Depressed Activities of Purine Enzymes in Lymphocytes of Patients Infected with Human Immunodeficiency Virus

Judith A. Renouf,¹,² Amanda Wood,¹ I. H. Frazer,³ Y. H. Thong,³ and A. H. Chalmers¹,⁴

Enzyme activities were studied in peripheral blood lymphocytes from patients infected with, or at risk for, infection with human immunodeficiency virus (HIV). No significant differences were observed in the HIV-infected and HIV-seronegative high-risk patients with regard to enzyme activities of hypoxanthine–guanine phosphoribosyltransferase (EC 2.4.2.8) and purine nucleoside phosphorylase (EC 2.4.2.1) in peripheral blood. Adenosine deaminase (EC 3.5.4.4) was significantly (P <0.02) depressed in asymptomatic HIV-seropositive patients and HIV-seronegative patients at high risk of HIV infection as compared with a healthy HIV-seronegative population. Adenosine kinase (AK, EC 2.7.1.20) was significantly increased in the asymptomatic seropositive (P <0.02) and also in the HIV-seronegative high-risk groups (P = 0.01) compared with the normal controls. AK activity was significantly lower in subjects with AIDS than in the asymptomatic (P <0.002) and high-risk groups (P <0.01). Taken together, these results indicate that adenosine deaminase and AK activities are influenced by the health of the patient, and that measurement of AK activity may prove useful in monitoring the clinical progress of patients with HIV infection.

Additional Keyphrases: adenosine deaminase • adenosine kinase • acquired immune deficiency syndrome

Acquired immune deficiency syndrome (AIDS) is a disease characterized by depleted numbers and impaired function of T-helper cells, resulting in a profound, irreversible loss of immunity (1, 2). AIDS is now known to be caused by infection with a virus currently called “human immunodeficiency virus” (HIV) (3, 4), and affected individuals are more susceptible to tumorigenesis (Kaposi’s sarcoma) and neurological dysfunction (5, 6). Profound immunosuppression is, however, the commonest feature of HIV infection.

The maximum likelihood estimate for the mean incubation period—the interval between HIV infection and onset of severe immunosuppression—is 7.8 years (90% confidence, with limits ranging from 4.2 to 15 y; 7). Some factors shown to correlate with the disease progression to AIDS include (a) a depressed T-helper (TH) cell count and antibody to HIV, (b) an increased T-suppressor (TS) cell count, (c) increased antibody titer to cytomegalovirus, and (d) sexual relations with a person who has AIDS (8).

In a recent study the disappearance of core antibody, the expression of antigen, and the presence of low TH cell counts were all shown to be predictors of AIDS (9). These authors noted, however, that these laboratory evaluations are not better predictors of disease progression than is careful clinical observation. We have attempted to find biochemical markers that may be more precise predictors of AIDS.

Congenital deficiencies of the enzymes adenosine deaminase (EC 3.5.4.4; ADA) and purine nucleoside phosphorylase (EC 2.4.2.1; PNP) are known to result in severely impaired immunity in young children (10, 11). Inhibition of these enzymes and hypoxanthine–guanine phosphoribosyltransferase (EC 2.4.2.8; HGPRT) also results in significant immunosuppression (12–14). Here we compare the activities of these enzymes in lymphocytes of patients seropositive to HIV with those of a seronegative homosexual group at high risk of HIV infection and an apparently healthy normal control group. In the HIV-infected group we compared enzyme activities in those patients with AIDS (CDC group IV.C + D; 15) or AIDS-related complex (ARC; CDC group IV.A) and those with asymptomatic infection (CDC groups II and III). We also measured adenosine kinase (EC 2.7.1.20; AK) activity, because, like ADA, this enzyme is involved directly in the metabolism of adenosine. Our findings indicate that the specific activity of these enzymes is altered in the course of HIV infection.

