Lymphocytes. 2 Differentiation

Differentiation of lymphoid precursor cells

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The diversity of immunoglobulin (Ig) molecules and antigen-specific receptors on lymphoid cells, the activation processes during the immune response, the clonal propagation of cells stimulated by antigens, and the sophisticated control mechanisms in the immune system are unparalleled in other biological systems (see A. R. Williamson at page 76, and P. C. L. Beverley at page 59). The emergence of early lymphoid precursors is nevertheless likely to be governed by developmental programmes similar to those observed in haemopoietic precursors and other differentiating cell types. The primary purpose of this paper is to summarise some of the salient observations about the development of the earliest identifiable human lymphoid precursors.

Definitions

There is no general agreement on the definition of terms such as differentiation, maturation, modulation, and activation. Differentiation has been used mainly in two different contexts (Weiss, 1973; Lajtha and Schofield, 1974). In the strict sense it is an irrevocable decision or commitment to follow a particular genetic programme. 'Differentiation' is also used in a wider sense to designate a controlled expression or fulfilment of this option; 'maturation' or 'development' seem to be more appropriate words to cover this meaning. The inherent dilemma is that although the analysis of differentiation may be the more interesting scientific question and the ultimate aim, the study of maturation within a given cell line is a prerequisite for this analysis. The appropriate experimental test system(s) for investigating cellular differentiation can be established only on the basis of a detailed knowledge of the relevant cell types (membrane marker characteristics, cell separation techniques for isolating 'clean' cell populations, etc.) and their potential maturation processes.

In experimental systems such as the differentiation of erythroblasts, myoblasts, chondrocytes, and mammary epithelium cells the decision for synthesising selected gene products (for example, haemoglobin, myosin, chondroitin sulphate, or

γ -lactalbumin, respectively) seems to be made in the mother cell although synthesis of the product starts only in daughter cells (Weintraub *et al.*, 1972; Abbott and Holtzer, 1968; Turkington *et al.*, 1971). Such expression of a given programme requires at least one cell cycle and is probably governed by certain gene rearrangements which are apparently very sensitive to substitution by bromodeoxyuridine (BudR) or other analogues (Weintraub *et al.*, 1972; Abbott and Holtzer, 1968).

These conclusions are in line with current concepts of haemopoietic precursor cell maturation. The cell populations in question are mixtures of cells at various stages of their commitments. In essence this is a 'three tier' system (Lajtha and Schofield, 1974). The multipotential stem cells have still more than one option and give rise to 'committed' precursor cell populations. These in turn generate recognisable (granulocytic, erythroid, etc.) bone marrow cell populations.

It is important to emphasise that lymphoid differentiation is different from lymphocyte activation. Lymphoid cells (both T and B lymphocytes) can be quickly activated by antigens and mitogens and express their predetermined functions before completing their first mitosis (reviewed in Oppenheim and Rosenstreich, 1975). This activation process is resistant to low doses of BudR, and is particularly readily studied within a subset of mouse B lymphocytes which develop plasmablast features and secrete large quantities of Ig in the presence of mitogens (Melchers and Andersson, 1974; Janossy *et al.*, 1976b). Lymphocyte activation is not the subject of this brief review.

Pluripotent and restricted stem cells of the myeloid and lymphoid systems

Chromosome marker studies in the mouse have shown that a common pluripotent stem cell can replenish both myeloid and lymphoid cells, including B and T lymphocytes (Micklem *et al.*, 1966; Abramson *et al.*, 1977). Clones deriving from restricted stem cells already committed to myeloid differentia-

tion only, B cell differentiation only, or T cell differentiation only have also been identified with the help of radiation-induced chromosome markers in the mouse (Abramson *et al.*, 1977). It is still unresolved whether a common stem cell for T and B lymphocytes (with no capacity for myeloid differentiation) or a common stem cell for myeloid cells and B lymphocytes (with no thymocyte/T cell capacity) exists. Surprisingly, perhaps, a common T + B lymphoid stem cell is yet to be detected, although there is already some circumstantial evidence in favour of a common precursor for myeloid cells and B lymphocytes. It has recently been shown that monoclonal mouse spleen colonies of proliferating myeloid cells (CFUc) contain B lymphoid colony-forming cells of identical chromosomal constitution (Lala and Johnson, 1978), and in man there is increasing evidence that in some cases of chronic granulocytic leukaemia B (but not T) lymphoid cells may be involved (Fialkow *et al.*, 1978; see below).

