Review Article

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 Seligmann showed that the leukemic cells from CLL patients often bear surface IgM (sIgM) 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 sIgM with such RF activity. Using 12-myristate 13-acetate (PMA), Broker et al. 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. Sthoeiger et al. 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 al. 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 cytopathogenesis 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), 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. 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 molecule. Figure 1. Generally, the first Ig gene rearrangements occur within the Ig heavy chain gene complex. More than two dozen minigenes, termed diversity segments (D), are positioned between six functional JH minigenes and 100 to 200 heavy chain variable region genes (VH genes). 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. Through Ig gene rearrangement, one or more D gene segments is joined with a single JH element. The resulting DJH complex may then rearrange with a VH gene to form a VH-DJH exon that may encode the variable portion of the antibody heavy chain. After successful VH-DJH rearrangement, one of approximately 70 VH genes may rearrange to one of five J, minigenes, thereby generating an exon that may encode a light chain variable region. Should this rearrangement fail to generate a functional gene, then one of approximately 70 λ light chain V genes may rearrange to one of four functional J-κ complexes to generate an exon that may encode a 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 genes. Somatic hypermutation, not triggered by the mere expression of Ig genes or B-cell proliferation, operates only in a subset of B lymphocytes at discrete times during an immune response to antigen. 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, ≤10−3/base pair/cell division). Thereafter, the mutation rates diminish as the B cells differentiate into memory or plasma cells. 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. Through this process, high-affinity antibodies can be generated against almost any antigen, including self-antigens. 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.

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 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 \( V_\kappa \) genes that are highly homologous with a conserved germline \( V_\kappa \) gene designated \( H_{\kappa 25} \). This highly conserved Ig \( V_\kappa \) gene is but 1 of 70 \( V_\kappa \) 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 \( V_{H1} \) gene frequently used by human fetal splenocytes designated 51p1. This highly conserved Ig \( V_\kappa \) gene is but 1 of 70 Ig \( V_\kappa \) 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 \( V_{H1} \) gene frequently used by human fetal splenocytes designated 51p1. However, despite expressing homologous \( V_{H1} \) genes, G6-reactive leukemic cells have been found to use \( J_{H3} \), \( J_{H4} \), \( J_{H5} \), or \( J_{H6} \)
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 resilient to substitutions within CDR3, but affected by differences in CDR1 and CDR2 that are found encoded by other VH genes of the V_H1 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 V_H1 subgroups, V_H4 and V_H5, and the single copy V_H6 gene are rearranged in CLL at frequencies that are disproportionate to the relative sizes of these subgroups in the germline DNA.5,9-97 On the other hand, the largest VH gene subgroup, the V_H3 gene family, seems underrepresented among the VH genes 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 V_H1 gene subgroup.93,94 Deane and Norton99 noted that 3 of the 9 Ig VH genes of 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 gene 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 J_H1 gene complexes.101 As 51p1 apparently is rearranged and expressed more often than V35, proximity to the D and J_H1 loci evidently does not determine the hierarchy at which VH1 genes are expressed in CLL. Finally, because 51p1 is but one 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 V_H 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 V_κ gene encoding the 17.109-CRI, Humkv325, to be involved in 8 of these nonproductive rearrangements. Another conserved V_κ3 gene, designated V_κ, was rearranged nonproductively in 3 of the λ-CLL. Similar to Humkv325, this conserved V_κ3 gene is frequently found to encode the κ light chain variable region of IgM, autoantibodies.78,104,105

