Henry Ford Hospital Medical Journal

Volume 32 | Number 1 Article 6

3-1984

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Henry Ford Hosp Med J
Vol 32, No 1, 1984

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Recent progress in our understanding of the immune system and the development of new techniques that permit the precise identification of lymphocytes have permitted a reexamination of lymphoid neoplasms. Most of the non-Hodgkin's lymphomas and lymphocytic leukemias have been characterized as T or B cell neoplasms and have been shown to possess features similar to those expressed by normal lymphoid cells at different stages of maturation. The clinical significance and therapeutic implications of these discoveries are now being explored. This paper presents a concise overview of the differentiation of human B lymphocytes, the surface and cytoplasmic markers that permit their recognition, and the diverse tumors that are now known to be malignant counterparts of normal B cell elements. Particular emphasis is placed on the utility of surface and cytoplasmic immunoglobulin as unique B cell markers and on the clonal nature of B cell tumors.

The last decade was a period of active discovery in the field of lymphoreticular neoplasia. New immunologic information and techniques made it possible to reexamine these tumors in a unique manner. These developments led to significant changes in our concepts and to a better understanding of the interrelation of these neoplasms (1-7). In this paper some of the major observations from these studies will be reviewed and correlated with representative examples of B cell neoplasms obtained from patients at Henry Ford Hospital. A concise review of our current understanding of B cell differentiation will also be presented.*

B Cell Ontogeny

Human B lymphocytes undergo an orderly maturation process known as B cell ontogeny (6,8-11). Lymphoid precursors in the fetal liver and bone marrow and in the adult bone marrow are derived from pluripotent stem cells (12-14). Those destined to develop into B lymphocytes and plasma cells undergo a series of genetic changes that culminate in the expression of cytoplasmic, membrane-bound, and ultimately secreted immunoglobulin (6,15,16). The signals for inducing these changes at the genomic level are currently being investigated; however, the consequences of these changes are increasingly being appreciated. This differentiation occurs in two phases: antigen-independent and antigen-driven (Fig. 1).

An early antigen-independent change in B cell precursors is the rearrangement of immunoglobulin genes to code for functional messengers. The DNA sequences that code for immunoglobulin heavy chains are located on chromosome 14, while those coding for kappa and lambda light chains are present on chromosomes 2 and 22, respectively (17-19). Nucleotide sequences that code for the constant and variable portions of immunoglobulin are consolidated and translated into messenger RNA that will instruct the synthesis of a complete heavy or light chain (16,20). A hierarchy exists within the cell which leads to initial synthesis of IgM heavy chains followed by kappa and then lambda light chain production (21,22). The IgM heavy chains initially are present only in the cytoplasm of the cell. Lymphoid cells containing cytoplasmic IgM but lacking surface immunoglobulin are designated pre-B cells and are the earliest recognizable B cells (9-11,23). They are present in the adult bone marrow where they constitute a pool of self-replicating B cell precursors. The IgM heavy chains are consolidated with either kappa or lambda light chains and inserted into the membrane of the young B lymphocyte where they function as antigen receptors. IgD synthesis then ensues, and these molecules are also inserted in the B cell membrane (6,9,11). Antigen

*Abbreviations used: Ia/DR (Immune-associated or D-related antigens are specific membrane antigens coded within the major histocompatibility complex); Fc (the crystallizable fragment is the non-antigen specific carboxy terminal end of an immunoglobulin molecule); ALL, acute lymphoblastic leukemia; CALLA, common acute lymphoblastic leukemia antigen; Tdt, terminal deoxynucleotidyl transferase; FAB, French-American-British; FCC, follicular center cell.

