Haematology and Blood Transfusion Vol. 28
Modern Trends in Human Leukemia V
Edited by Neth, Gallo, Greaves, Moore, Winkler
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B-Cell Malignancies: Origin and Extent of Clonal Involvement *

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Soon after it was established that normal lymphoid development proceeds along two distinct pathways of differentiation, it was recognized that lymphoid malignancies affected cells of either T or B lineage, and not both [1]. Studies conducted with animal models of lymphoblastic leukemias and lymphomas revealed that malignant T and B cells, like their normal counterparts, have their origin in central lymphoid tissues. The thymus is essential in the genesis of a variety of murine lymphoid malignancies that are induced by oncogenic viruses, ionizing irradiation, carcinogenic hydrocarbons, and hormones, or that arise spontaneously in AKR mice (reviewed [2]). Removal of the thymus prevents these lymphoid malignancies, and thymus transplants restore susceptibility [2–4]. This is due to an initial transformation of thymocytes with subsequent seeding or metastasis to peripheral tissues. On the other hand, the bursa of Fabricius is the source of malignant B cells in avian lymphoid leukosis [5, 6].

Avian lymphoid leukosis was the first model of a virus-induced B-cell malignancy and several of its features are relevant to the analysis of human B-cell malignancies. This B-cell lymphoma can be induced by infection of embryos or newly hatched chicks with avian group A leukemia retroviruses [7, 8]. The virus infects many cell types, but it selectively transforms B cells [6, 9]. Moreover, the virus-induced transformation only occurs at a very early stage in B-cell differentiation within the inductive bursal microenvironment. There are two distinctive phases in the evolution of this virus-induced malignancy of selected B-cell clones. First, one or more of the thousands of lymphoid follicles within the bursa exhibit lymphoblastic transformation. The transformed follicles are evident within 1–2 months after virus infection at hatching. The next phase usually occurs between 5 and 9 months of age, and involves widespread seeding and malignant growth of B cells, most of which do not become mature plasma cells. Bursectomy or physiological bursal regression prior to this second stage will abort the fatal B-cell malignancy [5, 6].

In the lymphoma cells, viral promoter sequences have been found to be integrated with a cellular *onc* gene called c-*myc* [10]. The activation of this transforming gene may be responsible for the initial transformation of lymphoid cells in the bursal follicles. However, the activated c-*myc* gene is unrelated to the transforming gene that has been detected by transfection with lymphoma DNA [11]. Activation of the latter *onc* gene could be responsible for the second step in the evolution of a malignant B-cell clone. Another hypothesis is that antigen-induced growth of transformed B cells may play a significant role in the malignant lymphomatosis phase [9]. The retrovirus itself could serve as the stimulating antigen for B-cell clones with appropriate immunoglobulin receptors [12].

We hypothesized that human B-cell malignancies would also involve an initial transformation of B-cell clones within the inductive microenvironment and that antigens could influence the subsequent be-

* This research was supported in part by Grants CA 16673 and CA 13148, awarded by the National Cancer Institute

havior of the affected clones. In this chapter we review the results of our studies on a spectrum of human B-cell malignancies within the context of normal B-cell differentiation. To identify the affected B cells we have used the immunoglobulins which they produce as clonal markers. Antibodies were prepared against immunoglobulin heavy and light chain isotypes, V_H subgroups and idiotypes, and these were used to diagnose and analyze pre-B leukemias, B-cell leukemias, Waldenström's macroglobulinemia, and multiple myelomas.

A. Normal B-Cell Differentiation

Cells of B lineage are unique in their expression of the immunoglobulin genes, and progression along this differentiation pathway can be discerned by determining which immunoglobulin genes are expressed. Immunoglobulin molecules consist of identical pairs of heavy and light chains. In the mouse, these are encoded by linked families of V_H, D_H, and J_H genes located 5′ to the heavy chain constant region (C_H) genes, the order of which is μ, δ, γ_3, γ_1, γ_{2b}, γ_{2a}, ε, and α on chromosome 12 [13–15]. The V_L and J_L genes are upstream from the $\varkappa$ and λ light chain genes on chromosomes 6 and 16, respectively [14, 16, 17]. The corresponding H, $\varkappa$, and λ immunoglobulin gene families in human are located on chromosomes 14, 2, and 22 [18–21].

