the same pattern of NA through week 24 had a steady level of plasma HIV RNA. Patients whose sera had a +/+ NA profile at entry and by week 24 became -/- showed some (though not significant) increases in viral load over the same time period.

**Effect of HIVIG on HIV infectivity of neutralization escape mutants.** The effect of HIVIG, a heterologous polyclonal neutralizing antibody preparation (AIDS Research and Reference Reagent Program, NIH), was tested for its effects on infectivity reduction in six clinical isolates from the patients with a -/- neutralization profile. HIVIG reduced the infectivity of the clinical isolates by more than 0.7 log$_{10}$ TCID$_{50}$ in five out of the six isolates tested (Fig. 2). These data suggest that patients with a -/- neutralization profile may benefit from HIVIG therapy.

**Effect of week 24 sera on the replication of autologous virus from baseline and week 24.** The week 24 sera from patients who had a negative autologous neutralization profile at entry and then became +/- by week 24 were examined for their neutralizing effect on autologous isolates from entry and week 24. As shown in Table 3, the week 24 sera from patients 5, 7, 12.
The sera of patients 11 and 12 were from baseline. Pt, patient. Indicates 90% neutralization. The sera of patients 2, 3, 4, and 6 were from week 24. The sera of patients 11 and 12 were from baseline. Pt, patient.

11, and 12 reduced the infectivity of the corresponding autologous isolates from baseline as well as from week 24. This indicates that therapy improved the patients’ neutralizing responses to the dominant isolates from baseline to week 24 of treatment.

DISCUSSION

In this study, a group of patients on different regimens of antiretroviral therapy were examined prior to initiation of treatment and 24 weeks into treatment for the NA of their sera against the MN strain and the autologous virus. The pattern of NA was compared to the phenotypic characteristics of the clinical isolate, CD4 cell counts, and HIV viral burden.

Prior to treatment, an NA pattern was observed that confirmed earlier reports indicating that sera from some HIV-infected patients failed to neutralize autologous isolates while maintaining some NA activity against laboratory-adapted strains (25, 31). In our cohort, 42% of the patients’ sera failed to neutralize either strain. This failure did not stratify with a particular range of absolute CD4 cell counts. However, there was a correlation between the double escape neutralization and HIV plasma and cellular viremia. The -/- sera were associated with cellular viremia of more than 1,122 IUPM and with a plasma HIV RNA level of more than 5 × 10^6 copies/ml.

The isolates from the patients who showed a -/- profile did not share a common viral phenotypic feature, such as an SI or NSI phenotype or lymphocyte or macrophage tropism (data not shown). These observations indicate that the neutralization profile of sera is independent of the phenotypic characteristics of the autologous virus strain. This is supported by published reports. For example, it was previously shown that heterologous serum pools neutralized chimeric LAI viruses with SI or NSI envelopes obtained from isolates from the same individual (13). More recently, NA was also shown to be unrelated to macrophage tropism (19) and to be independent of the coreceptor usage (5, 17, 21, 29). In these studies, the susceptibility to neutralization remained unchanged whether the virus strains used the chemokine receptor CXCR4 or CCR5. Almost all SI strains are T-cell tropic and use CXCR4, and NSI strains are macrophage tropic and use CCR5. The simultaneous loss of NA against both the autologous and the MN strains suggests that the limiting factor in this process is the availability of functional neutralizing antibody rather than the selection of HIV-resistant variants. This is supported by the fact that HIVIG was able to neutralize the double escape isolates. The failure of the sera from some patients to neutralize both the autologous and the MN virus strains is likely to have resulted from impaired T4 helper activity, which is necessary for proper B-cell function. We would like to point out that no correlation was observed between CD4 T-cell counts and NA. However, subjects who regained their NA by week 24 into therapy had a significant increase in their CD4 T-cell counts. Moreover, the change (increase or decrease) in CD4 T-cell counts over time predicted the pattern of NA of those subjects. Conversely, the viral load in plasma may have sequestered all the available neutralizing antibodies, which then can be reflected as a negative NA profile. However, the ability of heterologous plasmas from symptomatically infected patients to neutralize isolates resistant to neutralization by autologous plasmas argues against that (31).

The identification of three neutralization patterns (+/-, -/+ , and -/-) indicated that resistance to neutralization was progressive. The data indicate that this cascade can be halted or even restored in patients receiving effective antiretroviral therapy, as determined by the patients’ CD4 and viral burden responses. The fact that week 24 sera neutralized the baseline as well as the week 24 dominant virus isolates suggests that effective therapy limited the evolutionary changes in the neutralizing domain of gp120. This might be important for enhancing other immune activities that are dependent on neutralizing antibodies, such as antibody-dependent cellular cytotoxicity, complement-mediated virolyses, virus opsonization, and limiting the local spread of infection in vivo.

Knowing the autologous neutralization profile for a particular patient might be important in guiding treatment decisions, particularly in immunotherapeutic trials involving neutralizing monoclonal antibodies. It is conceivable that a patient with an autologous neutralization profile (+/+) would not benefit as much from this approach as another patient lacking the ability to neutralize his own virus strain. This may prove to be particularly beneficial to HIV-infected women for prevention of maternal-fetal transmission. A recent report associated the lack of autologous neutralizing antibodies to HIV and a lack of syncytium induction with the risk of mother-to-infant transmission (19).

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REFERENCES


