Elevated Adenosine Deaminase and Purine Nucleoside Phosphorylase Activity in Peripheral Blood Null Lymphocytes From Patients With Acquired Immune Deficiency Syndrome

By James L. Murray, Karin C. Loftin, C. Gwyneth Munn, James M. Reuben, Peter W.A. Mansell, and Evan M. Hersh

The purine metabolic enzymes adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) are important in lymphocyte differentiation, and genetic deficiencies of either enzyme have been associated with hereditary immunodeficiency states. Both ADA and PNP activity were measured in null cell-enriched and T cell-enriched peripheral blood lymphocytes from 16 patients with the acquired immune deficiency syndrome (AIDS), seven patients with the AIDS-related symptom complex (ARC), and seven asymptomatic homosexuals. ADA activity in nmol/10^8 lymphocytes/h was significantly elevated in null lymphocytes from AIDS (161 ± 12) as compared with 23 healthy heterosexual controls (127 ± 8; P < .025). PNP activity was also significantly increased in null lymphocytes from AIDS patients (96 ± 10; P < .005) as well as those from ARC patients (84 ± 11; P < .025) relative to controls (61 ± 6). No significant differences in enzyme activity were noted in T cell-enriched cells in any group. Along with elevated enzyme activity, AIDS patients had small yet significant increases in the percentages of HLA-DR (P < .025), terminal deoxynucleotidyl transferase (TdT) (P < .0001), and peanut agglutinin receptor (P < .0001) positive lymphocytes in the null fraction compared with controls. TdT-positive cells appeared morphologically as large lymphoblasts with irregular nuclei. The data imply that the cellular immune deficiency in AIDS is not a result of deficiencies in lymphocyte ADA or PNP activity, but is more likely associated with an increase in an immature and/or activated lymphocyte subset.

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A DENOSINE deaminase (ADA) is a purine enzyme that catalyzes the deamination of adenosine to inosine. Although it is widely dispersed in mammalian tissues, a specific deficiency of this enzyme has not been directly associated with disease states other than hereditary immunodeficiency.1 Both ADA and the enzyme terminal deoxynucleotidyl transferase (TdT) are relatively elevated in immature thymocytes and fall progressively as T cells mature.2 ADA and TdT levels are significantly increased in leukocytes of patients with acute lymphocytic leukemias, especially those of thymic (T cell) origin.3,4 Elevated levels of both enzymes have also been observed in lymphocytes activated by mitogens.5,6 Purine nucleoside phosphorylase (PNP) catalyzes the conversion of inosine to hypoxanthine. In the thymus, an inverse relationship between ADA and PNP activity has been demonstrated, with cortical thymocytes bearing the highest ADA activity and the lowest PNP activity.2 An inherited deficiency of PNP has been described in certain patients with T cell deficiency and normal B cell function.2 In addition, rat thymic cells infected with gross leukemia virus have high PNP levels and also contain high levels of TdT.8,9 Since 1981, severe opportunistic infections10,11 and malignancies, such as Kaposi’s sarcoma12 and non-Hodgkin’s lymphoma,13 have been reported in young homosexual males. These individuals have marked deficits in cellular immune function, including skin test anergy, decreased lymphocyte response to mitogens, and an absolute decrease in T helper cells.14,15 To determine whether there might be any relationships between the immunodeficiencies observed and the levels of ADA and PNP activity in peripheral blood lymphocytes, we measured these enzyme activities in purified null and T cell subpopulations from asymptomatic homosexuals, patients with acquired immune deficiency syndrome (AIDS)-related symptom complex (ARC), and patients with AIDS compared with a healthy heterosexual control group. Significant increases rather than deficiencies of both enzymes were noted in peripheral blood null cells from AIDS patients along with increases in the proportions of TdT-positive, PNA receptor-positive, and HLA-DR (Ia)-positive lymphocytes.