Subjects and Methods

Subjects

Seventy-eight HIV-seropositive homosexual men were investigated. Twelve were classified as having AIDS, 11 of whom had recovered from opportunistic infections and had a TH cell count <0.3 × 10⁹/L (CDC group IV.C), and one who had Kaposi’s sarcoma (CDC group IV.D) and a TH cell count of 0.8 × 10⁹/L. Ten patients had ARC (CDC group IV.A) and the remaining 56 were asymptomatic, HIV-seropositive, and healthy (CDC groups II and III); both groups had TH cell counts >0.6 × 10⁹/L. Twenty-two HIV-seronegative homosexual men who regarded themselves as having been at high risk for HIV infection were studied as controls. None of the patients or controls were receiving antiviral or antimicrobial chemotherapy at the time the tests were done. The patients ranged in age from 23 to 56 (x = 33) and the controls from 21 to 49 (x = 32) years.

Methods

From each subject we collected, with EDTA as anticoagulant, 20 mL of peripheral blood and separated the lymphocytes by density flotation, using lymphocyte separation
Lymphocyte enzymes and protein were measured as previously described, by spectrophotometric and radiochemical methods (16-18). Activities were expressed as nanomoles per hour per milligram (dry weight) of protein.

The study was done as a blind trial, with the analyst receiving coded samples and thus being unaware of the clinical status of the subjects. Immune-function tests were done as previously described (19). Delayed-type hypersensitivity was measured by use of the Multitest CMI skin test (Merieux, France).

The Mann-Whitney nonparametric ranking test for two-tailed probability was used in the statistical analysis (20). Product-moment correlation coefficients were calculated with a standard calculator and significances determined (20).

Results

The mean ADA activity was significantly lower in all HIV-seropositive groups and in a high-risk HIV-seronegative group than in the low-risk HIV-seronegative population (P <0.02) (Figure 1). Except between the AIDS and asymptomatic seropositive group (P <0.04), there was no significant difference in mean ADA activity when the

high-risk HIV-seronegative and other HIV-seropositive groups were compared.

In contrast, the mean AK activity was significantly higher in both the high-risk HIV-seronegative (P <0.01) and asymptomatic HIV-seropositive patients (P <0.02), than in the low-risk HIV-seronegative population. The group with AIDS had a significantly lower mean AK activity than did the asymptomatic HIV-seropositive (P <0.002) and high-risk HIV-seronegative groups (P <0.01). However, the mean AK activity in the AIDS group did not differ significantly from that in the healthy control group. There was also no significant difference in HGPRT and PNP activities between HIV-seropositive and high- or low-risk HIV-seronegative individuals (Figure 1).

Three HIV-seropositive subjects, all initially in CDC group II, were studied prospectively over two years. Two remained healthy, without significant changes in enzyme activities or immune function as measured by recall of delayed type hypersensitivity, TH cell counts, and TH/TS cell ratios. One subject who progressed to CDC group IV.C disease while under observation showed the decline in AK activity predicted by the results of the cross-sectional survey (Table 1). Unexpectedly, this patient’s ADA activities, which increased in progressing to CDC group IV.A, were inconsistent with the general depression of ADA in this ARC group (Figure 1). There was, however, a dramatic decrease in ADA when this patient was classified as CDC group IV.C (Table 1).

Correlation was sought between purine enzyme activity and the TH/TS cell ratio and helper TH count in blood, for the subjects in the cross-sectional survey. The TH cell count correlated with AK values among all HIV-positive patients, but not with any of the other enzymes (r = 0.40; P <0.01). None of the other markers correlated with any enzymes. Also within the disease category groups, no such correlation was observed for any enzyme (data not shown).

Discussion

Although this study has shown a depressed mean ADA activity in HIV-infected patients, it is unlikely that the depression of ADA is specifically linked to HIV infection, because the HIV-seronegative high-risk group had similarly depressed activities. Rather, the depressed ADA probably reflects more exposure to other intercurrent infections, because the high-risk groups have widespread bacterial and viral infections as compared with the heterosexuals (21, 22). It is also unlikely that the low ADA values are related to immune impairment, because high-risk HIV-seronegative and healthy HIV-seropositive groups do not

![Fig. 1. Lymphocyte purine enzyme values for persons seropositive to HIV who have (a) recovered from opportunistic infection (OF: CDC group IV.C); (b) AIDS-related complex (ARC: CDC group IV.A); (c) good general health and are asymptomatic (+: CDC groups II and III) HIV-seronegative homosexuals previously at high risk of HIV infection (term -) and healthy HIV-seronegative laboratory controls (N) are also indicated. The mean and standard deviation for each group is shown by the vertical bars. One patient with Kaposi’s sarcoma (CDC group IV.D, A) is in the OF group. Horizontal bars with significance for intergroup comparisons are shown at the top of the Figure. The absence of bars between groups indicates nonsignificance between these group comparisons](image-url)

