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Distribution of 5′-nucleotidase in human lymphoid tissues

(plasma membrane/thymocytes/lymphoblasts/purine metabolism/deoxynucleotides)

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ABSTRACT **Low activity of 5′-nucleotidase (5′-ribonucleotide phosphohydrolase, EC 3.1.3.5) in T lymphoblasts may explain the marked sensitivity of this cell to deoxynucleotide accumulation when compared to B lymphoblasts. The relevance of such observations with cultured cells to the normal immune system requires the demonstration of similar differences in the 5′-nucleotidase activity of normal human lymphocyte subpopulations. Sheep erythrocyte (E) rosette-forming cells from normal thymus, tonsil, and peripheral mononuclear cells have 5′-nucleotidase activities of 1.7, 11.3, and 21.2 nmol/hr per 10^6 cells. Non-E-rosette forming cells from the peripheral blood or tonsil have 5′-nucleotidase activity comparable to the higher levels found in the peripheral E-RFC. Increased levels of 5′-nucleotidase activity may be a marker for post-thymic T lymphocytes. T lymphoblasts have 5′-nucleotidase activity similar to values demonstrated for E-RFC in thymus, whereas cultured B lymphoblasts have 5′-nucleotidase activity 15 times greater than that of T lymphoblasts. On the basis of these observations, the 5′-nucleotidase deficiency in congenital agammaglobulinemia has been reevaluated. In these patients the data indicate that peripheral E-rosette forming cells have the enzyme deficiency, demonstrating an abnormality of T lymphocytes in this disorder of immunoglobulin production.**

Lymphocyte 5′-nucleotidase (5′-ribonucleotide phosphohydrolase, EC 3.1.3.5) may have an important role in the regulation of the human immune system. Decreased activity of this purine catabolic enzyme has been observed in patients with primary immunoglobulin deficiency (1–4). However, it remains unclear whether an etiological relationship exists between the immune dysfunction and this enzyme deficiency.

A marked difference in 5′-nucleotidase activity has been proposed as the molecular basis for the differential susceptibility of B and T lymphoblast lines to the toxicity related to deoxynucleoside accumulation (5–7). T lymphoblasts are killed by deoxyadenosine or deoxyguanosine, rapidly accumulate dATP or dGTP, and have very low levels of 5′-nucleotidase (5–9). In contrast, B lymphoblasts grow normally in deoxyadenosine or deoxyguanosine, accumulate only small quantities of deoxynucleoside triphosphates, and have higher 5′-nucleotidase levels than T lymphoblasts (5–9). Whether cultured human lymphoid cells can serve as a relevant model for normal cells is not yet known. If the hypothesis relating deoxynucleoside-induced cytotoxicity to 5′-nucleotidase activity is applicable to the human immune system, then similar differences in 5′-nucleotidase in normal lymphoid cells must be demonstrated.

To clarify the role of lymphocyte 5′-nucleotidase in the regulation of the immune system, we have examined the distribution of this enzyme in human peripheral mononuclear cells and lymphoid tissues. Our observations suggest that the difference in 5′-nucleotidase activity observed between cultured T and B lymphoblasts also occurs in certain populations of T and B lymphocytes, and the expression of this enzyme may be linked to T-cell ontogeny.

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METHODS

Materials. Adenosine, inosine, and AMP were purchased from Sigma. From Amersham, we purchased [8-^{14}C]AMP (50 mCi/mmol; 1 Ci = 3.7×10^{10} becquerels). Adenosine 5′-[α,β-methylene]diphosphate (p[CH_2]pA) was purchased from Miles. Ficoll-Paque for mononuclear cell preparation was obtained from Pharmacia. Sheep erythrocytes were purchased from Microbiological Associates (Walkersville, MD). We obtained carbonyl iron for macrophage depletion of mononuclear cell preparations from BDH Chemicals Ltd. (Dorset, England). All other chemicals were of the highest quality commercially available.

Cell Suspensions. A cultured T lymphoblast cell line, MOLT-4, was obtained from HEM Research, Rockville, MD. The MOLT-4 line is characterized by 23% E-rosette forming cells and the absence of cell surface immunoglobulins. A B lymphoblast cell line, MGL-8, was a gift from J. Epstein (Johns Hopkins University). Four other B lymphoblast lines (HSC-3, HSC-28, HSC-49, and HSC-58) and four T lymphoblast lines (CEM, Jurkat, MSB-2, and MOLT-3) were maintained and characterized as described (10).

Peripheral lymphocytes were obtained, separated into E-rosette forming cells and non-E-rosette forming cells, and characterized by described methods (11–13).

Lymphoid organs were obtained from children with no known immune dysfunction, and cells were separated from these tissues within 1 hr of surgery by using described methods (14). Thymus biopsies were performed during cardiac surgery and tonsils were obtained after their removal as a part of normal medical management. Monocytes were obtained by using the microexudate adhesion technique (15), and granulocytes were obtained from the buffy coat interface between erythrocytes and Ficoll during a standard Ficoll/Hypaque centrifugation.

