Autoantibodies in Chronic Lymphocytic Leukemia and Related Systemic Autoimmune Diseases

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LEUKEMIA B CELLS from patients with chronic lymphocytic leukemia (CLL) often make autoantibodies. Early studies by Preud'homme and Seligmann1 showed that the leukemic cells from CLL patients often bear surface IgM (slgM) that has rheumatoid factor (RF) activity, or binding activity for the Fc portion of human IgG. In another limited survey, the CLL cells from 4 of 13 patients (31%) expressed slgM with such RF activity.2 Using 12-myristate 13-acetate (PMA), Broker et al3 stimulated leukemia cells from 14 CLL patients to secrete IgM. Twelve (86%) of these cultures produced monoclonal IgM that reacted with one or more of a variety of different self antigens, including IgG, single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), histones, cardiolipin and/or cytoskeletal determinants. Sthoeager et al4 instead used pokeweed mitogen (PWM) and/or Staphylococcus aureus Cowan strain I (SAC) to induce neoplastic CLL lymphocytes to differentiate into antibody-secreting cells. Of the leukemia cell cultures from 17 CLL patients that had sufficient amounts of monoclonal Ig for testing, 9 (53%) had IgM RF and/or DNA-binding activity. Finally, Borche et al5 generated heterohybridomas with the leukemia cells from each of 23 CLL patients. Of the 12 CLL heterohybridomas that produced enough monoclonal antibody (MoAb) for testing, 7 (58%) produced IgM RF autoantibodies. Collectively, these studies indicate that the leukemia cells from most CLL patients express IgM autoantibodies, most notably RFs.

What is the significance of the frequent expression of autoantibodies in CLL? What implications, if any, does the type of antibodies expressed in CLL have for our understanding the cytogenesis and/or etiopathogenesis of this leukemia? What relationship do the autoantibodies produced by leukemia B cells have to the autoimmune diseases, such as autoimmune hemolytic anemia (AIHA) or immune thrombocytopenia (ITP)?6,7 that frequently afflict patients with this disease? This report will address several of these questions and review some of the recently acquired information regarding antibody expression in CLL and associated systemic autoimmune diseases. First, it is helpful to review the mechanisms that contribute to antibody diversity.

GENERATION OF ANTIBODY DIVERSITY

Ig gene rearrangement. The antibody molecule consists of two polypeptide chains that are encoded by gene complexes located on different chromosomes.8,9 During B-cell development, discontinuous elements within these gene complexes undergo a series of Ig gene rearrangements to form the exons that ultimately may encode the heavy and light chains of the antibody molecule10,11 (Fig 1). Generally, the first Ig gene rearrangements occur within the heavy chain gene complex. More than two dozen minigenes, termed diversity segments (D),16-19 are positioned between six functional JH minigenes20,21 and 100 to 200 heavy chain variable region genes (VH genes).22 Each VH gene belongs to one of at least 6 subgroups, with each subgroup comprised of V genes that share ≥80% nucleic acid sequence homology.23 Through Ig gene rearrangement, one or more D gene segments is joined with a single JH element.24,25 The resulting DJH complex may then rearrange with a VH gene17,22,26,27 to form a VH-DJH exon that may encode the variable portion of the antibody heavy chain.22,25 After successful VH-DJH rearrangement, one of approximately 70 κ V genes (Vκ genes)28-34 rearrange to one of five Jκ minigenes,21 thereby generating an exon that may encode a κ light chain variable region.21,35,36 Should this rearrangement fail to generate a functional gene, then one of approximately 70 λ light chain V genes (Vλ genes)37,38 may rearrange to one of four functional Jλ-Cλ complexes39-44 to generate an exon that may encode a λ light chain variable region. The final products of such genetic gymnastics are the somatically generated genes that encode the two polypeptide chains of the antibody molecule.

