Clin. exp. Immunol. (1982) **50**, 148–154.

Enzymes of purine metabolism in human peripheral lymphocyte subpopulations

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(Accepted for publication 7 May 1982)

SUMMARY

Ecto-5′nucleotidase (5′NT), adenosine deaminase (ADA), purine nucleoside phosphorylase (PNP) and deoxycytidine (CdR), deoxyguanosine (GdR), deoxyadenosine (AdR) and adenosine (AR) kinases have been measured in subpopulations of peripheral blood lymphocytes of eight healthy volunteers. The separation of B, T, T helper/inducer and T suppressor/cytotoxic cells was performed by means of density gradient centrifugation, E rosetting, passage through a nylon-wool column and antibody affinity chromatography utilising OKT8 and OKT4 monoclonal antibodies. ADA was significantly higher in T lymphocytes and 5′NT in B lymphocytes. Among T cell subpopulations, 5′NT activity was significantly higher ($P < 0{\cdot}01$) in T suppressor/cytotoxic ($OKT8^+$) cells (32·9 units/10^6 cells than in T helper/inducer ($OKT4^+$) cells (9·7 units/10^6 cells). Indeed, the 5′NT activity in T suppressor cells was similar to that in B cells. T helper cells tended, however, to have higher PNP and ADA activities than T suppressor cells but the differences were not statistically significant. No major differences were noted in kinase activities between any of the lymphocyte subpopulations.

INTRODUCTION

The importance of purine metabolism in lymphocyte function was suggested by the discovery of deficiencies of adenosine deaminase (ADA), purine nucleoside phosphorylase (PNP) and ecto-5′nucleotidase (5′NT) in some patients with congenital immunodeficiency (Giblett *et al.*, 1972, 1975; Johnson *et al.*, 1977).

In previous studies different thymic subsets have been found to show specific purine enzyme patterns (Barton *et al.*, 1980; Ma *et al.*, 1982). These differences are thought to be relevant to the different types of immune defects found in patients with congenital ADA or PNP deficiency.

T cells have been shown to be more sensitive than B cells to deoxynucleoside toxicity *in vitro* (Carson, Kaye & Seegmiller, 1978; Gelfand, Lee & Dosch, 1979). Functional studies of human peripheral blood lymphocytes *in vitro* have shown that the generation of antigen specific suppressor T lymphocytes is selectively affected by deoxyguanosine (GdR) (Gelfand *et al.*, 1979). This finding was confirmed in mice by Dosch *et al.* (1980). GdR was shown to be selectively toxic against T suppressor/cytotoxic (T-S/C) function, whereas T helper/inducer (T-H/I) function and B lymphocyte differentiation to plasma cells were not affected (Gelfand *et al.*, 1979; Dosch *et al.*, 1980). These differences in sensitivity may be explained by the variation in the activities of purine degradative and synthetic enzymes in B lymphocytes and subsets of peripheral T lymphocytes.

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0009-9104/82/1000-0148$02.00

The present study was undertaken to determine the levels of ADA, PNP and 5′NT, and the activities of nucleoside and deoxynucleoside kinases in B lymphocytes and phenotypic subsets of T lymphocytes, separated from normal human peripheral blood.

MATERIALS AND METHODS

One hundred and fifty to one hundred and eighty millilitres of fresh heparinized venous blood were obtained from eight healthy normal donors. Mononuclear cells were separated by Ficoll-Triosil density gradient centrifugation (Böyum, 1968). Monocytes were removed by the plastic adherent method (Messner, Till & McCulloch, 1973). The recovered lymphocytes were layered onto a pre-washed nylon-wool column (8×10^6 cells/100 mg/ml) (Greaves & Brown, 1974). Non-adherent cells (T lymphocytes) were eluted using warm medium. Adherent cells (mainly B lymphocytes) were eluted by mechanical agitation and further purified by depleting T lymphocytes using the E rosetting method. Non-adherent cells (T lymphocytes) were further separated into $OKT4^+$ T-H/I lymphocytes and $OKT8^+$ T-S/C lymphocytes. Cell–antibody affinity chromatography was used as previously described (Tidman *et al.*, 1981), the monoclonal antibodies OKT4 and OKT8 being used (1:500 dilution) for the affinity column separation.