Early lymphoid precursors in human bone marrow

Malignant cells continue to express differentiation-linked membrane antigens which are synthesised by normal cells during comparable stages of development (Boyse and Old, 1969; Akeson, 1977). Many leukaemias derive from haemopoietic precursors and appear to be blocked in an early stage of development. Antisera to leukaemic cells might, therefore, identify characteristic membrane antigens on early precursor cells (Greaves and Janossy, 1978).

Blast cells in the common form of acute lymphoblastic leukaemia (ALL) have an interesting phenotype (reviewed in Thierfelder *et al.*, 1977). This leukaemia is referred to as non-T, non-B ALL because the blast cells fail to express T lymphocyte, thymocyte, and B cell markers. Nevertheless, the blast cells react with anti-ALL, anti-Ia-like, and anti-TdT antisera (ALL⁺, Ia⁺, TdT⁺; see Table; reviewed by Greaves and Janossy, 1978), and biochemical assays for terminal transferase (TdT) show high enzyme levels in these leukaemic populations (Hoffbrand *et al.*, 1977). Leukaemic cells with the same ALL⁺, Ia⁺, TdT⁺ phenotype are also seen in Ph⁺-positive chronic granulocytic leukaemia (CGL) when the disease transforms into 'lymphoid' blast crisis. This suggests that the features common to the blast cells seen in these different diseases (that is, Ph⁺-negative common ALL and Ph⁺-positive 'lymphoid' blast crisis) may reflect the phenotype of the same or closely related precursor cell(s) involved in the leukaemic transformation rather than leukaemia-specific changes.

Attempts were made to find normal cells which

Table Some antisera reacting with human differentiation antigens on early lymphoid precursors and leukaemic cells¹

Anti-ALL serum^{1, 2}

Made in rabbits against acute lymphoid leukaemic blasts (non-T, non-B ALL) and reacts with a glycoprotein (MW 100 000) present on blast cells appearing in non-T, non-B ALL and during 'lymphoid' blast crisis of chronic granulocytic leukaemia (CGL). ALL⁺ cells in normal adult marrow are exceedingly rare but small ALL⁺ lymphocytes are present in small numbers in fetal and infant bone marrow. The number of ALL⁺ cells is increased during regeneration (e.g., after cessation of immunosuppressive treatment)³.

Anti-Ia-like serum⁴⁻⁶

Heteroantisera (made in rabbits and chicken) react with the species-specific common core antigens of 'Ia-like' ('B-cell associated') molecules. Ia-like molecules consist of two chains (28 000 and 33 000 M.W.). The synthesis of Ia-like molecules is probably governed by the HLA-D locus, the human equivalent of the I-region of the murine H-2 complex, and alloantisera to Ia-like antigens are used in histocompatibility testing to detect HLA-DR specificities.⁷ Ia-like molecules are important in modulating T cell responses (see McMichael, p. 30); anti-Ia antisera inhibit the mixed lymphocyte reaction.⁷ Ia-like molecules are present on B lymphocytes, a subset of macrophages and epithelial cells of various organs⁸ including thymus (see text). They are also present on non-T, non-B ALL, and some myeloid leukaemias⁴⁻⁶ but absent on thymocytes and the majority of T cells.

Antiserum to terminal deoxynucleotidyl transferase (TdT)⁹

Purified antibodies specifically bind to this peculiar nuclear enzyme which is present in the thymocytes of all species tested and in a small proportion (2-4%) of mammalian bone marrow 'lymphocytes'.¹⁰ Clinical interest in TdT was raised when the enzyme was discovered in the common form of non-T, non-B ALL¹¹, in 'lymphoid' blast crisis of CGL, and in thymic leukaemias (Thy-ALL, reviewed in ref. 12), but not in peripheral T-cell leukaemias or in myeloid and B-cell derived leukaemias.