Somatic hypermutation. After successful Ig gene rearrangement, a highly specialized process may introduce numerous mutations in the rearranged and expressed Ig V genes45-48 (Fig 1). This process, called somatic hypermutation, is not triggered by the mere expression of Ig genes or B-cell proliferation.49,50 Rather, somatic hypermutation apparently operates only in a subset of B lymphocytes at discrete times during an immune response to antigen.48,51,52 Structural analyses of the antibodies produced at different times in an immune response show that, at 6 to 14 days after antigenic stimulation, the Ig V genes expressed by antigen-reactive B cells may accumulate somatic mutations at rates nearly a thousand times higher than that of normal (eg, ≤10−7) base pair/cell division).45,46,53-56 Thereafter, the mutation rates diminish as the B cells differentiate into memory or plasma cells.56-59 Selection for B cells expressing antibodies of higher affinity for antigen enhances the cumulative number of nonconservative mutations at sites that encode parts of the antibody that bind to antigen.47,52,60-64 Through this process, high-affinity antibodies can be generated against almost any antigen, including self antigens.63,65 As a consequence, B cells are always capable of making potentially pathogenic high-affinity autoantibodies and self tolerance is never assured. Thus, immunoregulatory mechanisms must perpetually operate to prevent production of pathogenic autoantibodies.
Ig variable region structure. Comparison of the amino acid sequences from different antibody variable regions shows three discrete segments of extreme hypervariability. Affinity labeling and crystallographic studies substantiated earlier contentions that the hypervariable regions on both chains fold together to form the antigen-combining site. Hence, these regions of hypervariability are designated the complementarity-determining regions (CDRs). The third CDR is generated through the recombinatorial process joining the antibody light chain V gene with the J segment, in the case of the light chain, or the VH gene with the somatically generated DJH segment of the antibody heavy chain. The diversity in first and second hypervariable regions reflects, in part, differences between each of the various inherited antibody V genes. As discussed above, somatic hypermutation subsequent to antibody V gene rearrangement may also play an important role in increasing the amino acid sequence diversity noted within these regions.

Together, the CDRs of both the antibody heavy and light chains fashion the specificity of the Ig molecule. Owing to the need for antibodies to bind a diverse array of different pathogens, there exists great potential for diversity in the tertiary structures of these regions. This potential is reflected by the fact that the combining site of each antibody may possess determinants of unique specificity that, in turn, may be recognized by anti-idiotypic antibodies.

AUTOANTIBODY-ASSOCIATED CROSS-REACTIVE IDIOTYPES IN CLL

Despite the tremendous potential for diversity, the antibodies produced by leukemia B cells of unrelated CLL patients often share common idiotypic determinants. Initially identified using highly absorbed heterologous antisera, and then more recently using murine MoAbs, these common idiotopes, designated cross-reactive idiotypes (CRIs), were defined initially on IgM autoantibodies such as RFs. In an early study of more than 30 CLL patients, 5 of 20 (25%) with \( \kappa \) light chain-expressing CLL had malignant cells that expressed a CRI defined by 17.109, a mouse MoAb raised against an IgM RF paraprotein. Furthermore, approximately 20% of both \( \kappa \) and \( \lambda \) light-chain-expressing CLL were found to react with G6, an MoAb specific for an Ig heavy-chain-associated CRI present on several RF paraproteins. Yet, an additional MoAb specific for a distinct RF heavy-chain-associated CRI, named LcI, labeled neoplastic Ig-expressing B cells from 7 of 56 (13%) patients with CLL and related B-cell lymphomas. Another 2 cases (4%) in this study were found to have malignant cells reactive with B6, a third MoAb specific for an autoantibody heavy-chain-associated CRI that is distinct from either the G6-CRI or LcI-CRI. More recently, additional autoantibody-associated CRIs have also been found to be expressed frequently in CLL and related B-cell neoplasms.

Molecular characterization of several such CRIs shows that each may reflect expression of a conserved Ig V gene with little or no somatic mutation. For example, 17.109-reactive leukemia cells from unrelated CLL patients express Ig V genes that are highly homologous with a conserved germline V,3 gene designated Hunkv325. This highly conserved Ig V gene is but 1 of 70 Ig V genes found present in the human haploid genome. Similarly, nucleic acid sequence analyses of the Ig heavy chain genes expressed by G6-reactive leukemic cells of unrelated CLL patients shows that each is homologous to a VH1 gene frequently used by human fetal splenocytes designated 51p1. However, despite expressing homologous VH1 genes, G6-reactive leukemic cells have been found to use JH3, JH4, JH5, or JH6.
gene segments and markedly different D segments. Furthermore, comparisons of G6-reactive and G6-negative antibody heavy chains indicate that the G6-CRI may be relatively resistant to substitutions within CDR3, but affected by differences in CDR1 and CDR2 that are found encoded by other VH genes of the VHV1 subgroup. As such, the G6-CRI in CLL is an excellent marker for expression of 51p1 with little or no somatic mutation. Accordingly, the frequent expression of such CRIs in CLL implies that the leukemia B cells in this disease express a highly restricted repertoire of Ig V genes that have not diversified significantly from the germline DNA.

