Effects of Antibody on Viral Kinetics in Simian/Human Immunodeficiency Virus Infection: Implications for Vaccination

Lei Zhang, Ruy M. Ribeiro, John R. Mascola, Mark G. Lewis, Gabriela Stiegler, Hermann Katinger, Alan S. Perelson, and Miles P. Davenport

Department of Haematology, Prince of Wales Hospital and Centre for Vascular Research, University of New South Wales, Kensington, New South Wales 2052, Australia; Theoretical Biology and Biophysics, Los Alamos National Laboratory, Los Alamos, New Mexico 87545; Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892; BIOQUAL, Inc., Rockville, Maryland 20850; and Institute of Applied Microbiology, University of Agriculture, Vienna, Austria

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Passive antibody treatment of macaques prior to simian/human immunodeficiency virus infection produces “sterilizing immunity” in some animals and long-term reductions in viral loads in others. Analysis of viral kinetics suggests that antibody mediates sterilizing immunity by its effects on the initial viral inoculum. By contrast, reduction in peak viral load later in infection prevents CD4 depletion and contributes to long-term viral control.

Trials of antibody-inducing vaccines with primate models of human immunodeficiency virus (HIV) have demonstrated protection from infection (sterilizing immunity) and reduced viral loads with prolonged survival compared to controls (disease attenuation) (1, 5, 6, 12). However, these vaccines elicit both B-cell and T-cell responses to the immunogen, and the precise role each plays in preventing or controlling infection is unclear. Passive antibody treatment allows investigation of the role of antibody alone and has been successful in mediating both sterilizing immunity and disease attenuation in animals challenged via intravenous or mucosal routes (8, 9, 11). Understanding the mechanisms of protection after passive antibody administration is important in guiding rational design of antibody-based HIV vaccines in humans.

We have analyzed the kinetics of virus in two studies of passive antibody administration and intravenous (IV) (8) or intravaginal (IVAG) (9) challenge with simian/human immunodeficiency virus (SHIV) 89.6PD in macaques. The monoclonal antibodies 2G12 and 2F5, as well as HIV immunoglobulin, were administered either alone or in combination, and animals were challenged 24 h after antibody administration. Approximately half of the antibody-treated animals exhibited sterilizing immunity, and thus, these animals were not available for viral kinetic studies. We separately analyzed animals given a single antibody and those treated with multiple antibodies (Table 1). Weekly viral loads and CD4³ + T cells (as a percentage of CD3 + T cells) were obtained. We compared the kinetics of virus in control and antibody-treated animals in order to address the following questions. (i) What is the mechanism by which antibody mediates sterilizing immunity? (ii) How does the presence of passive antibody early in infection lead to disease attenuation even after the passive antibody is cleared (8)? (iii) What are the implications of this for understanding active vaccination in humans?

Sterilizing immunity. Sterilizing immunity may occur because either (i) antibody neutralizes the initial inoculum of virus before it has a chance to infect any cells, or (ii) some cells are infected by the initial inoculum of virus, but high levels of antibody reduce the early spread of the virus to other cells and the infected cells die (4). Although it is not possible to directly observe these very early kinetics in animals with sterilizing immunity, analysis of early viral kinetics in the antibody-treated animals that became infected provides insights into the effects of antibody.

Early viral loads (day 7) for multiple-antibody-treated animals were reduced around 700-fold from those for controls (P < 0.0001) (Table 1). Peak viral growth rates in acute infection were also reduced by ~25% for multiple-antibody-treated animals compared to controls (P = 0.014, Table 1). However, this reduced growth rate cannot account for the observed reduction in viral load on day 7, since a 25% reduction in the growth rate over 7 days would be expected to result in only ~8-fold less virus. This suggests that the dominant effect of antibody is to reduce the initial infection of cells by the challenge inoculum. Thus, antibody-treated monkeys have a small reduction in viral growth rates but a large reduction in the effective size of the initial inoculum that leads to fewer infected cells in the first round of infection. Moreover, the relatively small change in growth rate for multiple-antibody-treated animals suggests that once enough cells become infected, the virus spreads at similar rates. This spread might be predominantly cell to cell...
within lymphoid tissue and thus not easily inhibited by antibody (2, 13). Thus, sterilizing immunity is observed only in those animals in which initial infection is blocked (3).

