Expression of Adhesion Molecules and CD28 on T Lymphocytes during Human Immunodeficiency Virus Infection

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Adhesion molecules, which play a major role in lymphocyte circulation, have not been well characterized in human immunodeficiency virus (HIV) infection. T-lymphocyte populations, including CD3, CD4, CD28, and adhesion molecules (L selectin, LFA-1, VLA-4, and ICAM-1) were measured by flow cytometry in a cross-sectional study of 100 HIV-infected and 49 HIV-seronegative adults. HIV-infected adults had lower numbers of CD3⁺ lymphocytes expressing L selectin (P < 0.0001) and VLA-4 (P < 0.01) and higher numbers of CD3⁺ lymphocytes expressing LFA-1⁺ (P < 0.002) than did HIV-negative adults. By CD4⁺-lymphocyte count category (>500, 200 to 500, or <200 cells/µl), HIV-infected adults with more advanced disease had lower percentages of CD3⁺ lymphocytes expressing L selectin and VLA-4 and higher percentages of CD3⁺ lymphocytes expressing LFA-1⁺. The percentages of CD3⁺ CD28⁺ lymphocytes and of CD3⁺ L selectin⁻ lymphocytes were positively correlated (Spearman coefficient = 0.86; P < 0.0001), and the percentage of CD3⁺ CD28⁺ lymphocytes and the CD3⁺ LFA-1⁺-lymphocyte/CD3⁺ LFA-1dim⁺ lymphocyte ratio were negatively correlated (Spearman coefficient = −0.92; P < 0.00001). The results of this study suggest that HIV infection is associated with altered expression of adhesion molecules.

Human immunodeficiency virus (HIV) infection is characterized by progressive loss of CD4⁺ lymphocytes, increase in CD8⁺ lymphocytes, and major functional abnormalities in lymphocyte function (7). Prior to the development of AIDS there is homeostasis of CD3⁺ lymphocytes, in which CD8⁺ lymphocytes increase in number to compensate for the loss of CD4⁺ lymphocytes (16). The later progression of HIV disease is associated with major changes in T-lymphocyte populations, including loss of CD28 surface antigen from both CD4⁺ and CD8⁺ lymphocytes (5, 5).

Lymphectical adhesion molecules include the selectin, integrin, and immunoglobulin gene superfamilies (13, 22). L selectin (CD62L), which plays a major role in lymphocyte recirculation to peripheral lymph nodes, is normally expressed on approximately 70 to 80% of T lymphocytes (23). Lymphocyte function-associated antigen 1 (LFA-1), an integrin which is expressed on virtually all peripheral blood leukocytes, including T lymphocytes, is involved in lymphocyte-endothelial cell adhesion (13). The ligands for LFA-1 are two members of the immunoglobulin gene superfAMILY, intercellular adhesion molecules 1 (ICAM-1) and -2 (ICAM-2). Very late antigen 4 (VLA-4) is expressed on T cells and is involved in lymphocyte adhesion to endothelial cells. The expression of these adhesion molecules during HIV infection is not well understood. We conducted a clinic-based, cross-sectional study of major T-lymphocyte populations and adhesion molecules in HIV-infected adults.

MATERIALS AND METHODS

The study population consisted of a consecutive sample of HIV-seropositive and HIV-seronegative adults seen in the outpatient clinics at the Johns Hopkins Hospital, Baltimore, Md. The study design was cross-sectional. After written informed consent was obtained, blood samples were collected by vein puncture. All lymphocyte populations were studied from a single blood sample drawn from each subject. Peripheral blood mononuclear cells (PBMC) were separated from heparinized blood with Ficoll-Hypaque (Pharmacia, Piscataway, N.J.), washed in Hanks’ buffered salt solution, and resuspended in phosphate-buffered saline with 1% fetal bovine serum and 0.1% sodium azide (assay buffer). Conjugated monoclonal antibodies to human CD3, CD4, CD14, CD28, CD45, L selectin (Becton Dickinson Immunocytometry Systems, San Jose, Calif.), LFA-1, ICAM-1 (AMAC, Westbrook, Maine), and VLA-4 (Endogen, Boston, Mass.) were used in two-color (fluorescein isothiocyanate and phycoerythrin) analyses.

