B and T Lymphocyte Function in Disease

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The human body has among its many complex and functionally specific systems the ability to immunologically defend itself against invasion by foreign substances or antigens. Lymphocytes, the second most numerous leukocyte in the peripheral blood, are the essential mediators of the immunological response. Two subpopulations, T lymphocytes and B lymphocytes, have specific functions within this system, each acting separately and interdependently. T lymphocytes are responsible for cell mediated immunity which requires physical contact between the antigen and the T cell in order to be effective. B lymphocytes are the mediators of humoral immunity which involves antibody production by B cells in response to antigenic stimulus. However, despite its exceptional protective ability, the system itself is not immune to diseases which can alter lymphocytes and their functional ability. These defects can be manifested in one of three ways: stem cell defect, maturation defect, or functional defect. The study of these defects and the classification of the predominating lymphocyte subpopulation in diseases such as cancer and autoimmune disorders can aid in the diagnosis and possible therapy of these diseases.

The precursor of both T and B lymphocytes is thought to be a multipotential, immunologically incompetent stem cell which originates from the bone marrow or fetal hematopoietic tissues. This cell migrates to specific primary lymphoid organs where it can be directed to mature to either an immunologically competent B or T cell. In the case of T cells the lymphoid organ required
for maturation and functional ability is the thymus, hence they are referred to as thymic dependent lymphocytes. Mature cells produced here can then seed secondary lymphoid organs (the lymph nodes and spleen), peripheral blood and thoracic duct to establish complete protection. T cells are located in the paracortical regions of lymph nodes and the peria arteriolar sheaths of the spleen. They comprise 60 to 80% of the circulating lymphocytes in the peripheral blood and 85 to 90% of the lymphocytes in the thoracic duct.

Most thymic derived lymphocytes have a short lifespan of only five days because of the rapid renewal of immunologically incompetent lymphocytes in the thymus. T cells which do survive and seed secondary organs live for only a short period of time, probably never attaining the dormant, competent stage. Thus, only a small amount of the total cells formed become mature and competent. These cells can survive at this intermitotic stage up to ten years with an average lifespan of two to four years. T cells, having the ability to recirculate throughout the blood and lymphatics, can leave the lymph node by the efferant lymphatic duct and move into the blood stream where they may reside momentarily or enter another lymphatic tissue. This recirculation process continues until the T cell is stimulated by an antigen to divide and produce daughter cells. These cells can mount the immunological attack or reenter the circulation as memory cells. However, despite the great amount of recirculation that occurs, the system is kept in equilibrium at all times.
The B lymphocytes require the bone marrow as their specific maturation tissue and are referred to as thymic independent lymphocytes as a result of this. From the bone marrow the cells progress to seed secondary lymphoid organs to establish the final outpost of their immunological surveillance. B cells can be found in the germinal centers, subcapsular region and medullary cords of the lymph nodes and in the germinal centers, periphery of periarteriolar sheaths and red pulp of the spleen. Only 10 to 15% find their way to the thoracic duct. They comprise only 20 to 30% of circulating lymphocytes. B cells are relatively short lived and therefore, do not circulate as long or as often as T cells; most cells probably remain at the marrow production site. However, despite the differences in the percentages of cell types in the blood, in sublymphoid locations, and in lifespan and circulating capacity, recirculation of T and B lymphocytes is the key to total protection of the body. This allows dissemination of all lymphocytes into different organs of the body, enhancing their immunological capabilities simply through more direct contact with antigenic stimulus.  

T lymphocytes are responsible for cell mediated immunity which involves direct cellular contact between the antigen and the lymphocyte. This type of immunity is the major protective force for man against intracellular pathogens which include many bacteria, most viruses, protozoa and fungi. A small dormant lymphocyte becomes sensitized by one of these antigens and is provoked into transforming into an activated lymphoblast. This can proliferate into specific T cell subclasses and release
substances, collectively called lymphokines, necessary in the completion of the response. The blast can produce transfer factor, a low molecular weight substance, which is capable of transforming other nonsensitized lymphocytes into antigen specific lymphocytes, thus aiding in the proliferation of the response. Lymphotoxin, another mediator, causes local tissue injury which kills certain target cells implicated in the cause of the stimulation. Interferon is a potent, nonspecific agent against viruses which can be produced. Migration Inhibition Factor (MIF) is a substance which causes the localization of macrophages at the site of the antigenic stimulus. This substance also causes macrophages to be activated and to produce lysosomal enzymes which cause tissue injury and in turn, aid in the increased bacterial killing through phagocytosis. This processing of the antigen may be required before presentation of it to the T cells for active killing.2