<table>
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<tr>
<th>TH count, a</th>
<th>TH/TS ratio</th>
<th>AK</th>
<th>ADA</th>
<th>PNP</th>
<th>HGPRT</th>
<th>CDC category</th>
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<td>592</td>
<td>9200</td>
<td>10</td>
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<td>1293</td>
<td>nd</td>
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<td>13 500</td>
<td>18</td>
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<tr>
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<td>0.03</td>
<td>61</td>
<td>171</td>
<td>10 927</td>
<td>47</td>
</tr>
</tbody>
</table>

a Reference intervals: TH counts, >2.0-0.5 × 10^9/L; TH/TS 1.2-5.0.

b nd, not done.
exhibit demonstrably impaired immunity (19). These find-
ings are compatible with the observation that low ADA
activities are sufficient to restore immunity in partly de-
cient ADA patients (23, 24).

The increase in AK activity in high-risk seronegative
and healthy seropositive patients is similar to findings
of increased activities found in lectin-treated lymphocytes in
culture (25, and Renouf, Thong, Chalmers, unpublished
observation) and may indicate that these patients have
increased numbers of activated T-cells, presumably conse-
quently to the range of infections to which these groups are
exposed as a result of their life styles (21, 22). The progressive
decline in AK activities in ARC and AIDS groups
suggests that this occurs in association with immunosup-
pression. If so, this decline could prove clinically useful in
predicting the progression of immunosuppression in such
patients. It is unlikely that the decrease in AK activity per-
se in patients with AIDS is involved in the pathogenic
process of HIV infection, because AK values in patients
with AIDS are similar to those found in healthy controls
(Figure 1).

Other workers have also studied purine enzyme activi-
ties in patients with HIV infection. One study indicated
depressed ecto-5'-nucleotidase activity in lymphocytes of
sexually active homosexuals (26). However, this activity
was decreased equally in both healthy homosexuals (anti-
body status unknown) and in AIDS patients with opportu-
nistic infection and Kaposi's sarcoma, and therefore may
not be useful as a marker of disease progression. A recent
study showed normal ADA and PNP activities in erythro-
cytes of a patient with AIDS (27). Increases of serum ADA of
two- to threefold have been reported for CDC groups II to
IV patients, whereas high-risk seronegative homosexuals
had serum ADA activities similar to those measured in
normal, healthy controls (28). In a study using "null"
lymphocytes, Murray et al. (29) reported significantly in-
creased ADA and PNP activities in patients with AIDS as
compared with a normal, healthy group. In comparison,
they found no differences in the enzyme activities in en-
riched T-cells. It is difficult to relate these findings to the
present study, because different cell lines have been studi-
ed and depressed values for these enzymes, in general, are
related to immunodeficiency (10, 11, 23, 24). Our findings of
low mean ADA and AK activities are not likely to be due to
generalized malnutrition, because HGPRT and PNP activi-
ties, which might be expected to be similarly changed,
were not depressed in the AIDS group in this study (30).
Also, the finding of similar relative activities of PNP,
HGPRT, ADA, and AK in T-cell subsets and B-cells (31, 32)
would argue against an imbalance of lymphocyte subsets
being solely responsible for the depressed enzyme activities
seen in the AIDS group. A weak but significant correlation
found between TH cell numbers and AK would indicate
that loss of TH cells is contributing to the decreased AK
activities seen in CDC group IV.C. In vitro studies of T-
and B-lymphocytes with HIV would help elucidate whether
the depression of enzyme activities seen in our investi-
gation is specifically linked to a progressive HIV infection in pa-

ten. There is an urgent need for tests predictive of the clinical
outcome of HIV infection (8, 9, 19). Possibly ADA and AK
may prove useful in this scheme of tests already available
(8, 19, 33–35). Prospective studies are needed, but the main
problem with such a study would be the technical difficulty
of the tests themselves (thin-layer techniques) and the

expense of radioisotopes (16). We are currently developing
automated ADA and AK assays to facilitate these measur-
ing procedures.

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