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Nucleotide Metabolism and Enzyme Inhibitors in Thymic Acute Lymphoblastic Leukaemia *

A. V. Hoffbrand, D. D. F. Ma, and H. G. Prentice

A. Introduction

It is now clear that not only the immunological but also the biochemical characteristics of leukaemia cells are similar to those of the normal cells from which they arise. This has been most clearly established for the lymphoid malignancies and particularly in thymic-derived acute lymphoblastic leukaemia (Thy-ALL). Close similarities between Thy-ALL leukaemic blast cells and early cortical thymocytes in surface membrane antigen phenotype, in terminal deoxyribonucleotidyl transferase (TdT) content and in pattern of purine enzymes have recently been established [5]. These findings have helped to establish the exact cell of origin of Thy-ALL and made possible its diagnosis by single cell analysis in bone marrow or extra medullary sites. They have also led to the treatment of this disease with 2'deoxycoformycin (dCF), a specific inhibitor of the purine degradative enzyme, adenosine deaminase (ADA). Part of the stimulus to this research into the biochemistry of the thymus and the treatment of Thy-ALL with dCF has arisen from observations on children with severe defects of immune development due to congenital deficiency of ADA or of a second purine degradative enzyme, purine nucleoside phosphorylase (PNP). Lack of these enzymes causes absence of T- and B-lymphocytes or of T-lymphocytes respectively but other functions of the body, including haemopoiesis are largely unaffected.

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B. Purine Enzyme Patterns: Normal Tissues and Established Cell Lines

Studies in rats [1] and more recently in humans [12] have shown that the activities of several enzymes involved in purine metabolism differ widely between different lymphocyte subpopulations, both between B and T cells and in the B- and T-cell lineages, according to the degree of differentiation of the cell studied. ADA is concerned with the degradation of deoxyadenosine and adenosine to deoxyinosine and inosine respectively. It is present in all tissues, but its activity is highest in cortical thymocytes and decreases as T cells mature. In humans, the earliest cortical thymocytes (early cortical blasts or "prothymocytes") have the highest level of all, whereas in rats, immature cortical thymocytes and bone marrow prothymocytes have lower levels than the majority of thymic cortical cells. ADA activity in both humans and rats is higher in mature T cells than in B cells.

PNP is a consecutive enzyme with ADA in purine degradation, breaking down deoxyinosine and inosine to hypoxanthine, and also deoxyguanosine, guanosine and xanthosine to xanthine. In rat lymphocyte populations, a reciprocal relationship exists between ADA and PNP, since cortical thymocytes have high ADA and low PNP levels whereas medullary thymocytes and circulating T cells have high PNP and low ADA levels. Human thymocytes show a similar reciprocal relationship except in prothymocytes which have high PNP as well as high ADA levels.

A third enzyme, 5′-nucleotidase, exists on the surface of lymphocytes which is capable of degrading deoxynucleoside monophosphates to the corresponding deoxynucleosides. The exact biological function of this ectoenzyme is unclear. The activity amongst T-cell subpopulations in humans closely parallel that of PNP, being low in cortical thymocytes and higher in mature T cells [12]. 5′NT activity is, however, substantially greater on the surface of B- than T-lymphocytes and among mature T-lymphocytes; the activity is greater on T suppressor ($OKT8^+$) cells than on T helper ($OKT4^+$) cells [15]. Although 5′NT has been found low in the lymphocytes of patients with congenital agammaglobulinaemia, this is thought to be more a result of the lack of B cells than a cause of the condition. The activity of a recently described endonucleotidase [2] among different lymphoid populations is as yet unknown.

Other enzymes concerned in deoxynucleotide degradation are also more active in B cells than T cells. These include ecto-ATPase and thymidine phosphorylase. Moreover, studies with established cell lines have shown that immature T cells are unable to functionally compartmentalise thymine nucleotides into a degradative as well as a synthetic compartment, whereas other cell types have a degradative compartment for deoxynucleotides as well as a synthetic compartment destined to be incorporated in DNA [22]. Recent studies with established cell lines also show that as cells mature in B-cell development from c-ALL through pre-B-ALL to mature B cells, so the ability to degrade DNA precursors increases [23]. PHA-transformed T-lymphocytes, normal human bone marrow cells and myeloid cell lines all also have substantial degradative compartments. Virtually all the thymine nucleotides in Thy-ALL lines, and presumably in early thymocytes (although this has not been studied directly) are destined to be incorporated into DNA, however. This is thought to be due to the operation of a highly efficient multi-enzyme complex in Thy-ALL cells, synthesising thymine nucleotides and providing dTTP at the DNA replication fork without leakage of distal precursors to a degradative compartment. Such efficient complexes are likely to operate in supplying the other three deoxynucleoside triphosphates (dNTP), deoxyadenosine- (dA-), deoxyguanosine- (dG-) and deoxycytidine- (dC-) TP. Thus, Thy-ALL and normal thymic cortical cells have low levels of deoxyribonucleoside- and deoxyribonucleotide-degrading enzymes except for ADA. They also lack a degradative compartment for syphoning off excess deoxyribonucleotides. They are, therefore, peculiarly prone to dATP or dGTP toxicity if ADA of PNP are absent or inhibited, when excess amounts of dATP or dGTP respectively are built up.

