Clinical and Immunologic Aspects of B Chronic Lymphocytic Leukemia Associated with Autoimmune Disorders

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On behalf of the Israel Study Group on Chronic Lymphocytic Leukemia*

Key words: chronic lymphocytic leukemia, autoimmune disorders, autoimmune hemolytic anemia, immune thrombocytopenia

Abstract

Background: Autoimmune disorders often develop during the course of B chronic lymphocytic leukemia. The source of the autoantibodies is still uncertain: either uncontrolled production of the malignant B cells or disturbances of the residual normal B and T cells involved in the immune system.

Objectives: To evaluate immunologic parameters in B-CLL associated with autoimmune disorders. As a hypothesis we postulated that in those cases, the malignant B cells might disclose an activated phenotype pattern leading to the production of autoantibodies.

Methods: In the Registry of the Israel Study Group on CLL that includes 964 patients, we found 115 cases showing a single or a complex of autoimmune disorders. We evaluated the lymphocyte morphology, immunoglobulin G and beta-2-microglobulin serum levels and positivity of the CD38 and FMC7 markers, and compared these values with those of a matched CLL population without autoimmune disorder.

Results: The main autoimmune disorders encountered were autoimmune hemolytic anemia (55 patients), Evans’s syndrome (n=7), Hashimoto’s thyroiditis (n=15), vasculitis (n=5) and rheumatoid arthritis (n=4). We found atypical prolymphocytic morphology in 22%, high expression of the activation antigens CD38 and/or FMC7 in 30%, and high level of immunoglobulin G (> 1000 mg/dl) and beta-2-microglobulin in 57% and 78% respectively. When compared with a matched CLL population without an autoimmune disorder, these values were statistically significant.

Conclusions: Our data, which show activated lymphocyte morphology, high levels of IgG and beta-2-microglobulin, and increased expression of CD38 and/or FMC7 in a significant number of cases, suggest that some degree of activation of B cells may lead to the occurrence of an autoimmune disorder in CLL. IMJ 2006, 8:828–831

Autoimmune complications are common in chronic lymphocytic leukemia, occurring in up to 25% of patients during the course of their illness. Autoimmune hemolytic anemia occurs in 10 to 25% of cases, immune thrombocytopenia, Evans’s syndrome and pure red cell aplasia are less common. Several others autoimmune disorders have been observed to accompany CLL, including Sjögren’s syndrome, Graves’ disease, bullous pemphigoid, rheumatoid arthritis, ulcerative colitis, allergic vasculitis, systemic lupus erythematosus, pernicious anemia and nephrotic syndrome [1,2]. These associations have been extensively described, but several questions on the pathogenesis remain unresolved. The source of autoantibodies involved in autoimmune disorders associated with CLL is still uncertain, being either uncontrolled production of the malignant clone or disturbances in the immune system (residual normal B or T cells) [3-5]. In the Registry of the Israel Study Group on CLL that includes 964 patients, we found 115 cases showing a single or a complex autoimmune disorder. We undertook an immunologic analysis of those patients in an attempt to better clarify the mechanism of this association, and found that some degree of activation possibly leads to the production of autoantibodies.

Patients and Results

In the present analysis we included 964 patients registered in the database of the Israel Study Group on CLL. The patients were diagnosed between 1971 and 2006. The median age of the 561 men and 403 women was 69 years (range 20–92 years). There were 676 patients (69.2%) diagnosed in stage Binet A, 174 (18%) in stage B, and 75 (7.8%) in stage C. Another 48 patients (5%) were classified as de novo prolymphocytic leukemia [Table 1].

Direct antiglobulin test was found positive in 55 patients (5.7%) at diagnosis. Eleven patients (1.1%) presented with Coombs-positive autoimmune hemolytic anemia. During the follow-up another 43 patients developed AIHA. The distribution of AIHA over clinical stages was as follows: stage Binet A – 19 patients, stage B – 16, stage C – 19, and prolymphocytic leukemia – 1 patient. Six patients developed AIHA following fludarabine treatment. Immune thrombocytopenia was found in nine patients at diagnosis, all but two of whom were DAT-positive and therefore classified as having Evan’s syndrome. During the follow-up four patients developed immune thrombocytopenia and seven others Evan’s syndrome (two following fludarabine).
The other autoimmune features diagnosed at presentation or later during the follow-up were: Hashimoto’s thyroiditis – 15 patients, Graves’ disease – 3, Sjogren’s syndrome – 3, vasculitis – 5, SLE – 2, multiple sclerosis – 2, angioneurotic edema – 2, systemic sclerosis, paraneoplastic ichthyosis, pemphigus and other skin immune complications – 8, pure red cell aplasia – 4, rheumatoid arthritis – 4, and pernicious anemia, ankylosing spondylitis, ulcerative colitis, ulcerative colitis – one each. In addition, laboratory examination revealed rheumatoid factor in 43 of 370 patients tested (11.6%), antinuclear factor in 21 of 246 (8.5%), and decreased serum level of complement C3 or C4 in 59 of 385 patients tested (15.3%). Monoclonal peak was found in 78 cases (8.1%) [Table 2].

