Hypothesis: Differentiation of the Human Lymphoid System Based on Cell Surface Markers

By Stephen Davis

Human lymphocytes can be separated into distinct populations based upon receptors on their cell surface. Thymus-derived (T-cell) lymphocytes can be identified by their ability to form rosettes with sheep erythrocytes (SRBC); bone marrow-derived (B-cell) lymphocytes bear characteristic surface markers for immunoglobulin, complement, and the F<sub>r</sub> portion of IgG. Recently, populations of lymphocytes having either multiple markers or no detectable markers (null cells) have been observed. Based on studies of cell surface markers, a scheme is proposed that expands the known differentiation of the lymphoid cell to include subpopulations which represent developmental stages. It is suggested that lymphocyte maturation involves alloantigenic changes in a circulating stem cell-derived null cell, leading to a cell bearing markers for both T- and B-cells. It is from this latter cell that the classic T- and B-cells ultimately arise. Maturational defects which may explain the origin of primary lymphoproliferative diseases are discussed.

LYMPHOCYTES IN THE peripheral blood of mammals consists of a functionally heterogeneous population of cells responsible for antibody production and cellular immunity. A greater appreciation of this heterogeneity grew out of the observation that two cell types, thymus-derived (T-cell) and bone-marrow-derived (B-cell) lymphocytes not only could be distinguished functionally but also could be identified by specific markers on their surface. In the mouse the β-antigen defines the T-cell, whereas immunoglobulin (Ig) determinants are characteristic of the B-cell. The presence of surface Ig receptors is also an identifying feature of the human B-cell. In addition, the human B-cell has receptors for the third component of complement (C) as well as the capacity to bind aggregated Ig (agg. Ig) and antibody-antigen complexes, probably by means of surface receptors for the F<sub>c</sub> portion of Ig. Although the analogue of the β-antigen has not been identified on human T-cells, the identification of these cells is facilitated by their ability to form rosettes with sheep erythrocytes (SRBC), especially after treatment of either the T-cell or the SRBC with neuraminidase. Whether T-cells also contain surface Ig has not been entirely resolved; however, T- and B-cells can be distinguished by the use of standard membrane Ig fluorescence techniques. A more recent study has identified populations of lymphocytes having either multiple markers or no detectable markers. These cells cannot be precisely classified as T or B, suggesting the presence of transitional forms.

This article will attempt to correlate the results obtained from studies which use these immunologic methods to detect cell surface markers with a hypothetical model for lymphoid cell differentiation. I will show how maturational
defects in the differentiating lymphocyte may be pathogenetically linked to lymphoproliferative disorders.