Submitted for publication: February 2, 1984

Accepted for publication: March 1, 1984

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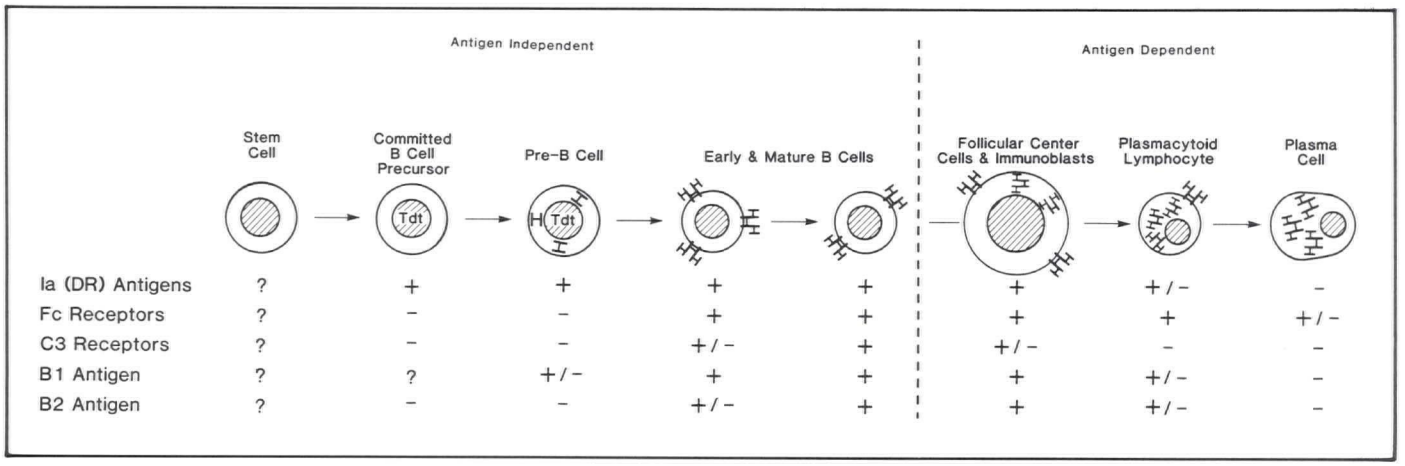

Fig. 1

Phenotypic Features of B Lymphocytes of Several Stages of Differentiation.

As a B cell differentiates, it acquires and loses certain characteristics that permit it to be recognized. Surface and cytoplasmic immunoglobulin (indicated by the line diagrams associated with the individual cells) are unique, intrinsic products of the B lymphocyte and are very specific markers.

Other features, eg, the B1 and B2 antigens, are believed to be B cell specific differentiation antigens. Their functional role is unknown. Finally, surface moieties like the C3 and Fc receptors are present on other lymphocyte and mononuclear cell populations and thus lack specificity. Detection of several of these features on a tumor cell population permits the tumor to be assigned a particular phase of B cell ontogeny.

recognition apparently triggers an additional series of DNA rearrangements at the heavy chain locus, a process referred to as heavy chain class switching. The latter result in the synthesis and expression of IgG, IgA, or IgE (6,9,15,24,25).

The membrane-inserted immunoglobulin of each B lymphocyte contains both common (class-specific) and unique (idiotypic) elements. Amino acid substitutions in the hypervariable regions of the heavy and light chains contribute to an antigen-specific receptor that will activate the cell and induce proliferation and differentiation when the complementary antigen is encountered (26). Each B cell expresses either kappa or lambda light chains, but not both. When a B cell clone expands, under physiologic or pathologic circumstances, the daughter cells and the immunoglobulin secreted by the more mature elements of the clone will possess the same light chains. This is the basis of the concept of monoclonality on which many diagnostic decisions about B cell neoplasia are based (4-6,15,27-29). The membrane immunoglobulin also provides a unique and convenient marker to identify B lymphocytes. When labeled anti-immunoglobulin antisera with specificity for the individual heavy or light chains are incubated with tissue sections or cell suspensions containing lymphocytes, the antisera will bind only to those cells possessing the complementary antigen, ie, immunoglobulin heavy or light chain of the same class or type. This method is used to identify, enumerate, and separate B lymphocytes from T lymphocytes and other mononuclear cells.

The membrane or surface IgM^+ B lymphocyte and the surface IgM^+ IgD^+ B cell are relatively immature cells. Most peripheral blood B lymphocytes belong to one of these two groups (30). Circulating B cells that express membrane IgG or IgA are uncommon, comprising no more than 2% or 3% of the circulating lymphocyte pool. B lymphocytes at various stages of maturation also populate the secondary lymphoid organs (lymph nodes, spleen, bronchial, and gut-associated lymphoid tissue) and the bone marrow (6,10,15,31-33). The follicular and medullary cord regions are the portions of the lymph nodes in which B cells predominate. B cells in each of these areas have distinctive characteristics.

Activation of a B lymphocyte is accompanied by transformation (follicular center cells and immunoblasts), proliferation, and differentiation (6,31,34,35). When B immunoblasts divide, the daughter cells become plasma cells or memory B lymphocytes. As the cells enter the preterminal and terminal stages of differentiation, they express more cytoplasmic and less membrane immunoglobulin. The mature plasma cell is rich in cytoplasmic immunoglobulin and has little membrane immunoglobulin. B cell ontogeny, therefore, includes changes in the relative amounts of membrane and cytoplasmic immunoglobulin expressed at various stages of differentiation.

