Absence of Lymphocyte Ecto-5'-Nucleotidase in Infants With Reticuloendotheliosis and Eosinophilia (Omenn’s Syndrome)

By Erwin W. Gelfand, Debbie McCurdy, C. Pandu Rao, and Amos Cohen

Lymphocytes from three infants with reticuloendotheliosis and eosinophilia (Omenn’s syndrome) and immunodeficiency were assayed for 5'-nucleotidase activity. B and T lymphocytes from all three patients were totally deficient in ecto-5'-nucleotidase activity, but had normal levels of cytoplasmic 5'-nucleotidase. In contrast, cultured lymphocytes expressed normal ectoplasmic and cytoplasmic activities, suggesting that the lymphocyte-restricted enzyme deficiency was not likely a primary genetic defect. The deficiency of lymphocyte ecto-5'-nucleotidase was not associated with any abnormality of deoxynucleoside metabolism. The absence of lymphocyte ecto-5'-nucleotidase may be a characteristic feature of this syndrome and may help to distinguish this disease from others with similar manifestations.

Three enzymes involved in purine metabolism, adenosine deaminase (ADA), purine nucleoside phosphorylase (PNP), and 5'-nucleotidase (5'-NT), are thought to be important in normal lymphocyte development. Patients with a deficiency of ADA and PNP, who have an associated immunodeficiency disease, have marked abnormalities of deoxynucleoside metabolism. On the other hand, the possible role of 5'-NT in deoxynucleoside metabolism in lymphocytes is controversial.2

Similar to ADA and PNP activities, the activity of the lymphocyte surface enzyme ecto-5'-NT also appears to correlate with T and B cell maturation and can serve as a marker for lymphocyte differentiation.1 Enzyme activities are low in immature T cells, thymocytes, and in cord blood mononuclear cells, but are significantly higher in mature T and B cells.3-6 In several diseases, 5'-NT activity also appears to correlate with the degree of lymphocyte maturity. For example, in severe combined immunodeficiency disease, the activity of ecto-5'-NT is decreased in circulating T cells, suggesting an abnormality of lymphocyte differentiation.7 T acute lymphoblastic leukemia cells7 and B chronic lymphocytic leukemia cells8,9 have low enzyme levels as well, likely reflecting their emergence at an early stage of differentiation. In the early phase of infectious mononucleosis, a deficiency of enzyme activity has also been reported.10

Although several immunodeficiency diseases have been characterized by partial deficiency of ecto-5'-NT,11,12 there has been no syndrome associated with a total deficiency of this enzyme. We have studied three patients with reticuloendotheliosis (Omenn’s syndrome)13 in whom B and T lymphocytes were totally deficient in ecto-5'-NT activity. All three patients were immunodeficient, suggesting a common pathogenetic event being expressed in susceptible individuals. The studies indicate that this enzyme may serve as a valuable marker in this condition, but they do not support a role for ecto-5'-NT in controlling deoxynucleoside-mediated inhibition of lymphocyte growth and proliferation.

Materials and Methods

Cell Preparations

Mononuclear cell preparations were obtained following Ficoll-Hypaque gradient centrifugation; T cell-enriched and B cell-enriched populations were isolated following E rosette depletion.14 T colony cells were obtained as described,15 and T cell growth was maintained for several months in the presence of T cell growth factor.13 B cell lines were established and maintained following Epstein-Barr virus infection.16

Immunologic Studies

Serum immunoglobulins were quantitated by a nephelometric technique or radioimmunossay (for IgE).

Circulating T cells were enumerated by E rosette formation using sheep erythrocytes17 or in indirect immunofluorescence assays using a panel of anti-T cell monoclonal antibodies (OKT3, 4, 6, 8, 11; Ortho Diagnostics, Raritan, NJ).18 Circulating B cells were identified using fluorescein-conjugated goat anti-human immunoglobulin antisera.14

T lymphocyte proliferative responses to phytohemagglutinin (PHA) and allogeneic cells were carried out as described14 using 5 x 10^6 mononuclear cells/well. In studies assessing the effects of added deoxynucleosides on PHA-induced proliferation, different concentrations of deoxyadenosine (dAdo) or deoxyguanosine (dGuo) (Sigma, St. Louis, MO) were added at the initiation of culture.19 In cultures containing dAdo, an inhibitor of deoxynucleosine deaminase (ADA), erythro-9-(2-hydroxyl-3-nonyl)adenine (EHNA), was also present.

