Combined immunodeficiency and thymic abnormalities

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Severe combined immunodeficiency (SCID), although a relatively rare congenital disorder, is an important disease because it has taught us much about the function and interactions of T and B lymphocytes. It is also the only disease where consistently encouraging results have been obtained with bone marrow transplantation. Furthermore, it was the first immunodeficiency disorder to be identified with an enzyme deficiency and is the first disease to be successfully treated by enzyme replacement therapy.

Isolated T cell defects may appropriately be discussed together with SCID, particularly since it is now apparent that some SCID patients have a predominantly T cell defect. Now that *in-vitro* tests are available to analyse both T and B cell functions separately in these patients the classification is likely to change and become more precise in the next few years. A major advance in understanding the differences between T and B cells at a biochemical level has come from the finding of a purine enzyme deficiency which causes a predominantly T cell defect. This should ultimately lead to the production of drugs which will selectively affect T or B lymphocytes.

Severe combined immunodeficiency

The characteristic clinical features are failure to thrive with severe bacterial, viral, fungal, and protozoal infections. Death is often due to *Pneumocystis carinii* pneumonia. There is an X-linked and autosomal recessive form of the disease but it may also occur sporadically. The condition is usually apparent within the first 3 months of life and most affected infants die before 2 years. Almost all patients have a severely depleted relative number of circulating T lymphocytes which, in absolute terms, may be exacerbated by a lymphopenia. The relative percentage of B lymphocytes is often expanded and many patients produce immunoglobulins. In fact, some patients have grossly raised serum immunoglobulin levels usually caused by a polyclonal, but

occasionally monoclonal, increase in one immunoglobulin class. However, the immunoglobulins produced appear to be non-functional in most cases.

In keeping with the virtual absence of T lymphocytes delayed hypersensitivity skin tests are negative, leucocyte inhibition factor is not produced *in vitro*, and lymphocyte transformation to phytohaemagglutinin (PHA) is severely depressed or absent. Nevertheless, the lymphocytes from some patients respond and proliferate in a mixed lymphocyte reaction (Seligmann *et al.*, 1974). The earlier explanation of this was that different subpopulations of lymphocytes responded to PHA and allogeneic stimuli. A more recent view is that the allogeneic cells may be supplying some essential metabolic function which is deficient in the patients' cells. Thymus glands examined at necropsy are often hypocellular with little differentiation between medulla and cortex, and no Hassall's corpuscles. Peripheral lymph nodes are small and hypocellular although the mediastinal nodes in some patients with raised serum immunoglobulin concentrations may be packed with plasma cells (Webster *et al.*, 1975).

AETIOLOGY

SCID is clearly a heterogeneous disease with more than one aetiology. Some patients have virtually no T or B lymphocytes and die within a few months, while others live up to 5 years and may even make some functional antibody. It is not known whether these two polar groups represent different diseases or reflect a spectrum of severity.

It is still unclear whether SCID is always a stem cell disorder (Buckley *et al.*, 1976; Incefy *et al.*, 1976). There is evidence in some cases of a basic abnormality of the thymus gland, which histologically often appears immature. Circulating lymphocytes from SCID patients have also been induced to form 'E' rosetting T lymphocytes when cultured *in vitro* with thymic extracts (Wara *et al.*, 1975). Furthermore, both the cellular and humoral immune defect in a few patients has been reconsti-

tuted by a thymus graft (Hong *et al.*, 1976). These findings can be interpreted on the basis of an underlying metabolic defect which affects both thymus and bone marrow.

Adenosine deaminase (ADA) deficiency

About half the cases with the autosomal recessive form of SCID are caused by a deficiency of the purine enzyme adenosine deaminase (ADA) (Meuwissen *et al.*, 1975). Patients with this defect cover the whole clinical and immunopathological spectrum of SCID, there being no recognisable subgroup. The disease conforms to the Lyon hypothesis with the heterozygote carriers having half normal ADA levels while the affected patients have less than 10% of normal activity. The enzyme is conveniently measured in red cells although it is deficient in all tissues of the body, including lymphocytes. Prenatal diagnosis is possible by measuring ADA levels in amniotic fluid cells obtained at amniocentesis.

