Elevated erythrocyte adenosine deaminase activity in patients with acquired immunodeficiency syndrome

(Murine nucleoside phosphorylase/lymphadenopathy retrovirus/trans-activation)

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ABSTRACT Acquired immunodeficiency syndrome (AIDS) is an often fatal disease caused by a retrovirus frequently resulting in malignancy and/or opportunistic infection. Because the immune deficiency in AIDS is similar to that in some purine enzyme deficiencies, we measured erythrocyte adenosine deaminase (ADA) and purine nucleoside phosphorylase activities in patients with AIDS, heterosexual controls, and a high-risk asymptomatic population. We found that erythrocyte ADA activity was significantly elevated in patients with AIDS (40 ± 11 nmol/mg of hemoglobin per hr, mean ± SD) relative to heterosexual controls (25 ± 10, P < 0.001). We also measured ADA activity in a group of individuals at high risk for AIDS and found that approximately half had significantly elevated ADA activities (45 ± 4, P < 0.002) that correlated with the presence of antibody to the lymphadenopathy retrovirus. Purine nucleoside phosphorylase activity was relatively normal in patients with AIDS as well as in individuals at risk for AIDS. Increased ADA appears to be a diagnostic marker of AIDS and may be useful in conjunction with antibody to the AIDS-related retrovirus in detecting the presence of infection in asymptomatic high-risk individuals. These data also suggest that, in addition to the lymphocyte, the erythroid cell line may also be infected by the AIDS-related retrovirus.

Acquired immunodeficiency syndrome (AIDS) was first recognized 5–6 years ago (1, 2). It is characterized by severe T-cell dysfunction and polyclonal hypergammaglobulinemia associated with life-threatening opportunistic infections, malignancies such as Kaposi sarcoma, and a variety of other clinical and laboratory manifestations (3, 4). The infectious etiology of AIDS is a retrovirus (5–7). The AIDS-associated retrovirus [human T-cell lymphotropic virus type III (HTLV-III), lymphadenopathy virus (LAV)] has an affinity for the T-helper/inducer subpopulation of lymphocytes, although other cell types, for example monocytes, can be infected (8).

The clinical and laboratory manifestations of AIDS are similar to those seen in children with inborn errors of purine metabolism, specifically adenosine deaminase (ADA) or purine nucleoside phosphorylase (PNP) deficiencies (9). Children with either of these inborn errors have severe T-cell immunodeficiency and, in those with PNP deficiency, hypergammaglobulinemia may also be present. For this reason we measured ADA and PNP activities in patients with AIDS or AIDS-related complex (ARC) and individuals at high risk for developing AIDS.

METHODS

Subjects. Patients with AIDS or ARC met the Centers for Disease Control criteria for these diagnoses (10) and were classified as having opportunistic infection, Kaposi sarcoma, or both, or lymphadenopathy syndrome. These patients were seen in the AIDS clinic at San Francisco General Hospital.

A second group of individuals (the high-risk group) were studied who were clinically healthy but had been exposed to the AIDS-related retrovirus either through sexual contact or through i.v. drug abuse. This population is part of an ongoing epidemiologic study of AIDS at San Francisco General Hospital.

Subjects with AIDS, ARC, or those at high risk were all seen as outpatients and had not received any blood transfusions for at least 3 months prior to this study. Specimens were obtained under procedures approved by the Committee on Human Research.

Immunological Studies. Following informed consent, peripheral blood samples were obtained. Heparinized blood was separated over Ficoll/Hypaque. Peripheral blood mononuclear cells were processed for T-cell subset analysis using monoclonal antibodies OKT4 (helper/inducer) and OKT8 (suppressor/cytotoxic) and in vitro lymphocyte responses to phytomem-gullin and pokeweed mitogen as described (11, 12).

Anti-Lymphadenopathy Virus (LAV) Antibody. Serum was assayed for antibody by using an ELISA-based procedure with an immunoblot confirmation (13), which was kindly performed in Luc Montagnier’s laboratory at the Institute Pasteur.

Purine Enzymes. After separation of the peripheral blood over Ficoll/Hypaque, the erythrocyte pellet was washed with phosphate-buffered saline and an extract was made in 50 mM potassium phosphate buffer (pH 7.4) by freeze-thawing four times in dry ice/acetone. The extract was frozen at −70°C until assayed.

ADA and PNP activities were measured using a spectrophotometric assay of uric acid production, which has previously been described (14). Briefly, for ADA activity, the reaction mixture consisted of 0.79 ml of phosphate buffer, 5 µl of xanthine oxidase (Sigma), 0.1 ml of 10 mM adenosine, 5 µl of PNP (Sigma) diluted 1:20, and 0.1 ml of a 1:10 dilution of extract. For PNP activity, the reaction mixture was similar except that inosine was used in place of adenosine, PNP was omitted, and the extract was diluted 1:40. The results are expressed as nmol of uric acid produced per hr per mg of hemoglobin. Hemoglobin concentrations were measured using the cyanomethanoglobin method.

RESULTS

Erythrocyte ADA activity is shown in Fig. 1. All of the patient groups with AIDS or ARC had significantly elevated

Abbreviations: AIDS, acquired immunodeficiency syndrome; HTLV-III, human T-cell lymphotropic virus type III; LAV, lymphadenopathy virus; ADA, adenosine deaminase; PNP, purine nucleoside phosphorylase; ARC, AIDS-related complex.