Indirect immunofluorescence was performed on separated lymphocyte populations (Tidman *et al.*, 1981). Monoclonal antibodies (McAbs), OKT3, OKT4 and OKT8 were used. Goat antiserum, FITC conjugated, against human immunoglobulins was used to identify B lymphocytes.

The radioactive thymidine autoradiographic procedure was used for cell cycle analysis. Cells with more than five grains were considered to be in S phase. The labelling index (LI) was calculated by counting at least 1,000 cells (Ma *et al.*, 1982).

All enzyme assays were performed radioisotopically utilizing the methods recently described in detail elsewhere. 5′NT was studied in intact fresh cells, whereas ADA, PNP, deoxynucleoside and nucleoside kinases were assayed in cell extracts prepared from lymphocytes and preserved at −70°C for not longer than 1 week. Briefly, 5′NT was measured by conversion of ^{3}H-AMP to ^{3}H-adenosine, ^{3}H-inosine and ^{3}H-hypoxanthine. Products were separated by paper chromatography in ammonium formate. PNP was assayed by the conversion of ^{3}H-inosine to ^{3}H-hypoxanthine, which were separated by paper chromatography in a mixture of methanol, boric acid and water. ADA was measured by the conversion of ^{3}H-adenosine to ^{3}H-inosine. Substrate and product were separated by chromatography in ammonium formate. Deoxyadenosine (AdR), deoxyguanosine (GdR), deoxycytidine (CdR) and adenosine (AR) kinase activities were measured by the conversion of ^{3}H-labelled nucleosides or deoxynucleosides to the corresponding nucleotides or deoxynucleotides which were then separated by ion exchange chromatography in ammonium formate.

Nucleated cell count was performed using an electronic cell counter (Coulter S, UK). Viability was performed using the trypan blue exclusion test.

Statistical significance was calculated using the Mann–Whitney *U*-test. Results were expressed as mean ± standard error. ADA activity was expressed in units/10^8 cells (1 unit = 1 μmol substrate converted/hr). PNP, 5′NT and kinase activities were expressed in units/10^6 cells (1 unit = 1 nmol substrate converted/hr).

RESULTS

Separation of lymphocyte subpopulations

Lymphocyte subsets were isolated from the peripheral blood samples of the eight healthy donors by means of the nylon-wool column. The non-adherent cell fraction contained an enriched population of T lymphocytes (83·5 ± 2·3%). Of this, 63 ± 2·7% were of the T helper/inducer phenotype ($OKT4^+$), whereas 20·6 ± 2·3% were of the T suppressor/cytotoxic cell phenotype ($OKT8^+$). There was invariably less than 3% of B lymphocytes in the non-adherent cell fraction.

Enriched B lymphocyte populations were obtained from seven healthy donors after passing their peripheral lymphocytes through nylon-wool columns and depletion of E rosette forming cells.

There were more than 93% B lymphocytes (surface immunoglobulin positive) with less than 5% T cell (OKT3$^+$) contamination.

In five cases, the T lymphocytes were further separated by antibody affinity chromatography using the monoclonal antibody OKT8. In the non-adherent cell fraction, there were $90 \pm 1{\cdot}5\%$ OKT4$^+$ T-H/I lymphocytes and in the adherent cell fraction there were $93 \pm 1{\cdot}1\%$ T-S/C lymphocytes (OKT8$^+$). In one case, the monoclonal antibody OKT4 was used and the recovered non-adherent cells were 85% OKT8$^+$ T-S/C lymphocytes, whereas in the adherent cell fraction there were 96% OKT4$^+$ T-H/I lymphocytes.

The viability in all lymphocyte subpopulations isolated was greater than 90%.