While immunoglobulin is a unique B cell marker, other features may be used to identify B lymphocytes. These markers include Ia (DR) antigens, receptors for the

third component of complement and the Fc portion of immunoglobulin, and a family of B cell antigens that are recognized by monoclonal antibodies (Fig. 1). However, none of these markers provide the clonal information obtained by light chain identification. For a fuller discussion of these markers, other papers may be consulted (4,6,36-41).

B Lymphocyte Neoplasms

Two concepts have dominated discussion of B cell neoplasia over the last decade. The first is the concept of monoclonality, ie, malignant tumors are a result of the uncontrolled growth of a single abnormal cell. Experimental support for this thesis has come from cytogenetics, glucose-6-phosphate dehydrogenase studies, and analysis of the immunoglobulin associated with B lymphocyte neoplasms (27-29,42,43). B cell neoplasms are ideally suited for these studies since each possesses a characteristic surface, cytoplasmic or secretory immunoglobulin product. The idiotypic determinants of each immunoglobulin molecule permit B cells to be discriminated at an individual cell level. The immunoglobulin associated with a B cell tumor, therefore, is a tumor-specific antigen. Recently, investigators have taken advantage of this feature and prepared monoclonal antibodies to the idiotypic determinants expressed on patients' B cell tumors (44,45). In some instances the response has been very encouraging, and additional work is under way.

Identifying and preparing antibodies to the idiotypic determinants is a complex, time-consuming process which is not in general use. Instead, advantage is taken of the single light chain expressed by all of the progeny of a clonally expanded B cell population. Therefore, in clinical terms, clonal B cell proliferation means demonstrating that all of the cells (or secreted immunoglobulin product) possess only kappa or lambda chains and not both (4-6,15,27-29,42,43).

The second feature associated with B cell neoplasia is the concept of "maturation arrest" introduced by Salmon and Seligmann in 1974 (8). They argued that various B cell tumors share many features with normal B lymphocytes and are expanded populations of malignant cells "frozen" at normal stages of B cell differentiation. While this concept has been extensively validated (4,6,15) and has provided a useful working model, it needs to be modified to accommodate new findings (46-53). Maturation arrest should be considered relative rather than absolute. Morphologists have recognized for some time that while most B cell tumors have a dominant morphologic and immunologic phenotype, many of these neoplasms are heterogeneous (47). However, it has required the techniques of modern cellular immunology and immunochemistry to establish that the diverse elements within a particular B cell tumor are all part of the same neoplasm, ie, they are monoclonal. Examples of this diversity within individual entities include: 1) increasing recognition of monoclonal proteins in the serum and urine of patients with non-Hodgkin's lymphomas and chronic lymphocytic leukemia (48-50); 2) the evolution of chronic lymphocytic leukemia into an aggressive, immunoblastic neoplasm (Richter's syndrome) that shares the same immunologic phenotype as the original tumor (51); and 3) the observation that the bone marrow and peripheral blood of some patients with classic multiple myeloma contain pre-B cells and small lymphocytes which express the malignant idiotype (52,53).

Case Reports

The following section contains the cases of patients with B cell tumors that demonstrate many of the features discussed earlier.

Case 1

A 13-year-old girl was admitted from the Emergency Room with a three-week history of irregular fevers, migratory body aches, anorexia, and weight loss. Physical examination revealed a febrile (38°C), pale child with a normal pulse and blood pressure. Neither lymphadenopathy nor hepatosplenomegaly were present. She complained of sternal, left femoral, and left tibial tenderness upon palpation. Initial laboratory studies revealed a hemoglobin of 11.1 gm/dl, white blood count of 8,600/mm^3, and a platelet count of 319,000/mm^3. The peripheral blood differential count contained 14 polymorphonuclear cells, 24 bands, 47 lymphocytes, 1 monocyte, 3 metamyelocytes, 3 myelocytes, and 8 blasts. A bone marrow aspirate and biopsy revealed 95% cellularity with diffuse replacement by lymphoblasts and immature lymphoid cells. The diagnostic impression was acute lymphoblastic leukemia. A portion of the bone marrow aspirate material was submitted for immunologic phenotyping. These studies revealed a leukemic cell phenotype with the following pattern: $CALLA^+$ Ia^+ $Tdt^{(+)}$ E^- $cIgM^-$ mIg^-. Bracketed ( ) results indicate the presence of the marker in a subset of the tumor cells and not in the entire population.

