Regular Review

Lymphocyte function and disease

A M DENMAN

Lymphocytes may all look alike, but studies in animals have shown that they may be classified on the basis of origin, ultrastructure, life cycle, surface markers, and function.[1] Human lymphocytes have attracted similar attention—and in addition they have been studied in the context of the many chronic inflammatory and degenerative disorders which appear to have an immunopathological basis. For clinicians, the questions that arise are, firstly, how far human lymphocyte subpopulations can now be identified and characterised and, secondly, how much insight this information provides about the nature and pathogenesis of disease associated with disordered immunity.

In man as in other species there are two major categories of lymphocyte, T and B.[1] T lymphocytes have several functions —discussed in detail below; B lymphocytes synthesise antibody. Thymus-dependent lymphocytes (T lymphocytes) are distinguished from the rest by the presence of surface receptors for sheep red cells and the absence of obvious surface immunoglobulin. In the laboratory T lymphocytes will proliferate after exposure to non-specific mitogens or to antigens to which these cells have been specifically sensitised. Nevertheless, T lymphocytes do not synthesise immunoglobulin—even when they divide in response to an immunological stimulus. Human T lymphocytes probably originate in different stem cells from those which give rise to B lymphocytes,[2] and early T cells in the cortex of the thymus possess an enzyme, terminal deoxynucleotidyl transferase (terminal transferase), which B lymphocytes lack at least during the later stages of differentiation. Though there is no formal proof in man that T lymphocytes can mature only in the microenvironment of the thymus, successful thymus grafting in children with congenital absence of the gland corrects their immunodeficiency—which is good evidence that the thymus performs this essential function.

But thymus-independent lymphocytes, by contrast, have no surface receptors for sheep red cells and most do carry surface immunoglobulin. Furthermore, in the laboratory human B lymphocytes will synthesise immunoglobulin in response to non-specific stimulation by pokeweed mitogen, or to specific antibody after challenge with antigens such as sheep red cells, diphtheria toxoid, or trinitrobenzene conjugated to a suitable carrier. Though this is more controversial, B lymphocytes probably carry the Ia (immune associated) structure which, as the product of the Ir or immune response gene, determines which antigen each B cell will bind, and thereby the precise antibody response it is programmed to produce. Ia determinants are also present on precursor cells of the granulocyte series—an observation which strengthens other evidence that B lymphocytes are the progeny of bone marrow stem cells and that these cells retain their pluripotential capacity in adult life. In birds, B lymphocytes differentiate under the influence of the bursa of Fabricius and human B lymphocytes were originally given their name because they were regarded as bursa dependent; but in fact no bursal analogue has been convincingly identified in man. Almost certainly the microenvironment of the tonsils, central lymphoid organs, and the lamina propria of the gut has the critical role in this differentiation.

A few circulating blood lymphocytes in man lack any obvious T or B cell markers and are referred to as "null" cells. For technical reasons estimates of their number differ widely. These cells should in any event be regarded not as a homogeneous group but as a mixed population which, among other cell types, includes pluripotential stem cells.

Except in the most extreme forms of immunodeficiency, counting T and B lymphocytes in the blood of patients with different diseases has not proved very profitable. Quite apart from technical difficulties in performing accurate counts on anything but whole blood,[3] the results are affected by physiological factors such as diurnal rhythm, the stages of the menstrual cycle, and the age of the individual concerned. Moreover, the numbers of T and B cells seem to be influenced by other non-immunological factors: for example, in 1973 American dairy farmers were exposed to polybrominated biphenyls, by consuming meat and dairy produce from cattle given contaminated feed.[4] The numbers of both T and B lymphocytes in the blood were greatly reduced and the proliferative response of these cells to mitogens was also impaired.

In animals the main populations of T and B lymphocytes have been classified into additional subpopulations on both functional and structural grounds. Human lymphocytes have been classified on a similar basis; and the relative concentrations of these subpopulations can be determined with reasonable accuracy provided that attention is given to technical detail.[5] There is, indeed, detailed information about the numbers of subpopulations of B lymphocytes in the circulation: the principal categories are cells with surface immunoglobulin molecules, cells with receptors for the Fc portion of the immunoglobulin molecule, and cells with receptors for the third component of complement (C3). Quite often B lymphocytes express two of these markers and occasionally all three markers are found on the surface of the same cell. These surface markers may represent different stages in the maturation of B lymphocytes or they may denote discrete populations; which is still not known.