DIFFERENTIATION OF LYMPHOID CELLS AND THE PATHOGENESIS OF LYMPHOPROLIFERATIVE DISEASES

The maturation scheme depicted in Fig. 1 introduces the concept of progressive and regressive changes in surface alloantigens, a process which has been well documented in mice. In man, both T- and B-cells appear to arise from a common bone marrow precursor. Schlesinger and Hurvitz and Basten et al. have demonstrated that stem cells in mice bear no detectable surface markers. The same appears to be true in man. The combined immunodeficiency syndrome, characterized by defects in both T- and B-cell functions due to the absence of primordial lymphoid tissue, may involve a block in maturation at the level of a stem cell. The fact that some children with the syndrome may be rendered immunocompetent by bone marrow grafts suggests a stem cell defect. Examination of blood from such patients reveals a paucity of cells bearing surface Ig, but an extensive study of lymphocyte markers has not been reported. An apparently analogous situation exists in the W/W mouse, which appears to be deficient in myeloid stem cells. Bone marrow cells of W/W mice fail to reconstitute the myeloid cells in an irradiated wild-type recipient; however, wild-type bone marrow is easily transplanted into the W/W strain.
The first step in the maturation of the stem cell is the production of a circulating null cell, i.e., a morphologically recognizable lymphocyte with no detectable surface markers. The transition of bone marrow stem cells to circulating null cells has not been documented experimentally; however, the finding that null cells form colonies in vitro suggests that they represent a lymphocyte precursor cell. The proposed model assumes that the null cell represents a single population of uncommitted cells; however, the null cell could conceivably be comprised of two cell populations, phenotypically similar, which are destined to become either T- or B-cells. Thymopoietin, a polypeptide hormone of the mammalian thymus, induces the differentiation of mouse precursor cells into T-cells only. Other agents, like cyclic AMP and polyadenylic-polyuridylic acid (poly A:U), appear to cause precursor cells to differentiate into both T- and B-cells. From these data, it was proposed that adenylcyclase inducers, like cyclic AMP and poly A:U, act on two distinct precursor cell populations to form both T- and B-cells, while thymopoietin acts as a specific inducer of T-cells. However, caution must be exercised in the interpretation of these findings since nonphysiologic doses of these inducers are used to convert less than 10% of the precursor cells present to T-cells. The existence of two null cell types could explain the rarities of (1) hypogammaglobulinemia in uncomplicated acute lymphocytic leukemia (ALL), a large percentage of cases which presumably represent a T-cell disease, and (2) depressed cellular immunity in most cases of agammaglobulinemia (believed to be a B-cell disease). Examination of the peripheral blood of healthy individuals has consistently revealed a distinct population of small lymphocytes bearing no detectable surface markers. High percentages of null cells can also be detected in most, if not all, cases of ALL.

Continued differentiation of the null cell would result in a cell capable of expressing both T- and B-cell markers. Combined phase and fluorescent microscopy have shown SRBC rosettes and agg. Ig markers simultaneously on individual lymphocytes of normal patients and (in higher concentrations) on lymphocytes of patients with agammaglobulinemia. By the same technique, I have studied two patients with ALL whose peripheral blood, respectively, yielded 7% and 18% blast cells with both markers. Other reports indicate that up to 20% of thoracic duct lymphocytes, supposedly 95% T-cells by rosetting techniques, bear markers for C-receptors. Further evidence for the multiple marker cell comes from a thymoma cell line produced in an irradiated Balb/c mouse. The cloned cells from this line possess simultaneously the ß-antigen and surface immunoglobulin receptors. In mice, 50% of thymocytes bear the ß-alloantigen, which is a cell surface antigen believed to represent the structural expression of a gene(s) located in the mouse major histocompatibility complex (H-2). This alloantigen was thought to be expressed preferentially on B-lymphocytes. From these data, I conclude that at some point before functional dichotomy occurs, a differentiating lymphocyte possesses surface markers of both T- and B-cells.

Maturing lymphocytes subsequently fall under the influence of the central lymphoid organs. Cells destined for the T-cell compartment migrate to the thymus and undergo alterations both in functional capacity and in cell surface
markers. The DiGeorge and Nezeloff syndromes are diseases in which the thymic influence on the differentiation of precursor cells into T-cells is defective because of congenital absence of the thymus. Consequently, infants so affected exhibit an inability to mount cell-mediated immune responses. Circulating lymphocyte levels are usually normal, as are serum immunoglobulin levels, but antibody production to thymus-dependent antigens is deficient. Although comprehensive cell surface marker studies in these syndromes have not been reported, 84% (normal, 20%-30%) of the circulating lymphocytes of an infant with thymic aplasia exhibited surface Ig receptors. More extensive data are available in an animal model, the nude (thymus-deficient) mouse. Work in our laboratory has shown that splenic lymphocytes from nude mice contain 1% θ-positive and 78% agg. Ig-positive cells. By the use of combined immunofluorescence with anti-θ-serum and anti-Ig serum we have been able to demonstrate multiple marker cells (2%-9%) in the splenic lymphoid population. The remainder of the splenic lymphocytes in the nude mouse appears to consist of null cells.