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erythrocyte ADA. Those individuals at high risk for AIDS could be divided into two groups, depending on whether they had serologic evidence for exposure to the AIDS-related retrovirus. Those who were positive had significantly elevated erythrocyte ADA activity \((P < 0.002)\) while those who were negative had normal activities.

Erythrocyte PNP activity was not significantly elevated compared to heterosexual controls except for the high-risk antibody-positive group, in which PNP was slightly greater than normal \((P = 0.04)\) (Fig. 2). Also, when the antibody-positive and -negative high-risk individuals were assessed, there was no correlation with PNP activity.

Lymphocyte phenotyping and in vitro responses to phytohemagglutinin and pokeweed mitogen are shown in Table 1. Data were available from all 20 patients with AIDS or ARC, 11 high-risk individuals, and 7 heterosexual controls. The AIDS patients had normal percentages of cells expressing the pan-T-cell marker OKT3 with depressed OKT4 and elevated OKT8. The lymphocyte responses to both PHA and PWM were significantly depressed in the patients with AIDS \((P < 0.02 \text{ and } P < 0.001, \text{ respectively})\). There were no significant differences between the high-risk group and the healthy heterosexual population for phenotype or in vitro lymphocyte responses to mitogens.

Finally, the purine enzyme activities and the results of the immunological studies are compared among the high-risk groups with high ADA and normal ADA, patients with AIDS/ARC, and healthy heterosexual controls in Table 1. Though the differences are not statistically significant, the T-cell immunity in those high-risk individuals who were positive for antibody to the AIDS-related retrovirus appears to be more abnormal than that in individuals who are antibody-negative. Also, erythrocyte PNP activity in this small subgroup of high-risk antibody-positive individuals with elevated ADA is elevated relative to that of healthy heterosexual individuals \((P = 0.04)\).

**DISCUSSION**

We have found an association between AIDS and elevated erythrocyte ADA. This appears to be relatively specific for ADA since the next enzyme in the purine metabolic pathway, PNP, is relatively normal in patients with AIDS. Furthermore, we have found that in individuals who are at high risk for exposure to the AIDS-related retrovirus but are otherwise clinically well, there appears to be a correlation between the presence of antibody to the retrovirus and significantly elevated erythrocyte ADA.

There are several possible explanations for the finding of increased ADA in AIDS. One is that this is a nonspecific elevation due to other factors, such as opportunistic or other viral infections, malignancy, or hematologic abnormalities. This is unlikely, since most of the patients with Kaposi sarcoma did not have opportunistic infections and those with opportunistic infections did not have malignancy. Also, we have measured ADA and PNP in children with immunodeficiency disease and disseminated viral infections and have found these enzymes to be normal (data not shown). Furthermore, in the two subgroups of high-risk individuals, the only distinguishing feature between them was the presence or absence of antibody to the AIDS-related retrovirus. Finally, we measured several hematologic abnormalities in these high-risk individuals including hemoglobin concentrations, erythrocyte counts, and mean corpuscular hemoglobin concentration (data not shown) and could find no differences between those with high ADA activity and those with normal ADA activity.

A second possible explanation for elevated erythrocyte ADA in these patients is that it is a result of the retrovirus infection. The elevated ADA activity could be due to either an alteration in the protein that would change the \(K_m\) or an increased amount of protein. In any event, these data suggest that the alterations in ADA metabolism occurred in the early bone marrow erythroid cells still capable of synthesizing protein and may be a result of retrovirus infection. It has recently been shown that HTLV-III has trans-activating capability and codes for a trans-activating factor that activates the expression of genes linked to the HTLV-III long terminal repeat \((15, 16)\). Early work suggests that this protein has structural features similar to other nucleic acid binding proteins. It is possible that trans-acting transcriptional reg-
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<table>
<thead>
<tr>
<th>Group (n)</th>
<th>% PBMC binding mAb</th>
<th>Lymphocyte response, cpm</th>
<th>Activity, nmol of uric acid/hr per mg of Hb</th>
<th>Cells binding</th>
<th>ADA</th>
<th>PNP</th>
<th>Cells binding</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>OKT3</td>
<td>OKT4</td>
<td>OKT8</td>
<td>Phytohemagglutinin</td>
<td>Pokeweed mitogen</td>
<td>ADA</td>
<td>PNP</td>
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<td>AIDS/ARC (20)</td>
<td>51 ± 14</td>
<td>11 ± 1</td>
<td>41 ± 13</td>
<td>5,611 ± 7,606</td>
<td>1,136 ± 1,829</td>
<td>40 ± 11</td>
<td>1961 ± 419</td>
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<td>High risk (11)</td>
<td>66 ± 8</td>
<td>35 ± 7</td>
<td>29 ± 6</td>
<td>15,906 ± 8,896</td>
<td>15,618 ± 5,394</td>
<td>45 ± 4</td>
<td>2359 ± 326</td>
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<td>High ADA (5)</td>
<td></td>
<td></td>
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<td>22 ± 4</td>
<td>2169 ± 372</td>
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<td>Normal ADA (6)</td>
<td></td>
<td></td>
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<td>25 ± 10</td>
<td>1863 ± 465</td>
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<td>Heterosexual (16)</td>
<td>51-73</td>
<td>39-53</td>
<td>18-27</td>
<td>22,097-11,783</td>
<td>10,039-9,899</td>
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</table>

Results represent mean ± SD or range. PBMC, peripheral blood mononuclear cells; mAb, monoclonal antibody.

Whether high-risk patients exist who have elevated ADA without antibody and whether this can be used to detect those individuals who may be infected but have not yet developed a positive antibody titer.

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