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REVIEW

Metabolic defects in immunodeficiency diseases

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INTRODUCTION

There are two ways to find the cause of primary immunodeficiency diseases. One approach is to start with the immune defect and work backwards, using *in vitro* techniques to define where the primary abnormality lies in the immune response. Immunologists favour this approach for obvious reasons; and it is not without virtue since, for example, it has shown that the defect in most cases of primary hypogammaglobulinaemia is due to a failure of B lymphocyte maturation.

The alternative approach is to screen empirically for defects in biochemical pathways in the hope of finding a clue which will eventually lead to the underlying disorder. This is a sensible approach in diseases which are clearly inherited (e.g. X-linked hypogammaglobulinaemia) but is less attractive in a disease such as late onset hypogammaglobulinaemia which is not obviously inherited. In practice, such procedures involve screening the urine for abnormalities in the quality or quantity of excreted compounds. Another way is to screen for abnormalities in organelle integrity by measuring the activity of various enzymes in subcellular fractions. In reality, the clue to the metabolic defect is usually discovered by accident, the prime example in our field being the discovery of adenosine deaminase (ADA) deficiency.

PURINE METABOLIC DEFECTS

Adenosine deaminase (ADA) deficiency

The discovery of the association of ADA deficiency with severe combined immunodeficiency (SCID), and the genetics of this disease, have already been extensively reviewed (Meuwissen *et al.*, 1975; Giblett, 1979; Hirschhorn, 1979a). Suffice it to say that the link between ADA deficiency and immunodeficiency was recognised by chance when red cell ADA isoenzymes were being measured in a SCID infant to help show whether a bone marrow graft had been successful. Since then it has become firmly established that ADA deficiency is the direct cause of one type of autosomal recessive severe combined immunodeficiency (ADA$^-$SCID). However there were some doubts about this relationship when two cases were reported of severe red cell ADA deficiency and normal immunity; although both these have subsequently been shown to have significant ADA activity in lymphocytes (Hirschhorn & Ratech, 1980).

Clinical features. Children with ADA$^-$SCID are clinically indistinguishable from other SCID patients with normal ADA. In most cases the disease starts in infancy and there is gross depletion of T lymphocytes with an associated failure to produce antibodies. Lymphopenia is common. Some patients have a normal or raised percentage of B lymphocytes in their peripheral blood and this may be associated with the production of immunoglobulin, and sometimes even specific antibody (Hirschhorn, 1979b). This heterogeneity is probably due to differences in the severity of the ADA

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deficiency; those with residual enzyme activity tending to develop clinical immunodeficiency later in life, although usually before the age of 2 years. Some of these patients may have previously been described as Nezeloff's syndrome (Webster *et al.*, 1975). It has been estimated in the United States that ADA deficiency accounts for about 20% of SCID patients, although only three affected infants have been found in Great Britain during the past 5 years. The implication is that severe combined immunodeficiency is under-diagnosed in this country or that cases are not being properly investigated.

Metabolic abnormalities. The substrates for ADA (EC 3.5.4.4), adenosine (Ad) and deoxyadenosine (dAd), accumulate in ADA deficiency and are excreted in the urine, where their presence is diagnostic (see Fig. 1). The concentrations in the plasma are about 1–2 μM (Hirschhorn *et al.*, 1980). An important question is why only lymphocytes are affected in this condition. The probable explanation is that lymphocytes, unlike other tissues, have an enzyme which phosphorylates dAd to produce deoxyadenosine monophosphate (dAMP), (see Fig. 2). Deoxynucleotides cannot move freely across the plasma membrane, so dAMP is 'trapped' inside the lymphocyte and is further phosphorylated to deoxyadenosine triphosphate (dATP) (Carson, Kaye & Seegmiller, 1977). Lymphocytes can also phosphorylate adenosine but so can other tissues such as the liver and lungs. For this reason, and because adenosine is relatively weak at inhibiting *in vitro* transformation of ADA deficient lymphocytes, deoxyadenosine is thought to be the metabolite responsible for the immunodeficiency (Carson, 1980).

It has not been possible to fully investigate the metabolic basis of the disease using lymphocytes from infants and children with ADA$^-$SCID. This is because many are severely lymphopenic; although a few experiments have been done on the ADA$^-$ lymphocytes obtained from a child who responded to red cell transfusions (see below). Most of the work has been done on human or mouse lymphoblastoid cell lines which are either deficient in ADA or in which the ADA has been inhibited by either erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) or deoxycoformycin.