C. Terminal Deoxynucleotidyl Transferase

This unusual DNA polymerase, like ADA, is present in high concentrations in prothymocytes and cortical thymocytes. It is absent from mature T cells. The only other normal cells containing TdT are a small proportion of bone marrow cells and these have been shown to exhibit the phenotype of c-ALL or pre-B-ALL [7], which have both been identified as early B cells because of the gene rearrangements they show. Thus TdT is a marker of early cells in the B- or T-cell lineages. The normal function of TdT is unknown, although it has been suggested to play a role in generation of immune diversity by altering the base composition of DNA. The optimum substrate for TdT is dGTP and Ma et al. [13] have recently speculated that in early thymocytes, TdT may polymerise excess dGTP or other dNTP to make single-stranded DNA polymers which if not incorporated into double-stranded DNA may be subsequently degraded with release of potentially toxic intracellular concentrations of the corresponding deoxynucleosides and deoxynucleotides.

D. ADA and PNP Deficiencies: Mechanisms of Cell Death

Children born with ADA deficiency show lack of T- and B-cell development. The lack of B cells may be due to absence of both suppressor and helper T cells needed

for B-cell maturation. The main mechanism by which ADA deficiency is toxic is thought to be dATP accumulation, due to failure of degradation of deoxyadenosine, with consequent allosteric inhibition of ribonucleotide reductase and failure of supply of the other three deoxynucleoside triphosphates with consequent cessation of DNA replication. Additional toxicity may be due to inhibition of *S*-adenosyl methionine (SAM) mediated methylation reactions because of inhibition of *S*-adenosyl homocysteine hydrolase by excess deoxyadenosine. Lowered ATP and raised cyclic AMP levels have also been found in ADA-deficient tissues and these may inhibit a wide variety of reactions in both replicating and non-replicating cells (see [6] for review). Most recently, Fox et al. [3] have shown that the combination of deoxyadenosine and ADA inhibition by erythro-9-(3-(2-hydroxynosyl)) adenosine leads to arrest of Thy-ALL lines in vitro in the G_1 phase of the cell cycle. This was associated with a rise in dATP in the G_1 phase, and this and the cell arrest could be prevented by deoxycytidine. Non-dividing T cells are also killed [8]. They have subsequently postulated that this may be due to incorporation of accumulated deoxyadenosine into the poly (A) tail of RNA with interference in the processing, transfer and transcription of messenger RNA [10].

PNP deficiency causes a much more selective lack of T cells with relative sparing of B cells and their function. Toxicity is thought to be mainly due to dGTP accumulation (at a later stage of T-cell maturation than in ADA deficiency) with inhibition of ribonucleotide reductase.

E. Enzyme Patterns in Leukaemic Cells

The pattern of purine enzymes and TdT in leukaemic cells shows a remarkably close similarity to the normal counterparts of these cells. Thus, Thy-ALL blasts, like early cortical thymocytes have, in general, high levels of ADA and TdT but lower concentrations of PNP and 5'NT [20]. On the other hand, more mature T-cell tumours (e.g. T-cell CLL, Sezary cells and T-prolymphocytic leukaemia) are TdT negative, have only moderately high ADA activity and show higher PNP and 5'NT levels than in Thy-ALL [14]. Although the normal bone marrow precursor cells from which AML and c-ALL arise have not been isolated in sufficient numbers and purity for biochemical analysis, it seems probable that the purine enzyme pattern and TdT content of blast cells in AML and c-ALL reproduce those of early bone marrow myeloid and lymphoid progenitors respectively, AML typically being TdT negative with moderately raised ADA, PNP and 5'NT and c-ALL being TdT positive with ADA lower, PNP and 5'NT higher than in Thy-ALL.