**Long-term viral control.** Animals treated with multiple antibodies that became infected showed lower long-term (>90 days) viral loads, compared with controls (Table 1). Since passive antibody has a short half-life (~3 to 13 days (8), only negligible amounts of antibody remain at this time. Thus, the effects of antibody during the acute phase of infection must somehow “program” the long-term outcome. Previous studies have suggested that antibodies may induce accelerated clearance of SHIV in acute infection (10). The maximum decay rate of virus following acute infection did not differ between controls and antibody-treated animals in our studies (Table 1), and the decay rate of virus was not correlated with long-term viral loads (P = 0.492 [Spearman]). However, the viral growth rate in acute infection was correlated with the viral load set-point in chronic infection (r = 0.416; P = 0.031 [Spearman]) (Fig. 1A).

Lifson et al. previously demonstrated a relationship between the viral growth rate in acute infection and the long-term outcome in macaques and concluded that intrinsic variation in the susceptibility of cells to infection in vitro determined this outcome (7). That is, the correlation occurred because animals with highly susceptible cells had both higher growth rates and higher long-term viral loads (not because high growth rates directly caused high long-term viral loads). In passive-antibody-treated animals, the short half-life of antibody means that the growth rate of virus is only transiently reduced, and thus, there is no reason why this should affect the long-term outcome. The most likely explanation is that antibody helps preserve the host immune response in early infection, which in turn mediates long-term viral control. Consistent with this, the early viral growth rate was significantly correlated with the level of CD4 depletion in acute infection (r = 0.501; P < 0.0078). The large depletion of CD4 T cells occurs around the time of peak viral load (8, 9, 11), and peak viral load in acute infection is also associated with the level of depletion of CD4 T cells (Spearman [r = 0.524; P = 0.005]) (Fig. 1B). Thus, passive antibody treatment may program the long-term outcome of infection if it reduces peak viral loads, allowing maintenance of CD4+-T-cell numbers and the host immune response. In this way, we observe that a reduction from ~90% CD4 depletion in control animals to only 30 to 40% in multiple-antibody-treated animals is associated with a 100-fold lower viral load several months later (Table 1).

**Implications for vaccination.** The above kinetic analysis of the effects of passive antibody administration on viral growth in SHIV infection identifies two distinct phases: (i) the effects of antibody on clearing or neutralizing the initial inoculum of virus and hence in reducing the number of initially infected cells and (ii) the effects of antibody on viral growth, peak viral load, depletion of CD4+ T cells, and programming the long-term outcome. By contrast, active immunization should lead to a low persistent level of antibody (5, 7). This persistent antibody will be present at the time of infection and if at sufficiently high levels may mediate clearance of the initial viral inoculum. In principle the necessary antibody levels could be titrated by using passive transfer experiments. If these levels are not attainable by immunization, sterilizing immunity may not occur. However, vaccines that are ineffective at preventing infection may still provide some benefits in slowing disease progression.

If sterilizing immunity results from neutralization of the initial inoculum, then the lower the inoculum, the more likely it is that antibody treatment would provide sterilizing immunity. Comparison of IVAG with IV infection in the control macaques shows a trend towards lower viral loads on day 7 in IVAG-infected animals (3.62 ± 0.16 versus 5.28 ± 1.3 log_{10} copies ml^{-1}; P = 0.081 [Mann-Whitney]), suggesting that IVAG infection may deliver a lower effective dose of virus. In addition, whereas nine of nine single-antibody-treated animals became infected after IV challenge, only two of four single-antibody-treated animals were infected after IVAG challenge (P = 0.038 [Fisher’s exact, one tailed]). This is consistent with