Lymphoblastoid cell lines. There is good evidence that ADA inhibited lymphoblastoid cell lines accumulate dATP when incubated with deoxyadenosine (Ullman *et al.*, 1978). T-derived cell lines accumulate more dATP than B-derived lines, although this difference is small and is only statistically significant when a number of T and B cell lines are compared (Carson, Kaye & Wasson,

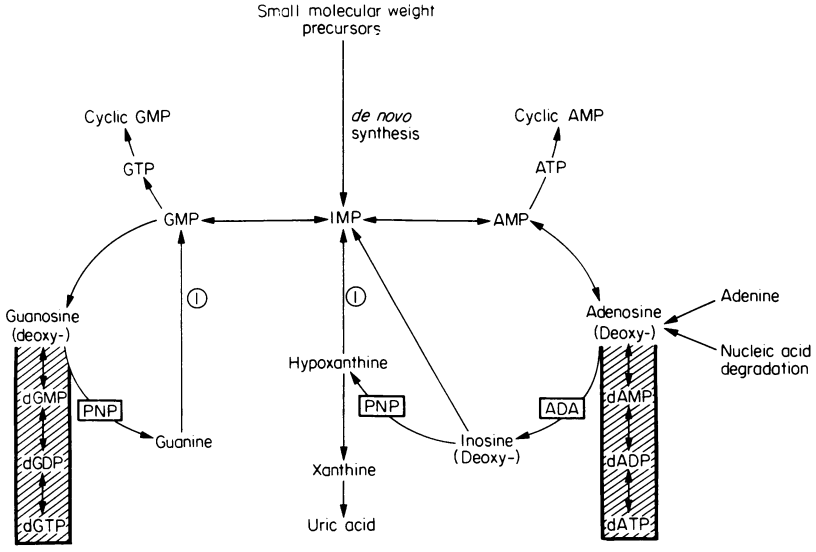

Fig. 1. Diagram to illustrate the relationship between *de novo*, interconversion and salvage pathways of purine metabolism. The rate of accumulation of dATP and dGTP (hatched areas), in the absence of ADA or PNP, depends upon the relative activities of kinases and phosphatases. (1) Hypoxanthine guanine phosphoribosyl transferase (HGPRT) is an important enzyme in the salvage of purines; but its absence does not lead to significant immunodeficiency.

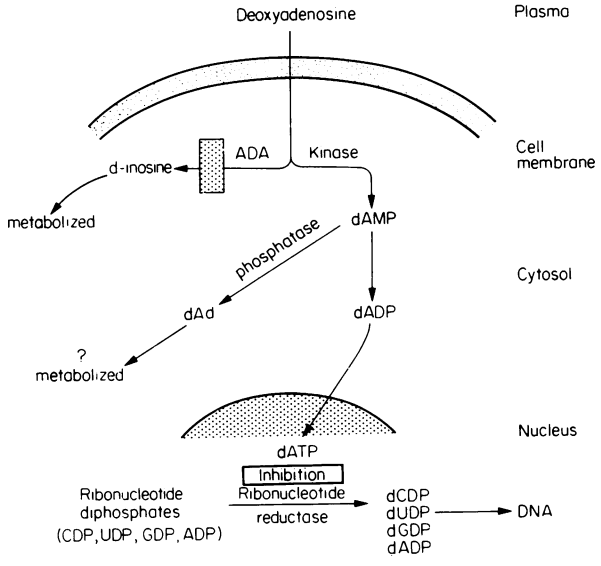

Fig. 2. Diagram to illustrate the metabolism of deoxyadenosine by lymphoblastoid cells. Deoxyadenosine (dAd) is normally converted to deoxyinosine which is then metabolized. In ADA deficiency, dAd is converted to dAMP by a lymphospecific kinase. Further phosphorylation produces dATP which inhibits ribonucleotide reductase, and consequently DNA synthesis B lymphocytes have a cytosol phosphatase which may prevent the accumulation of dATP. Theoretically, dAd will accumulate under these circumstances and should inhibit other important metabolic pathways. It is not clear why this does not happen in B lymphoblastoid cells. A similar sequence of events probably occurs in PNP^- lymphoblastoid cells exposed to deoxyguanosine (dG), although in this case dGTP accumulates.