Ig GENE REARRANGEMENT IN CLL

Analyses of CLL B cells not selected for expression of antibodies with particular binding specificities or CRIs show a generalized bias in the rearrangement and expression of select Ig V genes. Several investigators have noted that the Ig VH genes of the relatively small VH1 subgroups, VH14 and VH5, and the single copy VH6 gene are rearranged in CLL at frequencies that are disproportionate to the relative sizes of these subgroups in the germline DNA.5,95-97 On the other hand, the largest VH gene subgroup, the VH3 gene family, seems underrepresented among the VH gene used in CLL.96-98

Furthermore, within an Ig VH gene subgroup there may exist a bias toward the rearrangement of certain individual VH genes. As discussed, this may be exemplified by the G6-encoding gene 51p1, a member of the relatively large VH1 gene subgroup.93,94 Deane and Norton95 noted that 3 of the 9 Ig VH1 genes rearrangements they identified in CLL were homologous to 51p1. In contrast, these investigators did not find this gene used in a set of 9 VH1 gene rearrangements detected among pre-B acute lymphocytic leukemias, suggesting that the biased rearrangement of 51p1 is peculiar to CLL. In another survey of 44 patients with CLL, two-thirds (10 of 15) of the 15 leukemia samples found to have functionally rearranged VH1 genes were found to use VH1 genes homologous to 51p1.100 Two of the 15 (13%) were homologous to V35. This VH1 gene is distinctive in that it is the closest VH1 gene to the D and J9i gene complexes.101 As 51p1 apparently is rearranged and expressed more often than V35, proximity to the D and J9i loci evidently does not determine the hierarchy at which VH1 genes are expressed in CLL. Finally, because 51p1 is but only 1 of approximately 20 to 60 VH1 genes in the Ig heavy chain gene complex,25 these data indicate that VH1 gene rearrangement in CLL is not random.

At least two mechanisms may contribute to the frequent rearrangement and expression of certain Ig V genes in CLL. First, certain Ig V genes may undergo Ig gene rearrangement more frequently than other Ig V genes, possibly due to internal or neighboring recognition sequences for enzymes involved in Ig gene rearrangement.102 On the other hand, certain Ig V genes may encode antibodies that have peculiar binding specificities that are selected during leukemogenesis, resulting in a biased repertoire of Ig V genes used in CLL. These mechanisms need not be mutually exclusive.

To determine whether nonrandom Ig gene rearrangement occurs in CLL independent of selection for expressed Ig protein, we examined the abortive VH gene rearrangements in λ light chain expressing CLL (λ-CLL).103 Although most of these λ-CLL had rearranged both κ light chain alleles, 9 had rearranged only one, providing us with a total of 57 identified κ light chain gene rearrangements in a sample group of 33 λ-CLL. We found the Vκ gene encoding the 17.109-CRI, Humkv325, to be involved in 8 of these nonproductive rearrangements. Another conserved Vκ3 gene, designated Vg, was rearranged nonproductively in 3 of the λ-CLL. Similar to Humkv325, this conserved Vκ3 gene is frequently found to encode the κ light chain variable region of IgM, autoantibodies.78,104,105

In theory, any one of 70 Vκ genes may undergo Ig rearrangement.100 If Vκ gene rearrangement were random, then the chance of finding nonproductive Humkv325 or Vg gene rearrangements should be 1 in 70, or 1.4% of the number of Vκ gene rearrangements. Thus, the expected number of Humkv325 or Vg gene rearrangements in the sample group of 57 nonproductive Vκ gene rearrangements should be less than 1 (or 0.8). x² analyses show that the observed frequency of abortive Humkv325 or Vg gene rearrangements significantly exceeds that expected if Ig Vκ gene rearrangements were truly random (P = .0001 for Humkv325 and P = .0147 for Vg). Therefore, independent of Ig expression, there exists a significant bias in the rearrangement of certain Ig V genes in CLL.

Ig SOMATIC MUTATION IN CLL

Studies on the Ig V genes used by leukemia B cells have shown that the process of somatic hypermutation is not active in CLL. First, the Ig V genes expressed in any given CLL B-cell clone are homogeneous, or without significant "intraclonal diversity."92,93,106 This contrasts with non-Hodgkin's lymphomas (NHL) of follicular center cell origin. Analyses of these lymphomas show substantial intraclonal diversity in the expressed Ig variable regions suggestive of ongoing somatic hypermutation.108 Furthermore, the Ig V genes expressed in most CLL share extensive homology (>97%) with known germline Ig V genes.95,97,99,106,109,110 Collectively, these studies indicate that CLL may express germline Ig V genes with little or no somatic mutation.