In addition to the production of mediators, the T lymphoblast proliferates and matures into T cell subclasses of effector, suppressor and memory cells. The effector cells include helper cells which aid in recognition of the antigen and help instruct B cells in their humeral response, and killer cells which are the implicators of the cell mediated response and direct the actual "killing" of the antigen. Suppressor cells are responsible for cessation of the response when no more antigen is detectable. Memory cells are produced in this process for the quick recognition of the antigen at the next contact.2

T lymphocytes are specific for cell mediated immunity but,
through their production of helper cells are also interrelated to the humeral response. Humeral immunity is specific for encapsulated and pyogenic bacteria. In some cases the antigen requires processing by the T cells before antibody production can commence. At this point the T helper cells can "inform" the B cells the type of antigen they are dealing with and in turn, "instruct" them as to the necessary antibody to be produced. Hence, the B cell, through contact with either the antigen or the helper cell as determined by the nature of the antigen, becomes an activated lymphoblast which can mature into a sensitized B cell called a plasma cell. This cell is responsible for the actual production of the antibody, which is mostly of the IgG class. The sensitized B cell can also act as a memory cell which, like the T memory cell, aids in the secondary or anamnestic response to a specific antigen.\(^2\)

As with all biological systems the lymphocytic immune system is not immune to diseases and specific alterations in its structures. These alterations can cause malfunctions and the system becomes a disease or menace to the human body. Specific examples of this are lymphoproliferative diseases, such as carcinoma and leukemias, and autoimmune diseases. The possible lymphocytic defects can be classified into three basic categories: stem cell defect, maturation defect and functional defect. A stem cell defect involves the production of the pluripotential yet immunologically incompetent precursor cell of lymphocytes. A depressed bone marrow or hematopoietic tissue would lead to
a decreased production of stem cells and in turn, a decreased amount of lymphocytes available for maturation or the immune response. This situation would obviously result in a depressed immunological state.

Maturation defects involve the stages between the stem cell and the mature resting lymphocytes and may be manifested in one of two ways. First, a "block" in the maturation process can result in only cells from a single immature stage of development to be produced. An example of this is acute lymphocytic leukemia where the thymic or bone marrow lymphoblast is prevented from developing into the mature lymphocyte. Hence, there appears to be an increased production of lymphoblasts where there only is an inability to develop beyond that stage. Secondly, a "switch on" of the maturation process can cause an increased number of immature lymphocytes to mature to small competent lymphocytes and in turn, to be pushed out into the circulation despite the lack of increased demand. An example of this is Chronic lymphocytic leukemia where there is an abnormally increased number of small lymphocytes in the peripheral blood.

Functional defects appear in both subpopulations and their effects are often interrelated. These defects involve the stages of transformation of the mature lymphocyte to the activated lymphoblast responsible for immunity. B lymphocyte defects mainly involve the transformation of the blast to the plasma cell and the production of antibody from this final stage. An unknown stimulus of the humeral system, perhaps an increase in T helper cells
or a decrease in T suppressor cells, causes increased transformation of B cells to plasma cells. Antibody production could be increased or decreased depending on the effect increased transformation or cloning has on the B cell and plasma cell. This massive increase of plasma cells is the hallmark of multiple myeloma. An inability of the B cells to be stimulated to plasma cell transformation would result in a greatly decreased production. This could result from a decrease in T helper cells and/or an increase in T suppressor cells.

Autoantibody production is a defect that involves both lymphocyte subpopulations. T helper and suppressor cells are involved here also, being the main mediators of this antibody production. T helper cells may recognize an altered antigen on a self cell surface as foreign and instruct B cells to become reactive. Dormant T cells could recognize other T cells as foreign, causing their own activation and in turn, the activation of B cells through the resultant T helper cell production. To aid this defective process, T suppressor cells appear to be unable to suppress the T helper cell's activity. Hence, a balance of these two cells is needed to prevent autoimmunity.\(^5\),\(^7\)

In studying diseased states caused by malfunctioning lymphocytes it is always helpful to classify the disease according to lymphocyte subpopulation. Through this it is possible to elucidate the separate roles of each subpopulation in immunity to diseases, and as a result of this, ideas may arise as to how subpopulations may be manipulated to destroy diseases. To class-
ify lymphoproliferative diseases as to cell origin one must assume that they are of clonal origin and that the cells retain enough of their original features to be differentiated. These cells possess antigens which are absent or only present in small amounts on normal cells. This weak antigenicity allows the abnormal cells to "sneak through" the surveillance of the immune system. If abnormal cells are allowed to grow, they eventually may achieve a large enough size and number to activate the immune system. Yet, at this point the resulting tumor may be too large for the immunological attack to be successful.