1981). The explanation for this difference is that B cells have a high phosphatase activity in the cytosol which prevents the accumlation of toxic nucleotides (Fig. 1). Eriksson, Thelander & Akerman (1979) have shown that dATP allosterically inhibits ribonucleotide reductase which is thought to regulate DNA synthesis. Furthermore, the growth of mouse S49 mutant lymphoblastoid cells, whose ribonucleotide reductase is resistant to the allosteric effects of dATP, is not inhibited by deoxyadenosine; whereas growth is inhibited in other cell lines (Ullman *et al.*, 1980). There is therefore reasonable evidence in ADA deficient cell lines that the major effect of deoxyadenosine is to inhibit DNA synthesis. This may well explain the rapid disappearance from the circulation of leukaemic T lymphoblasts in patients treated with the ADA inhibitor, 2′-deoxycoformycin (Prentice *et al.*, 1980). However, lymphoblastoid cell lines and leukaemic lymphoblasts are very different from normal lymphocytes in that thay are programmed to repeatedly divide and presumably have no requirement for the normal triggering mechanisms for proliferation. For this reason, the findings in lymphoblastoid cell lines may be irrelevant to the aetiology of the immunodeficiency in ADA deficient children.

Findings in ADA^- blood lymphocytes. Most of the functional studies have been done on cells from normal subjects in which the ADA is inhibited with either EHNA or 2′-deoxycoformycin. Using such systems, it is clear the 1–2 μM deoxyadenosine inhibits the triggering of lymphocyte transformation in ADA inhibited human and rat T lymphocytes, and does not have any direct effect on DNA synthesis itself (Uberti, Lightbody & Johnson, 1979; Thuillier, Garreau & Cartier, 1981). B lymphocytes stimulated with Protein A are not as sensitive as T cells to these low concentrations of deoxyadenosine, although transformation is equally inhibited at dAd concentrations above 4μM. It is unclear whether these small *in vitro* differences can explain the presence of normal numbers of circulating B cells and serum immunoglobulins in some patients with ADA^-SCID. At all events, the studies outlined above show that the effect of dAd on ADA deficient lymphoblastoid cell lines is likely to be very different to that on lymphocytes under normal regulatory control. Interestingly, EB virus-induced proliferation and immunoglobulin synthesis by ADA^-B cells is usually unaffected by

deoxyadenosine concentrations below 10 μM. This is presumably because EBV bypasses the surface triggering events required for mitogen stimulation. It is likely that SCID infants are particularly susceptible to chronic EBV infection because they have very few cytotoxic T cells, and it is possible that polyclonal EBV stimulation accounts for the raised immunoglobulin levels and plasma cell proliferation seen in some ADA$^-$SCID patients.

Very little is known about the mechanisms involved in the triggering of lymphocytes by mitogens, and even less is known about how this could be upset in ADA deficiency. Polmar (1979) put forward some suggestions based on experiments with blood lymphocytes from an ADA deficient child. Such experiments were feasible because this child was one of the few who responded to red cell transfusions (see below). He showed that PHA stimulated lymphocytes were unusually sensitive to the inhibitory effects of prostaglandin E_1 or theophylline, implying that there was an over-production of cyclic adenosine monophosphate (cAMP). High concentrations of cAMP are known to inhibit lymphocyte transformation and it is likely that the ratio of cAMP/cGMP plays a role in cell triggering (Strom, Lundin & Carpenter, 1977). Although cyclic AMP levels were apparently raised in the lymphocytes of one ADA deficient patient (Schmalsteig *et al.*, 1977), this has not been investigated and confirmed in others. There is therefore still doubt about whether cAMP is directly involved in preventing lymphocyte proliferation in this disease.

There is general agreement that dATP levels are increased in the red cells of ADA deficient patients. ATP levels are also affected in both the red cells and lymphocytes, although the literature is confusing on this issue. The early reports claim that ATP levels are raised, although more recent evidence shows that they are depressed (Agarwal *et al.*, 1976; Polmar *et al.*, 1976; Simmonds *et al.*, 1982). These divergent findings presumably reflect difficulties in extracting and measuring these nucleotides. At all events, the likelihood is that red cell ATP is depressed, particularly since there is convincing evidence of low red cell ATP in patients given the ADA inhibitor 2′-deoxycoformycin (Siaw *et al.*, 1980). Data on the dATP and ATP levels in the lymphocytes of ADA$^-$SCID patients is hard to interpret because it is often not clear which cell populations are being studied.

Raised levels of adenosine or deoxyadenosine inhibit the metabolism of *S*-adenosylhomocysteine with consequent impairment of methylation reactions. This may be relevant since transmethylation of phospholipids may be involved in the triggering of lymphocyte proliferation (Maino, Hayman & Crumpton, 1975; Hirata & Axelrod, 1980).