Failure of lymphocytes in the thymus to mature properly might result in T-cell ALL, a disseminated disease, or in Sternberg’s lymphoma, a disease localized to the mediastinum initially but whose clinical course in its late stages may be indistinguishable from ALL. Demonstration of a thymocyte-specific enzyme, terminal deoxynucleotidyl transferase, in several cases of ALL and in a continuous cell line derived from a patient with ALL offers evidence for the thymic derivation of ALL. Unfortunately, no data pertaining to the surface markers in the described cases were provided, so that the relationship of this enzyme to null cells and T-cells remains open. Following the release of thymocytes to seed the peripheral lymphoid structures, T-cells lose their terminal transferase activity but retain their ability to bind the SRBC. It is possible that ALL can exist in two forms distinguishable by surface markers (i.e., null cell ALL and T-cell ALL). At present, no data is available regarding the clinical significance of this possible subdivision. However, certain membrane receptors such as cortisol binding sites may influence the course of ALL. It is well known that dramatic remissions occur in ALL following steroid therapy. In the mouse, T-cells with cortisol receptors are localized to the thymic cortex. The more differentiated T-cells in the thymic medulla are resistant to cortisone, presumably because they lack the appropriate receptors. The similarities in clinical course and surface markers of the neoplastic cells of Sternberg’s lymphoma and ALL suggest that the thymus, during its period of physiologic activity (early childhood), is the locus of malignant transformation for these diseases. This idea is further supported by observations that ALL rarely occurs at a time of relative thymic atrophy (adulthood). Thus, the T-cell pool in adulthood may be independent of an actively proliferating thymocyte. All that may be needed in the adult is a remnant of thymic epithelium, the site of production of thymopoietin. Understanding the role of this hormone in T-cell maturation at various ages could be crucial to understanding the pathogenesis of lymphoproliferative disease.

B-cells mature in a fashion similar to T-cells by developing from a multiple marker cell into cells endowed with markers for agg. Ig and C. Cell surface
markers for agg. Ig have been demonstrated in the absence of Ig determinants in chronic lymphocytic leukemia (CLL). The scheme depicted in Fig. 1 is consistent with the failure to find lymphocytes having surface Ig but lacking Fc receptors. The B-cell, therefore, acquires its markers in a sequential fashion, with surface Ig markers appearing after the markers for agg. Ig and C and before terminal differentiation of the lymphocyte to a plasma cell. It should be noted that a number of studies have shown a discordance in the percent of C-bearing and Ig-bearing cells, both in mice and in humans. As indicated in Fig. 1, the antigen-driven differentiation of B-cells into plasma cells may involve loss of surface receptors, in particular, those involved in the binding of complement.

Based on studies of lymphocyte surface markers, many investigators have classified CLL as a B-cell malignancy. However, the existence of T-cell CLL has been demonstrated recently. Work in our laboratory has pointed to a more fundamental abnormality in the pathogenesis of CLL. By the use of newly developed techniques for purifying human T- and B-cells by passage through nylon columns, we have studied the response of circulating CLL lymphocytes to mitogenic stimulation by phytohemagglutinin (PHA). Our results have shown the characteristic 3- to 5-day delay in proliferation, irrespective of whether the cells bore T- or B-cell markers on their surface. These findings suggested to us that both T- and B-cells are abnormal in CLL.

Recent studies have shown that IgD is present on about 15% of human cord blood lymphocytes. The majority of these cells also bear IgM. Kubo et al. have found that CLL lymphocytes which have IgM on their surfaces also bear IgD. This suggested that CLL cells are analogous to other tumor cells which may express fetal antigens on their surface. IgD, however, may not be a fetal antigen in the sensus strictus since it is also produced by the lymphocytes of normal adults. If the Ig type on a lymphocyte reflects the extent of differentiation of antibody-producing B-cells (i.e., $\delta \rightarrow \mu \rightarrow \gamma \rightarrow \alpha$), as suggested by Lawton and Cooper, the presence of IgD may reflect a poorly differentiated or dedifferentiated cell. Similarly, it has been clearly documented that, although CLL B-cells bear Ig determinants in the majority of cases, the density of these determinants is diminished.

