Elevated Levels of CD4 Antigen in Sera of Human Immunodeficiency Virus-Infected Populations

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CD4 antigen levels in sera from asymptomatic intravenous drug users and homosexuals and patients with lymphadenopathy, acquired immunodeficiency syndrome-related complex, or acquired immunodeficiency syndrome were quantitated. Like soluble CD8, CD4 antigen levels were elevated in human immunodeficiency virus-seronegative asymptomatic intravenous drug users and homosexuals, probably reflecting infections such as cytomegalovirus, Epstein-Barr virus, and hepatitis B virus infections. The sera from human immunodeficiency virus-seropositive groups of patients with human immunodeficiency virus infection also had elevated levels of anti-Leu-3a, presumably reflecting infections like cytomegalovirus and human immunodeficiency virus infections.

Acquired immunodeficiency syndrome (AIDS) is caused by human immunodeficiency virus (HIV) (7). HIV infection appears to be initiated by the binding of the viral envelope protein gp120 to the CD4 antigen present on T cells (2, 11, 13). HIV tropism is dependent on the expression of CD4, and the interaction of CD4 with gp120 eventually leads to CD4 cell dysfunction and ultimate destruction (16, 19, 20). The most characteristic laboratory feature of HIV infection is a decreased proportion and number of CD4 lymphocytes and an increased proportion and number of CD8 lymphocytes, resulting in an inversion of the ratio of CD4/CD8 lymphocytes (7). CD4 and CD8 glycoproteins belong to the immunoglobulin gene superfamily (12). We reported previously that soluble CD8 (SCD8) levels were elevated in patients with HIV infection (18). In this report, we present evidence to suggest that CD4 antigen levels are also elevated after HIV infection.

MATERIALS AND METHODS

Subjects. The subjects included 52 HIV-seronegative and 31 HIV-seropositive intravenous drug users (IVDU), 34 HIV-seronegative and 32 HIV-seropositive asymptomatic homosexuals, 68 patients with uncomplicated generalized lymphadenopathy, 11 patients with AIDS-related complex (ARC), 60 AIDS patients with *Pneumocystis carinii* pneumonia, 26 AIDS patients with Kaposi’s sarcoma, and 11 AIDS patients with both *P. carinii* pneumonia and Kaposi’s sarcoma who met the Centers for Disease Control surveillance definition of AIDS (1). Fifty normal control subjects who were HIV seronegative were also included in the study.

CD4 and CD8 lymphocytes. CD4 and CD8 lymphocyte proportions and absolute numbers were obtained as previously described (18).

CD4 antigen assay. CD4 antigen levels in serum were determined by using a Cell Free CD4 Test Kit (T Cell Sciences, Inc., Cambridge, Mass.). The standards or sera were added to the anti-CD4 monoclonal antibody-coated polystyrene microtiter plate wells. This anti-CD4 monoclonal antibody recognizes an epitope of the CD4 molecule similar to that of anti-Leu-3a of Becton Dickinson Immunocytometry Systems, San Jose, Calif. The CD4 epitope binding to anti-Leu-3a is of special interest because it blocks HIV infection of CD4+ cells, which suggests that it binds to the same region of the CD4 molecule as does the HIV gp120 envelope protein (19). After being incubated, the wells were washed to remove unreactive sample components. Horseradish peroxidase-conjugated anti-CD4 monoclonal antibody directed against a second epitope on the CD4 molecule, which would bind to the CD4 captured by the first CD4 antibody, was then added. This second anti-CD4 monoclonal antibody recognizes a different epitope of the CD4 molecule similar to that of the OKT4 monoclonal antibody of Ortho Diagnostic Systems, Raritan, N.J. After unbound enzyme-conjugated anti-CD4 was removed by washing, substrate solution was added to the wells. The reaction was terminated by the addition of stop solution (2 N H2SO4), and A490 was measured with an enzyme-linked immunosorbent assay reader. A standard curve was constructed from six standards by plotting concentration on the x axis versus absorbance on the y axis. Unknown values were determined from the resulting standard curve and expressed as units per milliliter.