MATERIALS AND METHODS

Subjects

Purine enzymes were studied in peripheral blood lymphocytes from 30 homosexual males. Seven were asymptomatic and were self-referred because of their concern about AIDS. Seven had two or more of the AIDS-related symptoms, and 16 had documented Kaposi’s sarcoma, opportunistic infections, or both (AIDS). No patients had received immunotherapy or chemotherapy prior to the study. Twelve healthy heterosexual males and 11 healthy females were self-referred because of their concern about AIDS.
served as controls. In addition, peripheral blood lymphocytes from 12 of the original 16 patients with AIDS and 15 controls were examined for immature or activated lymphocyte markers as described below.

**Lymphocyte Separation Techniques**

Peripheral blood mononuclear cells (PBMCs) were purified from defibrinated blood by Ficoll-Hypaque solution density centrifugation. PBMCs were depleted of monocytes and B cells and enriched for null and T cells by two methods. In the first method, nylon wool-purified lymphocytes were incubated for ten minutes with neuraminidase-treated sheep RBCs (SRBCs), pelleted by centrifugation, and placed on ice for 30 minutes. The E-rosette-containing pellet was gently resuspended, layered on Ficoll-Hyphaque, and centrifuged for 15 minutes at 800 g. Pelleted SRBCs were lysed with 0.14 Tnis-NH4Cl and resuspended in phosphate-buffered saline (PBS). In method 2, PBMCs were enriched for T cells by panning. An amount of 3 x 10^7 PBMCs in 3 mL of PBS was placed on F(ab')2 goat anti-human Ig (150 μg/mL) (Cappel Laboratories, Cochranville, Pa)-coated 20 x 100-mm polystyrene Petri dishes (Falcon). PBMCs were depleted of monocytes and B cells and enriched for null and T cells by two methods. In the first method, nylon wool-purified lymphocytes were incubated for ten minutes with neuraminidase-treated sheep RBCs (SRBCs), pelleted by centrifugation, and placed on ice for 30 minutes. The E-rosette-containing pellet was gently resuspended, layered on Ficoll-Hyphaque, and centrifuged for 15 minutes at 800 g. Pelleted SRBCs were lysed with 0.14 Tnis-NH4Cl and resuspended in phosphate-buffered saline (PBS). In method 2, PBMCs were enriched for T cells by panning.

**Lymphocyte Surface Markers in Null Cell- and T Cell-Enriched Subsets**

From 0.5 to 1 x 10^6 null cell-enriched and T cell-enriched lymphocytes from 15 controls and 12 homosexuals with AIDS were aliquoted into 12 x 75-mm glass tubes (Fisher Scientific, Houston). Ten microliters (1 μL) of the fluorescein-conjugated monoclonal antibodies anti-HLA-DR and anti-Leu 1 (Becton Dickinson, Mountain View, Calif) were added. Lymphocytes were incubated on ice for 30 minutes on ice. Five micrograms of anti-Leu 1 -treated cells at a concentration of 4 x 10^6 cells per milliliter and pretreated OEs were centrifuged at 300 g for five minutes and incubated on ice for 60 minutes. Following resuspension, rosette-positive lymphocytes were separated from rosette-negative lymphocytes by centrifugation at 500 g for 30 minutes in Ficoll-Hyphaque. Red cells were lysed using Tris-NH4Cl. Lymphocytes were washed twice in RPMI and resuspended in PBS. Null cell-enriched and T cell-enriched cells obtained by either technique were examined for monocyte contamination using fluorescein isothiocyanate (FITC)-conjugated polyvalent rabbit anti-human Ig (Cappel). Fluorescent-positive lymphocytes were enumerated by using a Leitz Ortholux (Wetzlar, Germany) phase contrast microscope.

**TdT Determination**

Cytoplasmic TdT was assessed according to the method of Bollum using an assay kit supplied by Bethesda Research Laboratories (Gaithersburg, Md).

