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Neutralization of Multiple HIV-1 Isolates from a Single Subject by Autologous Sequential Sera

Michel Tremblay and Mark A. Wainberg

Titers of neutralizing antibodies to different strains of human immunodeficiency virus type 1 (HIV-1), including five isolates sequentially obtained from one infected subject, were determined using sequential serum samples obtained from that individual. Neutralizing antibodies were detected against the HIV-IIIb laboratory strain of HIV-1 and against a clinical isolate from another HIV-1-infected individual. Sera from the subject under investigation possessed differential ability to exert viral neutralization, depending on which homotypic clinical isolate was used. In general, it appeared that effective neutralization capacity was present in serum against homotypic viral isolates of HIV-1 only if these isolates were obtained at or before serum collection. These data suggest that variants of HIV-1 in infected individuals may not be effectively neutralized by antibodies that have been generated in these same people against previously dominant viral strains.

Individuals infected with the human immunodeficiency virus type 1 (HIV-1), the pathogen of AIDS, develop antibodies to a variety of viral proteins. While neutralizing antibodies to HIV have been detected in a high proportion of HIV-1-infected individuals, they are generally not present at very high titers [1, 2]. Neutralizing antibodies are directed against the envelope glycoproteins of the virus [3].

The biologic significance of neutralizing antibodies remains unclear. Although one report has indicated that no relationship exists between levels of such antibodies and clinical status [4], most investigators claim that the presence of relatively high titers of neutralizing antibodies can be correlated with good short-term prognosis [4, 5].

We investigated whether sequential serum samples from one infected individual could effectively neutralize each of five serial viral isolates obtained from that same donor over time. In addition, we evaluated the ability of these sera to neutralize the HIV-IIIb laboratory strain of HIV-1 and a clinical variant isolated from a different patient.

Materials and Methods

Sera. Sequential serum samples were obtained from an adult infected with HIV-1. This individual remained asymptomatic throughout the study. Additional sera from healthy donors served as controls. All sera were stored at -20°C before study.

HIV-1. The well-characterized HIV-IIIb strain of HIV-1 was supplied (by Dr. R. C. Gallo, National Institutes of Health [NIH], Bethesda, MD) in the form of chronically infected H-9 cells [6]. Six clinical isolates of HIV-1, of which five were from the same patient who served as a sequential serum donor, were also studied. These viruses were isolated in our laboratory from patient peripheral blood mononuclear cells by coculture with phytohemagglutinin-stimulated cord blood mononuclear cells, as previously described [7].

Neutralization assay. The MT-4 cell line [8] was used as a target for infection by HIV-1 in a microtiter assay. Neutralization was measured as the ability of serum to completely block viral infection. All sera were heat-inactivated at 56°C for 30 min before assay and were then serially diluted twofold in growth medium. An equal volume (25 μl) of growth medium containing 250 TCID50 (50% tissue culture infectious dose) of test virus was added to each dilution, and the mixtures were incubated for 2 h at 37°C in the wells of a flat-bottomed microtiter plate. Infectious titers (TCID50) of each viral strain were calculated using the Kärber formula on the basis of viral cytopathicity for MT-4 cells.

In each assay, 5 × 10^5 MT-4 cells in log-phase were added to each well, and the cultures were incubated at 37°C. Controls included 250 TCID50 of virus inoculated in the absence of serum (medium alone) and in the presence of diluted serum from a HIV-1-seronegative donor. Cultures were followed for virus-induced cytopathic effects, which are well-characterized for MT-4 cells [8], and cells were scored by indirect immunofluorescence for the presence of viral p24 antigen. The neutralization titer was defined as the reciprocal of the highest dilution of serum that totally blocked viral infection. All experiments were performed in duplicate.

Indirect immunofluorescence. The detection of HIV-1 p24 antigen in these various cultures was determined using an indirect immunofluorescence assay. This involved the fixation of cells in 1:1 acetone-methanol for 30 min at room temperature, after which mouse monoclonal antibodies to the viral core protein p24 (supplied by Dr. R. C. Gallo, NIH) were used in conjunction with a fluorescein
isothiocyanate–conjugated goat anti–mouse immunoglobulin (Cappel Labs, Cochranville, PA) [9].

Results

Neutralization of HIV-1 variants. Neutralizing antibodies to the HIV-IIIb laboratory strain of HIV-1 and to a clinical isolate of HIV-1 isolated from an unrelated HIV-1–infected individual were detected in the sequential sera (table 1).

Neutralization of homotypic HIV-1 variants. When sequential HIV-1 isolates from the same patient who served as a multiple serum donor were studied, differential patterns of neutralization were observed. The neutralization antibody titers to the homotypic viral strain, isolated at week 6, were high (i.e., 1:64–1:128) for all sequential serum samples tested (table 2). In contrast, when homotypic HIV-1 variants isolated at week 36 or 42 were studied, high titers of neutralization activity could not be detected with each of four sera drawn before week 42. The same sort of delay in appearance of a strong neutralization response occurred when homotypic viruses isolated at each of weeks 27 and 72 were studied. In these instances, neutralizing antibody titers could not be demonstrated before weeks 27 and 72, respectively (table 2).

