Immune Complexes Containing Human Immunodeficiency Virus Type 1 Primary Isolates Bind to Lymphoid Tissue B Lymphocytes and Are Infectious for T Lymphocytes

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This study investigated the interaction of tonsil B lymphocytes with immune complexes containing human immunodeficiency virus (HIV IC) primary isolates and the infectivity of the B cell-bound HIV IC. Treatment of virus with a source of antibody and complement increased HIV IC binding to B cells by 5.6-fold. Most of the HIV IC that bound to B cells were not internalized but remained on the cell surface and were gradually released over 72 h. Cell-bound HIV IC were highly infectious for T cells while virus released by cultured B cells was only slightly infectious. Removal of HIV IC from the B-cell surface by protease treatment reduced the infection of T cells to near-background levels, indicating that infectious virus remained on the B-cell surface. These studies show that B lymphocytes can carry and transfer infectious HIV IC to T cells and thus suggest a novel mode of infection of T cells in lymphoid tissue that could be important for pathogenesis during HIV infection.

During infection with human immunodeficiency virus type 1 (HIV-1), plasma virus can reach levels as high as millions of virus particles/milliliter (12, 16), and a portion of this plasma virus is in the form of immune complexes (14, 15, 19, 20). High levels of HIV are also found in lymphoid tissues, including lymph nodes (reviewed in references 3 and 8), and the total amount of virus found in this compartment within infected individuals has been estimated at $5 \times 10^{10}$ virions (9). A large portion of this virus is associated with the surfaces of follicular dendritic cells (FDC) within follicles, and it is thought that FDC trap these HIV particles on their surfaces as immune complexes along the network of dendrites which express complement receptor 1 (CR1), CR2, CR3, and Fc receptors (7, 13).

Several studies suggest that FDC may play a role in the pathogenesis of HIV infection by transferring infectious immune complexes containing HIV (HIV IC) to T cells during cell-cell contact in follicles although it appears that FDC themselves do not become infected (5, 10, 17, 18). One study provided evidence that FDC may be particularly efficient in transferring HIV IC to T cells by showing that virus complexed with neutralizing antibody was not infectious when incubated with T cells but that the virus-antibody complexes were infectious for T cells when bound to FDC (10).

B lymphocytes within lymphoid tissues play critical roles in immune responses and are densely concentrated in and around the follicles of lymphoid tissue, where they interact with T cells and FDC to receive signals for clonal expansion, affinity maturation, and class switching (reviewed in reference 1). Since B cells in lymphoid tissues express CR1 and CR2 (CD35 and CD21, respectively) and the FcRIIB1 receptor (CD32) (4), which allow them to bind immune complexes, we reasoned that B cells might also be able to trap HIV IC and transfer them to T cells. Thus, in this study, we investigated several important features of the B-cell–HIV IC interaction, including (i) whether B cells from lymphoid tissues can bind HIV IC, (ii) the localization of the HIV IC after binding to B cells, and (iii) if the bound HIV IC are infectious for T cells. Cell-cell interactions such as these, which could result in the transfer of infectious HIV to T cells in vivo, are likely to contribute to HIV pathogenesis.

**Binding of primary isolate HIV IC to tonsillar B lymphocytes.** We first assessed the binding of HIV IC made with primary isolates (PI) of HIV-1 from three different patients to B cells isolated from tonsils. Autologous patient serum (taken from the same donor and at the same time as the virus isolate) was heat inactivated and used as an antibody source for each isolate, and the binding of HIV IC to B cells was assessed for virus treated with complement only, heat-inactivated complement (HIC) only, antibody plus complement, antibody plus HIC, and HIC incubated without antibody or complement. Previous studies have not investigated the interaction of B cells or FDC with HIV IC containing PI.

All three control-treated virus isolates bound at relatively low levels, with 7 to 31 pg of p24 bound to 2 × 10^6 B cells (Fig. 1). Treatment with HIC or autologous serum plus HIC did not significantly increase virus binding ($P > 0.05$, $t$ test). Treatment of virus with complement alone increased binding by an average of 2.4-fold (4.2-, 1.3-, and 1.9-fold for isolates 1, 2, and 3, respectively) ($P > 0.05$) while treatment with autologous serum plus complement significantly increased the amount of virus binding to B cells by an average of 5.6-fold (7-fold for isolate 1 and about fivefold for both isolates 2 and 3), compared to the level of binding of control-treated HIV ($P < 0.05$). The immunoglobulin G (IgG) in sera appeared to be responsible for the increased binding of HIV to B cells since treatment of PI 1 with complement plus protein G-purified IgG from serum sample 1 at 1 and 0.25 mg/ml increased p24 binding by 7- and 4.9-fold, respectively, over complement-alone treatment while IgG from an HIV-seronegative donor did not increase HIV binding (not shown). Taken together, these data indicate that treatment of virus with both complement plus the antibody source was necessary for the highest level of HIV IC binding since heat inactivation of complement reduced bound virus to control levels. These data also demonstrate that treat-
ment with complement alone induced some virus binding to B lymphocytes.