However, there are some exceptions to this generalization. Humphries et al110 noted that the leukemia cells from related CLL patients expressed extensively mutated Ig VH genes belonging to the relatively small VH5 subgroup. In addition, they noted that 30% of the CLL patients in their survey had leukemias that had rearranged one Ig VH5 gene, designated VH5251. These investigators also examined Ig VH5 gene-expressing CLL B-cell populations from 11 different patients.111 Because prior studies indicated that Ig VH5 genes of this small subgroup are highly conserved and nonpolymorphic,112 they compared the primary nucleic acid sequences of the expressed VH5 genes with that of known VH5 genes. In 9 of the 11 CLL, they found the nucleic acid sequences of the expressed Ig VH5251 genes to differ substantially (≥94% homology) from that of the known germline sequence. Importantly, the nucleic acid base differences resulted in amino acid substitutions were clustered primarily within the CDRs, regions that form the pocket of the antibody's antigen-combining site. As discussed, such substitutions often are
noted in the Ig selected in an antigen-driven secondary immune response. Accordingly, it appears that these CLL express antibodies that had been selected for their ability to bind some unknown antigen(s).

Expression of mutated Ig V<sub>H</sub> genes by these CLL may reflect a unique property of the Ig V<sub>H</sub>251 gene. This Ig V<sub>H</sub>5 gene, for example, is distinctive in that it drives relatively high rates of transcription in the germline nonrearranged configuration.113

To evaluate whether the Ig V<sub>H</sub>5 genes in CLL are distinctive, we recently examined for V<sub>H</sub>5 Ig gene rearrangements in leukemia cells from 68 patients that satisfied clinical and diagnostic criteria for common B-cell CLL.114 Southern blot hybridization studies with probes for V<sub>H</sub>251 and the J<sub>H</sub> locus showed that only 7 (10%) of the 68 monoclonal CLL cell populations had undergone Ig gene rearrangement involving V<sub>H</sub>5 genes. Two (3%) were found to have functionally rearranged V<sub>H</sub>5 genes that shared ≥98% sequence homology with 5-2R1, a V<sub>H</sub>251 gene isolated from a pre-B-cell acute lymphocytic leukemia. The other 5 CLL (7%) had functionally rearranged V<sub>H</sub>5 genes that each shared ≥99% nucleic acid sequence homology with a germline V<sub>H</sub>32 isolated from human sperm DNA. These data indicate that V<sub>H</sub>251 or V<sub>H</sub>32 also may be expressed by common CLL B cells with little or no somatic mutation. As such, these data conflict with those discussed above. Conceivably, the leukemia cells that express highly mutated Ig V genes may represent a unique subset of leukemias that differs from common CLL in ways other than just Ig V gene expression. Further work is necessary to determine whether there exist differences in the clinical and/or phenotypic characteristics of patients with leukemia cells that express mutated versus nonmutated Ig V genes. However, in general, CLL B cells that express highly mutated Ig V genes appear to be exceptional.

NORMAL CELL COUNTERPART TO THE CLL B CELL

**CD5 B cells—"B1 B cells."** CLL B cells coexpress B-cell–specific surface antigens and CD5 (Leu 1, OKT1).115-118 As such, the normal counterpart to these leukemia cells arguably is the “CD5 B cell.”119 These cells constitute a small subpopulation of human B lymphocytes in the lymphoid organs and peripheral blood of normal adults that coexpress B-cell differentiation antigens and CD5.120-125 Such cells are enriched for B cells that spontaneously may produce IgM autoantibodies126-128 and frequently may express autoantibody-associated CRls.125,129,130

Recently, a new nomenclature has been adopted for CD5 B cells.131 Because the CD5 surface antigen (1) may not be detected on the surface of B cells that otherwise have other developmental and/or phenotypic traits of “CD5 B cells,”125,132-134 (2) may be induced on non-CD5 B cells,135-138 and (3) can be reduced on “CD5 B cells” by treatment with various cytokines,139,140 it was argued that the term “CD5” (or “Ly-1”) was not adequate for this type of B cell. Therefore, participants at the New York Academy of Sciences meeting on “CD5 B cells,” held in West Palm Beach, FL (June 1991), proposed that “CD5 B cells” be designated B-1 B cells, and that “conventional” B cells be termed B-2 B cells.131