**Peanut Agglutinin (PNA) Receptor Assay**

From 0.5 to 1 x 10^6 null cell- and T cell-enriched lymphocytes were pelleted in 15 x 75-mm glass tubes (Fisher) and 50 μg of FITC-labeled PNA (Vector Laboratories, Burlingame, Calif) was added. Following a 20-minute incubation at 4 °C, cells were washed twice and pelleted, and a drop of cell suspension was placed on a microscope slide. The percentage of fluorescence-positive lymphocytes was enumerated by phase contrast (100 x oil immersion objective).

**Measurement of ADA and PNP**

ADA and PNP activity were measured in null cell- and T cell-enriched lymphocytes using a modification of the technique of Van Laarhoven et al. One-microliter aliquots containing from 1,000 to 3,000 lymphocytes in normal saline and 0.5% bovine serum albumin (BSA) (Sigma Scientific, St Louis) were added to individual wells of Terasaki microtiter plates (Falcon Plastics), frozen at −30 °C, and lyophilized. Five-microliter aliquots of ADA reaction mixture containing 0.26 mmol/L [8-14C]-adenosine (specific activity, 20 mCi/mmol; ICN Pharmaceuticals, Irvine, Calif), 0.5% BSA, 0.8 μg/mL streptomycin, 0.8 μg/mL penicillin in 0.17 mmol/L Tris-HCl buffer (pH 7.4) (GIBCO, Grand Island, NY), and PNP reaction mixture containing 0.17 mol/L Tris-HCl, pH 7.0; 0.5% BSA; 0.8% Pen-strep; 3.3 mmol/L EDTA; 4.7 mmol/L phosphate; and 0.26 mmol/L [8-14C]-inosine (Amersham, Arlington Heights, Ill; specific activity, 50 mCi/mmol) were added to each cell extract. Plates were incubated for 30 minutes (ADA) or two hours (PNP) in high humidity at 37 °C. Under these assay conditions, the amount of product formed was linear with respect to cell number and length of incubation. From 10% to 15% of substrate was converted to product over the specified time intervals. After incubation, plates were placed in ice, and 1-μL aliquots were removed and spotted on α-cellulose-coated thin-layer chromatography (TLC) plates (Analtech, Inc, Newark, Del). Plates were incubated for two hours in ADA solvent (butanol: NH4OH:H2O = 100:16:5) or PNP solvent (ethanol: NH4Ac = 4:1) and dried. Products were identified by ultraviolet (UV) fluorescence and were scraped off and added to 5-mL plastic counting vials (Curtin Matheson Scientific, Houston) along with 3 mL aquasol (Packard Instruments, Downers Grove, Ill). Radioactivity in cpm was measured using a β-scintillation counter (Packard Instruments) and the percentage of substrate that converted to product was calculated. Results were expressed as nmol of converted substrate/10^6 lymphocytes/h.

**RESULTS**

**Percent Yield and Purity of Lymphocyte Subpopulations**

The average yield of nylon wool column-purified lymphocytes expressed as a percentage of the total number of Ficoll-Hyphaque density gradient-purified mononuclear cells obtained from 60 mL of whole blood was significantly lower in ARC (49% ± 3%; mean ± SD) and AIDS patients (53% ± 6%) compared with controls (71% ± 10%; P < .005 and P <...
The percentage of yield of null cell-enriched lymphocytes obtained after neuraminidase-treated SRBC separation was significantly higher in ARC (37% ± 31%) and AIDS (22% ± 17%) patients as compared with controls (10% ± 8%; \( P < .005 \) and \( P < .025 \), respectively). Moreover, the percent yield of T cell-enriched lymphocytes in both ARC (38% ± 18%) and AIDS (35% ± 16%) patients was significantly lower than in controls (62% ± 9%; \( P < .005 \) and \( P < .001 \), respectively). No differences in yield of lymphocyte subpopulations were observed between controls and asymptomatic homosexuals.

