Review Article

Lymphocyte subpopulations

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Invertebrates possess an innate non-specific immune system that utilizes phagocytic cells to dispose of antigen. However, higher animals including man have developed an adaptive immune system which is specific in its reaction for a given antigen. Both humoral (antibody-mediated) and cell-mediated immune responses are dependent on lymphocytes which have the capacity to recognize and respond to antigen. Ninety five percent of peripheral blood lymphocytes (PBL) are small lymphocytes comprising both T and B lymphocytes which are derived from pluripotential stem cells. Although T and B cells are morphologically identical, the two groups differ in their cell surface structures and response to antigen. The remaining 5% of PBL are the so called third population of lymphocytes which include large granular lymphocytes (LGL).

Lymphocyte traffic

T cells mature immunologically during passage through the thymus while B cells acquire maturity and immunological competence in the fetal liver and adult bone marrow. Mature B cells migrate via the blood and lymphatic circulation to the secondary lymphoid organs, spleen, lymph nodes and the mucosal associated lymphoid tissue (MALT) of the gut, respiratory and genito-urinary systems. Within these organs the lymphocytes tend to be segregated into predominantly T or B cell areas, and here they can, in close co-operation, recognize and respond to antigen.

The lymphocytes recirculate non-randomly between the major lymphoid organs via the blood and lymphatic channels; the blood-borne lymphocytes enter the lymph nodes between the high cuboidal cells of the post capillary venules and leave via the efferent lymphatics. Following antigen entry into a draining lymph node there is a transient retention of lymphocytes in the node for approximately 24 hours. This mechanism is important because there is only a limited

number of lymphocytes that can react with a specific antigen and hence there is a maximization of the number of lymphocytes that come into contact with the antigen.¹

The majority of lymphoid tissues within the spleen reside in the white pulp which forms the sheath around the splenic arterioles known as the periarteriolar lymphatic sheath (PALS). T cells are located around the central splenic arterioles in the paracortex while the B cells are found in the cortex in either primary or secondary follicles. The PALS is surrounded by an important marginal zone which contains the slowly recirculating B cell population. In the medulla there are both T and B cells and also plasma cells.

Non-encapsulated lymphoid tissue is found in the gut, urogenital and respiratory mucosae and although the T cells are localized in discrete areas, the B lymphocytes can be either organized into follicles or distributed diffusely.

B lymphocytes

B cells develop in fetal liver but at about 8 weeks of gestation the bone marrow becomes the major site of production. The earliest recognizable B cell is the large pre-B cell with its characteristic cytoplasmic μ chain and these develop into B cells which exhibit surface IgM. The mature B cell can also express surface immunoglobulin of other classes, e.g. IgA or IgG, and these B cells can differentiate into plasma cells following exposure to antigen. During the immune response some memory B cells are generated which enable the more rapid and aggressive secondary humoral immune response on further antigen challenge. These memory B cells are located in the germinal centres of lymphoid organs.

Although B and T cells are morphologically similar, the characteristic feature of B lymphocytes is the surface expression of immunoglobulin which can be readily detected by immunofluorescence, as well as a number of other surface proteins including receptors for the Fc portion of IgG (FcR), Class II major histocompatibility complex (MHC) antigens and the

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complement receptors denoted CR 1–4. The presence of CR2 on the cell surface is currently thought to be essential for the entry of the Epstein-Barr virus.² All these surface receptors enable the cell to respond to a variety of stimuli and in particular the surface immunoglobulin acts as the antigen receptor on B cells. The specificity of the antigen receptor and the subsequently secreted immunoglobulin is maintained during B cell differentiation into plasma cells. The morphologically distinct plasma cell does not possess surface bound immunoglobulin but its function is to secrete large quantities of the antigen specific immunoglobulin.

T lymphocytes

Pre-T cells migrate from the marrow to the thymus and in that micro-environment they diversify and differentiate into a variety of T cell subpopulations. The acquisition of a number of cell surface antigens accompanies these changes and interestingly many of them are transient. The vast majority of cells within the thymus die before full maturation and only approximately 1% of the cells entering the thymus leave as mature immunocompetent cells. Many of the deleted pre-T cells are presumably auto-reactive.