Treatment. There was considerable optimism about the prognosis in ADA$^-$SCID patients when Polmar *et al.* (1975) first successfully treated an affected child with monthly red cell transfusions. The mechanism depends on the ADA in the transfused cells metabolizing the circulating deoxyadenosine to harmless deoxyinosine. The transfused red cells therefore act as a 'sponge' for the toxic metabolite. However, it is now known that less than 50% respond when treated with red cell transfusions. It is suggested that this reflects the severity of the ADA deficiency and the degree to which the thymus has been damaged; one affected child only responded to red cell transfusions when he was given additional thymosin (Rubinstein *et al.*, 1979). The concept that the thymus is particularly susceptible in ADA deficiency is supported by the finding that dATP accumulates in the thymus of mice treated with the ADA inhibitor 2′-deoxycoformycin (Nelson, LaFon & Lambe, 1979). Nevertheless, some patients are resistant to treatment with red cells and thymosin and then the only practical alternative is a bone marrow transplant. Deoxycytidine, which will prevent the growth inhibition *in vitro* of ADA$^-$ lymphocytes by dAd, has not yet been given a fair therapeutic trial in a ADA$^-$SCID patient.

Therapeutic use of ADA inhibitors. The finding that T lymphoblastoid cells are rapidly killed by 2′-deoxycoformycin indicated that this drug might be useful in the treatment of some forms of leukaemia. Early trials in patients with either terminal cancer or acute T lymphoblastic leukaemia showed that there was a profound depletion of normal T cells or lymphoblasts in most patients within 48 hr of giving the drug. The effect was reversible and there was a gradual recovery in T cell numbers over about 2 weeks (Smyth *et al.*, 1980). From the information summarized above it seems likely that leukaemic T lymphoblasts are killed predominantly through the effects of dATP on DNA synthesis. However, this is unlikely to be the explanation for the rapid disappearance of normal T cells from the circulation. The implication is that constant triggering of lymphocytes is required to maintain about 90% of the circulating T cells in normal subjects.

Purine nucleoside phosphorylase (PNP) deficiency

The prediction that other defects in purine metabolism would affect the immune system was realized within 3 years of the discovery of ADA deficiency. Giblett *et al.* (1975) found a deficiency of PNP in a child with severe depression of cellular immunity and very few circulating T cells. Unlike ADA⁻SCID, this child had normal antibody production following immunization. This in itself raises interesting questions about the relevance of helper T cells for immunoglobulin production *in vivo*. Since then about nine PNP deficient patients, some showing clear evidence of an autosomal mode of inheritance, have been reported (Ammann, 1979; Asherson & Webster, 1980). From a clinical point of view, these patients have less frequent infections than those with ADA⁻SCID, but are prone to chronic and potentially fatal cytomegalovirus and *Varicella zoster* viral infections. One patient has also died of vaccinia. Two patients have suffered from a progressive neurological disorder with spastic tetraplegia (Watson *et al.*, 1981) and at least two have developed an autoimmune haemolytic anaemia.

Metabolic abnormalities. The substrates for PNP (E.C.2.4.2.1), guanosine and deoxyguanosine (dG), accumulate in the plasma and are excreted in the urine (Fig. 1). The plasma levels of these compounds reach about 5 μM (Stoop *et al.*, 1980). Work on human PNP deficient lymphoid cell lines and on mutant PNP deficient mouse lymphoblasts show that these cells convert dG to deoxyguanosine triphosphate (dGTP) (Ochs *et al.*, 1979; Ullman *et al.*, 1979). This in turn inhibits DNA synthesis which probably explains why PNP deficient T lymphoblastoid cells are very sensitive to growth inhibition by as little as 5 μM dG. On the other hand, guanosine has no significant effect at similar concentrations and is therefore unlikely to have any relevance to the immunodeficiency.

From an immunologist's point of view, the fundamental interest in this condition concerns the differential effect on T and B cells. Paralleling ADA deficiency, PNP⁻ T cell lines are very sensitive to growth inhibition by dG, in contrast to B cell lines which are relatively resistant (Ochs *et al.*, 1979). One possible explanation for this dichotomy is that B cells fail to 'trap' dG because they are unable to phosphorylate this compound. However, this is not the case since both human B lymphoblasts and normal blood B cells are as good as T cells at phosphorylating dG (Carson *et al.*, 1981; North, Newton & Webster, 1981). The explanation for the insensitivity of B lymphoblasts to dG parallels that for deoxyadenosine in ADA deficiency; here again the B derived lines seem to be protected from accumulating dGTP by having a relatively high phosphatase activity (Fig. 2).