One unit of CD4 antigen is equivalent to 10 pg of recombinant soluble CD4. The within-run coefficient of variation was 12%, and the between-run coefficient of variation was 13%.

Soluble CD8 assay. Soluble CD8 levels in serum were determined as described previously (18).

Antibodies to HIV. An enzyme-linked immunosorbent assay and Western blots (immunoblots) against purified HIV proteins were performed as previously described (18).

RESULTS

The CD4 antigen level in the serum specimens from 50 normal controls was 11.3 ± 0.7 U/ml (mean ± standard error), compared with 28.6 ± 4.0 U/ml in the serum specimens from 52 HIV-seronegative asymptomatic IVDU (Table 1). This difference is statistically significant (P < 0.001). The mean CD4 antigen level in serum specimens from 34 HIV-seronegative homosexuals was 15.9 ± 1.3 U/ml, which was significantly different from the control value. The CD4 antigen level in the sera of HIV-seropositive IVDU was 67.5 ± 37.9 U/ml (one patient in this group had a very high level [1,200 U/ml] of CD4 antigen in circulation; when this patient was excluded, the mean CD4 antigen level in this group was
TABLE 1. CD4 antigen and soluble CD8 levels and lymphocyte subsets in asymptomatic IVDU and homosexuals and patients with lymphadenopathy, ARC, or AIDS

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of subjects</th>
<th>HIV antibody</th>
<th>Lymphocytes/μl&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SCD8 (U/ml)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>CD4 antigen (U/ml)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>CD4</td>
<td>CD8</td>
</tr>
<tr>
<td>Normal controls</td>
<td>50</td>
<td>Negative</td>
<td>1,884 ± 79</td>
<td>860 ± 40</td>
<td>463 ± 29</td>
</tr>
<tr>
<td>Asymptomatic IVDU</td>
<td>52</td>
<td>Negative</td>
<td>2,568 ± 117</td>
<td>919 ± 46</td>
<td>625 ± 35</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>Positive</td>
<td>1,831 ± 107</td>
<td>488 ± 38</td>
<td>669 ± 51</td>
</tr>
<tr>
<td>Asymptomatic homosexuals</td>
<td>34</td>
<td>Negative</td>
<td>2,311 ± 109</td>
<td>653 ± 37</td>
<td>640 ± 36</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>Positive</td>
<td>1,931 ± 131</td>
<td>539 ± 55</td>
<td>568 ± 47</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>68</td>
<td>Positive</td>
<td>1,938 ± 109</td>
<td>422 ± 35</td>
<td>762 ± 62</td>
</tr>
<tr>
<td>ARC</td>
<td>11</td>
<td>Positive</td>
<td>2,046 ± 229</td>
<td>623 ± 176</td>
<td>1,301 ± 148</td>
</tr>
<tr>
<td>AIDS with:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. carinii pneumonia</td>
<td>60</td>
<td>Positive</td>
<td>794 ± 76</td>
<td>129 ± 19</td>
<td>321 ± 39</td>
</tr>
<tr>
<td>Kaposi's sarcoma</td>
<td>26</td>
<td>Positive</td>
<td>1,481 ± 150</td>
<td>305 ± 42</td>
<td>532 ± 62</td>
</tr>
<tr>
<td>P. carinii pneumonia</td>
<td>11</td>
<td>Positive</td>
<td>784 ± 104</td>
<td>137 ± 26</td>
<td>295 ± 54</td>
</tr>
<tr>
<td>and Kaposi's sarcoma</td>
<td></td>
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<sup>a</sup> Mean ± standard error.
<sup>b</sup> P value, compared with normal controls, according to Mann-Whitney U test.
<sup>c</sup> P value, compared with HIV-seronegative IVDU.
<sup>d</sup> P value, compared with HIV-seronegative IVDU, is not significant.
<sup>e</sup> P value, compared with HIV-seronegative homosexuals.