T cells can be characterized by a fortuitous reaction with sheep red blood cells (SRBC) whereby their combination *in vitro* causes red cells to form a rosette around the T cell. The advent of monoclonal antibodies having specificities for T cell surface structures has enabled characterization of the repertoire of T cell receptors. The SRBC receptor has been defined as the OKT11 (CD2) glycoprotein and the OKT3 (CD3) determinant defines a mature T cell population which comprises the majority of total circulating lymphocytes. The OKT4 (CD4) determinant is associated with a subset of T cells which help or induce other lymphocytes, e.g., B cells and some T cells, and are hence called T helper cells (T_h). The OKT8 (CD8) positive subset of cells was classically thought to be involved with cytotoxic and suppressor functions, but the allocation of suppressor function to only T8 bearing cells is currently debated. The transferrin receptor (CD9) is recognized by the monoclonal OKT9.

T lymphocytes function by 'dual recognition', that is, that they will only recognize foreign antigen when it is associated with cell surface glycoproteins coded for by genes within the major histocompatibility complex (MHC). The antigen-specific T cell receptor comprises a disulphide linked dimer of alpha and beta subunits. Each receptor is unique and is associated with the OKT3 (CD3) glycoprotein. In man the MHC is situated on the short arm of chromosome 6 and this gene complex encodes two groups of cell surface

proteins, namely the class I antigens (HLA A, B and C) and the class II antigens (HLA DP, DQ and DR). The cellular distribution of class I and class II antigens differs enormously in that class I antigens are expressed on the surface of all nucleated cells whereas the distribution of class II antigens is very much more restricted, being expressed on B lymphocytes, macrophages, monocytes, activated T cells and some endothelial cells.³ T_h cells recognize antigen only in association with HLA DR whilst cytotoxic cells recognize antigen in association with class I antigens.

The T helper population is characterized by the OKT4 (CD4) antigen. Activation of T helper cells is shown schematically in Figure 1 and the antigen presenting cell (APC) (macrophages and dendritic cells) present antigen and MHC class II in concert. The T_h cell, on recognizing the antigen, is induced to enter the early G_1 phase. This G_1 T_h cell has interleukin 1 (IL-1) receptors and also secretes γ -interferon which then activates the APC. This activated APC produces IL-1 to further stimulate the IL-1 receptor bearing T helper cells which then move into the late G_1 phase and themselves secrete interleukin 2 (IL-2), which causes proliferation of the antigen-specific T helper and cytotoxic T cells, ultimately allowing full T cell activation to occur.

Cytotoxic T cells (T_c) reside in OKT8 (CD8) population and recognize and kill target cells independently of antibody by direct contact, probably inducing an alteration in cellular permeability leading to osmotic lysis. Human T_c cells will lyse virally infected cells by recognizing the viral antigens in close association with class I antigens on the cell surface. The advantage of this dual recognition is that the cytotoxic T cells are unable to recognize free viral antigen, but will recognize and lyse virally infected cells thereby combating the infection at its source. T_h cells are essential in T_c generation; they recognize the same antigen in association with class II glycoproteins on an antigen-presenting cell surface and cause clonal expansion of T_c by releasing IL-2.

B and T cell activation

B lymphocytes recognize the surface configuration of an antigen rather than a particular polypeptide sequence. This recognition occurs via the B cell surface immunoglobulin receptor and they also require a second signal which is provided by a T_h cell which, as described above, recognizes antigen and the class II antigen on the APC. Lymphokines secreted by activated T_h cells help to stimulate B cells to differentiate further into plasma cells or to become memory B cells.

Third population lymphoid cells

A third population of lymphocytes exists which do not possess the surface marker characteristics of T or B cells and they have been given several names including K cells, null cells and unclassified cells. All have FcR, are non-phagocytic and esterase negative. The majority of third population cells are morphologically large granular lymphocytes (LGL) and mediate natural killing (NK) and antibody-dependent cellular cytotoxicity (ADCC) functions. Surface marker analysis with monoclonal antibodies and functional assays define overlapping subpopulations, but the best marker to date for LGL remains $\text{Leu } 11^+ \text{ Leu } 7^-$ (monoclonal typing).⁴ The NK activity within this population is probably mediated in a manner similar to that of cytotoxic T cells and is usually demonstrated against tumour cells and virally infected cells. ADCC requires antibody to confer specificity, the cell being armed via FcR with target specific antibody, though the products of the cell are necessary for the actual lysis of the target.