Enzyme assays

Peripheral T lymphocytes had significantly higher ADA activity ($5{\cdot}4 \pm 0{\cdot}6$ units/10^8 cells) than B lymphocytes ($2{\cdot}6 \pm 0{\cdot}8$ units/10^8 cells) ($P < 0{\cdot}05$). In contrast, 5′NT activity was higher in B lymphocytes ($37{\cdot}9 \pm 2{\cdot}3$ units/10^6 cells) than in T lymphocytes ($22{\cdot}9 \pm 10^6$ cells) ($P < 0{\cdot}01$) (Fig. 1). The kinase activities were similar in T and B lymphocytes except for GdR and AR kinases, which were higher (not significantly) in T lymphocytes (Table 1).

ADA was higher (not significantly) in T-H/I ($7{\cdot}4 \pm 1{\cdot}0$ units/10^8 cells) than in T-S/C lymphocytes ($4{\cdot}5 \pm 1{\cdot}0$ units/10^8 cells) (Fig. 2). PNP was also higher in T-H/I than in T-S/C cells but the difference was of borderline significance ($P = 0{\cdot}05$). In contrast, 5′NT activity was three times higher in T-S/C lymphocytes ($32{\cdot}9 \pm 3{\cdot}0$ units/10^6 cells) compared to T-H/I cells ($9{\cdot}7 \pm 0{\cdot}9$ units/10^6 cells) ($P < 0{\cdot}01$).

In order to check that the monoclonal antibody used in separation did not affect surface 5′NT activity, the separation was carried out with OKT4 rather than OKT8. ADA, PNP and 5′NT results were identical when T-H/I and T-S/C lymphocytes were separated using OKT4 instead of OKT8 (data not shown). Moreover, 5′NT enzyme activity in T lymphocytes incubated with McAb OKT8 for 30 min was not different from activity of control lymphocytes incubated alone (data not shown).

Kinase activities were similar in T-H/I and T-S/C populations (Table 1).

No proliferative activity was detected in all the fractionated lymphocytes (LI < 0·1%).

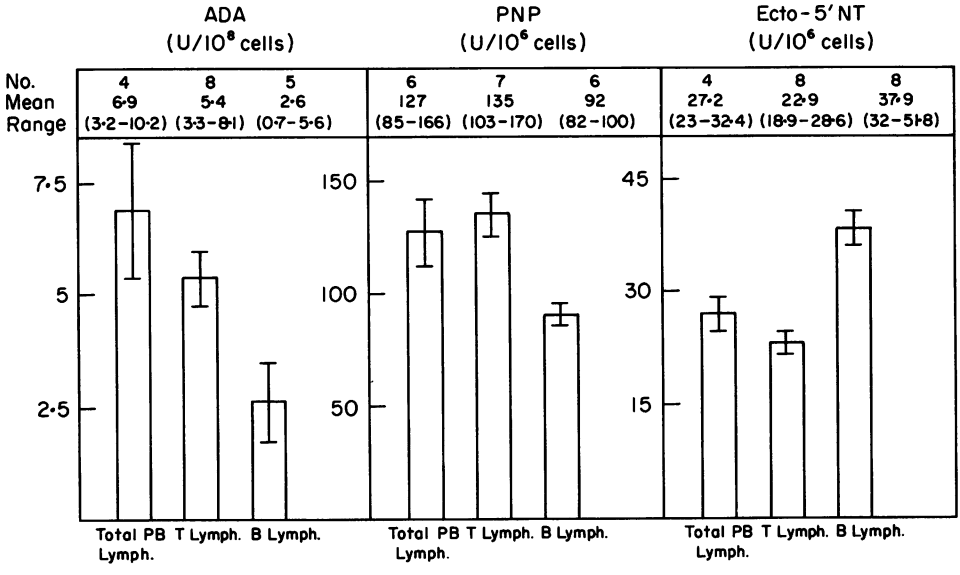

Fig. 1. Purine degradative enzymes in peripheral lymphocyte subpopulations.