Antisera reacting with human T cell/thymocyte antigens (HuTLA)¹³

These are conventional rabbit antisera reacting with a number of T cell/thymocyte specific membrane structures, including receptors for sheep erythrocytes. These sera do not react with myeloid cells or B lymphocytes and are also negative on myeloid, B lymphoid, and non-T, non-B ALL leukaemic cells.

Monoclonal antibody NA1/34 to human thymus antigen (HuThy)¹⁴

This is directed against a major cell surface protein (M.W. 45 000) present exclusively on cortical thymocytes and absent in all other cell types including medullary thymocytes, T cells, and bone marrow lymphoid cells. This membrane protein might be equivalent to murine TL antigens.

Antisera to various human immunoglobulin (Ig) isotypes

B lymphocytes carry surface Ig (sIg) and transform into plasma cells which synthesise and secrete large quantities of Ig. Immediate precursors of B lymphocytes (pre-B cells) do not exhibit surface Ig but synthesise small amounts of cytoplasmic Ig which can be detected with purified antibodies to IgM¹⁵ (see J. J. T. Owen at page 1, and A. R. Williamson at page 76).

¹Greaves and Janossy, 1978; ²Greaves *et al.*, 1975; ³Greaves *et al.*, 1978; ⁴Schlossman *et al.*, 1976; ⁵Winchester *et al.*, 1977; ⁶Janossy *et al.*, 1977a; ⁷Bodmer, 1978; ⁸Wiman *et al.*, 1978; ⁹Bollum, 1975; ¹⁰Bollum, 1978; ¹¹McCaffrey *et al.*, 1975; ¹²Hoffbrand *et al.*, 1977; ¹³Janossy *et al.*, 1977b; ¹⁴McMichael *et al.*, 1979; ¹⁵Cooper *et al.*, 1977.

exhibit the ALL⁺, Ia⁺, TdT⁺ phenotype. Multiple labelling techniques have identified this cell type in normal infant bone marrow (Fig. 1; Janossy *et al.*, 1979) and also in adult patients undergoing bone marrow regeneration (Bradstock *et al.*, 1979a).

The affiliation of this interesting cell remains an

enigma but a few relevant observations may be quoted.

Firstly, the earliest recognisable precursors of B lymphocytes are large- to intermediate-sized lymphoid cells that contain small amounts of cytoplasmic IgM (cyIgM) and do not bear detectable surface Ig (sIg) of any class ('pre-B' cells; see J. J. T. Owen at page 1; Cooper *et al.*, 1977; Gathing *et al.*, 1977). About 1-2% of human pre-B cells express small amounts of TdT (cyIgM⁺, TdT⁺; Fig. 2) and appear to represent a short overlap during the maturation of ALL⁺, TdT⁺, Ia⁺, cyIgM⁻ cells to ALL⁻, TdT⁻, Ia⁺, cyIgM⁺ pre-B cells (Fig. 3).

Secondly, more than 30 cases of non-T, non-B ALL have been demonstrated to exhibit small amounts of cyIgM but no sIg (Vogler *et al.*, 1978; Greaves *et al.*, 1979). These are the leukaemic counterparts of the rare TdT⁺ pre-B cell shown in Fig. 2. Most of these cases show high levels of TdT and contain a variable mixture of ALL⁺, cyIgM⁻; ALL⁺, cyIgM⁺; and ALL⁻, cyIgM⁺ blast cells (Greaves *et al.*, 1979).

Thirdly, three patients with Ph⁺-positive leukaemia developed 'lymphoid' blast crisis (ALL⁺, TdT⁺, Ia⁺) with blast cells synthesising cyIgM. It is important to note that these patients had originally presented with typical Ph⁺-positive chronic granulocytic leukaemia (Minowada *et al.*, 1979; Greaves and Vogler, personal communication). The simplest explanation for this intriguing clinical course is that in these patients the Ph⁺ chromosome aberration had taken place in a common precursor of myeloid and

B lymphoid cells which had a full maturation capacity to develop into myeloid cells and a limited maturation capacity to develop into pre-B lymphoid cells (Minowada *et al.*, 1978; 1979).