By analogy with ADA deficiency, the work on lymphoblastoid cell lines may be misleading and the mechanism of the immunodeficiency may be due to a failure of lymphocyte triggering. This can now probably be investigated *in vitro* using the new specific PNP inhibitor, 8-aminoguanosine (Kazmers *et al.*, 1981).

Treatment. Red cell transfusions were effective in raising the blood T cell numbers in a PNP deficient patient in the Netherlands (Zegers *et al.*, 1979). However, this had no effect on the central nervous system disease in this case and another in England, (A.R. Watson—personal communication). As in ADA deficiency, the red cells act as a 'sponge' although in this case they apparently metabolize dG and prevent this from interfering with lymphocyte function.

Deoxycytidine should theoretically compete with dG for deoxycytidine kinase in the cytosol, thus preventing the accumulation of dGTP. Although this happens *in vitro*, deoxycytidine therapy has not helped affected patients. This may be because it is not possible to reach high enough concentrations of deoxycytidine *in vivo*, and because there is a mitochondrial kinase which preferentially phosphorylates dG (Gower, Carr & Ives, 1979).

MISCELLANEOUS METABOLIC ABNORMALITIES

Ecto 5'Nucleotidase (5'-N)

Hypogammaglobulinaemia. While investigating lymphocyte plasma membranes (Johnson *et al.*, 1977), we found that the lymphocytes of patients with late onset primary hypogammaglobulinaemia

had low activity of the enzyme 5'nucleotidase. Subsequent work showed that this enzyme was located on the surface of the plasma membrane and that it had low activity in both the T and B cells of patients with primary hypogammaglobulinaemia (Webster *et al.*, 1978; Rowe *et al.*, 1980). The T cells of patients with X-linked hypogammaglobulinaemia also had low ecto 5'-N suggesting that these patients had a more generalized lymphocyte disorder than had previously been realized. B lymphocytes have about four times as much 5'-N activity as T cells in normal subjects, although it is interesting that the monoclonal B cells in chronic lymphatic leukaemia have extremely low activity (Quagliata *et al.*, 1974).

It was soon established that inhibiting the 5'-N activity with α, β-methylene adenosine diphosphate had no effect on *in vitro* lymphocyte transformation, immunoglobulin production or cytotoxicity. It is therefore extremely unlikely that the enzyme defect itself has any relevance to the hypogammaglobulinaemia (Webster *et al.*, 1979). Work on animal testicular and cartilage cells suggests that 5'-N appears in the plasma membrane when cells mature and differentiate but disappears when they proliferate (Roden, Bourret & Cutler 1977; Adams & Harkness, 1976). Most workers now feel that its absence on lymphocytes reflects immaturity, and this is supported by other studies which suggest that both the T and B cells of patients with late onset hypogammaglobulinaemia are immature (Matamoros *et al.*, 1979; De Gast *et al.*, 1980).

The question still remains why lymphocytes, and not other blood elements, have such high activity of ecto 5'-N. The main problem is to identify the origin of the physiological substrate. A probable candidate is adenosine diphosphate (ADP) released from platelets during aggregation, which can then be converted by lymphocyte ecto-ADPase to adenosine monophosphate (Smith *et al.*, 1981). Thus lymphocytes have the capacity to convert ADP to adenosine, the latter having the property of de-aggregating platelets and dilating blood vessels (Fig. 3). The significance of this ecto enzyme system, which is similar to that found on the endothelial cells of blood vessels, is unclear but it may be involved in facilitating lymphocyte traffic to and from sites of inflammation. At all events, it is interesting that the lymphocytes of patients with primary hypogammaglobulinaemia have normal ecto ADPase activities, showing that the low ecto 5'-N activity is highly selective and not due to a generalized plasma membrane defect.

Esterases and lactate dehydrogenase. Apart from 5'-nucleotidase deficiency, various other biochemical abnormalities have been found in the T lymphocytes of patients with late onset hypogammaglobulinaemia. The circulating T cells in most patients lack non-specific esterase granules (Matamoros *et al.*, 1979) and have very low activity of the cytosol enzymes, malate and lactate dehydrogenase (Shah, Webster & Peters, 1981). The latter appears to be mainly due to an absolute deficiency of the isoenzyme LDH-1. It is not yet clear whether any of these findings merely reflect immaturity or are clues to an underlying metabolic defect.