In theory, any one of 70 V_κ genes may undergo Ig rearrangement.104 If V_κ gene rearrangement were random, then the chance of finding nonproductive Humkv325 or V_κ gene rearrangements should be 1 in 70, or 1.4% of the number of V_κ gene rearrangements. Thus, the expected number of Humkv325 or V_κ gene rearrangements in the sample group of 57 nonproductive V_κ gene rearrangements should be less than 1 (0.8). χ² analyses show that the observed frequency of abortive Humkv325 or V_κ gene rearrangements significantly exceeds that expected if Ig V_κ gene rearrangements were truly random (P = .0001 for Humkv325 and P = .0147 for V_κ). 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 "intrachain diversity."99-102 This contrasts with non-Hodgkin’s lymphomas (NHL) of follicular center cell origin. Analyses of these lymphomas show substantial intrachain 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 IgV genes.99,106,107,109,110 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 al95 noted that the leukemia cells from related CLL patients expressed extensively mutated Ig VH genes belonging to the relatively small V_H5 subgroup. In addition, they noted that 30% of the CLL patients in their survey had leukemia cells that had rearranged one Ig VH5 gene, designated V_H5251. 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 CLLs, 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. To evaluate whether the Ig V<sub>H</sub>5 genes in CLL are distinctive, we recently examined for vH5 hybridization studies with probes for VH251 and the JH 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 lymphoblastic 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<sup>+</sup> B cells—"B1 B cells."  CLL B cells coexpress B-cell-specific surface antigens and CD5 (Leu 1, OKT1).<sup>115-118</sup> As such, the normal counterpart to these leukemia cells arguably is the "CD5 B cell."<sup>119</sup> 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.<sup>120-125</sup> Such cells are enriched for B cells that spontaneously may produce IgM autoantibodies<sup>126-128</sup> and frequently may express autoantibody-associated CRIs.<sup>129,130</sup>

Recently, a new nomenclature has been adopted for CD5 B cells.<sup>131</sup> 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,"<sup>125,132-134</sup> (2) may be induced on non-"CD5 B cells,"<sup>135-138</sup> and (3) can be reduced on "CD5 B cells" by treatment with various cytokines,<sup>139,140</sup> 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.<sup>131</sup>

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 origin.<sup>124,132,141-144</sup> (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).<sup>141</sup> In contrast, B-1 B cells constitute a major lymphoid subpopulation in the murine peritoneal cavity.<sup>146</sup> B-1 B cells also share several physical and developmental properties with monocytes and macrophages.<sup>133,147,148</sup> Studies indicate that mouse B-1 cells frequently may produce IgM autoantibodies and use a restricted set of Ig variable region genes (V genes).<sup>143,149-151</sup> In addition, some investigators have noted that B-1 B cells may influence the repertoire of other B and T lymphocytes.<sup>152-154</sup> indicating that these cells have potential immunoregulatory function. Finally, most B-1 B cells originate in early ontology and are long-lived.<sup>132,143,155</sup> 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.<sup>151,156</sup> These studies show a paucity of nontemplated N-region sequence insertions in the V<sub>H</sub>-D<sub>H</sub> and D<sub>H</sub>-J<sub>H</sub> junctions in the 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 deoxynucleotidyl transferase (TdT), the enzyme responsible for the addition of N-region sequences during VDJ gene rearrangement.<sup>146</sup> 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.<sup>94</sup> 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.<sup>146</sup> Operationally then, expression of CD5 re-

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<th>Table 1. Proposed B-Cell Subpopulations</th>
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<tr>
<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 ontology</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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<tr>
<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 identi-
ifying 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 Vc
gene expressed by this CLL differed substantially (<96% ho-
monology) from that of any known germine Vc gene.158,159 Fur-
thermore, nucleic acid sequence analyses of independent Vc
gene clones showed substantial intraclonal variation in the
Vc genes expressed by this leukemia cell population.160 Sim-
ilar intraclonal diversity recently was observed in a case of
small lymphocytic lymphoma (SLL) that also failed to express
CD5,160 and in another CLL cell population that also was
CD5+.110 These findings contrast with those made in analyzing
conventional CD5 B-cell CLL.89,92,93,106,107,110 As discussed
above. Collectively, these studies suggest that the expres-
sion 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.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 κ-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 κ light chain-bearing cells,
and from 46% to 97% (mean = 77% ± 16%) of the cells
expressing κ light chains of the Vκ11β subgroup. G6-positive
cells expressed either κ or λ 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 ton-
sillar 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.125 In fact, in more than 5,600
nucleotide bases of the Ig Vc 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 Vc gene se-
quencies 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 homolo-
gous Vc 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.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.161,162 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 CD10.125 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 sub-
population of mantle zone lymphocytes.125