T lymphocytes can be divided into clearly different functional categories. In mice (and to a lesser extent in other species) each category bears a distinctive pattern of surface antigens. A similar heterogeneity is also apparent in human T lymphocytes, but suitable markers have been less clearly defined. Cytotoxic T lymphocytes are specifically sensitised to kill target cells bearing antigens induced, for example, by virus infection. These cells are of considerable clinical importance:

they contribute to host defence in a variety of viral, bacterial, and parasitic infections[6] and this response also provokes many of the deleterious, immunopathological consequences of acute or persistent virus infection. Furthermore, in susceptible individuals the cytotoxic T lymphocyte response may also be provoked by drugs and other simple haptens bound to cell membranes and tissue proteins. In mice the cytotoxic reactions mediated by T lymphocytes kill target cells bearing viral antigens only if the sensitised T lymphocytes and the target cells have crucial histocompatibility antigens in common.[7] Similar restrictions govern the cytotoxicity of T lymphocytes for chemically modified target cells.

There are two hypotheses which could explain these observations: either cytotoxic T lymphocytes possess two surface receptors, one of which recognises a virus-coded or chemically determined antigen and the other a histocompatibility antigen; or the killer lymphocytes recognise a histocompatibility antigen modified by virus or by other forms of immunogen. In virus infections the molecular changes which render infected target cells susceptible to lysis by killer cells have been elucidated to some extent. For example, vesicular stomatitis virus induces the formation of a membrane glycoprotein in the target cell which is indispensable for the lytic process.[8] Whatever the precise mechanism, the cytotoxic reactions are at least partially directed against self-antigens.

Similar restrictions seem to govern the generation of cytotoxic responses by human T lymphocytes. For example, sensitised T lymphocytes mount an efficient cytotoxic attack against cells infected by influenza virus only when the killer cells and target cells share HLA antigens.[9] In man, however, this is not yet established as a universal principle and, indeed, cytotoxic reactions against measles-infected cells are not governed by this restriction.[10] Nevertheless, should the principle be clearly established that cytotoxic lymphocytes in man react primarily with "altered self" antigens, this would go some way towards explaining genetically determined susceptibility to many immunological disorders. An illustration of this principle was provided by a girl with aplastic anaemia who received a bone marrow graft from her histocompatible brother. Eight months later she developed measles followed by chronic sclerodermatous changes in the areas of the original exanthem[11]—lesions possibly induced by lymphocytes in the donor marrow reacting with minor histocompatibility antigens of the recipient modified by measles virus.

The results of studies in animals also suggest that cytotoxic T cells first learn to recognise histocompatibility antigens during a period of maturation in the thymus and that this education enables cytotoxic T lymphocytes to recognise altered self-antigens after these cells have migrated to the periphery.[12] If found applicable to man these findings may help in the treatment of immunodeficiency: they would, for example, predict that if a grafted thymus and its recipient are not histocompatible the cytotoxic T cells which mature in the graft will fail to recognise altered self-antigens and will therefore be unable to limit many forms of virus infection.[13]

The importance of T lymphocytes in virus infections is shown in the context of persistent infection by DNA herpesviruses. Most individuals become infected by herpes simplex, cytomegalovirus, and EB virus during their lifetime, but, while the primary infection may produce transient illness, the subsequent carriage of these viruses is usually of little clinical consequence. Young children who develop clinically important cytomegalovirus infections seem to have a specific defect in cell-mediated immunity to this virus which results in impaired killing of virus-infected cells.[14] T lymphocytes also limit the extent to which B lymphocytes are infected by EB virus and the subsequent proliferation of the infected cells.[15] The consequences of uncontrolled B lymphocyte proliferation after primary infection with EB virus are vividly portrayed in an X-linked recessive lymphoproliferative disease[16] in which affected family members succumb to the immediate effects of infectious mononucleosis or to ensuing agammaglobulinaemia or lymphoma.

T lymphocytes also function as "helper" cells in inducing antibody responses. This conclusion was foreshadowed by the experimental restoration of normal immune responses in animals deprived of immunological competence; it has been amply confirmed in laboratory studies of human antibody synthesis. The synthesis of both immunoglobulin and specific antibody by B lymphocytes seems critically dependent on the presence of adequate numbers of T lymphocytes in the cultures. The mechanism of this "help" is still debated, but it may depend on such factors as the manner in which antigen is presented to B lymphocytes and the minimal requirements for antigen to trigger their proliferation and differentiation.[1] The discovery that helper T lymphocytes are also required for the maturation in vitro of progenitor cells of the erythroid series[17] provides a strong hint that the helper function of at least some T lymphocytes is not necessarily immunological. There is some evidence that human helper T lymphocytes possess a distinctive marker in the form of surface receptors for IgM,[18] thereby providing a welcome correlation between structure and function.