Acute Lymphoblastic Leukemia (ALL)

Acute lymphoblastic leukemia is a generic disorder that may be subcategorized into five groups on the basis of immunologic markers and nuclear enzyme studies (Table I, 54-56). The largest single group is the "common" or "non-T, non-B" type of ALL. Although these leukemic cells do not express typical T or B cell features, they are usually positive for Ia, CALLA, and nuclear Tdt. Recently, the DNA from several of these leukemias was

TABLE I
Immunologic Phenotypes of Acute Lymphoblastic Leukemia

	Incidence (%)[1]	Ig Genes[2]	cIg[3]	mIg[4]	T cell[5] markers	Tdt[6]
Common	50-70	+	–	–	–	+
Pre-B cell	5-30	+	+	–	–	+
B cell	2-5	+	–	+	–	
T cell	15-35	–	–	–	+	+/–
Undetermined	5-25	?	–	–	–	+/–

[1]Approximate figures based on several reported series
[2]A positive indicates immunoglobulin gene rearrangement is present
[3]Cytoplasmic immunoglobulin
[4]Membrane immunoglobulin
[5]Rosette formation with sheep red blood cells and/or reaction with anti-T cell antibodies
[6]Terminal deoxynucleotidyl transferase

examined by DNA hybridization methods, and the immunoglobulin genes were observed to be rearranged, suggesting an abortive commitment to the B cell lineage (56). The child presented in Case 1 is an example of "common" ALL. These patients have a good prognosis and usually respond to therapeutic intervention.

The incidence of pre-B ALL varies. In early reports it represented less than 10% of all cases (57), but as its features are becoming better understood, it is being reported more often (58). Morphologically, pre-B ALL cannot be distinguished from the other types of ALL. The immunologic marker profile is similar to that of common ALL with one exception. The distinguishing feature of pre-B ALL is the presence of cytoplasmic IgM, usually detected by direct immunofluorescent examination of the leukemic cells. These cells lack cytoplasmic light chains and membrane immunoglobulin (23,57,58).

B-cell ALL is an uncommon entity occurring primarily in children (54,55,59,60). It is characterized by the presence of membrane immunoglobulin, often of the IgM class, on cells with morphologic features of ALL. Some investigators have suggested a relationship to Burkitt's lymphoma. This is the one type of ALL that may be discriminated morphologically; it often falls into the L3 group of the FAB classification (61).

T cell neoplasms comprise 15-35% of ALL cases (54,55). They are characterized by their ability to form rosettes with unsensitized sheep red blood cells and/or their reaction with T cell specific antisera. Many cases have an associated mediastinal T cell neoplasm at some stage of the disease. The incidence of ALL cases that cannot be determined or classified varies from series to series, depending partly on the extent to which a reporting laboratory has attempted to phenotype these tumors. The variability also reflects our limited knowledge. In most adult ALL series, the group of undetermined cases is larger, a fact which suggests that some differences in this condition may exist between adults and children.

Case 2

A 69-year-old man presented with cervical and axillary lymphadenopathy. Hepatosplenomegaly was not present, and the initial laboratory studies were unremarkable. A cervical lymph node biopsy was performed. The pathologic diagnosis was non-Hodgkin's lymphoma, poorly differentiated lymphocytic type, nodular and diffuse pattern. A cell suspension prepared from a minced portion of the biopsy tissue was submitted for immunologic marker studies. These studies revealed a presumably monoclonal population of B lymphocytes with the membrane phenotype: IgM^+ $IgD^{(+)}$ $Lambda^+$ Ia^+ $B1^+$ $B2^+$. The percentages of IgM and IgD positive cells were 77% and 21%, respectively.

Neoplasms derived from follicular center cells comprise the largest single group of non-Hodgkin's lymphomas (1-7,15,33,42). These tumors include all of the nodular and many of the diffuse, poorly differentiated lymphocytic, mixed, and large cell lymphomas of the modified Rappaport scheme (62) as well as the cleaved and many non-cleaved tumors in the Lukes-Collins classification (1,2). Immunologic phenotyping studies have demonstrated that these tumors are B cell neoplasms (4,5,15,27,29,33,40-42,63-65). Most tumors express membrane IgM, often associated with IgD (4,6,15,63,64); less often, membrane IgG or IgA is the dominant membrane immunoglobulin.