It should be noted that slgD-bearing cells constitute only
37% to 87% of the tonsillar B cells.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 fre-
quencies 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 Vc gene repertoire of
circulating B cells in adult peripheral blood may also be highly
restricted.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.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.105
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 JH3 se-
gments. The JH3 segment use of G6-reactive CLL (JH3 [31%],
JH4 [15%], JH5 [8%], or JH6 [46%]) apparently favors the use
of JH3 compared with that of normal G6-reactive tonsillar
lymphocytes or normal circulating adult B cells.165 D segment
use also is skewed compared with that of normal circulating
B cells,165 with XP4, XP1, and XP1 accounting for approxi-
mately 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.125 Rather, the D segment use of
G6-reactive tonsillar B cells mimics that observed for normal
circulating B cells.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 coun-
terpart 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, cardiolipin, actin, thyroglobulin, and/or cytoskeletal components. Such 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 antibodies 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 antibodies, 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. The 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 SMIVH1 gene flanked by unique restriction enzyme sites. This enabled us to exchange several different functional VH-DJH 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 VH-DJH 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 popularity 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 blood 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-I 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 or Ig light chains in any one patient. Furthermore, Ig isotypes of such autoantibodies usually differ from that of the Ig expressed by the malignant B-cell clone. In addition, the circulating levels and the clinical significance of such autoantibodies do not relate directly to the duration or severity of the underlying lymphoproliferative disease. Moreover, immunosuppressive therapy may result in clinical remission of pathologic autoimmunity without affecting the overall leukemia cell burden. Collectively, these observations suggest that the cells producing pathogenic autoantibodies may originate from bystander B cells that are not related to the leukemic cell clone.

Although difficult to detect, especially in patients with high leukemia cell counts, such bystander B cells may be present in the peripheral blood of patients with CLL. We noted human antierythrocyte autoantibodies in mice with severe combined immunodeficiency (SCID) that were engrafted with peripheral blood leukocytes (PBL) from patients with CLL. SCID mice fail to develop functional T or B lymphocytes due to an autosomal, recessive mutation. Leukemia cells injected into the peritoneal cavity of these animals may survive for several weeks in vivo. Eight to 16 weeks after receiving PBL from patients with CLL, SCID mice develop human IgG autoantibodies to human red blood cells and/or high serum levels of human Ig. Soon thereafter, these animals develop lethal human B-cell tumors. In contrast to the original CLL cells, these human B-cell tumors are CD5+, have genomic DNA of EBV, express antigens associated with latent EBV infection, and have distinctive Ig gene rearrangements by Southern. We conclude that bystander B cells, originally present at ≤0.1% of the injected PBL, may generate tumors in CLL-reconstituted SCID mice that emulate the EBV-associated lymphoproliferations noted in SCID mice reconstituted with normal human PBL.

Similar to the pathogenic autoantibodies that develop in patients with CLL, the human IgG antierythrocyte autoantibodies of such reconstituted mice apparently are produced by circulating bystander B cells that are not clonally related to the transferred CLL B-cell population. First, in addition to having different Ig isotypes, the antierythrocyte Ig may have 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 antierythrocyte autoantibodies. Finally, the emergence of antierythrocyte autoantibodies in such SCID mice coincided with the development of high serum levels of human Ig. This invariably preceded the development of B-cell lymphomas that have clonal origins distinct from that of the original leukemia B-cell population. The data suggest that polyclonal activation and/or lymphoproliferation of bystander B cells may account for the antierythrocyte antibodies detected in these animals.

Conceivably, the occurrence of antierythrocyte or anti-platelet autoantibodies in CLL patients also may be associated with dysregulation of the cellular immune mechanisms that control latent EBV infections. Alternatively, the development of such autoantibodies 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.

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 requirement for the self-antigen(s) to be physically associated with or processed by either normal or leukemic B-1 B cells. Clearly, more studies are required to define the mechanism(s) that accounts for the oftentimes pathogenic autoantibodies that may arise in CLL. Such studies may contribute to our understanding of normal B-1 B cells and how they potentially may contribute to the maintenance of immunologic tolerance and/or to the development of systemic autoimmunity.
CONCLUSIONS

Common CLL is a B-1 B-cell malignancy. The leukemia cells often make polyclonal autoantibodies and use a restricted repertoire of nonmutated Ig V genes. Recent studies indicate that somatic selection of B-1 B cells for polyclonal autoantibody production 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 cytototoxic 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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