Clinical value of estimating lymphocyte sub-populations

There now exists an extensive body of information concerning lymphocyte sub-populations in health and disease. Studies of PBL sub-populations have yielded

important information about the role of the immune system in the pathogenesis of various diseases. However, it should be noted that surface phenotype, assessed by monoclonal antibodies, does not always correspond to the predicted function, and circulating lymphocytes at a single time point are a transitory population and may not be indicative of the tissue based lymphocytes.

Systemic lupus erythematosus (SLE), an autoimmune disease, is characterized by a variety of autoantibodies⁵ which are the result of a generalized B cell hyper-reactivity. It is still, however, debated whether these abnormalities are the result of a primary B cell defect⁶ or a T cell regulatory deficit. A T_H cell deficiency has been demonstrated both qualitatively and quantitatively,⁷ although paradoxically a T_H deficiency, both functionally and pheno-typically,⁸ has also been reported.

In Type I (insulin dependent) diabetes mellitus, the reported lymphocyte abnormalities include an increase in T_C cells, a reduction in T_H cells, with a normal helper/suppressor ratio but with reduced suppressor cell function.⁹⁻¹³ A histological study of a pancreas from a patient dying within 24 hours of diagnosis revealed that the predominant type of infiltrating cells were T lymphocytes bearing the activated T_C/T_S phenotype.¹⁴ Interestingly an increase in circulating activated T cells had been previously reported not only in recent onset type 1 diabetics but

Figure 1 T cell activation (see text for abbreviations)

also in 50% of non-diabetic siblings,^{15,16} suggesting that the peripheral blood abnormalities could reflect the infiltrative process within the islets of Langerhans.

Probably the most important clinical application of lymphocyte subpopulation analysis is in the diagnosis and management of white blood cell malignancies,^{1,22} such as undifferentiated acute myeloid leukaemia (AML) and acute lymphocytic leukaemia (ALL). With the advent of specific polyclonal and monoclonal antisera these can now be accurately typed and the appropriate treatment instituted. Furthermore the use of monoclonal antibodies against lymphocyte surface determinants has allowed the B cell differentiation pathways to be accurately mapped.

Lymphocyte analysis is important in the investigation of the patient presenting with a peripheral blood lymphocytosis where differentiation of a T cell from a B cell lymphocytosis is a relatively simple task with anti-CD2 and antibodies to surface immunoglobulin respectively. In the absence of a circulating paraprotein, evidence of B cell monoclonality requires further surface phenotype analysis looking for a significant deviation from the normal ratio of κ -bearing to λ -bearing lymphocytes normal range (1:1–5:1). Evidence of T cell monoclonality is considerably more demanding and requires the demonstration of T cell receptor gene rearrangement^{11,19} and currently there are only a few institutions which can perform this analysis.

Both acute allograft rejection and graft versus host

disease are thought to be mediated by T cells.^{20,21} Monoclonal antibodies directed at T cell-specific surface determinants have been used to eliminate T cells for donor marrow and the recipients of renal allografts to reduce the requirements for immunosuppression.^{22–26}

An example of the limitations of lymphocyte subpopulation analysis is in the investigation of the acquired immunodeficiency syndrome (AIDS). Full-blown AIDS is characterized by a T_h (CD4) lymphopenia but normal cytotoxic/suppressor (CD8) lymphocyte numbers, leading to a characteristically reversed helper/suppressor ratio.²⁷ However other virus infections such as mononucleosis syndromes and herpes virus infection²⁸ can lead to a rise in the CD8 + lymphocyte count and hence a reversed CD4/CD8 ratio.^{29,30} Human immunodeficiency virus seropositive homosexual individuals who showed low T₄ levels were shown to be more likely to develop subsequent AIDS.³¹ However, the estimation of PBL sub-populations has no diagnostic value and little discriminatory capacity in the investigation of patients thought to have AIDS.

In conclusion, although many different patient groups have been studied, the clinical value of lymphocyte sub-population estimations remains unproven except in the investigation of some lymphoid neoplasms. However, their measurement has certainly enhanced our understanding of the underlying mechanisms of many diseases.

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