One of the first steps in differentiation is the assembly by chromosomal rearrangement of one each of the V_H, D_H, and J_H genes and the transcription of the V-D-J set along with the $C\mu$ gene [22, 23]. A cell expressing such μRNA is known as a pre-B cell, and at this stage few of the μ chains reach the cell surface [24–29]. Next, one set of V_L and J_L genes is productively rearranged and a complete IgM molecule is expressed on the cell surface. This differentiation event marks the birth of an immature B-lymphocyte, and the point at which antigens may begin to influence the cell's behavior.

The foregoing stages, stem cell $\rightarrow$ pre-B $\rightarrow$ immature B, occur initially within inductive microenvironments of the fetal liver and thereafter in the bone marrow. Subsequent stages in B-cell differentiation entail changes in the expression of the C_H genes, without alteration in expression of the V_H–D_H–J_H set or the light chain gene. Intermediate stages in B-lymphocyte differentiation are marked by the expression of a variety of cell surface proteins involved in regulating migration, growth, and differentiation of B cells into terminally differentiated plasma cells with the associated shift from surface expression to secretion of immunoglobulin molecules.

Immature B cells first express surface IgM and later coexpress IgD with the same V_H–D_H–J_H and light chains. Some members within each B cell clone undergo a further switch, from IgM (and IgD) to IgG, IgA, or IgE [30], and all members of the clone will of course share the same antibody specificity and idiotype. Current views on heavy chain isotype switching mechanisms, sequence, and regulation are reviewed elsewhere [31].

The number of B-cell clones within an individual is very large, probably well over a million. Each expresses a unique antibody specificity and idiotypic pattern, but may share cross-reactive idiotypes with other clones [32].

B. Pre-B Leukemias

Approximately 20% of all acute lymphocytic leukemias of childhood can be recognized as pre-B leukemias by the presence of intracytoplasmic μ chains, absence of surface immunoglobulin (Ig), characteristic lymphoid morphology with lobulated nucleus and marrow cytoplasmic rim, surface expression of B-cell differentiation antigens, and the absence of T-cell and myelomonocytic antigen markers [33–36]. Another large segment of acute lymphocytic leukemias, perhaps 50%–60%, can be recognized as "pre" pre-B cells by detecting rearrangements of immunoglobulin heavy chain genes [28] and the expression of B-cell surface antigens [37–39]. Other characteristic features are the expression of HLA-DR, common ALL antigen, and terminal deoxynucleotidyl transferase activity [33–36].

There is suggestive evidence that the more differentiated μ^+ pre-B leukemias have a worse prognosis than the μ^- pre-B

leukemias [40]. Neither follows the relentless and rapid downhill course of the childhood B-cell leukemias, which are featured by surface IgM expression.

The target cell for the oncogenic events appears to be an Ig^- bone marrow precursor cell. Even in the μ^+ pre-B leukemias, some members of the leukemic clone do not express μ chains. More compelling evidence comes from the study of individuals with chronic myelogenous leukemia. Analysis of chromosomal markers (i.e., the Philadelphia chromosomal aberration on chromosome 22 and the G6PD isoenzymes, or alleles, encoded on the X chromosomes) has revealed that normal blood cells in these patients are derived from the same pluripotent stem cell as the myelogenous leukemia cells [41, 42]. More relevant to our theme here are the patients who undergo conversion from chronic myelogenous leukemia to acute lymphocytic leukemia of pre-B phenotype. Chromosomal marker analysis indicates that both lines of malignant cells are sequentially derived from the same multipotent stem cells [43–45].

The patterns of immunoglobulin gene expression in pre-B leukemia clones are also informative. Most pre-B leukemia cells express μ chains but no light chains, a finding that is consistent with the asynchronous onset of heavy and light chain expression observed in normal pre-B cells [46–50]. Unlike normal pre-B cells, however, subpopulations of pre-B cells within the leukemic clones may express heavy chain isotypes other than μ [33, 35]. In order to examine further the heavy chain isotope switching in leukemic pre-B cells, we have used monoclonal antibodies in immunofluorescence assays to allow unambiguous assignment of the heavy chain isotopes expressed by individual leukemic cells. Switching in leukemic pre-B clones from 11 childhood leukemias invariably led to expression of γ_1 heavy chains, and less often to expression of γ_4 and α [51]. The observed frequencies of isotype switches, μ to $\gamma_1 > \gamma_4 \gg \alpha$ and the absence of δ, γ_2, γ_3, and ε, indicate a preferential order for the switching process in leukemic pre-B cell clones. Since these cells lack surface immunoglobulins, these data favor a stochastic model for isotype switching rather than an antigen-induced switch mechanism.