Case 2 is an example of the discordance between IgM and IgD expression. Although the reasons are unknown, under physiologic circumstances, IgD disappears from the B cell membrane after it has been activated with antigen. Tumors with less membrane IgD may represent neoplastic equivalents of antigen-activated B cells. The studies cited indicate that the FCC lymphomas represent neoplastic equivalents of B cells from the early, antigen-independent stage through the mature, antigen-activated B lymphocyte. FCC lymphomas express other markers in addition to membrane immunoglobulin,

including Ia antigens, C3 and Fc receptors, B1 and/or B2 antigens, and in some instances, CALLA (4,5,15, 40-42,63,64). Some cases also have variable amounts of cytoplasmic immunoglobulin-positive cells. Attempts to correlate histologic subtypes with immunologic phenotypes have only been partially successful, and considerable heterogeneity exists within either category (65). A leukemic phase accompanying FCC lymphomas is being recognized more frequently as immunologic methods are combined with flow cytometric methods (66,67). It is possible to understand peripheral blood involvement better by these methods than by classical means. Clinical studies are currently being undertaken to evaluate the significance of these findings.

Case 3

An 88-year-old man with many medical problems presented with a left groin mass and edema in his lower extremities. A computerized tomographic (CT) scan of the abdomen showed extensive retroperitoneal lymphadenopathy, and serum protein immunoelectrophoresis revealed an IgM, kappa monoclonal protein. An excisional biopsy of the mass was performed. The pathologic diagnosis was non-Hodgkin's lymphoma, nodular and diffuse, large cell type with prominent plasmacytoid features. The pathologist described a large, non-cleaved lymphoid cell as the predominant cell type and commented upon the exceptional number of plasma cells. Immunologic phenotyping of a portion of the biopsy tissue revealed a population of presumably monoclonal B lymphocytes with the following features: IgM^{+} IgD^{-} $Kappa^{+}$ $Ia^{(+)}$ $B1^{(+)}$.

Large cell lymphomas are a heterogeneous group of disorders that include neoplasms of B cell, T cell, or true histiocytic lineage (4,5,68,69). In most series approximately one third to one half of the large cell lymphomas are B cell tumors with a phenotype similar or identical to that described for FCC neoplasms. Substantial numbers of large cell tumors do not express either surface or cytoplasmic immunoglobulin, although they may express Ia antigens and C3 or Fc receptors. The latter have been referred to as "null" cell tumors since they lack typical B or T cell characteristics. With the advent of more B and T cell specific monoclonal antibodies and DNA hybridization techniques, new insights into the nature of these tumors should be available soon.

Serum from the patient in Case 3 contained an IgM, kappa monoclonal protein. This feature is being observed more often as laboratory methods to detect these proteins become more sensitive and widely applied (48,70). In most such cases the presumption is that the serum or urine monoclonal protein is a product of the tumor cell population, just as it is in multiple myeloma. However, this has not been formally established in most instances. Where a monoclonal protein is associated with a case of non-Hodgkin's lymphoma, it may provide a useful marker to monitor the tumor burden.

Case 4

A 68-year-old man presented with generalized lymphadenopathy, but his past medical history was unremarkable. Physical examination revealed that the adenopathy was accompanied by a moderately enlarged spleen. The white blood cell count was 118,000/mm^3 with 85% lymphocytes, 10% atypical lymphocytes, and 3% prolymphocytes. The hemoglobin was 9.0 gm/dl, and the platelet count was 156,000 mm^3. Bone marrow study revealed a diffuse infiltration of the marrow elements with mature lymphocytes. The clinical and morphologic impression was chronic lymphocytic leukemia, Rai stage 4. A sample of peripheral blood was submitted for immunologic marker studies. They revealed a presumably monoclonal population of B lymphocytes with the membrane phenotype IgM+ IgD(+) Kappa+ Ia+ B1+ B2–. High resolution agarose gel electrophoresis, and immunofixation of the serum and urine revealed monoclonal free kappa light chains in the beta region.