5'-Nucleotidase Assays

The assay for detecting ecto-5'-NT activity was carried out using intact cells and adenosine monophosphate as substrate, as described.20

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Clinical Features

During the course of their illness, all three patients presented with the typical features of reticuloendotheliosis, including a diffuse erythematous rash, hepatomegaly, lymphadenopathy, and eosinophilia similar to that previously described, only patient 3 did not have an enlarged spleen. The age of onset was between 2 wk and 5 mo, and survival was less than 1 yr. The parents of the third patient were consanguineous (second cousins) and there was a previously affected sibling in the first family. Of particular interest was that each patient appeared to have a different underlying primary condition with different degrees of humoral and cellular immunodeficiency. A partial description of patient 1 has been previously presented. His illness progressed, culminating in a T cell lymphoproliferative disorder. Patient 2 was diagnosed clinically and radiologically as a patient with cartilage-hair hypoplasia with Hirschsprung’s disease during the first week of life, prior to the onset of symptoms characteristic of Omenn’s syndrome. Patient 3 was initially diagnosed as a variant of severe combined immunodeficiency disease.

Laboratory Evaluation

Mean serum immunoglobulin determinations are given in Table 1. Levels of IgG were low in patients 1 and 3 and remained low, whereas patient 2 had normal levels for her age at 2–5 mo. IgM levels were normal in patient 1, elevated in patient 2, and low in patient 3. All three consistently demonstrated markedly elevated serum IgE levels. Circulating surface immunoglobulin positive (sIg+) B lymphocytes were present in normal numbers. Specific antibody responses to administered antigens (diptheria-pertussis-tetanus and polio) were not detected in any of the patients, and in two of the patients (patients 1 and 2), there was no response to the administration of bacteriophage \( \theta \chi 174 \).

Absolute lymphocyte counts varied with the stage of the illness (Table 1). All of the patients were lymphopenic initially, with absolute lymphocyte counts of 1,300, 300, and 350/cu mm, respectively. Over a period of time, numbers of lymphocytes increased dramatically in patient 1 to >100,000/cu mm, >5,000/cu mm in patient 2, and 1,500–2,500 in patient 3. During the latter stages of their illness, all formed normal numbers of rosettes with sheep erythrocytes, and lymphocytes from patients 2 and 3 showed normal reactivities to a panel of T cell monoclonal antibodies. They were, however, unable to mount delayed hypersensitivity reactions to several antigens (Candida albicans, dermatophyton, streptokinase-streptodornase) and failed to reject an allogeneic skin graft.

The proliferative responses to PHA were clearly abnormal in patient 3 and initially in patient 2; although subnormal, patient 1 initially had a significant response. The responses in patients 1 and 2 were normal subsequently (Table 1).

Thymic biopsies in patients 1 and 3 and necropsy on patient 2 were identical: the thymus contained few cells, only scattered lymphocytes, and an infiltration of

Table 1. Familial Reticuloendotheliosis—Immunologic Evaluation

<table>
<thead>
<tr>
<th></th>
<th>Patients*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Humoral immunity</strong></td>
<td></td>
</tr>
<tr>
<td>Serum immunoglobulins†</td>
<td></td>
</tr>
<tr>
<td>IgG (mg/dl)</td>
<td>200</td>
</tr>
<tr>
<td>A (mg/dl)</td>
<td>&lt;5</td>
</tr>
<tr>
<td>M (mg/dl)</td>
<td>57</td>
</tr>
<tr>
<td>E (ng/ml)</td>
<td>4,000</td>
</tr>
<tr>
<td>Percent sIg-bearing B cells‡</td>
<td>5</td>
</tr>
<tr>
<td><strong>Cell-mediated immunity</strong></td>
<td></td>
</tr>
<tr>
<td>Lymphocyte number (/cu mm)§</td>
<td>1,300</td>
</tr>
<tr>
<td>Percent E-rosettes</td>
<td></td>
</tr>
<tr>
<td>PHA-induced proliferation¶</td>
<td>17,000</td>
</tr>
</tbody>
</table>

*The ranges of patient values over a period of several weeks were always within ± 20% of the reported mean.
†Means of at least 3 determinations. (Normal range IgG 420–1,310 mg/dl, IgA 20–150 mg/dl, IgM 45–170 mg/dl, IgE <100 ng/ml).
‡Means of at least 2 determinations (normal range 5%–15%).
§Means of at least 4 determinations.
¶Means of at least 4 determinations (normal range 42%–70%).
§§Means of at least 4 determinations (normal range 40,000–80,000 cpm).
**Early refers to tests carried out prior to the onset of the skin rash and late studies after the appearance of the rash.
5'-NUCLEOTIDASE DEFICIENCY IN OMENN'S SYNDROME