The remaining subpopulation of T lymphocytes, termed suppressor cells, is concerned with regulating immune responses. The immune system consists of a network of cells[19] some of which initiate and others limit specific immune responses. This second function is served by suppressor T lymphocytes; but, though the existence of these cells has been shown repeatedly, their mode of action is still conjectural. Suppressor cells may act by recognising the specific combining sites for antigen on the surface of immunologically activated lymphocytes—whose unique structure, like that of any exogenous protein antigen, is recognised by T lymphocytes. These combining sites, termed idiotypes, stimulate an anti-idiotype antibody response which regulates the extent and duration of the initial response. In the laboratory human suppressor T lymphocytes can be shown to limit reactions such as the proliferative response to mitogens and immunoglobulin synthesis. In contrast with helper lymphocytes, suppressor T lymphocytes bear receptors for IgG.[18] Again in the laboratory human suppressor cells can be induced which limit the antibody response to defined antigens[20]; Broder and his colleagues[21] have described a clinical parallel in which a child with T lymphocyte leukaemia was found to have cells which depressed the capacity of normal B-lymphocytes to synthesise immunoglobulin—a malignant clone of suppressor cells.

Clearly, if autoantibodies are induced in the course of normal immune responses, then autoimmune disease may develop simply from a failure of suppressor cells to limit this response. Evidence in favour of this hypothesis is provided by the discovery that autoantibody-producing lymphocytes may easily be detected in human tissues; and they can readily be induced in vitro.[22] More pertinently, the hypothesis would predict a deficiency of suppressor T lymphocytes in some autoimmune diseases and particularly in systemic lupus erythematosus, a disease characterised by an abnormal proliferation of B lymphocytes with a wide range of activity against both autoantigens and exogenous antigens. Deficiencies

of this nature have indeed been reported in systemic lupus erythematosus,[22] though the assays used are still cumbersome. Nevertheless, the more direct approach of counting the numbers of T lymphocytes with receptors for IgG (suppressor cells) and IgM (helper cells) in the blood of patients with autoimmune diseases has indicated that the distribution of these cells is indeed abnormal.[23] In contrast, excessive T suppressor-cell function has also been implicated in the pathogenesis of myelomatosis, some immunodeficiency diseases, and lymphoproliferative disorders[24]—but the biological complexities of the techniques engender a cautious interpretation of the results.

B lymphocytes undergo a stepwise differentiation associated with sequential changes in membrane surface receptors.[1] Little is known about human B cell maturation, and the analysis of circulating B lymphocyte subpopulations has had little clinical application. Nevertheless, in malignant lymphoproliferative disease detailed analysis of surface markers can distinguish malignant T and B cell lymphomas and leukaemias.[25] This information has proved of clinical importance as a guide to prognosis and to the likely response to chemotherapeutic agents in individual patients.

In contrast with the specific cytotoxicity mediated by T lymphocytes mononuclear cells may attack target cells through non-specific mechanisms. For example, mononuclear cells with receptors for the Fc portion of the immunoglobulin are attracted to target cells coated with antibody[1] and their attachment leads to cell death. This "K" (killer) cell activity of lymphocytes which bear Fc receptors has also been attributed to a special class of mononuclear cells which lack other population markers. As is so often the case with immune mechanisms, this form of cytotoxicity contributes to the host defences in a variety of infections.[6] It is also implicated in the pathogenesis of tissue damage accompanying many immunological disorders.[1] Normal individuals also possess a lymphocyte subpopulation which has the capacity to kill certain tumour cell lines in vitro. These cells, most of which have receptors for the Fc portion of IgG, have been designated "natural killer" cells and they may play a part in tumour surveillance.[26]

Better understanding of the immune system has led research workers to a sober awareness of its complexities, but they are still confident that analysing human lymphocyte subpopulations in terms of structure and function will unravel the mechanisms of the immunological diseases characterised by persistent inflammation, autoimmunity, and strong associations with histocompatibility antigens. The techniques for analysing these populations are now vastly improved.[27] Above all, the need to obtain pure preparations of the lymphocyte subpopulations which are to be characterised is now generally appreciated; and cell separation techniques have steadily improved in line with this awareness.