Chronic Lymphocytic Leukemia (CLL)

Case 4 is a typical example of the immunologic phenotype observed in CLL. Usually of B cell origin in more than 90% of cases (28,42,71,72), it is considered by most to be a neoplastic expansion of immature B lymphocytes. The membrane immunoglobulin on CLL cells is weakly expressed and at times may not be evident. IgM and varying amounts of IgD are the predominant heavy chain classes observed. Kappa light chain positive cases outnumber lambda positive cases by about 2:1. CLL cells also express C3 receptors and the B1 and B2 antigens in most instances (72). Classically, CLL has been described as a "nonsecretory" B cell tumor with monoclonal proteins reported in less than 10% of cases. However, experimental studies have shown that CLL cells cultured in vitro usually secrete free light chains of a single type and identical to that expressed on the surface of the leukemic cells (73). Introduction of high resolution agarose gel electrophoresis and immunofixation has enhanced our ability to identify these secretory products in body fluids (50,70). In a consecutive series of 35 CLL patients studied in our laboratory, we identified a serum or urine monoclonal protein in approximately one half of the patients. Thus, the incidence of monoclonal proteins in CLL is much higher than anticipated. One group of workers used the monoclonal urinary light chains from CLL patients to prepare tumor-specific anti-idiotypic antibodies for clinical investigation and possible therapeutic intervention (74).

Case 5

A 55-year-old man was transferred from another hospital with a history of fever, weakness, and malaise of 10 days' duration. He had been in good health until two years before the present admission. At that time, he also complained of weakness and malaise. He was pancytopenic, and a bone marrow examination reportedly showed myelofibrosis. Six months before his current illness, he experienced weakness and fever. Splenomegaly was noted, and a bone marrow examination again revealed myelofibrosis. No specific treatment was given, and his fever was not explained.

Immediately before his transfer, cultures from several sites and several radiologic studies were reported to be negative, and he was treated empirically with antibiotics. On admission he was febrile (38.2°C) and was experiencing shaking chills. Physical examination revealed a chronically ill man with mobile axillary lymph nodes and marked splenomegaly. Crusting perioral herpetic lesions were present. He was pancytopenic (hemoglobin 8.4 gm/dl, white blood cell count 600/mm^3, platelet count 61,000/mm^3) and had a relative lymphocytosis (79%). No abnormal cells were observed on peripheral blood examination. Multiple blood, urine, sputum, and cerebrospinal fluid cultures and stains were negative. A splenectomy was performed after considerable consultation and discussion. The spleen was diffusely enlarged (1,800 gm) and a deep red color. No discrete lesions were noted on gross examination. Histologic examination revealed two conditions: hairy cell leukemia and caseating microgranulomata. Special stains revealed acid-fast organisms in the granulomatous areas, and cultures of the splenic tissue subsequently were positive for Mycobacterium kansasii, a member of the atypical Mycobacteria family. A portion of the tissue submitted for immunologic studies revealed a presumably monoclonal population of B lymphocytes with the membrane phenotype IgM^- IgD^+ IgG^- $Kappa^+$ Ia^+.

Studies conducted in the last decade have established that most examples of hairy cell leukemia are B cell expansions (75-77). However, well-described examples of T hairy cell leukemia and other variants have been reported (78). In contrast to CLL, IgM or IgD are not present as often (77). This evidence has been used by some to argue that hairy cell leukemia is a more mature condition than CLL with regard to the ontogenic scheme described earlier. Hairy cells have avid Fc receptors but often lack C3 receptors. They express Ia and a variety of other antigens recognized by monoclonal antibodies. Efforts to identify the normal equivalent of the hairy cell have not been successful. The case presented is typical of this condition. Patients frequently present with pancytopenia, and the characteristic morphologic features may not be obvious upon initial examination. Infections with low-grade pathogens, particularly the atypical mycobacteria, are also characteristic of this disorder and remain unexplained (79).

Case 6

A 67-year-old woman was in good health until one month before admission when she noted a swelling in the left submandibular and cervical areas. Physical examination revealed nodal enlargement in the left cervical, occipital, and submandibular regions. The spleen extended below the left costal margin, and a mass was palpable in the right lower quadrant. A CT scan of the abdomen revealed massive retroperitoneal lymphadenopathy with mesenteric and small bowel involvement. A left supraclavicular lymph node was biopsied. The pathologic diagnosis was non-Hodgkin's lymphoma, diffuse large cell type (immunoblastic) sarcoma. Immunopathologic examination of a representative portion of the biopsy material revealed a presumably monoclonal population of B lymphocytes with the membrane phenotype IgA^+ IgM^- IgD^- $Kappa^+$ Ia^+ $B1^+$ $B2^-$.

Immunoblastic tumors of the B and T lineages have been described (1-5,80,81). B cell neoplasms, despite their appearance, are neoplastic equivalents of the postfollicular immunoblast, a B cell at an advanced stage of differentiation. These tumors grow in a diffuse pattern. The characteristic cell is a large, transformed lymphocyte with considerable cytoplasm containing many ribosomes and frequently a perinuclear hof. The nuclei are round to oval, often eccentrically located, and contain one or two prominent nucleoli (1,2,81).