Finally, it has been shown in the mouse that in a subset of TdT⁺ bone marrow cells the expression of Thy-1, a thymus-associated antigen, can be induced with thymopoietin, a thymic hormone (Silverstone *et al.*, 1976). Conceivably, therefore, some human TdT⁺ cells also include prothymocytes, but no direct evidence supports this possibility. Human ALL⁺, TdT⁺ cells fail to express thymus antigens (HuTla⁻, HuThy⁻; see Table) and no mixed leukaemias of non-T, non-B ALL, and thymic ALL have been reported (apart from one interesting but unconfirmed communication: Koike *et al.*, 1978). Interestingly, however, some of the ALL⁺, TdT⁺ cells in normal infant bone marrow lack Ia-like antigens. These cells might be candidates for emigration into the thymus because thymocytes are also TdT⁺, Ia⁻ (see below; Janossy *et al.*, 1979).

The essential point in this discussion, therefore, is that an interesting human bone marrow cell has been identified by anti-ALL serum (Greaves and Janossy, 1978) and by additional markers (Bollum, 1978; Janossy *et al.*, 1979). This cell shows the phenotype of the common form of ALL (ALL⁺, Ia⁺, TdT⁺) and is therefore clearly important for studies of leukaemogenesis and immunodiagnosis of leukaemia. In addition, the available scanty information suggests that this cell and/or its close relatives are placed at critical 'bifurcation points' of human

Fig. 1 Normal infant bone marrow: a few cells of lymphoid appearance express ALL-associated antigens, Ia-like antigens on their membrane, and TdT in the nucleus (large arrow). Leukaemic blast cells in non-T, non-B ALL, and in Ph⁺-positive 'lymphoid' blast crisis of CGL express the same phenotype suggesting that the ALL⁺, Ia⁺, TdT⁺ precursor cell might be involved in both diseases. The same field was photographed with phase contrast and with selective filters for TRITC (ALL antigen) and FITC (nuclear TdT stain + ring membrane Ia stain, white arrow; Janossy *et al.*, 1979).

Fig. 2 Cells from normal infant bone marrow stained for cytoplasmic Ig (red, TRITC) and nuclear TdT (green, FITC). Most small TdT⁺ cells are clearly distinct from the large pre-B cells (*). Note, however, that a small proportion of pre-B cells were stained for both cytoplasmic IgM (red, TRITC) and TdT (nuclear TdT, green FITC). One of these cells is depicted (arrow; Janossy *et al.*, 1979).

lymphoid-myeloid cell differentiation (Janossy *et al.*, 1976a, 1977c) or may represent multipotential (pre-B – pre-T; or pre-B – pre-myeloid?) stem cells. Since the isolation of this cell type is feasible (using anti-ALL serum and the fluorescence-activated cell sorter; Greaves and Capellaro, personal communication) key experiments regarding early events of lymphocyte differentiation can be planned and differentiation signals analysed.

Furthermore, permanent cell lines of the non-T, non-B ALL phenotype (ALL⁺, TdT⁺, Ia⁺, cyIgM⁻) and pre-B ALL phenotype (ALL⁺, TdT⁺, Ia⁺, cyIgM⁺) exist and are available for large scale biochemical genetic studies (Minowada *et al.*, 1978,

1979; Janossy *et al.*, 1978; see also A. R. Williamson at page 76).