So far the order of human C_H genes on chromosome 14 [18] has only been partially elucidated. Cμ and $C\delta$ are thought to be next in line 3′ to the J_H genes as is the case in mice [52]. $C\gamma_2$ is 5′ to $C\gamma_4$ [53, 54]; $C\gamma_1$ appears to be 5′ to $C\gamma_3$ [54]; and $C\varepsilon$ genes are thought to be located 5′ to the $C\alpha_1$ and $C\alpha_2$ gene [55]. Although our observations would fit with a gene order in man of μ, δ, γ_1, γ_3, γ_2, γ_4, ε, and α, the data indicate that the switch sequence cannot be explained solely by the C_H gene order.

The results of two-color immunofluorescence analysis indicated that individual pre-B cells within the leukemic clones express as many as three or even four heavy chain isotypes [51]. The stability of these phenotypic patterns has not been examined yet, but the presence of multiple heavy chain isotypes in individual pre-B cells might be explained by the hypothesis of a preliminary switch mechanism involving a large primary transcript of all the C_H genes and differential RNA splicing [56, 57]. This hypothesis does not, however, simplify the problem of ordered switching in the leukemic pre-B cells.

Another remarkable finding in our studies was the expression of $\varkappa$ light chains by almost all of the leukemic pre-B cells exhibiting heavy chain switches. This preference for $\varkappa$ over λ expression might be expected in view of evidence which suggests that $V\lambda$–$J\lambda$ gene rearrangement for expression with $C\lambda$ regularly follow nonproductive rearrangements of $\varkappa$ genes on both chromosomes [58–60]. However, the consistent acquisition of a productive $\varkappa$ gene by switching pre-B cells is unprecedented and suggests that these genetic events, occurring on chromosomes 14 and 2 [18–20], are coupled by a regulatory mechanism that remains to be elucidated.

Occasional clones of leukemic pre-B cells appear to continue differentiation into B-lymphocytes. One such example is il-

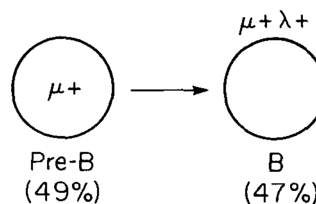

Fig. 1. Cell phenotypes in the transitional form of acute lymphocytic leukemia

lustrated in Fig. 1. Approximately half of the leukemic cells in this patient were μ^+ pre-B cells and the other half IgMλ-bearing B-lymphocytes. This suggests that the transformation process per se does not necessarily preclude continued differentiation beyond the pre-B compartment, and this principle has been confirmed in the following studies of B-cell leukemias and multiple myelomas.

C. B-Cell Leukemias

B-cell leukemias are monoclonal lymphoproliferative disorders marked by the expression of surface immunoglobulin (reviewed in [61]). Most of them express surface IgM, or IgM and IgD together. Less frequently the leukemic B cells express IgG or IgA. B-cell leukemias are closely related to B-cell lymphomas; their distinction rests primarily upon the predominant migration pattern of the involved B-cell clone, i.e., lymphoid tissues versus circulation. The affected B cells usually fail to differentiate into mature plasma cells, although some malignant B-cell clones contain mature antibody-secreting members, and many can be induced to differentiate into plasma cells *in vitro* [62–64].

An ideal marker for malignant B cells is the idiotype (Id) of the immunoglobulin that they express. Anti-Id antibodies have been difficult to prepare for B-cell leukemias and lymphomas, because they produce so little of their immunoglobulin product. However, hybridoma technology now makes it feasible to make monoclonal antibodies to the Id determinants expressed by malignant B-cell clones. We have prepared monoclonal antibodies specific for the Id determinants on leukemic B cells from selected patients, and have used these anti-Id antibodies to trace the extent of clonal involvement.

Ninety percent of the circulating mononuclear cells (18,000/mm^3) in one such patient were small lymphocytes bearing IgMλ and IgDλ molecules. Virtually all of these were reactive with a monoclonal anti-Id antibody tailor made against her leukemic B cells [65]. IgG and IgA B cells were very rare in this woman but, of these, 40% and 25% were reactive with the same monoclonal anti-Id antibody. This suggests that a few members of the leukemic clone have undergone heavy isotype switches, the frequency of which was governed by rate-limiting feature of the switch process.