Table 2. Quantitation of Lymphocyte 5'-Nucleotidase Activity

<table>
<thead>
<tr>
<th>Patient</th>
<th>Ecto-5'-Nucleotidase*</th>
<th>Endo-5'-Nucleotidase*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mononuclear Cells</td>
<td>Lymphocytes</td>
</tr>
<tr>
<td></td>
<td>T B Colony Cells</td>
<td>T B Mononuclear Cells</td>
</tr>
<tr>
<td>1</td>
<td>&lt;0.5 &lt;0.5 9.9 ± 2.9</td>
<td>— — —</td>
</tr>
<tr>
<td>2</td>
<td>&lt;0.5 &lt;0.5 &lt;0.5</td>
<td>— — 7 ± 3.2</td>
</tr>
<tr>
<td>3</td>
<td>&lt;0.5 — —</td>
<td>3 36 ± 9.4</td>
</tr>
<tr>
<td>Controls</td>
<td>20.8 ± 3.4 18.7 ± 4.5 23.4 ± 5.3</td>
<td>18.0 ± 4.1 2.0 ± 0.6 28.6 ± 11.4</td>
</tr>
</tbody>
</table>

The studies of ecto-5'-NT were carried out on at least 5 separate occasions, and twice on T and B cells and T cell colonies. On at least one occasion, adherent cells were depleted from the mononuclear cell preparation prior to assay in patients 1 and 2. Preparations of T cells contained greater than 95% E rosetting cells. B cell preparations contained 55%-75% surface immunoglobulin bearing cells. The T cell line was studied once and the B cell lines were studied on at least two occasions. The endo-5'-NT values represent the means ± SD of two separate determinations carried out in duplicate.

*Expressed as nmole/10⁶ cells/hr.

There was no corticomedullary demarcation, and Hassall's corpuscles were not seen.

5'-Nucleotidase

Screening of the patient lymphocytes for several purine pathway enzymes revealed normal levels of ADA and PNP activities. The activity of ecto-5'-NT was below the level of detection on peripheral blood mononuclear cells from all three patients (Table 2). In patients 1 and 2, separated T and B cells were similarly devoid of ecto-5'-NT activity. On the other hand, activity of the cytoplasmic 5'-nucleotidase assayed in patient lymphocytes was normal. Studies of enzyme activity on patient fibroblasts (range 75–110 nm/10⁶ cells/hr) and granulocytes (0.6–2.8 nm/10⁶ cells/hr) were within the normal range, as were activities on mononuclear cells from all three patients (Table 2). In patient lymphocytes, normal T cells were incubated in the presence of patient serum, patient cells, or cell lysates, but no inhibitors of normal ecto-5'-NT were detected.

Following culture for 7 to >100 days, patient lymphocytes demonstrated significant ecto-5'-NT activities (Table 2). For example, T colony cells in patient 1, a T cell line in patient 3, and B cell lines from patients 2 and 3 had essentially normal activities. It was of interest that initially (4–8 wk) the B cell line from patient 2 had no enzyme activity, but subsequently, enzyme activity was detected (Table 2).

Inhibition of Lymphocyte Proliferation in the Presence of Deoxynucleosides

To test for possible metabolic consequences of ecto-5'-NT deficiency on T cell growth, we determined if the absence of ecto-5'-NT affected the PHA-induced proliferative response of patient T cells when incubated in the presence of dAdo or dGuo. Figure 1 summarizes the results of these studies. Despite the absence of ecto-5'-NT and the inhibition of ADA by EHNA, the degree of dAdo- or dGuo-mediated inhibition of the PHA-induced proliferative response of patient or normal cells was not significantly different. Further, in patient 1, where sufficient cells were available, the addition of dGuo did not result in a significant accumulation of dGTP (data not shown).

DISCUSSION

The patients described in this report demonstrate all the clinical, laboratory, and pathologic characteristics of the syndrome originally described by Omenn, including repeated infections, eosinophilia, hepatosplenomegaly, lymphadenopathy, and eosinophilia. The many descriptions of this syndrome have emphasized the variable nature of the accompanying immunodeficiency. This was observed in our patients who, with the full manifestations of the disease, had normal numbers of T and B cells, had normal responses to PHA and mixed lymphocyte reactivity in two of the patients, but failed to reject a skin graft or mount specific cell-mediated or antibody responses to administered antigen. The findings of normal T cell numbers, normal T cell subset distribution, and partial function in the face of a grossly abnormal thymus is confusing. This combination of findings has been previously described in patients with combined immunodeficiency, and in graft-versus-host disease (GVHD). All of the patients had markedly elevated concentrations of serum IgE, a finding previously described in patients with abnormalities of T cell function as well as in patients with GVHD. Indeed, this clinical syndrome may be confused with GVHD, and patients with acute or chronic GVHD may have diminished but not absent levels of ecto-5'-NT (unpublished observations). GVHD was eliminated as a possible explanation of our findings, as HLA typing and karyotyping of peripheral blood mononuclear cells and cell lines from our patients did not
manifestations of the disease; patient 2, on the other hand, was studied on several occasions prior to the onset of the syndrome, and the immunologic findings were clearly different when compared to later studies (Table 1). 5'-NT was not assayed prior to the onset of the rash in patient 2.