I suggest that CLL involves a defect acquired early in the maturation of the lymphocyte. All of the observed phenomena, including defective ribosome processing in CLL cells, might simply reflect a metabolic alteration acquired by a precursor cell. The defect would then be reflected in inadequate development of antigen (and PHA)-recognizing surface determinants which result eventually in failure of terminal differentiation. A precursor cell defect could explain our data which suggest that both T- and B-cells are involved. This view is compatible with the clinical manifestations of hypogammaglobulinemia and autoimmune phenomenon seen in the disease.

The Sezary syndrome is a rare condition characterized by erythrodermia and generalized pruritis with an associated increase in large, abnormal cells in the skin and peripheral blood. Morphologic and ultrastructural studies have confirmed the lymphocytic origin of these Sezary cells. Recently, a small cell variant of the Sezary syndrome has been described which, by morphologic and
clinical criteria, may be difficult to distinguish from the CLL cell. However, in contrast to the circulating cell seen in the majority of patients with CLL, most abnormal cells of the Sézary syndrome form rosettes with SRBC (60%-90%) and lack agg. Ig and C receptors, suggesting a T-cell disease. It is worth noting that, in all patients studied to date, the number of SRBC attached to the Sézary cell as a rosette has been lower than that observed with normal T-lymphocytes. The Sézary cell therefore appears to be analogous to that of CLL where surface Ig determinants are present but diminished in the majority of cases. Studies on the responsiveness of Sézary cells to PHA have shown variable results. Crossen et al. have found a normal response, whereas Labaze et al. have found Sézary cells unresponsive to PHA stimulation. We have found the maximum lymphocyte response to PHA in two cases of small cell variant Sézary syndrome to be delayed to 120 hr (normal, 48-72 hr). These findings were similar to those obtained in studies of two cases of CLL with erythrodermia (S. Davis and A. D. Rubin, unpublished data).

The immunologic and morphologic similarities between the abnormal lymphocytes infiltrating the skin in Sézary syndrome and mycosis fungoides (MF) have been confirmed; most of the tumor cells in MF represent T-cells. In addition, Sézary syndrome may become clinically indistinguishable from MF. In both diseases patients may present with multiple, painful tumor masses composed of neoplastic lymphocytes. Accordingly, it has been suggested that these two clinical entities represent two forms of a single disease process.

Cell surface marker studies in cases of nodular lymphoma (NL) have consistently shown Ig-determinants in normal amounts on tumor cells. Based on these findings, there seems little doubt that NL is a B-cell malignancy. Jaffe et al., who studied biopsy material by immunologic methods, concluded that most cases of NL arise in the germinal centers of isolated lymphoid organs. Our data support such a conclusion; circulating lymphocytes from patients with NL responded to PHA with two peaks of ³H-uridine incorporation, reflecting a population of normal lymphocytes responding at 48 hr and a delayed population responding at 168 hr. Unlike CLL, which appears to involve a diffuse replacement of lymphoid tissues by a monoclonal proliferation of neoplastic lymphocytes, NL most likely involves unicentric or multicentric proliferations of neoplastic B-cells.

Burkitt's lymphoma appears to be analogous to NL. Of 35 Burkitt's lymphoma biopsies, van Furth et al. found 21 which secreted Ig, mainly IgM. In addition, those human lymphoblastoid lines that carry the Epstein-Barr virus genome, e.g., Burkitt's lymphoma and infectious mononucleosis, have receptors for Ig. This evidence suggests that Burkitt's lymphoma might also be a B-cell malignancy.

At the present time the origin of the malignant cell in Hodgkin's disease is unknown. Based mainly on histologic criteria, various studies have suggested that the Reed-Sternberg (RS) cell is the malignant cell. Tindle et al. have suggested that the RS cell arises as the result of an abnormal proliferation of plasma cell precursors (B-cells), whereas Thomson has suggested that this cell arises as the result of an abortive attempt at the formation of Hassall's corpuscles in the thymus. Rappaport states that the RS cell is a malignant histo-
cyte. Recently, Leech performed cell surface immunofluorescent staining for Ig on a biopsied lymph node from a patient with mixed-cellularity Hodgkin’s disease and found that 78% of RS-like cells showed positive staining for IgG. Obviously, confirmatory data is needed, but these preliminary findings suggest that the RS cell in Hodgkin’s disease is of B-cell origin.