Additional information can be obtained by study of bone marrow in addition to the blood cells in individuals with B-cell leukemia. This is illustrated by our findings in an elderly man with acute lymphoblastic leukemia cells that expressed surface IgA$_1\kappa$ molecules [66]. All of his leukemic cells were reactive with one of a panel of four monoclonal anti-V_H subgroup antibodies [67]; a monoclonal anti-Id antibody (WF) was prepared which reacted with all of the leukemic IgA B cells and <1% of normal B cells. None of the plasma cells found in this patient expressed the homologous idiotype, suggesting that the leukemic B-lymphocytes did not complete differentiation. The expression of the WF idiotype by cells in the circulation was restricted to the IgA$_1\kappa$ leukemic B cells; no T cells or IgM and IgG B cells with the WF Id could be found.

The picture was different in the bone marrow of this patient (see Fig. 2). Here we found a small subpopulation of IgMκ lymphoblasts that expressed the WF idiotype and the same V_H subgroup as the IgA$_1\kappa$ lymphoblasts. Pre-B cells containing α, γ, and μ chains of the same V_H subgroup were also present in the bone marrow. The lineal relationship between the μ, γ, and α pre-B cells was indicated by presence of both μ and γ, and of γ and α together in some of these pre-B cells. Light chain expression was not evident in these pre-B cells; this precluded identification with the monoclonal anti-Id antibody, because it recognized an idiotope formed by the heavy and light chains combined. When the bone marrow sample was depleted of B-lymphocytes and placed in culture, IgA$_1\kappa$ B cells with the WF Id were generated. These results suggest that this leukemic clone was transformed prior to the heavy chain switch and before κ light chain expression. The basis for the preferential expansion of the IgA$_1$ B-cell subpopulation is unclear. These cells did not display translocations on chromosomes 14 or 2 as have been observed in IgMκ Burkitt's lymphomas [21, 68]. It is noteworthy, however, that examination of

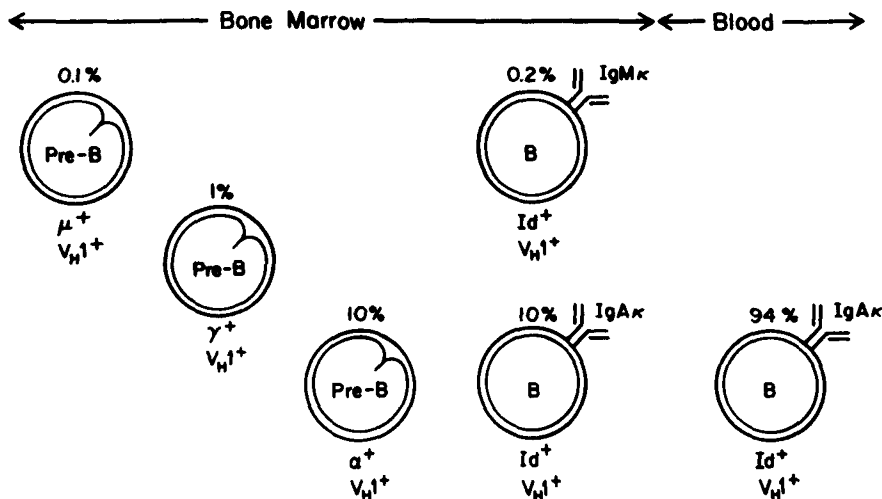

Fig. 2. Extent of clonal involvement in patient W.F. with acute lymphocytic leukemia

the DNA from the IgA_1 leukemic cells failed to reveal deletion of all of the γ genes (J. Ellison, unpublished). Deletion of C_H genes 5′ to the expressed C_H gene on one or both chromosomes has been a consistent feature in mouse myeloma cells, but this would appear to be the first attempt to examine this switch event at the B-lymphocyte level.

D. Plasma Cell Malignancies

Multiple myeloma is a B-cell malignancy that has classically been thought to involve bone marrow plasma cells. This viewpoint has been modified by the demonstration of an increase in circulating B-lymphocytes bearing the homologous idiotype. In two patients with IgA myelomas, we found expression of the homologous Id on IgM^+/IgD^+ B-lymphocytes as well as on IgA B-lymphocyte precursors. Moreover, a few Id^+ cells of the μ^+ pre-B phenotype were identified in the bone marrow [69]. Similar observations were made in studies of a patient with an IgD myeloma [65]. Hence, we have proposed that even multiple myelomas have roots within the pre-B cell compartment, and the bone marrow predilection of the myeloma population may be due to its genesis from marrow stem cells.