### Table 1. Comparison of viral kinetics

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. Infected</th>
<th>Viral load (log_{10} copies ml^{-1})</th>
<th>Viral Growth (day^{-1})</th>
<th>Viral Decay (day^{-1})</th>
<th>CD4 Depletion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 7</td>
<td>Peak</td>
<td>Final</td>
<td></td>
</tr>
<tr>
<td>IV infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6/6</td>
<td>5.28 ± 1.30</td>
<td>7.17 ± 0.61</td>
<td>5.07 ± 0.50</td>
<td>1.19 ± 0.21</td>
</tr>
<tr>
<td>One Ab</td>
<td>9/9</td>
<td>3.78 ± 0.71</td>
<td>6.59 ± 0.72</td>
<td>4.41 ± 1.36</td>
<td>0.90 ± 0.30</td>
</tr>
<tr>
<td>Multi Ab</td>
<td>5/9</td>
<td>2.43 ± 0.29*</td>
<td>6.26 ± 0.71</td>
<td>2.88 ± 0.75*</td>
<td>0.86 ± 0.28</td>
</tr>
<tr>
<td>IVAG infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5/5</td>
<td>3.62 ± 0.16</td>
<td>7.21 ± 0.76</td>
<td>5.05 ± 0.50</td>
<td>1.14 ± 0.22</td>
</tr>
<tr>
<td>One Ab</td>
<td>2/4</td>
<td>3.02</td>
<td>6.30</td>
<td>2.30</td>
<td>0.69</td>
</tr>
<tr>
<td>Multi Ab</td>
<td>4/10</td>
<td>2.44 ± 0.17*</td>
<td>6.72 ± 0.52</td>
<td>2.54 ± 0.48</td>
<td>0.86 ± 0.21</td>
</tr>
</tbody>
</table>

* Viral loads (mean ± standard deviation) at different times, as well as maximum growth and decay rates of virus in infected animals following antibody (Ab) treatment and IV or IVAG challenge. The viral load on day 7 and the final viral load were significantly different between controls and antibody-treated animals in each group. Viral growth rates were also significantly different between controls and multiple-antibody-treated animals when data from both groups is considered. P values for Kruskal-Wallis test for comparison between control, single-antibody-treated, and multiple-antibody-treated animals were determined. P values for IV infection were as follows: day 7, 0.0028; peak, not significant; final, 0.029; viral growth and decay, not significant; percentages of CD4 depletion, 0.516. P values for IVAG infection were as follows: day 7, 0.013; peak, not significant; final, 0.016; viral growth and decay, not significant; percentages of CD4 depletion, 0.0227. P values for two-way analysis of variance on ranks for effect of treatment, comparing control, single- and multiple-antibody-treated animals with IV and IVAG infections were as follows: day 7, <0.0001; peak, not significant; final, 0.0001; viral growth, 0.0142; viral decay, not significant; percentages of CD4 depletion, 0.0003.

* Number of animals infected/total number challenged.

* Standard deviations not shown (sample size of two).

* *, significantly different from control animals (P < 0.05) infected by same route (Dunn’s multiple comparisons).

* Data for three of six control animals and four of nine multiple-antibody-treated animals only available.
a model where IVAG infection delivered a lower effective inoculum of virus and was thus easier to protect against. The challenge dose of HIV in sexual transmission is likely to be extremely small (based on the low probability of transmission after active vaccination, could provide some effective sterilizing immunity.

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REFERENCES

FIG. 1. Effect of passively administered antibody on long-term outcome: (A) Correlation \( r = 0.416; P = 0.031 \) [Spearman] between maximum growth rate of virus in acute infection and long-term viral load (day 98 for IV [\( \bullet \) ] infection and day 140 for IVAG [\( \square \) ] infection). (B) Correlation \( r = 0.524; P = 0.005 \) between peak viral load in acute infection and the CD4\(^+\)-T-cell depletion \( \frac{\text{[preinfection % CD4]}}{\text{(peak viral load % CD4)}} \). In both cases, ANCOVA was performed to confirm that the data from the IV and IVAG infection studies could be pooled. Negative values for CD4\(^+\)-T-cell depletion are shown as zero.