An alternative mechanism of the attack against tumors is "immune modulation". Here, in the face of an immunological attack, the tumor cells lose their surface antigens by moving them into their fluid surface membrane. Once the surveilling lymphocytes abandon their attack the antigens reappear and the tumor continues to grow. The tolerance or unresponsiveness of the immune system in both "sneaking through" and "immune modulation" depends on the dose of the antigen, its nature and strength, its form of presentation to the system and the amount of suppression of the immune system already present in the host.

Lymphoproliferative diseases are examples of maturation defects. Those involving B cell proliferation are more common than the T cell type. Chronic lymphocytic leukemia is most often a monoclonal proliferation of normal, small B cells accompanied by a decrease in antibody production. Both T and B cells may be involved early in this disease. The B cell clone may be
frozen at a stage in development or correspond to a clone of a B cell which could mature uninterrupted to an IgG secreting plasma cell.\(^3\) The fact that some cells are frozen at a pre-plasma cell stage may explain why antibody production is decreased. Multiple myeloma is the most mature of B cell proliferations having many monoclonal plasma cells being produced. However, as in chronic lymphocytic leukemia, the antibody production is down as a result of the abnormal cloning process. Incomplete antibodies (free light chains) are produced by the plasma cells and may appear in the urine as Bence Jones Protein.\(^2\)

T cell proliferative diseases include Hodgkin's lymphoma and acute lymphocytic leukemia. Hodgkin's is a malignant lymphoma which first involves invasion of the lymph nodes by T cells which later disseminate into the lungs, liver and bone marrow. In these advanced stages cellular immunity is impaired but humoral antibodies are still functioning properly.\(^2\) Acute lymphocytic leukemia produces a proliferation of cells, 25 to 30% of which show T cell surface features while 65 to 70% have no B or T cell type surface markers.\(^3\) One possible explanation is that the malignant blast forms were blocked at a point in the maturation sequence before they had developed subpopulation characteristics. Another explanation is that there are two different types of acute lymphocytic leukemia; a B cell type and a non B or T cell type.\(^3\)

Conversely to the proliferation of lymphocytes in the above diseases, B and T cell deficient states also occur. Those involving B cells are usually due to a functional defect and are
called agammaglobulinemias. Here, a normal count of B lymphocytes is present but they completely fail to functionally transform into Ig secreting plasma cells. B cells producing all three antibody types, IgG, IgM, and IgA, may be affected or only one or two specific Ig secreting B cells may be affected.\(^7\)

T cell deficiencies are diseases involving a maturation defect in the precursor stem cell due to a maldeveloped thymus which is unable to allow the cell to mature. Hence, a decreased number of T cells is produced or, the defect can be so pronounced as in Di George's syndrome, that no T cells are produced.\(^7\) A severe combined immunodeficiency of both B and T cells is the product of a stem cell defect causing lymphopenia of both types. Either the stem cell production is decreased or, the cells are unable to mature to either type due to an inability to migrate to a primary lymphoid organ or an inability to mature once there. This state would cause great vulnerability to rampant infections. A less severe variation of this disease is hypogammaglobulinemia where there is a defect in the T cell production along with a decreased production of B cells.\(^7\)

Autoantibody production is another functional defect and includes such diseases as systemic lupus erythematosus (SLE), rheumatoid arthritis, and thyroiditis. Through an error of recognition, B cells (or T helper cells if the "antigen" needs to be processed) assume some parts of the human body to be foreign and in turn, make antibodies against. In cases where the antibody production is turned on by T helper cells, it seems that the
counterbalancing T suppressor cell is present in functionally decreased numbers and is unable to complete its tasks. In SLE the antibodies are directed against nucleoproteins of cells causing a disseminated inflammatory disease.\(^5\) B cells seem to predominate, although it is not known whether T cells are involved. Immune complexes containing complement block \(C_3\) receptors on the cells causing difficulties in testing for the presence of surface Ig necessary to differentiate cell types. These complexes could very easily coat T cells causing them to appear as B cells.\(^5\) Therefore, more research in testing needs to be done to resolve this problem.