Assay. Assay of 5′-nucleotidase was carried out on intact lymphocytes within 2 hr of venipuncture or cell preparation. Incubations were performed in duplicate at 37°C in a total reaction volume of 100 μl containing approximately 0.25×10^6 lymphocytes. The lymphocytes were added to a mixture containing 150 mM NaCl, 10 mM Tris·HCl (pH 7.4), 3 mM $MgCl_2$, and 200 μM [8-^{14}C]AMP. The incubations were carried out at 37°C for 30 min, and the reaction was stopped by heating to 85°C for 5 min. Determination of enzyme activity has been described (2).

RESULTS

Unfractionated cells from different normal lymphoid organs were examined (Table 1). Mononuclear cells from peripheral blood and tonsil had similar 5′-nucleotidase activities. The 5′-nucleotidase content of human thymocytes was substantially

Abbreviations: E, sheep erythrocyte; p[CH_2]pA (AOPCP), adenosine 5′-[α,β-methylene]diphosphate.

Table 1. Activity of 5′-nucleotidase in cells of the human immune system

Cell source	5′-Nucleotidase,* nmol/hr per 10^6 cells		
	Unfractionated cells	E-rosette forming cells†	Non-E-rosette forming cells‡
Peripheral blood mononuclear cells (9)§	21.9 (12.8–47.1)	21.2 (11.9–33.6)	24.0 (12.0–43.2)
Tonsil (5)	25.9 (13.0–34.4)	11.3 (5.9–23.5)	34.5 (15.8–55.8)
Thymus (4)	1.7 (1.4–2.1)	1.7 (1.4–2.1)	—
Peripheral monocyte (3)	—	—	4.2 (3.6–4.8)
Peripheral granulocyte (3)	—	—	1.9 (1.5–2.3)
Cultured T lymphoblast (5)	1.8 (1.2–2.0)	—	—
Cultured B lymphoblast (5)	26.1 (6.7–47.2)	—	—

* Enzyme levels are the mean values for multiple determinations with the range of values in parentheses.
† These fractions contain more than 90% E-rosette forming cells.
‡ These fractions contain less than 10% E-rosette forming cells. In peripheral blood, approximately 80% of the cells were esterase, sIg, or C_3 positive. In tonsil, 90% of the cells were C_3 or sIg positive and only 5% were esterase positive.
§ Number of individual determinations performed in duplicate.

lower than that of either peripheral blood or tonsil and similar to that observed in continuous T lymphoblast lines.

E-rosette forming cells from thymus had low 5′-nucleotidase activity identical to that of the unfractionated cells from that organ. Peripheral blood and tonsillar E-rosette forming cells had substantially higher enzymatic activity. The mean 5′-nucleotidase activity for tonsillar E-rosette forming cells was approximately half of the value for peripheral blood mononuclear cells. However, there was considerable overlap of 5′-nucleotidase values between these two tissues.

The 5′-nucleotidase activity in non-E-rosette forming cells from peripheral blood and tonsil was higher than that of E-rosette forming cells. The enzyme activity in non-E-rosette forming cells from tonsil exceeded the activity of peripheral blood non-E-rosetting cells. These tissues had 5′-nucleotidase activity similar to cultured B lymphoblasts (Table 1). In contast, peripheral monocytes and granulocytes had low values for 5′-nucleotidase.

To assess whether the AMP dephosphorylating activity measured by our enzyme assay on the lymphocyte surface is specific for 5′-nucleotidase or is a nonspecific phosphatase, inhibition of dephosphorylating activity by 25 μM p[CH_2]pA was performed. p[CH_2]pA, an ADP analog, is an inhibitor of 5′-nucleotidase but not of the nonspecific phosphates (16). Therefore, that portion of dephosphorylating activity inhibited by p[CH_2]pA represents 5′-nucleotidase (Table 2), whereas the uninhibited activity represents nonspecific phosphatase. The degradation of AMP was inhibited by greater than 85% in peripheral lymphocytes, in lymphocytes from tonsil and thymus, and in cultured B lymphoblasts. Cultured T lymphoblasts, peripheral monocytes, and granulocytes all demonstrated less than 25% inhibition of dephosphorylating activity with p[CH_2]pA. These observations show that, although thymocytes and T lymphoblasts have similar low plasma membrane dephosphorylating activity, the enzyme responsible for the hydrolytic activity in the two cell populations appears to be different.