Since more than 95% of tonsil B cells express CR2 and since this receptor is important for binding immune complexes to B cells, CR2 was studied for its role in binding HIV IC produced by the incubation of virus with autologous serum and complement. The PI and serum from patient 1 was used to make HIV IC in all further experiments since this combination yielded the greatest increase in binding to B cells in the presence of complement (Fig. 1). Preincubation of B cells with anti-CR2 monoclonal antibody OKB7 blocked 76% of the binding of HIV IC to B cells (Fig. 2). However, anti-LFA-1 antibody, which also binds to B cells, did not substantially block HIV IC binding (Fig. 2). Thus, although antibodies directed to LFA-1 have been shown to reduce HIV infectivity at the initial virus-cell interaction as well as at later stages of infection (11), anti-LFA-1 antibodies did not significantly inhibit the binding of HIV IC to tonsil B lymphocytes.

Localization of HIV IC after binding to tonsil B lymphocytes. The next studies determined the localization of B-cell-bound HIV IC in cultures. Tonsil B cells were incubated with HIV IC made with PI 1 plus autologous serum and complement. Cells were then washed and cultured for 72 h. The amount of HIV IC associated with B cells decreased from 189 pg of p24 (100%) bound at time 0 to approximately 124 pg (66%) at 15 h, 113 pg (60%) at 24 h, 47 pg (25%) at 48 h, and 38 pg (20%) at 72 h (Fig. 3). Surprisingly, most of the HIV IC appeared to remain on the surfaces of the B cells since the treatment of cells with proteinase K at each time point substantially reduced detection of the virus (Fig. 3), while the protease treatment did not decrease the cell number or viability (data not shown). However, at all time points there was a small amount of protease-resistant p24 associated with B cells, suggesting some internalization (Fig. 3).

At 15 h, 87 pg (46%) of the p24 had been released into the culture medium, and this amount increased to 111 pg (59%) at 48 h. Most of the released p24 appeared to be retained within an intact virus membrane, since the majority of this released p24 was undetectable in the absence of detergent. Thus, at 15, 24, 48, and 72 h there was 51, 59, 79, and 51 pg of p24 antigen, respectively, released from B lymphocytes that appeared to be intact virus.

Infection of PBMC by B-cell-bound HIV IC. The above studies indicated that HIV IC could remain on the surfaces of tonsil B lymphocytes for up to 3 days and that a portion of the released p24 may have intact membranes. We thus investigated whether the released or cell-bound virus remained infectious for phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC). HIV IC were prepared by the treatment of PI 1 with autologous serum and complement and incubation with B cells, and the B cells were cultured as described in the Fig. 2 legend. The B cells and B-cell culture...
Cells were washed and cultured for 72 h in complete medium in 24-well plates (2 × 10^6 cells/ml). At 0, 15, 24, 48, and 72 h, cells were washed and lysed with 0.5% Triton X-100 and the amount of cell-associated virus was determined by p24 ELISA. The total amount of virus released was also measured in the supernatant after treatment with 0.5% Triton X-100. The amount of intact released virus in a supernatant was calculated by subtracting the free p24 measured in the absence of detergent from the total amount of released p24. Some aliquots of B cells were treated with proteinase K (1.0 μg/ml; Sigma) in serum-free medium for 10 min before the cells were washed and lysed with 0.5% Triton X-100 to assess the amount of protease-resistant p24 associated with B cells. The means of results from three experiments ± the standard errors of the means are shown.

TABLE 1. Infection of T cells by HIV IC bound to tonsil B cells

<table>
<thead>
<tr>
<th>Time after HIV IC bound (h)</th>
<th>Level of virus replication (pg of p24/ml) in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B cell-bound HIV IC</td>
</tr>
<tr>
<td>0</td>
<td>16,787 ± 1,487</td>
</tr>
<tr>
<td>24</td>
<td>17,616 ± 1,899</td>
</tr>
<tr>
<td>48</td>
<td>19,007 ± 1,791</td>
</tr>
<tr>
<td>72</td>
<td>20,106 ± 1,576</td>
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\(^a\) Tonsil B cells were incubated with HIV IC made with PI 1 plus autologous serum and complement. Autologous serum alone at a 1:30 dilution did not significantly neutralize PI 1 (data not shown). Cells were washed and cultured for 72 h, and at 0, 24, 48, and 72 h of culture, cells and culture supernatants were harvested. Cells were treated with or without proteinase K as described in the Table 1 legend. Cells and supernatants were cultured with 2 × 10^6 PHA-stimulated PBMC per well in 24-well plates, and HIV replication in PBMC cultures was assessed by measuring the p24 core antigen in the supernatants after 12 additional days of culture. The mean level of p24 detected in cultures of B cells incubated with HIV IC in the absence of PBMC-derived T cells on days 7 and 12 were 62 and 77 pg/ml, respectively. The means ± standard errors of the means of results from triplicate cultures are shown. Results shown are representative of two experiments.

\(^b\) NA, not applicable.
HIV IC to T cells and thus suggest a novel means of infection of T cells in vivo.

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REFERENCES