ADA deficiency leads to high circulating levels of adenosine and deoxyadenosine, these metabolites also appearing in high concentrations in the urine.

Lymphocytes are thought to be preferentially affected in this disease because they have specific kinases which 'trap' deoxyadenosine by converting it to deoxyadenosine triphosphate (dATP) (Carson *et al.*, 1977). Circulating lymphocytes in ADA deficiency contain high concentrations of dATP (Donofrio *et al.*, 1978), which is a potent inhibitor of ribonucleotide reductase—an enzyme that regulates DNA synthesis (Figure). The concept of lymphocytes trapping metabolites produced by other tissues is novel and explains why red cell transfusions can be effective in treating these patients.

TREATMENT

Tissue grafting

Bone marrow transplantation is the only curative procedure currently available. Results have been good using sibling donors compatible at the A, B, and D loci of the histocompatibility complex (Buckley, 1977). Nevertheless, other related or unrelated matched donors are worth considering, although one has to be prepared for a potentially fatal graft-versus-host (GVH) reaction. GVH disease is the main hurdle in this procedure and often occurs even when matched siblings are used as donors. It is caused by the grafted lymphoid cells reacting against the tissues of the recipient with the skin, liver, gut, and bone marrow being mainly involved. In severe cases death usually results from neutropenia and thrombocytopenia (Bortin and Rimm, 1977). GVH disease can sometimes be controlled with immunosuppressive drugs such as corticosteroids, cyclophosphamide, and methotrexate. More recently, cyclosporin A appears promising as an alternative and less toxic drug.

Fetal liver grafts have been used as an alternative to bone marrow, since they contain lymphoid and haemopoietic stem cells. T cell function was restored in about half the cases in one series (O'Reilly *et al.*, 1978) although B cell restoration was less successful. GVH reactions can usually be avoided if liver is taken from a fetus aged not more than 12 weeks.

Fetal thymus grafting has been tried in a few patients but has been generally unsuccessful (O'Reilly *et al.*, 1978). However, Hong *et al.* (1976) restored both humoral and cellular immunity by grafting thymus glands removed from children at open heart

Figure Schematic diagram of probable effects of ADA and PNP deficiency on lymphocyte DNA synthesis. ADA = adenosine deaminase. PNP = purine nucleoside phosphorylase. dATP = deoxyadenosine triphosphate. dGTP = deoxyguanosine triphosphate. RR = ribonucleotide reductase.

surgery. Culturing these glands for three weeks before grafting seems to prevent a GVH reaction.

Enzyme replacement for ADA deficiency

Bone marrow transplantation is still the best treatment for ADA-deficient SCID patients provided a suitable sibling donor is available. The immunological defect, however, can be reversed in about half the patients by regular red cell transfusions (Polmar, 1977). Transfusion is needed about once a month and the red cells should be washed, frozen, and irradiated to avoid transfusing viable lymphocytes that might cause a GVH reaction. One such child has already been kept in good health for over a year on this regimen. The transfused red cell ADA, which is not taken up by the recipient's lymphocytes, is thought to metabolise circulating deoxyadenosine, thus preventing it from accumulating in the lymphocytes. The multiple transfusions carry the potential of iron overload, and it is current practice to venesect these children regularly.

Isolated T cell defects

THYMIC APLASIA

This extremely rare condition, originally described by Di George (1965), is caused by developmental failure of the third and fourth fetal branchial arches with consequent absence of the thymus and parathyroid glands. There may be associated abnormalities of the heart and thoracic vessels (for example, Fallot's tetralogy) and a characteristic facies with a fish-shaped mouth and low-set ears. Affected infants present with hypocalcaemic tetany or cardiovascular features, some children dying from the latter before the diagnosis is made. The disease is not usually inherited although one family is described with an autosomal dominant form of the condition (Steele *et al.*, 1972). There is a wide spectrum of severity with some infants developing recurrent bacterial, fungal, and viral infections while others remain healthy. This is probably explained by the presence of residual thymic tissue in those that do well. This may also account for the gradual restoration of T cell function over the first few years of life seen in some affected infants.