Plasmacytoid features are readily apparent, and in some instances a spectrum of cells from the immunoblast to the mature plasma cell is present. B immunoblastic sarcomas express many of the immunologic features described in this paper. As noted, they often have plasmacytoid features and express cytoplasmic immunoglobulin of a clonal nature. Case 6 is of interest in this regard because IgA was expressed as the sole membrane immunoglobulin, and the B1 but not the B2 antigen was expressed. Both features are consistent with an advanced stage of B cell maturation (Fig. 1). These tumors, which are commonly observed in immunocompromised hosts (80), may present de novo or evolve during the course of other B cell immunoproliferative disorders, eg, CLL and multiple myeloma (51,82).

Case 7

A 56-year-old man presented with a three-month history of night sweats, a 10-pound weight loss, and increasing fatigue. Physical examination revealed bilateral axillary lymphadenopathy and slight hepatomegaly. Initial laboratory studies revealed a hemoglobin of 9.7 gm/dl, a white blood cell count of

9,200/mm^3, and a platelet count of 299,000/mm^3. The total protein was 9.9 gm/dl. Quantitative immunoglobulin levels were: IgG, 393 mg/dl; IgA, 121 mg/dl; and IgM, 9,790 mg/dl. Serum protein electrophoresis revealed a prominent band in the mid-gamma region which, upon immunoelectrophoretic analysis, was established as an IgM, kappa monoclonal protein. Plasma and whole blood viscosity levels were elevated. A test for serum cryoglobulins was negative. An axillary lymph node was removed. The pathologic diagnosis was malignant lymphoma, well-differentiated lymphocytic type with plasmacytoid features, diffuse patten, consistent with Waldenström's macroglobulinemia. Immunologic marker analysis of the biopsy material revealed a presumably monoclonal population of B lymphocytes with the membrane phenotype IgM^+ IgD^- $Kappa^+$ $Ia^{(+)}$. Immunoperoxidase studies for cytoplasmic immunoglobulin revealed a large number of lymphocytes and plasmacytoid cells that stained positively for IgM and kappa.

Waldenstrom's Macroglobulinemia

The final stages of B cell maturation culminate in the production of antibody secreting cells, which include the plasma cells and plasmacytoid lymphocytes. Morphologically, this phase is characterized by a mixture of small lymphocytes, plasmacytoid lymphocytes and plasma cells, often with an admixture of immunoblasts. The plasmacytoid lymphocyte, a cell with features of both the lymphocyte and the plasma cell, presumably represents a transitional form between the two elements. Immunocytologic studies have demonstrated that these cells contain cytoplasmic immunoglobulin and may also express surface immunoglobulin (83). Significant numbers of these cells are present in the blood and tissues of individuals with Waldenstrom's macroglobulinemia, a classic preterminal B cell neoplasm (84,85). In the early stage this disorder is characterized by an IgM serum monoclonal protein and a lymphoplasmacytic infiltrate in the bone marrow and peripheral blood. Clinical symptoms may result from the hyperviscosity syndrome created by high levels of the IgM monoclonal protein. Later, lymphadenopathy, hepatosplenomegaly, and anemia become prominent and contribute to the clinical features (86). Lymphomas with histopathologic features similar to that observed in Waldenstrom's macroglobulinemia, and associated with IgG or IgA monoclonal proteins or with no obvious secretory component, have been reported (87,88). These are slowly progressive tumors that do not come to clinical attention until a frankly lymphomatous condition exists. While immunopathologic studies of these tumors are sparse, the presence of a clonal population of cells rich in cytoplasmic immunoglobulin has been documented.

Case 8

A 67-year-old retired man complained of a six-month history of intermittent chest and low back pain exacerbated by exercise. Physical examination was unremarkable except for tenderness to palpation in the lumbar region. Laboratory studies included a hemoglobin of 10.5 gm/dl, a white blood cell count of 3,500/mm^3 with a normal differential, and a platelet count of 380,000/mm^3. The serum calcium, creatinine, and urea nitrogen were 9.1, 1.0, and 9.0 mg/dl, respectively. Serum protein electrophoresis revealed diffuse hypogammaglobulinemia and no evidence of a monoclonal protein. The serum IgG level was 225 mg/dl; IgA, 17 mg/dl; and IgM, 17 mg/dl. A 24-hour urine collection contained 1.0 gm of protein. Urine electrophoresis and immunoelectrophoresis revealed a prominent free kappa light chain monoclonal protein and traces of albumin. A bone marrow aspirate contained 42% plasma cells including many immature forms. Radiologic studies revealed compression fracture at T10-12 and L3-4. The clinical diagnosis was multiple myeloma, kappa light chain type.