Although essentially limited in the scope of their clinical manifestations, the primary agammaglobulinemias (AG) have shown considerable variability in the percentages of lymphocyte subpopulations detectable by cell surface markers. Most studies have reported decreased numbers of Ig-bearing cells in AG, but there have been recent demonstrations of normal levels of circulating Ig-bearing cells. Dickler et al., with the simultaneous rosette-agg. Ig technique, has found cases of AG involving high percentages of null cells. From these studies it appears that AG, unlike NL, may be a more heterogeneous disease, with possible defects occurring at multiple sites along the proposed pathway of B-cell differentiation (Fig. 1). From the work of Wernet et al. it appears that the defect in certain cases of AG may be due to the absence of some external signal with resultant failure in Ig synthesis. These investigators have shown that a factor present in normal serum could induce lymphocytes from a patient with AG to produce Ig in vitro. Certain cases of AG, therefore, may result from blocked B-cell proliferation due to the absence of some extrinsic factor.

There remain to be discussed two diseases where immunologic deficits play a major role in the clinical course, but, because of our limited knowledge, no concrete pathogenetic defect in my scheme can be assigned. Ataxia-telangiectasia (A-T) is a hereditary multisystem disease characterized clinically by cerebellar ataxia, oculocutaneous telangiectasia, frequent sinopulmonary infections, and various gastrointestinal and endocrine dysfunctions. A-T patients, in addition, exhibit partial defects both in cellular and humoral immunity that show progressive deterioration with time. The immunologic abnormalities consistently present in this disease are low or absent serum levels of IgA and an absent or poorly developed thymus gland. The nature of the fundamental abnormality in A-T is obscure, but it appears that impaired thymic development may not be involved. McFarlin et al. have suggested that A-T involves a developmental defect of tissue differentiation and interaction between primitive entodermal and mesodermal cells. Still other investigators incorporate the concept of a basic thymic abnormality with a secondary autoimmune phenomena. An increased incidence of autoantibodies has been reported in these patients, and the central nervous system pathology has been described as demyelinating as well as degenerative. The Wiskott-Aldrich (W-A) syndrome is another hereditary disease characterized clinically by eczema, thrombocytopenia, and recurrent infection. Histopathologically, the thymus has features of secondary atrophy with decreased numbers of lymphocytes and indistinct corticomedullary differentiation. This corresponds to the lymphopenia observed as the patients age and is reflected in a progressive decrease in cell-mediated immune responses. Young children with the disease may respond normally to immunologic testing, but, as immunologic attrition occurs, they classically have an inability to process polysaccharide antigens.
generalized antibody-producing defect is also usually seen. Kuramato et al. have linked the thrombocytopenia and immune deficiency to a single defect in the alpha-granules of platelets and macrophages resulting in decreased platelet survival and the inability of macrophages to respond to specific antigens. However, Oppenheim et al., by the use of in vitro techniques, have shown W-A macrophages to be functionally normal. It is interesting that the ability of lymphocytes to undergo blastogenesis following exposure to PHA is normal in W-A; it is possible that W-A represents a condition characterized by immunologic competence to mitogens but a failure of antigenic recognition. But any theory of the basic defect in W-A must await adequate explanation of the associated clinical findings. Surface marker studies have not helped in the elucidation of an immunologic defect since no consistent abnormality can be found.

In this report I have attempted to expand the differentiation of the lymphoid system to include subpopulations of cells based on results obtained from studies on characteristic cell surface markers. It is suggested that maturational defects arising in this scheme may explain the pathogenesis of lymphoproliferative diseases in which a lymphoid abnormality is suspected as being etiologic. Conceivably, the classification of lymphoid diseases with regard to proposed sites of origin will be of diagnostic and therapeutic importance.

ACKNOWLEDGMENT

The author wishes to thank Dr. Arnold D. Rubin for his helpful discussions during the preparation of this manuscript.

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