In contrast to the above, the yield of nonadherent lymphocytes obtained following panning (method 2) was similar in ARC patients (68% ± 9%), AIDS patients (58% ± 27%), and asymptomatic homosexuals (69% ± 10%) as compared with controls (70% ± 9%). The yield of null cell-enriched cells following ox erythrocyte rosetting remained significantly higher in ARC patients (23% ± 5%) and AIDS patients (30% ± 8%) compared with controls (16% ± 3%; \( P < .025 \) and \( P < .005 \), respectively). There were no differences, however, in the yield of T cell-enriched lymphocytes between groups. Hence, overall yield was lower following the nylon wool column procedure, most likely because of a loss of adherent T cells on the column. Despite this, the purity of null and T lymphocyte subfractions with respect to monocyte and B cell contamination as well as T cell enrichment was similar regardless of the separation procedure used (Table 1).

**ADA and PNP Activity in Null Cell- and T Cell-Enriched Subpopulations**

Both ADA and PNP activity expressed as nmol/10⁶ lymphocytes/h are shown in Table 2. Mean ADA activity was slightly higher in null lymphocytes from controls (\( P = .051 \)) and markedly higher in null lymphocytes from all homosexuals combined (\( P < .001 \)) than in T lymphocytes from each group respectively. ADA activity in null cell-enriched cells from AIDS patients was significantly elevated (161 ± 8; \( P < .025 \)) as compared with controls (127 ± 8; \( P < .025 \)) and asymptomatic homosexuals (114 ± 11; \( P < .025 \)). There were no significant differences in ADA activity in null cells between patients with AIDS and patients with ARC. Neither was ADA activity significantly increased in patients with ARC compared with controls.

**Table 2. ADA and PNP Activity in Null Cell- and T Cell-Enriched Lymphocytes**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Null ADA</th>
<th>T ADA</th>
<th>Null PNP</th>
<th>T PNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>23</td>
<td>127 ± B*</td>
<td>107 ± 6t</td>
<td>61 ± 5</td>
<td>62 ± 5</td>
</tr>
<tr>
<td>Homosexuals</td>
<td>30</td>
<td>146 ± 8</td>
<td>109 ± 5t</td>
<td>85 ± 75</td>
<td>68 ± 3</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>7</td>
<td>114 ± 11</td>
<td>82 ± 10</td>
<td>53 ± 13</td>
<td>68 ± 3</td>
</tr>
<tr>
<td>ARC</td>
<td>7</td>
<td>139 ± 16</td>
<td>104 ± 5</td>
<td>84 ± 115</td>
<td>62 ± 3</td>
</tr>
<tr>
<td>AIDS</td>
<td>16</td>
<td>161 ± 125</td>
<td>121 ± 7</td>
<td>98 ± 10</td>
<td>72 ± 4</td>
</tr>
</tbody>
</table>

*Activity expressed as nmol/10⁶ lymphocytes/h; mean ± SEM.
†Control T cell-enriched v control null cell-enriched; \( P = .051 \).
‡Homosexual T v null; \( P < .001 \).
§Homosexuals v controls; \( P < .025 \).
||\( P < .005 \).

Enzyme activity per milligram of protein was not determined on all patient cells because of the low lymphocyte yields. However, in three AIDS patients and three controls in whom protein determinations were performed, the mean milligram of protein per 10⁶ null cells in AIDS (30.1 mg) did not differ from controls (30.6 mg). This indicates that the elevated enzyme activity in AIDS null cells was probably not caused by the presence of large blastlike cells with high protein content, but rather was increased on an individual cell basis. T cells also had similar protein content (AIDS, 34.1 mg; controls, 41.5 mg).