Maturation of thymocytes

The thymus develops from an epithelio-mesenchymal rudiment that originates from the pharyngeal region of the digestive tract. Moore and Owen (1967), Owen and Ritter (1969), and Le Douarin (1977) showed that neither the epithelium nor the mesenchyme of the thymus primordium are able to differentiate into thymocytes, and that the lymphoid stem cells of the thymus derive from haemopoietic precursors. During early embryonic life the stem cells arrive in

Fig. 3 The first 'recognisable' stem cell of the B lymphocyte series is the pre-B cell (cytoplasmic IgM⁺, sIg⁻, Ia⁺). A few pre-B cells are weakly stained with anti-TdT antibodies suggesting that these cells might represent a brief overlap in maturation of TdT⁺, CylgM⁻ cells into TdT⁻, CylgM⁺ pre-B cells. Thus ALL⁺, TdT⁺, Ia⁺ cells could be primitive stem cells of the lymphoid pathway (multipotential stem cell?). Further studies are essential to analyse the maturation of these cells in culture systems in vitro.

successive waves, and eventually the bone marrow becomes the established source of 'prothymocytes'. Little is known about the phenotype of these cells. In athymic (nude) mice 3-6% of spleen and bone marrow cells express Thy-1 antigen (and the proportion of these cells can be further increased by incubation on thymic epithelium (Sato *et al.*, 1976)). It has also been shown in rat bone marrow that an increase in the percentage of TdT⁺ cells can be induced by thymosin (Goldschneider *et al.*, 1979). A monoclonal antibody to cortical thymocytes has been produced in man (McMichael *et al.*, 1979; Table). Fetal and juvenile bone marrow cells were analysed and no HuThy⁺ cells were seen (< 0.01%) in samples that contained up to 4% TdT⁺ cells and up to 2% HuTLA⁺ lymphocytes. This suggests that human prothymocytes are probably not reactive with anti-HuThy serum (Bradstock *et al.*, 1979b). No attempts were made to induce HuThy expression with thymic hormones or epithelial cells.

In the thymus the demarcation line between the cortex and medulla is rather sharp (Fig. 4) and the phenotypes of lymphoid cells in these areas are distinctly different (Fig. 5). *Cortical thymocytes* are immunologically incompetent, fail to respond to antigens and mitogens, and are sensitive to the cytolytic effects of steroid (reviewed by Greaves *et al.*, 1973). These cells express remarkably low amounts of HLA-A and HLA-B antigens (Brown *et al.*, 1979; McMichael *et al.*, 1979) and lack Ia-like (HLA-D) antigens (Schlossmann *et al.*, 1976). They form particularly strong rosettes with sheep erythrocytes (E⁺), exhibit large quantities of HuThy and HuTLA antigens in their membranes (Table), and TdT enzyme in their nuclei (Fig. 6) and sometimes in the cytoplasm (Barton *et al.*, 1976). It is interesting that *medullary thymocytes* reside in the thymus and

do not recirculate (Elliott, 1973). Thus they are thought not to be transitional forms between cortical thymocytes and peripheral T lymphocytes but rather a special form of thymic lymphocytes with unknown function. These cells have the same characteristics as peripheral T cells: they are immunologically competent, antigen and mitogen responsive, and relatively resistant to steroids. Most T cells lack Ia (HLA-D) but exhibit HLA-A and B antigens and have lost the HuThy and TdT. T cells also form rosettes and show moderate to low amounts of HuTLA. (For subset heterogeneity of peripheral T cells see P. C. L. Beverley at page 59.)

Even this short account of the phenotype characteristics of thymocytes clearly suggests that at least two distinct differentiation steps take place during the development of lymphoid stem cells into peripheral T lymphocytes (that is, the 'bone marrow stem cell → thymocyte' step and the 'thymocyte → T lymphocyte' step; Fig. 6). It is therefore unlikely that thymic hormones (thymopoietin and thymosin) could induce the full maturation process in a short-term experiment (in 2-6 hours) as is sometimes suggested. More probably thymic hormones can influence a number of different maturation steps in this sequence of events: an increased synthesis of Thy-1 and TL antigens in murine prothymocytes (which seems to be associated with only a very modest increase in immune competence; Scheid *et al.*, 1975; Basch and Goldstein, 1975) and the induction of immunocompetence in post-thymic T lymphocytes (reviewed in Stutman, 1977). The immunological characterisation of human T-lineage cells by use of multiple markers (Fig. 6) may help to phrase the appropriate questions and to investigate the effects of thymic hormones at a cellular level in various immunodeficiency syndromes.