Multiple Myeloma

Multiple myeloma is undoubtedly the most frequent B cell neoplasm. As a plasma cell tumor it represents the final stage in the spectrum of B cell neoplasia. The plasma cell is a short-lived cell with little proliferative capacity which is organized to produce large amounts of specific antibody for secretion into body fluids. Multiple myeloma is a neoplastic B cell disorder with a predilection for bone marrow rather than nodal or peripheral blood involvement. Plasma cells represent the most obvious cellular element in myeloma. However, a growing body of experimental evidence indicates that malignant transformation occurs in an immature B cell precursor that retains the capacity to differentiate into a plasma cell (52,53,89,90). Studies with the murine plasmacytoma, MOPC-315, have demonstrated that the malignant plasma cells differentiate from less mature precursors while under the influence of the host's immunoregulatory apparatus (89). Experiments performed in intraperitoneal diffusion chambers containing the malignant cells have shown that mature myeloma cells rapidly die and are replaced from a pool of lymphoid cells contained in the chamber. This observation suggests that the oncogenic event occurred, and is perpetuated, in an earlier cell (90). In a series of elegant experiments using cells from selected myeloma patients and a patient with an unusual B-ALL, Kubagawa, Mayumi, and their colleagues demonstrated considerable intraclonal diversity (52,53). These investigators prepared anti-idiotypic antibodies to the monoclonal protein associated with the malignant tumor and examined bone marrow and peripheral blood cells from the same patients with these antibodies, which had been labeled

with fluorescein. They found pre-B cells in the bone marrow and circulating, normal appearing B lymphocytes with membrane immunoglobulin containing the idiotype of the malignant clone. In the context of this discussion, these observations indicate that B lymphocytes at several stages of differentiation are present and express the idiotype associated with the myelomatous or leukemic cell population. These studies support the hypothesis that myeloma cells are derived from an earlier, malignant B cell.

The case presented is an example of light chain myeloma, a condition in which monoclonal free light chains are secreted without accompanying heavy chains (91,92). Approximately one of every five cases of multiple myeloma secrete free light chains exclusively; these cases pose certain diagnostic problems (93). The pattern of red blood cell aggregation known as rouleaux is not observed in this type of myeloma. Moreover, since the secreted light chains are rapidly cleared by the kidney and either reabsorbed and catabolized by the proximal tubular cells or excreted in the urine, there is usually no serum monoclonal protein demonstrated by electrophoresis. Instead, a moderately severe, diffuse hypogammaglobulinemia is found. Urine electrophoretic and immunoelectrophoretic studies are necessary to identify and characterize the free light chains and should be ordered whenever a patient suspected of having multiple myeloma does not have a typical serum monoclonal protein. These patients may have severe bone or renal disease, and amyloidosis is common, particularly in those individuals secreting lambda light chains (92). Bone marrow examination usually reveals the typical morphologic features of multiple myeloma.

Conclusion

The evidence cited in this paper supports the assumption that neoplastic B lymphocytes retain a large amount of the genetic information and maturation potential of normal B lymphocytes. Such investigations have deepened our understanding of normal and pathologic processes, provided a potentially quantitative and reproducible basis for the classification of B cell neoplasms, and stimulated the development of new therapeutic approaches that will be fully explored in the next decade. Since these immunologic advances can now be combined with our rapidly increasing understanding of oncogenes, we are able to examine the specific molecular events that lead to malignant transformation and explore the genetic and environmental factors that influence malignant cells. The B lymphocyte system is ideal for studying many of the general questions about essential features of the neoplastic process. Additional progress will come as a result of the creative use of recombinant DNA technology, flow cytometry, cell cloning, high resolution cytogenetics, and still undiscovered technologic and intellectual concepts.

Acknowledgments

I would like to thank the many physicians and surgeons who have assisted in these studies. Particular thanks to members of the Divisions of Hematology and Medical Oncology, Department of Internal Medicine, and to my colleagues in the Department of Pathology who frequently brought the material for these studies to my attention. I would also like to thank Dr. Hajime Hayashi, Dr. Carolyn Barth, and other members of the Immunopathology Division who have participated in the development and performance of the immunologic marker studies. Final thanks to Ms. Alice Taran for typing the manuscript.

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