DNA Repair. Most patients with primary hypogammaglobulinaemia have some circulating IgM and IgG, so the coding genes for immunoglobulin are present. It is therefore reasonable to propose that the defect involves some aspect of the splicing and rearrangement of DNA sequences. This would fit in with current concepts on the mechanisms for generating immunoglobulin isotypes

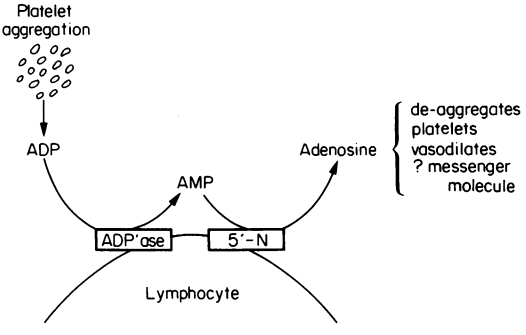

Fig. 3. Possible role for the two lymphocyte ecto-enzymes, adenosine diphosphatase (ADPase) and adenosine monophosphate hydrolase (5'-N) in converting ADP, released during platelet aggregation, to adenosine.

(Davis, Kim & Hood, 1980). Moreover, selective IgA deficiency is a common feature of ataxia telangiectasia where there is an associated DNA repair defect (reviewed by Asherson & Webster, 1980). We have recently described a dwarfed girl with IgA deficiency and depressed cellular immunity who appears to have a defect in the final (ligation) stages of repairing DNA (Webster *et al.*, 1981). Very little is known about the biochemistry of DNA repair, but it is clear that many enzymes and cofactors are involved. Theoretically, any abnormality in this system will affect antibody production.

Miscellaneous defects

Zinc deficiency. Zinc is an important cofactor for numerous enzyme reactions and severe deficiency leads to characteristic skin lesions, diarrhoea and infections (acrodermatitis enteropathica). Hypogammaglobulinaemia may occur but T cell function is mainly affected (Julius *et al.*, 1973). In mice, zinc is required for the normal development of T cytotoxic cells and T cell help for immunoglobulin production by B cells; and a severe deficiency leads to atrophy of the thymus, depletion of T cells and ultimately to failure of antibody production (Good *et al.*, 1980). Inadequate absorption or dietary intake of zinc may therefore play a part in the immunodeficiency associated with malnutrition or malabsorption.

Transcobalamin II deficiency. Transcobalamin II (TCII) promotes the uptake of vitamin B_{12} into cells. One well investigated case of inherited TCII deficiency had severe neutropenia and lymphopenia, hypogammaglobulinaemia and megaloblastic anaemia, all of which developed during the first 6 months of life (Hitzig *et al.*, 1974). These abnormalities responded to vitamin B_{12} injections although the tendency to pyogenic infections remained. This appeared to be due to a failure of neutrophil bactericidal killing which improved when additional *N5*-formyltetrahydrofolate was given (Seger *et al.*, 1980). It is suggested that the resulting deficiency of certain cobalamin containing co-enzymes might lead to a failure of phospholipid transmethylation, a process which is thought to be important in both bactericidal killing and chemotaxis.

Biotin-dependent carboxylase deficiencies. There are a number of recently recognised disorders of branched chain amino acid metabolism, where the common link seems to be abnormal metabolism of biotin (Theone *et al.*, 1981). Affected children have retarded growth, excrete abnormal organic acids in the urine and have a lactic acidosis. Alopecia, ataxia, seizures, kerato-conjuctivitis and mucocutaneous candidiasis are common features. Some affected children have had depressed humoral and/or cellular immunity (Cowan *et al.*, 1979). The importance of recognising the syndrome is that treatment with biotin may be helpful.

CONCLUSION

The investigation of patients with ADA and PNP deficiency has shown us that T and B lymphocytes are not equally affected by certain metabolic disturbances in purine metabolism. We still do not know the mechanism of the immunodeficiency in these diseases, but there is good evidence that the triggering of lymphocyte proliferation is mainly affected. Further work in this area should help us understand the triggering mechanism itself, and tell us whether this is different in T and B lymphocytes.

A variety of other enzyme defects have been described in this review. The area most likely to expand in the near future concerns DNA repair mechanisms, and their relation to the production of immunoglobulin isotypes. There are some clues which suggest that patients which immunoglobulin deficiencies do have defects in DNA repair. There are also a number of biochemical abnormalities in the lymphocytes of these patients which are probably secondary to a failure of maturation. However, some of these abnormalities may be important clues to the primary defect.

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