Fig. 4 Thymus cortex densely populated with cortical thymocytes reacting strongly with anti-HuThy antibodies (A, C) and anti-HuTLA serum (B). Cortical thymocytes are surrounded by processes protruding from thymus epithelial cells. These processes contain large amounts of Ia-like molecules (D) as well as HLA antigens (not shown). The thymic medulla is loosely populated with thymic lymphocytes. Most of these cells react with anti-HuTLA serum (B) but not with anti-HuThy antibodies (A). The Ia-positive epithelial cells (and possibly macrophages) in the medulla are abundant (not shown). The implications of these observations are discussed in the text (from Pizzolo and Janossy, in preparation). A, B $\times 450$; C, D $\times 300$ (original magnifications).

Fig. 5 Thymocytes mostly showing nuclear TdT (green FITC) and membrane staining with anti-HuThy serum (red, FITC). These cortical thymocytes are found exclusively in the thymus and not in the bone marrow or in the peripheral lymphoid organs (Bradstock et al., 1979b). 1 = TdT⁻, HuThy⁻ (medullary) thymocyte; 2 = only TdT⁺ cell.

Fig. 6 Distinctive phenotypes of cortical thymocytes and peripheral T lymphocytes (see also Table and text). The phenotype of prothymocytes is unknown; preliminary studies suggest that these cells are probably HuThy⁻.

Role of thymic epithelium

There are two main complementary lines of evidence suggesting that thymic epithelial cells not only release thymic hormones and provide a suitable microenvironment for multiplication of cortical thymocytes but also deliver selective differentiation

signals to thymocytes through direct cell-cell interactions.

The first line of evidence (in the mouse) is that attempts to restore T cell functions in athymic 'nude' mice with thymus enclosed in cell-impermeable diffusion chambers have been completely unsuccessful (Pierpaoli and Besedovsky, 1975; Stutman,

1977) despite the fact that 'nude' mice possess pro-thymocytes. This indicates that the flow of pro-thymocytes through thymus tissue is essential for the generation of T cell competence.

The second line of evidence shows that during maturation lymphoid stem cells apparently acquire recognition structures with specificity for major histocompatibility antigens, and this process takes place in the thymus (Zinkernagel *et al.*, 1978; reviewed by P. C. L. Beverley at page 59). These experiments have been carried out in chimaeric mice, using T-cell cytotoxicity against virus-infected target cells as a detection system. The essential point here is that the radio-resistant (non-lymphoid) part of the thymic gland propagates only T lymphoid cells which seem to possess recognition structures for the major histocompatibility antigens of the thymus, while it apparently does not support the maturation of T cells which lack these recognition structures. As a result of this peculiarly selective propagation peripheral T cells will 'see' foreign antigens—for example, virus—in conjunction with the recognised histocompatibility antigen ('altered self' or 'self + X') but will be unable to recognise the same viral antigen in conjunction with other unrelated histocompatibility antigens.

It is not yet clear how this experimental model (described by Zinkernagel *et al.*, 1978) relates to other immunological phenomena such as tolerance to 'self' ('unaltered self') and reactivity to allo-antigens. The answer probably depends on whether T cells have one receptor for 'altered self' or two receptors, one for 'self' and one for X antigen. The important point, however, is that these studies have already established that radioresistant cells in the thymus seem to regulate thymocyte maturation very precisely and that to accomplish this function these cells have to exhibit the major histocompatibility antigens of the individual in a form which is clearly 'visible' to cortical thymocytes.

Two further observations are relevant here. Firstly, the contiguous processes of thymic epithelial cells exhibit abundantly large amounts of HLA-A and HLA-B antigens as well as HLA-D antigens (Ia-like antigens, detected in tissue sections by heterologous anti-Ia-like antisera (Table, Fig. 4)). Similar findings have also been reported in the guinea-pig thymus (Wiman *et al.*, 1978). Secondly, the cortical thymocytes themselves express histocompatibility antigens very poorly. This has been shown in the mouse (reviewed by Greaves *et al.*, 1973) and in man for HLA-A, HLA-B, as well as HLA-D (Ia-like) determinants (*cf.* Brown *et al.*, 1979; Schlossmann *et al.*, 1976). This arrangement may facilitate the monitoring role of epithelial cells in thymocyte maturation.