PBMC were prepared according to the Centers for Disease Control and Prevention guidelines for leukocyte immunophenotyping in HIV infection (4). One million PBMC were placed in 1.5-ml microcentrifuge tubes, pelleted, and resuspended in 50 µl of assay buffer. The PBMC were stained with combinations of monoclonal antibodies for 20 min at 4°C. All samples were kept at 4°C and in 1% sodium azide throughout the staining process to minimize the likelihood of cell activation. The PBMC were washed twice with 1 ml of assay buffer, fixed with 1% paraformaldehyde in phosphate-buffered saline, and analyzed by direct two-color immunofluorescence. Isotype-matched monoclonal antibodies from the same commercial suppliers served as controls for fluorescence marker settings and identification of nonspecific staining.

Flow cytometry was performed by using two-color (FACStar Plus; Becton Dickinson) analysis. Fluorescent microbeads (Simply Cellular and Quantum Simply Cellular beads; Flow Cytometry Standards Corporation, Research Triangle Park, N.C.) were used to calibrate the flow cytometer daily and to control for reproducibility of the assay. For each sample, 20,000 ungated events were collected. The lymphocyte population was selected by backgating for CD45⁺ events in the profile of the sample stained with fluorescein isothiocyanate-anti-CD45-phycocerythrin anti-CD14 such that >95% of the forward-scatter versus side-scatter lymphocyte gate was CD45⁺ and CD14⁺. Thus, >95% of the lymphocyte gate contained lymphocytes. Quadrant analysis cursors were set from each individual and adjusted from the isotype control sample so that >95% of the gated cells were double negative. Dot plots, histograms, and raw statistical information were generated from this gated lymphocyte population for each analyzed sample. Any shift in population and the presence or absence of dim cells versus bright cells were noted. The raw subset percentage value was divided by the CD45⁺CD14⁺ lymphocyte percentage to correct for nonlymphocyte events in the lymphocyte gate. The CD4⁺ lymphocyte count was calculated by multiplying the absolute lymphocyte count by the correct CD4⁺-lymphocyte percentage from flow cytometric analysis. The absolute lymphocyte count was measured by an automated cell counter (Coulter Diagnostics, Hialeah, Fla.) in the clinical laboratory of Johns Hopkins Hospital. In subjects with large unclassified cells, the lymphocyte count was adjusted after a manual count of a blood

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TABLE 1. Expression of adhesion molecules on CD3⁺ lymphocytes

<table>
<thead>
<tr>
<th>Marker</th>
<th>HIV-seronegative adults</th>
<th>HIV-seropositive adults</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>No. (cells/μl)</td>
<td>P value</td>
</tr>
<tr>
<td>L selectin&lt;sup&gt;high&lt;/sup&gt;</td>
<td>49</td>
<td>1,242 (1,104-1,412)</td>
</tr>
<tr>
<td>L selectin&lt;sup&gt;low&lt;/sup&gt;</td>
<td>246 (186-339)</td>
<td>0.007</td>
</tr>
<tr>
<td>VLA-4⁺</td>
<td>22</td>
<td>1,501 (1,371-1,659)</td>
</tr>
<tr>
<td>VLA-4⁻</td>
<td>22 (16-33)</td>
<td>0.005</td>
</tr>
<tr>
<td>LFA-1&lt;sup&gt;high&lt;/sup&gt;</td>
<td>49</td>
<td>382 (239-538)</td>
</tr>
<tr>
<td>LFA-1&lt;sup&gt;low&lt;/sup&gt;</td>
<td>1,097 (891-1,283)</td>
<td>0.0001</td>
</tr>
<tr>
<td>ICAM-1⁺</td>
<td>13</td>
<td>547 (336-739)</td>
</tr>
<tr>
<td>ICAM-1⁻</td>
<td>882 (732-963)</td>
<td>0.004</td>
</tr>
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</table>

<sup>a</sup> n, number (n) of patients.
<sup>b</sup> NS, not significant.