In mice, “B-1 B cells” can be distinguished readily from “B-2 B cells” by their anatomic localization, phenotype, functional characteristics, gene expression, and developmental stage of origin124,132,141-144 (Table 1). Although demonstrable as a rare subpopulation in the spleen of most normal inbred mouse strains, murine B-1 B cells normally are not found in the lymph node (LN), blood, or bone marrow (BM).145 In contrast, B-1 B cells constitute a major lymphoid subpopulation in the murine peritoneal cavity.144 B-1 B cells also share several physical and developmental properties with monocytes and macrophages.133,147,148 Studies indicate that mouse B-1 cells frequently may produce IgM autoantibodies and use a restricted set of Ig variable region genes (V genes).143,149-151 In addition, some investigators have noted that B-1 B cells may influence the repertoire of other B and T lymphocytes,152-154 indicating that these cells have potential immunoregulatory function. Finally, most B-1 B cells originate in early ontogeny and are long-lived.132,143,155 This latter point may be shown through analyses of the rearranged Ig heavy chain variable regions expressed by B-1 B cells in adult mice.151,156 These studies show a paucity of nontemplated N-terminal sequence insertions in the V<sub>H</sub>-D<sub>H</sub> and D<sub>H</sub>-J<sub>H</sub> junctions in rearranged Ig heavy chain variable regions of B-1 B cells. Such Ig gene rearrangements are typical of those made by early fetal B cells before the developmental expression of terminal deoxynucleotransferase (TdT), the enzyme responsible for the addition of N-region sequences during VDJ gene rearrangement.24,157 As such, the absence of N-sequence insertions serves as a developmental clock, implying that these adult B-1 B cells may have undergone Ig gene rearrangement and differentiation during early B-cell ontogeny.

With some exceptions, human B-1 B cells share many phenotypic and developmental characteristics with murine B-1 B cells (Table 1). However, the enzyme TdT apparently is active during the early human B-cell differentiation, resulting in human fetal Ig heavy chain gene rearrangements that have junctional N-sequence insertions.94 As such, one cannot use the relative absence of N-sequence insertions to characterize Ig V genes that have rearranged during early human B-cell development. Also, human CD5 B cells coexpress higher levels of sIgD and CD23 than do their murine counterparts.144 Operationally then, expression of CD5 re-

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<th>Table 1. Proposed B-Cell Subpopulations</th>
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<td>B-1 B cell</td>
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<td>Enriched for B cells that constitutively express CD5</td>
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<td>Originate in early ontogeny</td>
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<td>Long-lived with slow cell turnover</td>
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<td>Low rates of Ig V gene somatic mutation</td>
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<td>Enriched for cells that make &quot;natural&quot; IgM autoantibodies</td>
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<td>B-2 B cell</td>
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<td>CD5 - unless induced</td>
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<td>Adult bone marrow derived</td>
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<td>Short-lived and cycling</td>
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<td>Capable of Ig V gene somatic hypermutation</td>
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mains one of the best markers for distinguishing human B-1 B cells.

In fact, CD5 still may serve as a useful marker for identifying human B-1 B-cell malignancies. More than 90% of patients with B-cell CLL have leukemia cells that express CD5. Patients with leukemia B cells that do not express this surface differentiation antigen may have a distinct disease that differs from common CLL in its etiopathogenesis and/or its expression of Ig genes. For example, we examined the Ig V genes expressed by the neoplastic cells of an unusual CLL patient that did not express CD5 but still secreted an IgM RF autoantibody. The nucleic acid sequence of the V\_H gene expressed by this CLL differed substantially (<96% homology) from that of any known germline V\_H gene.\textsuperscript{158,159} Furthermore, nucleic acid sequence analyses of independent V\_H gene clones showed substantial intraclonal variation in the V\_H genes expressed by this leukemia cell population.\textsuperscript{160} Similar intraclonal diversity recently was observed in a case of small lymphocytic lymphoma (SLL) that also failed to express CD5,\textsuperscript{160} and in another CLL cell population that also was CD5\^-\textsuperscript{160} These findings contrast with those made in analyzing conventional CD5 B-cell CLL\textsuperscript{89,92,93,106,110} as discussed above. Collectively, these studies suggest that the expression of CD5 may help distinguish conventional "B-1 B-cell" CLL from other types of B-cell leukemias that differ physiologically in the ways they process their expressed Ig V genes.

**Mantle zone B cells.** To evaluate for normal B cells that also may express Ig V genes similar to those often observed in B-cell CLL, investigators have examined lymphocytes in nonmalignant human lymphoid tissue for reactivity with MoAbs specific for autoantibody-associated CRIs.\textsuperscript{125,126} Flow cytometric analyses of tonsillar lymphocytes from 19 subjects, for example, showed that the lymphocytes binding 17.109 are a subpopulation of Ig \(\kappa\)-bearing B cells comprising 1.3% to 6.3% (mean = 3.9% ± 1.2%) of the total lymphocytes, 3% to 16% (mean = 10% ± 3%) of the \(\kappa\) light chain-bearing cells, and from 46% to 97% (mean = 77% ± 16%) of the cells expressing \(\kappa\) light chains of the V\_H1b/h subgroup. G6-positive cells expressed either \(\kappa\) or \(\lambda\) light chains and comprised ≤0.1% to 4.5% (mean = 2.2% ± 1.2%) of the total lymphocytes. Cells expressing both the 17.109 and G6 CRI comprised a very small subpopulation constituting less than 0.1% to 1% (mean = 0.4% ± 0.3%) of the total lymphocyte population.