Nucleoside incorporation studies in blast cells of Thy-ALL have shown a pattern distinct from the blast cells in other types of acute leukaemia. Incorporation of deoxycytidine is raised and of thymidine low, so that the ratio of uptake of deoxycytidine to thymidine is higher in Thy-ALL than in c-ALL or AML blasts [17]. The levels of all four deoxynucleoside triphosphates (dNTP) are also usually considerably higher in Thy-ALL than in other acute leukaemias. This may partly be due to the larger number of cells in cycle in Thy-ALL, but the particularly high levels of dNTP suggest that this may also be due to greater synthetic and less degradative capacity of Thy-ALL blasts for deoxyribonucleotides. Normal thymocytes also show high concentrations of the dNTP [16].

F. Deoxycoformycin Therapy

The known dependence of early thymocytes on ADA led to the development of a specific ADA inhibitor 2'deoxycoformycin (dCF) as a potential immunosuppressive agent and for the treatment of thymic-derived tumours. A number of groups in the United Kingdom and the United States have used dCF to treat Thy-ALL and found the drug to be effective in obtaining a remission in the majority of cases, even those resistant to other forms of chemotherapy. In our own experience, a remission was obtained in 7 of 12 patients using a 5-day course of the drug at 0.25 mg/ kg each day. Two cases proved resistant and in three a partial remission

was obtained with one or two courses [18]. Patients with other T-cell tumours, e.g. T-prolymphocytic leukaemia and mycosis fungoides have also responded to dCF whereas cases of c-ALL and AML have, in our hands, proved resistant. Others, however, have obtained responses in c-ALL [4] and even in B-cell CLL [9].

The mechanism of cell killing has been analysed by serial biochemical studies. Blast cell death more closely correlates with dATP rise than with *S*-adenosyl homocysteine hydrolase inhibition [19]. Indeed, a predictive test for response based on the degree of dATP rise in blasts incubated with dCF and deoxyadenosine in vitro has been devised [18, 21].

G. Side Effects

The effects of dCF therapy in Thy-ALL on tissues other than the leukaemic cells could not have been predicted from observations on ADA-deficient children. Iritis, hepatic abnormalities, haemolysis (in 9 of 17 patients studied by Prentice et al. [18]), central nervous system toxicities and renal abnormalities including renal tubular necrosis have all been described. The mechanisms for these toxicities are not clear. Reduced red cell ATP concentrations have been demonstrated and postulated to lead to haemolysis. Interference with cyclic AMP and SAM-mediated reactions are further possibilities. Hyperuricaemia was a problem in early studies, but since the use of allopurinol this has been prevented.

H. In Vitro Removal of Thy-ALL Blasts

The selectivity of dCF therapy for Thy-ALL with sparing of haemopoiesis has aroused interest in the possibility of using dCF in vitro to remove selectively residual Thy-ALL blasts from bone marrow prior to autologous bone marrow transplantation. However, studies in cell lines have shown that ADA inhibition alone in vitro does not lead to death of T cells or other cell types. On the other hand, deoxyadenosine is toxic to cells in vitro and T cells are susceptible at lower concentrations than B cells or other cell types. Studies of the combination of dCF ($10^{-5}M$) and deoxyadenosine ($10^{-4}M$) have shown toxicity to Thy-ALL lines in vitro with considerable selectivity, growth of c-ALL, B- and myeloid cell lines being far less inhibited [11].

The use of the combination of dCF and deoxyadenosine in vitro for selective killing of residual Thy-ALL blasts prior to autologous bone marrow transplantation has not yet been used because of the long incubation period necessary to achieve substantial cell killing. For established Thy-ALL cell lines, 72 h incubation at 37 °C is needed to achieve over 80% cell death and studies of blast cells from individual patients with Thy-ALL have shown a similarly long incubation period to be necessary (Ma, Sylwestrowicz and Hoffbrand, unpublished observations).

It is not considered practical to maintain bone marrow in culture at 37 °C in vitro for 3 days free from contamination and with sufficient preservation of normal haemopoietic stem cells to ensure successful engraftment.

I. Conclusion

Many of the biochemical features of Thy-ALL reproduce those of early cortical thymocytes, and result in Thy-ALL blast cells being exquisitely dependent on adenosine deaminase to degrade deoxyadenosine. These observations have led to the use of deoxycoformycin, a specific ADA inhibitor, in treatment of Thy-ALL. Further studies of the biochemical make-up of the blast cells in different types of leukaemia, particularly of the organisation of DNA and RNA synthesis and degradation, are needed in order to improve the design of chemotherapy with antimetabolite and other drugs in these diseases.

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