Rheumatoid arthritis results from the production of an antibody of the IgM class against antibodies of one's own IgG class, causing severe pain and stiffening in joints. Large numbers of T cells are present in the synovial fluid but it seems their function is impaired, especially in severe cases.\(^5\) The B cells present produce increased amount of the IgM rheumatoid factor. Specific testing for lymphocyte subpopulations suffers in this case from the same problems that plague typing in SLE. Hence, the true breakdown of cell types in rheumatoid arthritis is yet to be known.\(^5\)

Thyroiditis, either Graves's or Hashimoto's, involves increased antibodies against the cells of the thyroid. There is an increase in T cell numbers and an increase in the Migration Inhibition Factor which is produced by them. This suggests some type of cell mediated autoimmunity. B cell count is normal but
antibody production is increased, possibly because the T cells require antibody production as an auxiliary attack system. Hence, in this disease the two lymphocyte subpopulations work hand in hand.5

In the classification of B and T cells in disease states one must remember that abnormal lymphocytic cells may have abnormal phenotypes and surface characteristics making them more difficult to type than normal cells.10 Daughter cell lines divide slower and may be different from rapidly dividing lines and hence, difficult to type.10 Also, it must be determined whether the cell in question is a reactive or malignant type lymphocyte or just a dormant cell. Despite these characteristics, though, there are still successful assays for lymphocytic typing available. These may be divided into two categories on the basis of techniques involved; detection of surface markers (receptors or) by rosette techniques and, detection by specific antisera conjugated to a marker such as fluorochrome, enzymes, isotopes, or particles.11

The major technique for typing T lymphocytes is the E-rosette method. This is accomplished through the spontaneous adherence of sheep erythrocytes to T cells, making them microscopically distinct from other cells. The test is quite variable however, and lacks specificity. It has been found that many factors, too numerous to discuss here, see ref. 11 can affect the percentage of recovery of T cell rosettes from the assay. Other cells such as fibroblasts and parenchymal cells from the lung, liver
and parathyroid can also form rosettes with sheep erythrocytes. Although the technique is simple, the test may be affected by many factors and it is therefore necessary to cautiously interpret the results.

The most reliable marker for B cells is the detection of surface Ig by use of anti-immunoglobulin conjugated to a specific marker. The test can be done by the direct method using labeled antihuman Ig directed against Ig on the B cell surface, or indirectly by using goat antihuman Ig to attach to the B cell and then adding labeled immunoglobulin against goat to attach to this antigen antibody complex (sandwich technique). Reliability, as in all immunologically based assays, will depend on the sensitivity and specificity of the antisera. Monocytes and other lymphocytes may nonspecifically absorb the antibody causing false positive results. Incubation at 37°C for 2 to 24 hours may prevent this by allowing the immune complexes to be shed from non-lymphocytic cells. Thus like the E-rosette technique, many factors must be considered in both performance and interpretation.

The technique that appears to be the most promising of all in the future is one which determines differential antigenic characteristics for each cell type through specific antisera directed against specific surface antigens. This not only has the potential to simply type T and B cells, but also may be able to differentiate subclasses of cells within these types. This could lead to an even better and more specific classification of lymphocytic diseases and a better key to their treatment.
The importance of lymphocyte classification in specific diseases has just recently been realized with the introduction of T and B cell assays. Classification is the first step toward an in-depth understanding of the two immune systems. This in turn can eventually lead to lymphocyte manipulation to both enhance normal functioning immunity and to cure or perhaps prevent diseases causing malfunctions in these systems. At some point, drugs may be linked to antibodies produced by these systems to improve their efficiency. T cells may be manipulated to efficiently survey the body for possible malignant cells and, with their increased potency, destroy the abnormal cells before they are allowed to grow and multiply. B cell antibodies may be produced to favor this T cell cytotoxicity by adhering to and clearing out any other cross reacting material in the serum.

It seems ironic that the systems responsible for man's immunity to foreign substances can become altered in such ways that these systems themselves become the very thing that they try to prevent, invasive disease. But, through classification and manipulation, man may eventually become immune to his own lymphocytic alterations and in the process produce an overall better immune system.
REFERENCES