Table 2. Inhibition of 5′-nucleotidase by p[CH_2]pA

Cell source	% inhibition with p[CH_2]pA (25 μM)
Peripheral blood	
Mixed mononuclear cells	96
E-rosette forming cells	91
Non-E-rosette forming cells	91
Monocytes	14
Granulocytes	20
Thymus	
E-rosette forming cells	87
Adenoid	
Mixed mononuclear cells	97
Cultured cells	
T lymphoblast	24
B lymphoblast	97

The different tissue activities for 5′-nucleotidase may have bearing on the observations of 5′-nucleotidase deficiency in primary immunoglobulin deficiency (1–4). Because abnormal T lymphocyte subpopulations may underlie the pathogenesis of the immune deficiency in these diseases (17, 18), we compared the 5′-nucleotidase activity in peripheral E-rosette forming lymphocytes from nine normal subjects and five patients with congenital agammaglobulinemia (Table 3). We observed enzyme activities of 21.2 (11.9–33.6) nmol/hr per 10^6 cells in normal subjects and 7.1 (3.3–11.8) nmol/hr per 10^6 cells in these patients with congenital agammaglobulinemia. These observations suggest that the enzyme deficiency originally

Table 3. Peripheral mononuclear cell 5′-nucleotidase in congenital agammaglobulinemia

	5′-Nucleotidase,* nmol/hr per 10^6 cells	
	Normal subjects (9)†	Congenital agammaglobulinemia (5)†
Unfractionated cells	21.9 (12.8–47.1)	5.9 (3.3–11.8)
E-rosette forming cells	21.2 (11.9–33.6)	7.1 (3.3–11.8)
Non-E-rosette forming cells	24.0 (12.0–43.2)	6.6 (2.8–8.4)

* Enzyme levels are mean values for multiple determinations with the range of values in parentheses.
† Number of individual determinations performed in duplicate. All five patients were characterized by infantile-onset agammaglobulinemia and the absence of circulating B lymphocytes.

described in mixed peripheral mononuclear cells (2) also involves T cells and is not simply due to the absence of B cells in these patients.

DISCUSSION

The enzyme 5′-nucleotidase is localized to the plasma membrane and catalyzes the dephosphorylation of 5′-nucleotide monophosphates (AMP, IMP, GMP) by a hydrolysis reaction. The large difference in 5′-nucleotidase activity between T and B lymphoblasts (6, 7) has prompted the measurement of this enzyme activity in human lymphoid tissues. The current observations indicate that normal human thymocytes have a low value for 5′-nucleotidase similar to that seen in T lymphoblasts. Peripheral blood mononuclear cells and tonsillar cells have a value for 5′-nucleotidase similar to B lymphoblasts. Observations by others in human and other mammalian lymphoid tissue support these data (19–24).

The difference between 5′-nucleotidase activity in thymocytes and peripheral circulating T lymphocytes suggest that this enzyme may be a marker for post-thymic T lymphocytes. The acquisition of greater plasma membrane 5′-nucleotidase activity may be one of many T-lymphocyte surface modifications occurring during T-cell differentiation (25). The E-rosette forming cells (T cells) from the peripheral blood display a similar range of values as that seen in unfractionated cells. The tonsillar T lymphocytes have a mean 5′-nucleotidase activity that is intermediate between peripheral T cells and thymocytes, although there is considerable overlap between the peripheral blood and tonsillar cells. Non-E-rosette forming cells tend to have higher values for 5′-nucleotidase than E-rosette forming cells. This is especially true in the non-E-rosette forming cells from the tonsil, which contain a greater proportion of B lymphocytes than a comparably prepared population from the peripheral circulation.

Inhibition of 5′-nucleotidase activity by adenosine-α,β-methylene diphosphonate is specific for 5′-nucleotidase but does not affect nonspecific phosphatase (2, 16). Our observations indicate that the activity measured in peripheral mononuclear cells, thymocytes, and B lymphoblasts is specific 5′-nucleotidase. The activity in monocytes, granulocytes, and T lymphoblasts is mainly nonspecific phosphatase.

If the hypothesis relating low 5′-nucleotidase activity and lymphocyte sensitivity to deoxynucleoside toxicity is correct (5, 6), then the low activity of 5′-nucleotidase in human thymocytes should indicate high susceptibility to deoxynucleoside toxicity. Indeed our observations of nucleoside toxicity in thymocytes support this conclusion (unpublished results). In addition, adenosine deaminase inhibition blocks the maturation of precursor T cells to T lymphocytes, but has no effect on mature T cells or the maturation of precursor B cells to immunoglobulin-producing cells (26, 27). These observations are compatible with the proposed role of 5′-nucleotidase in lymphocyte sensitivity to deoxynucleosides, because it appears that the immature T cells are the lymphocyte population with low 5′-nucleotidase.

The decreased 5′-nucleotidase activity in peripheral blood mononuclear cells from patients with primary immunoglobulin deficiency (1–4) has been reconsidered in the light of the distribution of 5′-nucleotidase in normal lymphoid tissues. Deficient activity of 5′-nucleotidase in these patients could posssibly result from absent B cells, increased monocytes, or decreased T-lymphocyte 5′-nucleotidase. Both the peripheral T and non-T lymphocytes from patients with congenital agammaglobulinemia and 5′-nucleotidase deficiency have decreased 5′-nucleotidase activity when compared to normal peripheral lymphocyte subpopulations (Table 3). Thus, the previously observed enzyme deficiency in congenital agammaglobulinemia cannot be accounted for entirely by a shift of mononuclear cell populations. These findings support the hypothesis that abnormal T lymphocytes may be responsible for the perpetuation of disease in certain patients with primary immunoglobulin deficiency (17, 18).

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