The diagnosis is confirmed by finding severely depleted or absent circulating T lymphocytes, often with a relative expansion of the B cell population. Delayed hypersensitivity and *in-vitro* lymphocyte transformation to mitogens and allogeneic stimuli are also severely depressed. Serum immunoglobulin concentrations are normal and most patients produce functional antibody. When test immunised some patients give unusually high primary antibody responses which fade rapidly. Such responses are

explained by a lack of T cell regulation. Some reported patients with SCID who have B cells and normal immunoglobulin levels are difficult to distinguish from the Di George syndrome. A functional analysis of the B cells in such patients should enable a better classification to be made. For example, measuring immunoglobulin production by pokeweed-stimulated patient's B cells in the presence of T cells from a normal donor should help reclassify the syndrome (Seeger *et al.*, 1976).

PURINE NUCLEOSIDE PHOSPHORYLASE (PNP) DEFICIENCY

Nine infants and children from five families have been reported with PNP deficiency. They have suffered from recurrent upper and lower respiratory tract infections and some have had severe infections with varicella, vaccinia, and cytomegalovirus. Megaloblastic changes in the bone marrow have been found in two cases and another case had central nervous system features with spastic tetraparesis. The disease can have a variable onset, as shown by one member of an affected family developing infections at the age of 6 years while his brother's disease started in infancy (Biggar *et al.*, 1978).

All the patients have very low numbers of circulating T lymphocytes with consequent severe impairment of T cell function. A thymic shadow is often absent on the chest radiograph, the tonsils are atrophic, and the lymph nodes show depletion of thymic dependent areas. As in Di George's syndrome, the serum immunoglobulin concentrations are normal and exaggerated antibody responses may occur after immunisation (Amman, 1979).

PNP deficiency leads to high concentrations of circulating guanosine and deoxyguanosine. The latter is thought to be the relevant 'toxic' metabolite, which is probably trapped in lymphocytes by a mechanism similar to that which operates in ADA deficiency, except that deoxyguanosine triphosphate (dGTP) is produced (Figure). The B lymphocytes are spared in this condition, which presumably means that either deoxyguanosine is preferentially trapped by T cells or that B cells are resistant to high concentrations of dGTP.

The disease probably has an autosomal recessive mode of inheritance. Heterozygote carriers have normal red cell and lymphocyte PNP concentrations but their enzyme may have molecular abnormalities, a feature that may help in genetic counselling of affected families.

TREATMENT

Thymus grafting has been successful in treating thymic aplasia. The procedure is relatively simple—a fetal thymus of about 12 weeks' gestation is inserted

beneath the rectus sheath. Lymphocyte transformation usually improves within 48 hours, which supports the view that the graft secretes a 'hormone' which influences the patient's own lymphocytes. GVH reactions may occur after thymus grafting, although not so commonly as after bone marrow transplantation. More recently, patients have been successfully treated with thymus extracts (thymosin) given by injection (Wara and Amman, 1978). Before thymosin is used, it is recommended that an *in-vitro* experiment is performed to see if 'null' cells from the patient can be converted to 'E' rosetting T cells when incubated with thymosin.

Patients with PNP deficiency are not as severely ill as those with SCID, and the risk of bone marrow grafting may not be acceptable. There is not enough experience yet with thymic grafting to assess this approach. One patient has been treated successfully with regular red cell transfusions (Zegers *et al.*, 1979), and this is probably the treatment of choice at present.

Conclusion

Each case of SCID needs to be investigated in detail if we are to gain any further insight into the wide spectrum of the disease. Techniques are available to study blood T and B lymphocyte function although a severe lymphopenia will sometimes preclude such tests. In expert hands thymic biopsies can now be safely performed in life (Borzi *et al.*, 1979), which should be an improvement over trying to interpret the secondary changes often present in necropsy specimens.

It is hoped that other enzyme defects will be discovered to account for the remaining cases of SCID with normal ADA levels. This, together with the rapidly expanding knowledge of ADA and PNP, should lead to the production of drugs which will specifically inhibit lymphocyte subpopulations. For example, a drug that inhibits nucleoside phosphorylase would be virtually specific for T lymphocytes and might be very useful in controlling graft rejection or treating certain autoimmune diseases. Deoxycoformycin, an inhibitor of ADA, is already being used to treat leukaemia with promising results (Smyth, 1979).

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