In a woman with Waldenström's macroglobulinemia, most of the circulating B cells had surface IgM with the homologous idiotype. In addition, 25% of her circulating IgA B cells expressed the same idiotype, suggesting that these cells belonged to the malignant clone as well [65]. The IgA^+ members of the clone were different from their sister IgM cells in that they apparently did not complete differentiation, i.e., we could find no IgA^+ Id^+ plasma cells and no serum IgA paraprotein.

These results contrast with the extent of clonal involvement in another patient with a serum IgM paraprotein that had binding specificity for intermediate filaments (IMF). The involved clone also included IgG_1, IgG_3, IgA_1, and IgA_2 plasma cells which were identified by the homologous idiotype, V_H subgroup, and antigen specificity (A. Landay, H. Kubagawa, and M. D. Cooper, unpublished).

It is puzzling that different members of a malignant B clone can behave so differently with regard to proliferation and differentiation. It is of course possible that, like normal B cells, they are influenced differently by antigens and immunoregulatory T cells (e.g., see chapter by Gershon). The problem is usually complicated by the unknown antibody specificity of the immunoglobulins made by malignant B cells. In the above example, however, the antibodies were directed against a highly conserved determinant present on all IMF forms. Since it is on a basic cellular constituent, this antigen would be released with cell injury and hence available to stimulate immunocempetent cells. This could explain why individuals with hepatitis often produce high titers of antibodies

to IMF [70]. The mere fact that 5%–10% of the IgM paraproteins in humans have IMF specificity [71] may in itself imply a role for antigens in the malignant behaviour of transformed B-cell clones.

E. Conclusions

These results are consistent with the idea that while B-cell malignancies show great variability in their progression along normal differentiation pathways, they undergo in common an initial transformation process within the bone marrow environment (Fig. 3). An important corollary of this hypothesis is that the events involved in initiation of normal differentiation would also be engaged in the genesis of malignant B-cell clones.

Our data further suggest that the initial transformation process is not always immediately followed by exaggerated overgrowth of the B cells belonging to the affected clone. Lane and her co-workers have obtained evidence in DNA transfection studies suggesting that different transforming genes may be activated in neoplasms featuring pre-B, B, or plasma cells [72]. Their results indicate that specific transforming genes are activated in neoplasms corresponding to specific stages of differentiation within the cell lineage. The hypothesis that human B-cell malignancies involve the sequential activation of at least two transforming genes, as may be the case in the avian lymphoma model [11], would easily accomodate both sets of observations.

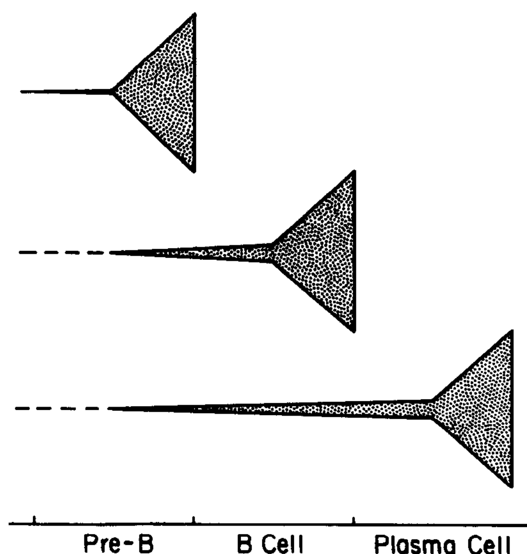

Fig. 3. Extent of involvement and clonal expansion in pre-B, B, and plasma cell malignancies

Still to be explained is the great variability in the growth and differentiation behavior of different members within neoplastic B-cell clones, and why B-cell clones with certain antigen specificities are more frequently involved. In view of these features, and the demonstration that immunoregulatory T cells can modify growth and differentiation of neoplastic B-cell clones [73, 74], it is still plausible that antigens and T cells may be significant modifiers of human B-cell tumors. It should be mentioned, however, that pre-B cell leukemias represent a clear exception to the idea that antigens may influence growth and differentiation of neoplastic B cells, and since the pre-B leukemia cells lack the surface immunoglobulin with which to see antigen, they would not be expected to be clonally regulated by the usual immunoregulatory controls.

Acknowledgments

We thank Mrs. Ann Brookshire for preparing this manuscript.

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