Thus, in general, appearance of a neutralizing antibody response for a particular homotypic variant of HIV-1 occurred only at around the time of isolation of that variant.

Discussion

This work confirms earlier reports on the presence of neutralizing antibodies in the sera of HIV-1–infected individuals. Our assay involved the use of the MT-4 cell line as a target for HIV-1. This cell line was used because of its sensitivity to infection by HIV-1, as assessed by virus-induced cytopathic effect and the rapid induction of HIV-1 antigens after infection.

As previously reported, neutralization titers can vary considerably from one assay to another, depending on what is used as a criterion of neutralizing activity. Some laboratories have relied on levels of neutralization amounting to 40% or 80% of viral infectivity, while others have insisted on 90%–100% inhibition of infection. Our study, which evaluated total blockade of infection by HIV-1, yielded neutralization titers consistent with reports from others. Our use of a fairly high challenge dose of HIV-1 (i.e., 250 TCID_{50}) gave rise to neutralization titers ranging from 1:2 to 1:256.

The finding of detectable, constant levels of neutralizing antibodies, over time, to either laboratory strains or clinical isolates of HIV-1 has previously been demonstrated [10]. However, this is the first report to our knowledge to compare serial isolates of HIV-1 from the same patient for determination of levels of neutralization activity by his own sera studied longitudinally at different times after infection.

It has previously been shown that the generation of neutralizing antibodies usually occurs several weeks after seroconversion [11]; this may imply a lag between time of viremia

Table 1. Neutralizing antibody titers to HIV-1 in sequential serum samples from one subject.

<table>
<thead>
<tr>
<th>Time of serum collection (weeks)*</th>
<th>Neutralization titer†</th>
<th>Clinical isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>64</td>
<td>128</td>
</tr>
<tr>
<td>21</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>30</td>
<td>64</td>
<td>128</td>
</tr>
<tr>
<td>42</td>
<td>32</td>
<td>128</td>
</tr>
<tr>
<td>72</td>
<td>16</td>
<td>128</td>
</tr>
<tr>
<td>108</td>
<td>64</td>
<td>128</td>
</tr>
</tbody>
</table>

* After initiation of study.
† Reciprocal of serum dilution that completely prevented viral infection.

Table 2. Neutralization capacity of sequential serum samples from one HIV-1–infected subject for five homotypic viral isolates.

<table>
<thead>
<tr>
<th>Time of serum collection (weeks)*</th>
<th>Neutralization titer† against HIV-1 isolates obtained after no. of weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>128</td>
</tr>
<tr>
<td>12</td>
<td>64</td>
</tr>
<tr>
<td>21</td>
<td>64</td>
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<td>24</td>
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<td>42</td>
<td>128</td>
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<tr>
<td>72</td>
<td>NT</td>
</tr>
<tr>
<td>84</td>
<td>128</td>
</tr>
<tr>
<td>108</td>
<td>64</td>
</tr>
</tbody>
</table>

NOTE. NT = not tested.

* After initiation of study.
† Reciprocal of serum dilution that completely inhibited viral infection.
and formation of neutralizing antibodies. We have detected the same type of delay with regard to appearance of neutralizing antibodies to homotypic HIV-1 isolates. This result may be due to the ability of individual viruses to mutate within the env gene and hence escape the effects of neutralizing antibodies. Polymorphism in the hypervariable regions of viral envelope glycoproteins, which represent the major target for neutralizing antibodies, have been reported [12, 13]. This genomic divergence results, in part, from in vivo immunologic pressure on the part of neutralizing antibodies; indeed, supportive evidence for this hypothesis has been generated in vitro [14, 15]. Single base point mutations were shown in those studies to generate antibody-resistant strains.

The current study offers further evidence of the high rate of HIV-1 mutability in infected individuals over time and suggests that seroselection of neutralization-resistant viral variants can occur. Although detailed results are presented on only one subject, less extensive studies have also been performed in our laboratory on two other individuals; the data obtained from these analyses are consistent with the findings reported here (tables 1 and 2). Future experiments will attempt to amplify known hypervariable domains of the env gene of these isolates by polymerase chain reaction to determine the locations of the sites that account for neutralization resistance.

The clinical relevance of our findings remains to be determined. The culture conditions we used may favor the isolation of fast-growing viruses that adapt well to in vitro growth; such viruses may not necessarily be representative of viruses present in vivo. Nonetheless, it is possible that the absence of an effective neutralization titer to individual homotypic isolates may restrict the ability of the immune system to control the spread of HIV and ultimately lead to disease progression.

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References