The association of severe immunodeficiency with deficiency of ADA and PNP has demonstrated the unique role of purine metabolism in lymphocyte differentiation and maturation. Reduced levels of ecto-5'-NT have been associated with a variety of immune disorders. In several of these conditions, the low enzyme level is correlated with an immature stage of lymphoid differentiation. In others, such as congenital or acquired hypogammaglobulinemia or infectious mononucleosis, the partial deficiency is less clearly explained by abnormalities of differentiation but may reflect an imbalance in the proportions of lymphocyte subsets. The consistent finding in our three patients with Omenn's syndrome was the failure to detect ecto-5'-NT activity on circulating T or B cells. (In patient 2, lymphocytes obtained from a lymph node biopsy had no activity as well.) This was not likely a reflection of immaturity, as by all other marker criteria, the cells were mature in phenotype. The absence of this enzyme was restricted to freshly isolated lymphocytes; normal activities were observed on patient granulocytes, fibroblasts, and cultured T and B cells. In addition, lymphocytes obtained from both parents expressed normal enzyme levels. Because cultured lymphocytes did express enzyme activity, we can presume that this lymphocyte-restricted enzyme deficiency is not a primary genetic defect, but is possibly host-environment related. Although we were unable to detect circulating or cell-bound inhibitors in various mixing studies, this does not totally exclude some form of inhibitor. Unfortunately, antibodies to 5'-NT were not available to determine if the enzyme was indeed present but functionally inactive.

5'-NT converts nucleoside monophosphates to the corresponding nucleosides. Earlier models utilizing established T cell lines suggested a critical role for this enzyme in the metabolism or prevention of lymphotoxicity in the presence of deoxynucleosides. In our studies, the absence of ecto-5'-NT on patient T cells did not render them more sensitive to dAdo or dGuo when PHA-induced proliferation was measured. The similar sensitivities of patient and normal cells to dAdo in Fig. 1A, where 5'-NT was absent and ADA was inhibited, confirm the results of Boss et al., who assayed dAdo-induced growth inhibition of 5'-NT and ADA-deficient B cell lines. The finding of normal cytoplasmic or endo-5'-NT in these cells may be more

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**Fig. 1.** Deoxynucleoside-mediated inhibition of the PHA-induced proliferative response. Peripheral blood mononuclear cells (5 x 10⁷) were cultured in the presence of varying concentrations of deoxyadenosine or deoxyguanosine for 72 hr. Results are expressed as the percent thymidine uptake observed in cultures not containing added deoxynucleoside. (A) Deoxyadenosine-mediated inhibition. All cultures contained 7.5 μM EHNA; (○) normal cells, (△) cells from patient 2. (▪) patient cells in the presence of 7.5 μM EHNA alone. (●) control cells in the presence of 7.5 μM EHNA alone. (B) Deoxyguanosine-mediated inhibition. (□) Normal cells. (◇) cells from patient 1. (♦) cells from patient 2.

detect extraneous antigens or suggest the presence of foreign cells.

The variability in immunologic findings may be linked to the stage of the disease. Absolute lymphocyte counts, T cell numbers, and PHA-induced proliferation (patients 1 and 2) increased with time. When initially evaluated, patients 1 and 3 had all the clinical
relevant to the metabolism of these deoxynucleosides than the activity of the plasma membrane enzyme.\(^2\)

The absence of ecto-5'-NT may be a characteristic feature of this particular syndrome, labeled Omenn's syndrome or reticuloendotheliosis. As a diagnostic test, it may help to distinguish this disease from others that have similar manifestations. Of additional interest is the seemingly acquired nature of the syndrome. All three patients appeared healthy at birth and expressed the clinical features after a period of time. Similarly, the inability to express ecto-5'-NT did not appear to be due to an intrinsic defect of their lymphocytes. The clinical features and the absence of detectable enzyme activity may represent the consequences of an, as yet undeclared, extrinsic process (e.g., a viral infection). The presentation of this syndrome in three entirely different clinical settings—a patient with a T cell lymphoproliferative disorder, one with cartilage-hair hypoplasia, and one with combined immunodeficiency disease—may support a common (pathogenetic) event being expressed in immunocompromised individuals, leading to a lymphocyte-restricted enzyme deficiency.

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