A M DENMAN

Consultant Immunologist, Clinical Research Centre,
Northwick Park Hospital,
Harrow, Middx

[1] Roitt, I M, *Essential Immunology*, 3rd edn. Oxford, Blackwell, 1977.
[2] Christensen, B E, *et al*, Traffic of T and B lymphocytes in the normal spleen, *Scandinavian Journal of Haematology*, 1978, **20**, 246.
[3] Druguet, M, and Pepys, M B, Enumeration of lymphocyte populations in whole peripheral blood with alkaline phosphatase-labelled reagents, *Clinical and Experimental Immunology*, 1977, **29**, 162.
[4] Bekesi, J G, *et al*, Lymphocyte function of Michigan dairy farmers exposed to polybrominated biphenyls, *Science*, 1978, **199**, 1207.
[5] Bankhurst, A D, *et al*, Human lymphocyte subpopulations defined by double surface markers, *Journal of Laboratory and Clinical Medicine*, 1978, **91**, 15.
[6] Allison, A C, Interactions of antibodies, complement components and various cell types in immunity against viruses and pyogenic bacteria. *Transplant Review*, 1974, **19**, 3.
[7] Doherty, P C, *et al*, Models for recognition of virally modified cells by immune thymus—derived lymphocytes, *Immunogenetics*, 1976, **3**, 517.
[8] Hale, A H, *et al*, Vesicular stomatitis virus glycoprotein is necessary for H-2 restricted lysis of infected cells by cytotoxic T lymphocytes, *Proceedings of the National Academy of Sciences of the United States of America*, 1978, **75**, 970.
[9] McMichael, A J, *et al*, HLA restriction of cell-mediated lysis of influenza virus-infected human cells, *Nature*, 1977, **270**, 524.
[10] Ewan, P W, and Lachmann, P J, Demonstration of T-cell and K-cell cytotoxicity against measles-infected cells in normal subjects, multiple sclerosis and subacute sclerosing panencephalitis, *Clinical and Experimental Immunology*, 1977, **30**, 22.
[11] Fenyk, J R, *et al*, Sclerodermatous graft-versus-host disease limited to an area of measles exanthem, *Lancet*, 1978, **1**, 472.
[12] Howard, J C, H-2 restriction, the thymus and the immune response, *Nature*, 1978, **272**, 11.
[13] Zinkernagel, R M, *et al*, Cytotoxic T cells learn specificity for self H-2 during differentiation in the thymus, *Nature*, 1978, **271**, 251.
[14] Gehrz, R C, *et al*, Specific cell-mediated immune defect in active cytomegalovirus infection of young children and their mothers, *Lancet*, 1977, **2**, 844.
[15] Rickinson, A B, Crawford, D, and Epstein, M A, Inhibition of the *in vitro* outgrowth of Epstein-Barr virus-transformed lymphocytes by thymus-dependent lymphocytes from infectious mononucleosis patients, *Clinical and Experimental Immunology*, 1977, **28**, 72.
[16] Purtilo, D T, *et al*, Immunodeficiency to the Epstein-Barr virus in the X-linked recessive lymphoproliferative syndrome, *Clinical Immunology and Immunopathology*, 1978, **9**, 147.
[17] Nathan, D G, *et al*, Human erythroid burst-forming unit: T-cell requirement for proliferation *in vitro*, *Journal of Experimental Medicine*, 1978, **147**, 324.
[18] Moretta, L, *et al*, Functional analysis of two human T-cell subpopulations: help and suppression of B-cell responses by T-cells bearing receptors for IgM or IgG, *Journal of Experimental Medicine*, 1977, **146**, 184.
[19] Siegal, F P, Suppressors in the network of immunity, *New England Journal of Medicine*, 1978, **298**, 102.
[20] Uytede Haag, F, Heynen, C I, and Ballieux, R E, Induction of antigen-specific human suppressor T lymphocytes *in vitro*, *Nature*, 1978, **271**, 556.
[21] Broder, S, *et al*, Characterisation of a suppressor-cell leukaemia, *New England Journal of Medicine*, 1978, **298**, 66.
[22] Talal, N, ed, *Autoimmunity: Genetic, Immunologic, Virologic and Clinical Aspects*. New York, Academic Press, 1978.
[23] Moretta, L, *et al*, Imbalances in T cell subpopulations associated with immunodeficiency and autoimmune syndromes, *European Journal of Immunology*, 1977, **7**, 696.
[24] Siegal, F P, Siegal, M, and Good, R A, Role of helper, suppressor and B-cell defects in the pathogenesis of the hypogammaglobulinemias, *New England Journal of Medicine*, 1978, **299**, 172.
[25] World Health Organisation Technical Report, Immunological diagnosis of leukaemia and lymphoma, *British Journal of Haematology*, 1978, **38**, 85.
[26] Jondal, M, Spina, C, and Targan, S, Human spontaneous killer cells selective for tumour-derived target cells, *Nature*, 1978, **272**, 62.
[27] Melchers, F, T cell hybrids; shortcut or dead end? *Nature*, 1978, **271**, 9.