RESULTS

Expression of adhesion molecules on CD3⁺ lymphocytes. The percentages and absolute counts of CD3⁺ lymphocytes expressing L selectin, VLA-4, LFA-1, and ICAM-1 are shown in Table 1. HIV-seropositive individuals had significantly lower percentages and absolute counts of CD3⁺ lymphocytes expressing L selectin and VLA-4 than did HIV-seronegative individuals. The percentages of CD3⁺ lymphocytes expressing LFA-1<sup>high</sup> and ICAM-1 were significantly higher in HIV-seropositive individuals. There was no significant difference in the absolute numbers of CD3⁺ lymphocytes expressing ICAM-1 between HIV-seronegative and HIV-seropositive individuals; however, HIV-seronegative individuals had significantly higher absolute numbers of CD3⁺ ICAM-1⁺ lymphocytes than did HIV-seropositive individuals. Representative immunofluorescence histograms of adhesion molecules on CD3⁺ lymphocytes are shown in Fig. 1.

The expression of adhesion molecules on CD3⁺ lymphocytes was examined according to the CD4⁺-lymphocyte count (>500, 200 to 500, or <200 cells/μl) among HIV-seropositive individuals (Fig. 2). Lower proportions of CD3⁺ lymphocytes expressing L selectin were found in HIV-seropositive individuals with lower CD4⁺ lymphocyte counts. The proportion of CD3⁺ lymphocytes expressing VLA-4 tended to decrease slightly among HIV-seropositive individuals with lower CD4⁺ counts. With decreasing CD4⁺-lymphocyte counts among HIV-seropositive adults, the proportions of CD3⁺ lymphocytes expressing LFA-1 increased.

Expression of CD28⁺ on CD3⁺, CD4⁺, and CD8⁺ lymphocytes. Expression of CD28⁺ on CD3⁺, CD4⁺, and CD8⁺ lymphocytes was examined according to the CD4⁺-lymphocyte count (>500, 200 to 500, or <200 cells/μl) among HIV-seropositive individuals (Fig. 3). Lower proportions of CD3⁺ lymphocytes expressing CD28⁺ were found in HIV-seropositive individuals
with lower CD4+ lymphocyte counts. The proportions of CD4+ lymphocytes expressing CD28+ were similar among HIV-seropositive individuals with CD4+ counts of >500 and 200 to 500 cells/µl but were lower among those with CD4+ lymphocyte counts of <200 cells/µl. There was no clear trend for the proportion of CD8+ lymphocytes expressing CD28+ by CD4+ lymphocyte count.

The percentages of CD3+ CD28+ lymphocytes and CD3+ L selectin+ lymphocytes were positively correlated (Spearman coefficient = 0.86; P < 0.0001), as shown in Fig. 4a. The percentage of CD3+ CD28+ lymphocytes and the CD3+ LFA-1bright lymphocyte/CD3+ LFA-1dim lymphocyte ratio were negatively correlated (Spearman coefficient = −0.92; P < 0.00001), as shown in Fig. 4b.

**DISCUSSION**

The results of this study suggest that the expression of L selectin and VLA-4 on CD3+ lymphocytes by HIV status and CD4+ lymphocyte count category. SN, seronegative; HIV SP, HIV seropositive. The bars represent the medians (horizontal) and the 25th and 75th percentiles (vertical).

<table>
<thead>
<tr>
<th>Subset</th>
<th>HIV-seronegative adults</th>
<th>HIV-seropositive adults</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=</td>
<td>No. (cells/µl)</td>
</tr>
<tr>
<td>CD4+ CD28+</td>
<td>49</td>
<td>1,228 (1,074-1,348)</td>
</tr>
<tr>
<td>CD3+ CD28+</td>
<td>305 (215-425)</td>
<td>0.0005</td>
</tr>
<tr>
<td>CD4+ CD28+</td>
<td>27</td>
<td>807 (723-1,015)</td>
</tr>
<tr>
<td>CD4+ CD28+</td>
<td>88 (46-183)</td>
<td>0.0004</td>
</tr>
<tr>
<td>CD8+ CD28+</td>
<td>27</td>
<td>281 (208-357)</td>
</tr>
<tr>
<td>CD8+ CD28+</td>
<td>198 (162-252)</td>
<td>0.0001</td>
</tr>
</tbody>
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* n, number (n) of patients.
The present study corroborates the finding that advanced HIV infection is associated with loss of both CD4+ lymphocytes and CD3+ LFA-1bright/CD3+ LFA-1dim lymphocyte ratio, suggesting that CD3+ CD28+ lymphocytes do not express LFA-1.

ACKNOWLEDGMENTS

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REFERENCES