Molecular studies indicate that such CRI-expressing tonsillar lymphocytes may express Ig V genes similar to that used by CRI-expressing CLL B cells. G6-reactive tonsillar lymphocytes, for example, are found to express 51p1 with little or no somatic mutation.\textsuperscript{125} In fact, in more than 5,600 nucleotide bases of the Ig V\_H genes from 13 independent G6-reactive tonsillar B-cell clones, there were only three base differences detected. The absence of even nonconservative base changes indicates that the conserved Ig V\_H gene sequences are not the mere consequence of selection for cells having reactivity for the G6 MoAb. Rather, these data indicate that G6-reactive tonsillar B cells generally express homologous V\_H1 genes that have not diversified through somatic mutation.

B cells that express such autoantibody-associated CRIs are confined to the mantle zones surrounding the germinal cen-
ters of secondary B-cell follicles.\textsuperscript{98,130} Tonsillar B cells can be delineated into subpopulations based on the differential expression of slgD, CD10, and receptors for peanut agglutinin (PNA). Mantle zone B cells bear slgD, but fail to bind PNA or MoAbs specific for CD10 (CALLA), whereas B cells within the germinal center generally lack slgD, express low levels of CD10, and avidly bind PNA.\textsuperscript{161-163} Consistent with their mantle zone distribution, greater than 95% of CRI-positive B cells express slgD but fail to bind PNA or MoAbs specific for CD10.\textsuperscript{125} Furthermore, multiparameter analyses indicate that human tonsillar B cells that express CD5 also have this phenotype, indicating that B-1 B cells may constitute a subpopulation of mantle zone lymphocytes.\textsuperscript{125}

It should be noted that slgD-bearing cells constitute only 37% to 87% of the tonsillar B cells.\textsuperscript{125} As such, the 17.109-reactive or G6-reactive cells, respectively, may constitute up to 17% or 12% of the mantle zone lymphocytes. These frequencies approach those noted for expression of these CRIs in B-cell CLL and argue that the Ig V gene repertoire of mantle zone B cells may be comparably restricted. Recent studies suggest that the expressed Ig V\_H gene repertoire of circulating B cells in adult peripheral blood also may be highly restricted.\textsuperscript{164} It is conceivable then that transformation of lymphocytes such as those found in the mantle zone may account for the high frequency expression of autoantibody-associated CRIs in CLL.

However, it seems that CLL B cells may originate from only a subset of such B cells. For one, there is an apparent bias for coexpression of both 17.109 and G6 by leukemia cell populations that is not noted in most tonsillar lymphocyte populations studied.\textsuperscript{86,125} Biased coexpression of such CRIs reflects a predilection for CLL B cells to express certain pairs of heavy and light Ig chains.

Furthermore, there exists an apparent bias in the structure of the CDR3 of certain Ig heavy chains expressed in CLL.\textsuperscript{100} Nucleic acid sequence analyses of 13 G6-CRI-positive CLL cells that express 51p1 shows each to have an unusually long CDR3 of 30 codons (± 4, SD). These CDR3 are encoded by germline D segments, N-sequence insertions, and J\_H segments. The J\_H segment use of G6-reactive CLL (J\_H3 [31%], J\_H4 [15%], J\_H5 [8%], or J\_H6 [46%]) apparently favors the use of J\_H3 compared with that of normal G6-reactive tonsillar lymphocytes or normal circulating adult B cells.\textsuperscript{165} D segment use also is skewed compared with that of normal circulating B cells,\textsuperscript{165} with XP4, XP1, and XP1 accounting for approximately three-fourths of the D segments used in G6-reactive CLL. However, the heavy chain variable regions expressed by 14 independent normal G6-reactive tonsillar B-cell clones did not show such a bias.\textsuperscript{125} Rather, the D segment use of G6-reactive tonsillar B cells mimics those observed for normal circulating B cells.\textsuperscript{165} Also, in contrast to the length of the CDR3 of G6-reactive CLL, CDR3s of the G6-positive tonsillar B cells are significantly shorter, averaging only 24 codons in length (± 5, SD).

Conceivably, the bias in the composition of CDR3 may reflect the time in development when the normal cell counterpart to CLL had rearranged its Ig genes. Alternatively, apparent constraints in the Ig CDR3 and the bias in Ig chain pairing may reflect restrictions on the Ig repertoire of normal
B cells that are subject to malignant transformation in CLL. For example, certain pairs of heavy and light Ig chains that have appropriate CDR3s may generate antibody molecules with specificities that increase the likelihood for B-cell malignant transformation. If so, then the finding that the Ig expressed by CLL B cells so frequently have autoantibody activity may be relevant to the etiopathogenesis of this disease.