29.7 ± 3.6 U/ml, and in the sera of HIV-seropositive asymptomatic homosexuals, the level was 20.3 ± 4.1 U/ml, compared with the control value of 11.3 ± 0.7 U/ml. These increases are statistically significant. Similar increases were observed for patients with lymphadenopathy or ARC and for patients with AIDS with P. carinii pneumonia or Kaposi's sarcoma or both. The range of values was broad in these groups (Fig. 1). As reported previously (18), SCD8 levels were also elevated in the risk groups and HIV-seropositive groups, compared with levels in controls (Table 1).

We also examined correlations between CD4 antigen and SCD8 and absolute CD4 and CD8 counts. There appears to be a negative correlation between absolute CD4 cells and CD4 antigen levels (r = −0.1; P < 0.05) and a positive

![Fig. 1. CD4 antigen levels in serum specimens from asymptomatic IVDU and homosexuals and patients with lymphadenopathy, ARC, or AIDS. +VE, Positive; –VE, negative.](http://jcm.asm.org/)
correlation between CD4 antigen and SCD8 levels (r = 0.09; P < 0.05).

DISCUSSION

HIV infection results in a defect in cell-mediated immunity. This includes lymphopenia, a decreased number of CD4 cells, an increased number of CD8 cells resulting in a decreased CD4/CD8 ratio, cutaneous anergy, and decreased T- and B-lymphocyte function (7). Furthermore, increased circulating levels of β₂-microglobulin, neopterin, soluble interleukin-2 receptor, acid-labile human leukocyte interferon, and soluble CD8 have been reported (3, 9, 17, 18). The results of the present study demonstrate that CD4 antigen levels in serum were also elevated in HIV-seropositive asymptomatic IVDU and homosexuals and in patients with lymphadenopathy, ARC, or AIDS.

Our results also indicate that CD4 antigen levels in serum increased significantly in HIV-seronegative asymptomatic IVDU and homosexuals, compared with levels in the normal control group. Abnormally elevated CD4 antigen in asymptomatic HIV-seronegative IVDU and homosexuals may be due to a variety of infections that these individuals contract, such as cytomegalovirus, Epstein-Barr virus, and hepatitis B virus. Chronic exposure and reinfection with these agents may lead to a transient increase in CD8 cells and soluble CD8 in circulation (4-6, 18). Although these patients were HIV seronegative, some of them may still be infected with HIV, since it has been reported recently that HIV-seronegative patients were carriers of viral DNA, as demonstrated by the polymerase chain reaction (10). The mechanism for CD4 antigen elevation is unclear, although these infections may also cause CD4 antigen elevation in circulation.

HIV infection is generally associated with a decrease in the number of CD4 cells, which continue to decrease by a mean of 60 to 100/μl per year after infection (14). The CD4 depletion may be due to a direct cytopathic effect of HIV in vivo, since HIV infection of T cells in vitro results in cell death (2, 7). In addition, the depletion of CD4 cells may be due to the loss of uninfected CD4 cells after fusion with infected CD4 cells expressing gp120 or through cytotoxicity by gp120-specific cytotoxic T cells following uptake of soluble gp120 into CD4 cells (20-22). However, we observed an increase in CD4 antigen in circulation after HIV infection and in patients with ARC and AIDS. The mechanism for this increase in circulation is not understood, but this may represent the CD4 molecules released into circulation after CD4 cell death caused by HIV infection or increased synthesis of CD4 molecules in a manner similar to soluble CD8 synthesis from alternately spliced mRNA (8, 15). This mRNA for soluble CD8 is alternately spliced so that an exon encoding a transmembrane domain is deleted, which gives rise to a 30,000-dalon soluble CD8 molecule. Whether serum CD4 antigen is encoded by alternate splicing or by some other mechanism remains to be seen.

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LITERATURE CITED