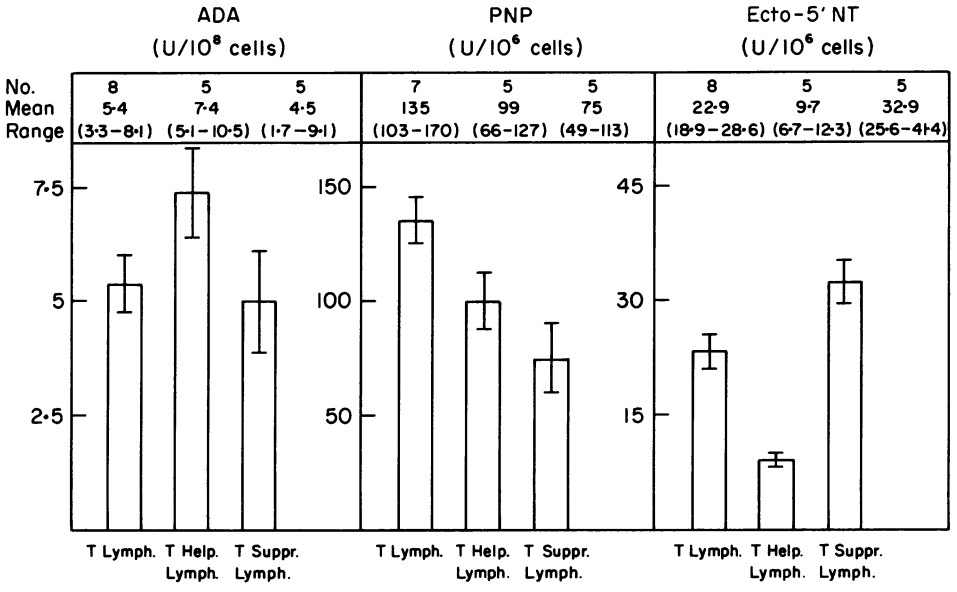

Fig. 2. Purine degradative enzymes in peripheral T lymphocyte subsets.

Table 1. Kinase activities in peripheral lymphocyte subpopulations

	GdR-K	AdR-K	AR-K	CdR-K
	(U/10⁶ cells)*			
Unseparated peripheral blood lymphocytes	0·6±0·1†	0·4±0·2	1·7±0·7	0·5±0·06
T lymphocytes	1·2±0·3	0·8±0·2	2·5±0·6	0·3±0·06
B lymphocytes	0·5±0·2	0·8±0·3	1·5±0·3	0·5±0·1
T helper lymphocytes	0·2±0·05	0·2±0·08	0·3±0·1	0·3±0·08
T suppressor lymphocytes	0·3±0·01	0·2±0·08	0·1±0·04	0·3±0·08

* U = nmol/hr; † mean ± s.e.

DISCUSSION

In this study, normal peripheral blood T lymphocytes were found to have higher adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) activities but lower ecto-5'nucleotidase (5'NT) activity than B cells. These findings are in agreement with previous reports (Thompson *et al.*, 1979; Rowe *et al.*, 1979; McDermott, Tritsch & Formeister, 1980). In the analysis of the T lymphocyte subsets, T helper/inducer (T-H/I) cells were found to have slightly higher ADA and PNP activities but remarkably lower 5'NT activity than T suppressor/cytotoxic (T-S/C) lymphocytes. Zachowski *et al.* (1981) reported that murine thymomas of T-H/I phenotype exhibit 5'NT activity, whereas thymomas of T-S/C phenotype lack 5'NT activity. However, 5'NT activity shows an opposite pattern in normal T and B rat lymphocytes compared to human lymphocytes (Barton & Goldschneider, 1978). The significant difference in 5'NT activity found here between T-H/I and T-S/C populations was not due to membrane binding by McAb OKT8 affecting the

enzyme assay because, when McAb OKT4 was used in the separation, the same differences in 5'NT activity between T-H/I and T-S/C cells were found.