The expression of HLA antigens on thymocytes (while these cells are at 'close encounter' in the crowded thymic cortex) would almost certainly interfere with the relevant cellular interactions between thymocyte receptors for 'self' (or 'altered self') and the HLA molecules on the thymic epithelium. Thus the thymus cortex provides an appropriate microenvironment where thymic epithelial cells can govern the differentiation and maturation of early thymocytes, probably through direct interactions between epithelial cell membrane antigens and thymocyte receptors, without much interference. The mechanism of these interactions and the site of differentiation into helper and suppressor type of T lymphoid cells (see P. C. L. Beverley at page 59) is unknown.

A role for terminal transferase (TdT)?

TdT is an unusual enzyme which inserts mononucleotides into the 3'-OH end of DNA without template direction (Bollum, 1978). Its physiological role is unknown. Its peculiar tissue distribution in the thymus and in 'immature' bone marrow lymphoid cells (which may include some pre-B cells; see above) has led to suggestions that it may act as a somatic mutagen in B lymphocytes (Baltimore, 1974) and in T lineage cells (Bollum, 1978). Nevertheless no TdT⁺ cells were found in the chicken bursa (Sugimoto and Bollum, 1979) where the generation of B cell diversity seems to be a genetically determined process with an exact 'time-table' for the multifocal appearance of different clonotypes (Lydyard *et al.*, 1976). Similar observations were made in mouse bone marrow (Klinman *et al.*, 1977) and the general importance of TdT in B cell diversification is doubtful (see A. R. Williamson at page 76).

The presence of TdT in the thymocytes of all vertebrate species studied is, however, interesting. Jerne (1971) has suggested that thymocytes with specificity for 'self' will proliferate on contact with self-histocompatibility antigens and in some way become inactivated or destroyed, while the thymocytes which mutate to express a receptor for slightly 'altered-self' will be released from the thymus. Some experimental evidence supported this hypothesis (Pfizenmaier *et al.*, 1976; Zinkernagel, 1976) but recent observations show that lymphoid stem cells (e.g. from strain A) are genetically restricted in developing a recognition site for self A (or 'altered-A') and cannot generate T cells which would recognise B (or 'altered-B'; Zinkernagel *et al.*, 1978).

Thus thymocytes cannot revoke major genetic commitments by extensive somatic mutations. These observations do not exclude, however, that TdT may play a role in introducing point-mutations to

generate diversity in genes coding for the still elusive antigen specific T cell receptor. This is a difficult question to analyse, and one approach to investigating the putative role of TdT in lymphocyte diversification would be to analyse immunodeficiency conditions attributable to TdT enzyme defects. Meanwhile, until such conditions are described TdT serves as a useful biological 'marker' for lymphocyte precursors and acute lymphoid leukaemias.

Conclusions

Insight into differentiation of early lymphoid precursors is important for the analysis of leukaemias, immunodeficiency syndromes, and autoimmune diseases. The relevance for leukaemia is that these cells represent the primary targets for leukaemic transformation (reviewed by Greaves and Janossy, 1978). The relevance for immunodeficiency is that enzyme defects (see A. D. B. Webster at page 10) as well as deficient differentiation signals can probably lead to immunodeficiency. An analysis at cellular level seems to be a prerequisite for understanding these disease conditions. The observations reviewed also influence clinical judgments on reconstituting immunodeficient patients with thymic and bone marrow grafts (Zinkernagel *et al.*, 1978). In autoimmune diseases defective control of lymphocyte diversity and self-recognition may play a role. An age-dependent loss of the functional and morphological characteristics of thymic epithelium has recently been demonstrated in the New Zealand mice which develop a systemic lupus erythematosus-like syndrome (Gershwin *et al.*, 1978).

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