**TdT, PNA, and Surface Marker Expression in Null Cell- and T Cell-Enriched Lymphocytes**

We and others have previously determined that patients with AIDS have significantly elevated percentages of lymphocytes expressing the surface antigens T10 and HLA-DR along with a decreased percentage and absolute number of OKT4-positive lymphocytes.²¹ Because the highest enzyme levels were noted in the null cell-enriched cells from AIDS
patients, we measured the percentages of cells expressing the immature surface antigens, T10, and HLA-DR as well as Leu 1, PNA receptor, and the enzyme TdT in both null cell- and T cell-enriched subpopulations in 15 controls and in 12 of the 16 patients with AIDS (Table 3). In controls, a higher percentage of OKT10- and HLA-DR-positive lymphocytes were found in the null fraction (OKT10, 24 ± 6; HLA-DR, 11 ± 2) compared to the T fraction (OKT10, 8 ± 2; HLA-DR, 5 ± 1), whereas AIDS patients had similar percentages of OKT10- and HLA-DR-positive lymphocytes in each fraction. However, AIDS patients had significantly elevated percentages of HLA-DR-positive ($P < .025$), TdT-positive ($P < .0001$), and PNA-positive ($P < .0001$) lymphocytes in the null fraction as compared with controls. Significantly elevated percentages of OKT10- and anti–HLA-DR positive cells were also observed in the T cell-enriched fraction of AIDS patients compared with the T cell-enriched fraction of controls. No TdT-positive or PNA-positive lymphocytes were seen in the T cell-enriched fraction of any group studied.

As seen in Figs 1 and 2, the TdT-positive cells observed in the null fraction of AIDS patients were generally large lymphocytes (ie, greater than 12 μm) with an increased nuclear to cytoplasmic ratio. Only cells with both cytoplasmic and nuclear staining for TdT were counted.

### DISCUSSION

Although the pathophysiology of AIDS is not entirely clear, the findings of this report shed additional light on several hypothetical considerations. ADA and TdT are elevated in human and rat cortical thymocytes. Activity of both enzymes decreases pro-

<table>
<thead>
<tr>
<th>Table 3. Lymphocyte Surface Marker and TdT Expression in AIDS Patients</th>
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<tbody>
<tr>
<td><strong>Marker</strong></td>
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<tr>
<td>-------------</td>
</tr>
<tr>
<td>Null cell-enriched</td>
</tr>
<tr>
<td>OKT10</td>
</tr>
<tr>
<td>Anti–HLA-DR</td>
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<tr>
<td>Anti–TdT</td>
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<tr>
<td>PNA</td>
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<td>T cell-enriched</td>
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<tr>
<td>Anti–TdT</td>
</tr>
<tr>
<td>PNA</td>
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<tr>
<td>OKT3</td>
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</tbody>
</table>

NS, not significant.

*Student’s t test; controls v AIDS.
†Expressed as mean percentage of positive cells ± SEM.

Fig 1. (A) RPMI 8402 TdT-positive cell line (positive control) labeled with rabbit anti–TdT antibody followed by FITC-F(ab')2, goat anti-rabbit IgG. Nuclear as well as perinuclear staining is evident. (B) TdT-positive "null" cell from AIDS patient. (C) Null cells from AIDS patient stained with normal rabbit IgG followed by FITC-F(ab')2, GARlGg as above (negative control). Although moderate background fluorescence occurred, fluorescence intensity was less as compared with positive test (phase contrast; original magnification ×375).
gressively as cortical thymocytes mature into medul-
ary thymocytes and peripheral T cells.2 Hence, it is
possible that an increase in ADA activity in null cells
from AIDS patients, along with an increase in the
percentages of TdT-positive lymphocytes, could reflect
abnormalities in T cell differentiation. This hypothesis
is attractive in light of recent evidence that demon-
strates thymic atrophy in AIDS patients23 along with
elevated levels of thymosin-α.24