Deficiency of 5'NT found in some patients with congenital hypogammaglobulinaemia is believed not to be the cause but rather to reflect the stage of lymphoid differentiation at which T cell development is arrested (Webster *et al.*, 1979; Thompson *et al.*, 1979). This is supported by the observations that lower levels of 5'NT occur in cortical thymocytes, compared to functionally more mature medullary thymocytes and peripheral T lymphocytes (Edwards *et al.*, 1979). An increase in T-S/C population has been found in the peripheral blood in infectious mononucleosis (De Waele, Thielemans & Van Camp, 1981). These T-S/C cells are found to express large amounts of Ia antigen (De Waele *et al.*, 1981) but low 5'NT activity (Quagliata *et al.*, 1974). Occurrence of Ia antigen in T-H/I and T-S/C lymphocytes is thought to be a marker of activation (Reinherz *et al.*, 1979). In the present study, B lymphocytes as well as T-S/C lymphocytes expressed high 5'NT activity. The explanation for this apparent discrepancy probably arises because we have investigated non-proliferating lymphocytes as indicated by their low labelling index (LI). It appears that low 5'NT activity may be related not only to developmental immaturity of lymphocytes but also to proliferative activation of subsets of mature T lymphocytes.

High levels of 5'NT are thought to protect against deoxynucleoside toxicity (Wortman *et al.*, 1979). In spite of the high level of 5'NT in T-S/C lymphocytes, their function is highly sensitive to GdR toxicity (Gelfand *et al.*, 1979). The present findings demonstrate that T-S/C precursor cells do not have a purine enzyme pattern similar to thymocytes as suggested by Dosch *et al.* (1980) but their susceptibility to GdR toxicity may be expressed only during their activation, which is proliferative dependent (Dosch & Gelfand, 1979; Siegal & Siegal, 1977). Our preliminary data show that GdR, AR, AdR and CdR kinase activities are increased in T lymphocytes after mitogen stimulation and 5'NT is decreased (Massaia, unpublished observations).

Recently Carson, Kaye & Wasson (1981) described a soluble deoxynucleotidase activity which may play a more important role than ecto-5'NT in protection from deoxynucleoside toxicity. This endo-deoxynucleotidase activity is distinguishable from ecto-5'NT, and correlated better than 5'NT with AR sensitivity.

In the present study, CdR and AR kinase activities were measured as well as GdR and AdR kinase activity. CdR kinase is thought to play a role in AdR toxicity because mouse T lymphoma cells lacking CdR kinase are AdR resistant (Ullman *et al.*, 1979). However, AR kinase is thought to be the physiologically important kinase, phosphorylating AdR (Ullman *et al.*, 1981). In the present study, GdR and AR kinase activities were found to be slightly higher in T than in B lymphocytes but differences were insignificant. North, Newton & Webster (1980) reported no difference in GdR kinase activity between T and B cell lines, T and B tonsil lymphocytes and between T and B peripheral lymphocytes. In the present study, the activities of all the kinases measured were found to be very similar in T-H/I and T-S/C lymphocytes.

Cells exhibiting high levels of ADA and PNP activities are thought to be particularly dependent on these enzymes for their survival and function (Barton & Goldschneider, 1979). Deoxycoformycin treatment in Thy-ALL was based upon this theoretical rationale (Prentice *et al.*, 1980). In the present study we found little differences in ADA or PNP activity between T-H/I and T-S/C lymphocytes, the latter tending to have a lower level. Simpkins, Stanton & Davis (1981) claimed ADA activity was higher in Tγ lymphocytes than in non-Tγ lymphocytes. These results, however, are not necessarily in conflict with our data because little correlation exists between T cell subsets defined by McAb OKT8 and by Fc receptor for IgG (Reinherz *et al.*, 1980). Although the OKT4 population consists mainly of 'helper' cells, it also contains a subset of T lymphocytes with suppressor activity on the pokeweed mitogen induced B cell differentiation test (Thomas *et al.*, 1981). It would obviously be of interest to examine the various purine enzyme activities, in particular 5'NT activity, in this OKT4$^+$ suppressor subpopulation.

M.M. is supported by Comitato G. Ghirotti, Italy. D.D.F.M. is supported by the Clothworkers' Foundation and the travelling fellowship of the Royal Australasia College of Physicians. T.A.S. is supported by The Wellcome Trust. Monoclonal antibodies OKT3, OKT4 and OKT8 were a gift from the Ortho Diagnostic Systems Ltd. We are grateful to Miss J. Allaway for the typing of the manuscript.

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