**SOMATIC SELECTION OF CLL AUTOANTIBODIES**

An important feature of the autoantibodies expressed in CLL is their "polyreactivity," or binding activity for two or more seemingly disparate self antigens. RF autoantibodies made by CLL B cells, for example, generally also are found to bind other "self" antigens, such as ssDNA, dsDNA, histones, cardioliopin, actin, thyroglobulin, and/or cytoskeletal components. These polyreactivity is a feature also noted for antibodies produced during early B-cell development, even in animals raised in apparently germ-free environments. These types of autoantibodies are not a priori pathogenic in that they can be detected in the sera of all individuals and appear physiologic. Because of this, several investigators have used the term "natural autoantibodies" to describe these types of antibodies.

Polyreactive IgM autoantibodies may be encoded by Ig V genes that are present in the germline DNA. Accordingly, the frequent expression of polyreactive IgM autoantibodies in CLL may be a direct consequence of the frequent use of such Ig V genes, with little or no somatic mutation. As noted, a high proportion of CLL patients have leukemia cells expressing Ig V genes that encode CRIs frequently found to be associated with IgM autoantibodies, particularly RFs. Moreover, more than 90% of the monoclonal IgM paraproteins that bear both 17.109 and G6 CRIs are noted to have RF activity. As such, the simple pairing of a 17.109-reactive Ig light chain with a G6-reactive Ig heavy chain may produce an Ig with polyreactive anti-self activity. If this were so, then frequent autoantibody activity of the Ig expressed by CLL B cells may be an epiphenomenon.

However, this model minimizes the contribution of the third complementarity determining region (CDR3) to the autoreactive binding activity of the Ig expressed in CLL. As discussed, this region of the Ig heavy chain is encoded by the D and JH gene segments that undergo recombination and N-terminal nucleic acid base insertion immediately before VH gene rearrangement. Accordingly, the sequence of the CDR3 is generally idiosyncratic to each Ig VH gene rearrangement, as has been noted with all G6-positive heavy chains sequenced to date. (T. Johnson, S.F. Duffy, T.J. Kipps, unpublished observations). In view of the large potential for diversity in the CDR3 of G6-positive heavy chains, the random pairing of a 17.109-positive k light chain with any G6-positive Ig heavy chain may not be anticipated to form an autoantibody if the CDR3 is critical to autoantibody-binding activity (Fig 2).

To examine this, we generated murine transfectomas to pair the 17.109-positive k light chains of SMI, a 17.109/G6-positive CLL B-cell population, with each of several different G6-positive heavy chains expressed by normal or leukemic B cells. For this, we generated pRTM1, a human μ-chain expression vector that contains the functionally rearranged SM1 VH1 gene flanked by unique restriction enzyme sites. This enabled us to exchange several different functional VH1DJH exons of other G6-positive leukemia cells or tonsillar lymphocytes. Each of the rearranged VH genes within these genes shared more than 99% nucleic acid sequence homology to 51p1, a VH1 gene expressed during early fetal development. In contrast, these exons differed markedly in the CDR3. We found that the myeloma cells cotransfected with the original pair of Ig heavy and light chain genes of SMI secrete polyreactive IgM, RF autoantibodies. However, myeloma cells cotransfected with the SM1 k light chain gene and any 1 of 10 different G6-encoding VH1DJH exons produced 17.109/G6-reactive IgM, that failed to have such autoantibody activity (Fig 2). This indicates that such polyreactive binding activity of these natural autoantibodies is dependent on the somatically generated CDR3.

These results imply that the polyreactive binding activity of natural autoantibodies is a selected specificity. In human fetal spleen, B cells frequently express autoantibody-associated CRIs, such as G6 and 17.109. Genetic mechanisms may enhance rearrangement of Ig V genes that encode CRI-bearing IgM autoantibodies. However, random pairing of such Ig light and heavy chains only infrequently may give rise to polyreactive autoantibodies. Because more than 90% of Ig paraproteins that bear both CRIs have autoantibody activity, it appears that autoreactivity is a selected specificity.