ADA levels in immature thymocytes have been
reported to be five- to tenfold higher than in peripheral
blood T cells.2 Because ADA levels in null lymphocytes
were only 13-fold higher than T cells in this study, it is
most likely that we are dealing with a very small subset
of immature and/or activated T cells within the null
cell fraction. An alternative explanation for the in-
crease in enzyme activity in AIDS might be the
selective loss of the OKT4-positive subpopulation that
could have very low ADA and PNP activity. However,
this is unlikely in view of similar percentages of con-
taminating anti-Leu I/OKT3-positive lymphocytes in
the null fraction of both AIDS patients and controls (17% ± 3% vs 11% ± 5%, respectively). Moreover,
in preliminary studies, we have measured ADA and
PNP levels in OKT4-positive cells in both AIDS
patients (ADA, 55 ± 37; PNP, 26 ± 11) and controls
(ADA, 36 ± 31; PNP, 27 ± 11) without noting signifi-
cant differences.

Unlike ADA and TdT, PNP levels are low in
cortical thymocytes and increase with subsequent
maturation.28 PNP is elevated over ADA in bone
marrow null cells26 as well as various T, B, and null
lymphoblastoid cell lines.27 Therefore, a more likely
explanation for the findings observed could be that a
normal compensatory bone marrow response is occur-
ing secondary to thymic, lymph node, or peripheral
destruction of helper T cells. The presence of immature
lymphocyte subsets expressing “activation” antigens
T10 or DR in association with thymic atrophy has also
been observed in disease states in which regeneration
of the lymphoid system is occurring, such as following
bone marrow transplantation.27 Hence, this hypothesis
is also feasible and would explain the occasional
appearance of TdT- and PNA-positive stem cells in the
peripheral blood. Also, in preliminary experiments,
we have not seen increased percentages of OKT9-
positive or OKT6-positive lymphocytes in AIDS,
which suggests that changes are occurring at the
prethymic level.

Both ADA and TdT levels have been shown to be
transiently increased in mitogen-activated lympho-
cytes.5,6 The increased percentage of large, blastlike
cells seen in AIDS could reflect T cell tropism sec-
dary to viral infection. In the last several years, consid-
erable evidence has been presented linking the cause
of AIDS to infection by a retrovirus of the human T cell
leukemia family, HTLV III.29,30 A virus of similar
serologic reactivity designated LAV has also been
isolated from AIDS and pre-AIDS patients in
France.34 HTLV III is cytopathic for helper (OKT4)
lymphocytes; T cell lines that have been infected with
virus and grown in the presence of interleukin 2
transform into large cells with basophilic cytoplasm
and indented nuclei. Occasional multinucleated giant
cells and numerous cells in mitosis are also seen.30 In
addition, from 35% to 45% of infected lymphocytes
collected from sperm samples in AIDS patients express
HLA-DR antigens and demonstrate reverse tran-
scriptase activity.35 Hence, the increases in enzymes
along with the presence of occasional TdT- and PNA
receptor-positive cells in the null cell fraction of AIDS
patients could be a direct result of infection by virus. It
is also possible that an association could exist between
T cell infection by HTLV III and thymic dysplasia.
For example, recent evidence demonstrates the pres-
ence of an antigen (P19) on thymic epithelium which
cross-reacts with an internal core protein of HTLV.36

Significantly elevated percentages of OKT10- and
HLA-DR-positive lymphocytes were also noted in the
T lymphocyte fraction of AIDS patients as compared
with controls (Table 3), yet significant increases in
enzymes, TdT-positive or PNA receptor-positive lym-
phocytes were not observed. These findings could most
likely represent a small subset of T cells which are
coeXpressing activation antigens. Nicholson et a137
have reported an increase in Ia-positive cells in ARC
patients that were of the suppressor (OKT8) pheno-
type. Additional studies using double immunofluores-
cent labeling techniques are needed to confirm these
observations.
In summary, the findings in this study confirm that the cellular immunodeficiencies manifested in AIDS are the result of, or are associated with, abnormal lymphocyte differentiation or activation rather than due to deficiencies of either enzyme. Additional studies should be directed toward elucidating the interactions between cytopathic viral infections in this disease, and their subsequent influence on thymic and bone marrow physiology.

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