In addition, several studies indicate that more than half of all CLL patients have leukemia cells that can be stimulated to secrete polyreactive autoantibodies. This proportion greatly exceeds that noted for polyreactive B cells in normal embryonic tissues, cord blood, or adult peripheral blood. Even when analyzing B cells greatly enriched for B-1 type B cells (eg, neonatal or fetal B cells), Guigou et al noted that only 11% to 16% of all Epstein-Barr Virus (EBV)-transformed Ig-secreting B-cell clones expressed polyreactive antibodies. The high prevalence of CLL patients with leukemia cells that make polyreactive Ig thus may not be reflective of the repertoire expressed by the normal B-1 B-cell population. Rather, this may reflect the possibility that expression of polyreactive Ig...
may enhance a B cell’s risk for malignant transformation into CLL.

SYSTEMIC AUTOIMMUNE DISEASES ASSOCIATED WITH CLL

Patients with CLL frequently may develop intermittent autoimmune disease. 

Soon after CLL popularly became recognized as a distinct clinical entity, investigators noted an association between CLL and various autoimmune diseases, such as AIHA and ITP. Less frequently, patients with CLL are noted to have pure red cell aplasia or neutropenia secondary to a presumed autoimmunity against precursor cells in the bone marrow.

Larger retrospective surveys confirmed the association between certain types of autoimmune disorders and CLL. Duhrsen et al. studied the records of nearly 1,000 patients with either myeloproliferative or lymphoproliferative diseases for clinical manifestations of autoimmune pathology. Included in this survey were 104 patients with CLL. As a group, patients with CLL had the highest incidence of associated autoallergic hematologic diseases, accounting for more than 60% of all cases observed to have hemolytic anemia (9 of 14) or autoimmune thrombocytopenia (3 of 5). Similarly, Hamblin et al. studied the prevalence of autoantibodies in 195 patients with B-cell CLL. Fifteen patients (7.7%) tested positive in the direct antiglobulin test for antierythrocyte autoantibodies. One of these 15 had high titer anti-IgM, MoAbs produced by the leukemia cell clone. The other 14 had polyclonal IgG anti-red blood cell autoantibodies, 10 of which produced clinically detectable hemolytic anemia. Four patients (2.1%) had ITP, 1 patient (0.5%) had pure red blood cell aplasia, and 1 patient (0.5%) had isolated neutropenia thought to be secondary to antineutrophil autoantibodies. The incidence of autoantibodies increased later in more advanced stages of CLL. The incidences of such autoantibodies were significantly higher than in a similarly sized group of age- and sex-matched control subjects.

ORIGIN OF PATHOGENIC AUTOANTIBODIES IN CLL

Although pathogenic autoantibodies occasionally may be made by the clone of malignant B cells, generally such autoantibodies appear to be made by bystander B cells. Similar to studies on autoantibodies that arise in systemic autoimmune disease, one can discern autoantibodies with Ig light chains that differ from that of the Ig expressed by the population of injected leukemia B cells. Second, although all mice received comparable numbers of PBL from patients with CLL, only a fraction of the mice developed antineutrophil autoantibodies. The data suggest that polyclonal autoantibodies arise not from polyclonal B-cell stimulation, but rather from a pathologic immune response to self antigen. Whatever hypothesis is advanced must take into consideration the peculiar predilection of CLL patients to develop autoantibodies primarily against hematologic cells (eg, erythrocytes, platelets, etc.) This may reflect a more generalized defect in the homeostatic mechanisms that prevent generation of antiseif humoral immunity. According to this hypothesis, autoantibodies arise not from polyclonal B-cell activation, but rather from a pathologic immune response to self-antigen.
Common CLL is a B-1 B-cell malignancy. The leukemia cells often make polyreactive autoantibodies and use a restricted repertoire of nonmutated Ig V genes. Recent studies indicate that somatic selection of B-1 B cells for polyspecific autoreactivity may play an important role in shaping the repertoire of Ig expressed by these cells. However, chronic stimulation of such long-lived B-1 B cells by ubiquitous self antigens also may increase their risk for neoplastic transformation into CLL.

Even though CLL B cells often make autoantibodies, the Ig expressed by leukemia B cells generally do not contribute directly to the types of autoimmune diseases that frequently are observed in patients with this disease. CLL-associated autoimmune diseases generally result from the intermittent production of pathogenic autoantibodies that most commonly react with antigens present on human hematopoietic cells (eg, red blood cells or platelets). These autoantibodies are apparently produced by polyclonal bystander B cells as a consequence of an immune dysregulation associated with B-cell CLL.

The fact that CLL cells express a limited repertoire of Ig V genes that have not diversified substantially from the germline DNA implies that the surface Ig may be a convenient target antigen for passive or active immunotherapy. Current efforts to prepare additional anti-CRI MoAbs, to induce cytoxic T lymphocytes reactive with idiotypic antigens on CLL cells, and to develop improved delivery systems for Ig-directed immunotherapy may yield important advances in the treatment of this disease.

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AUTOANTIBODIES IN CLL


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