We postulated that in CLL, the B cells might disclose an activated phenotype pattern, leading to production of the autoantibodies. The parameters that we studied which define a status of B cell activation included the atypical prolymphocytic morphology, serum immunoglobulin G level > 1000 mg/dl, more than 20% CD38 and/or FMC7 antigen expression and beta-2 microglobulin > 1900 ng/ml. A matched population with CLL and without autoimmune disease was studied from the Registry for comparison.

We found an atypical morphology of the cells in 22% of the cases with autoimmunity compared to 6% in the group without autoimmunity, 30% of CLL with autoimmune disorder disclosed CD38 and/or FMC7 in comparison to 10% in the group without autoimmunity. The level of IgG was high in 57% of the cases in comparison to 30% with no autoimmune disorder. Finally, beta-2 microglobulin level was high in 78% compared to 40% in the group with no autoimmune disorder. The difference in all these values was statistically significant (P < 0.05).

**Discussion**

In this discussion, we would like to review the clinical and immunologic pattern of autoimmune disorder in CLL and, including our data, debate on the pathogenesis of autoimmune disorders in CLL.

*SLE = systemic lupus erythematosus*

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**Table 1. Patients’ characteristics**

<table>
<thead>
<tr>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population of the Israel Study Group on CLL</td>
<td>964</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>561</td>
</tr>
<tr>
<td>Women</td>
<td>403</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>69</td>
</tr>
<tr>
<td>Range</td>
<td>20-92</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
</tr>
<tr>
<td>Binet A</td>
<td>667</td>
</tr>
<tr>
<td>Binet B</td>
<td>174</td>
</tr>
<tr>
<td>Binet C</td>
<td>75</td>
</tr>
<tr>
<td>Prolymphocytic leukemia</td>
<td>48</td>
</tr>
</tbody>
</table>

**Table 2. Autoimmune phenomena**

<table>
<thead>
<tr>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIHA at diagnosis</td>
<td>11</td>
</tr>
<tr>
<td>DAT-positive without AIHA at diagnosis</td>
<td>55</td>
</tr>
<tr>
<td>AIHA during follow-up</td>
<td>43</td>
</tr>
<tr>
<td>AIHA fludarabine-related</td>
<td>6</td>
</tr>
<tr>
<td>Immune thrombocytopenic purpura</td>
<td>9</td>
</tr>
<tr>
<td>Evan’s syndrome</td>
<td>2</td>
</tr>
<tr>
<td>Hashimoto’s thyroiditis</td>
<td>15</td>
</tr>
<tr>
<td>Graves’ disease</td>
<td>3</td>
</tr>
<tr>
<td>Sjogren’s syndrome</td>
<td>3</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>5</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>2</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>2</td>
</tr>
<tr>
<td>Angioneurotic edema</td>
<td>2</td>
</tr>
<tr>
<td>Skin complications</td>
<td>8</td>
</tr>
<tr>
<td>Pure red cell aplasia</td>
<td>4</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>4</td>
</tr>
<tr>
<td>Pernicious anemia</td>
<td>1</td>
</tr>
<tr>
<td>Ankylosing spondylitis</td>
<td>1</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>1</td>
</tr>
</tbody>
</table>

There are several hypotheses about the source of autoantibodies. The simplest explanation is that the CLL clone is responsible for their production. Stevenson et al. [7] demonstrated the secretion of small amounts of autoreactive idiotypic IgM by CLL cells. Sthoeer and co-workers [3] described antibodies in sera with the same light-chain type as the surface IgM of the CLL cells, which therefore was not the product of contaminating normal B cells. Considering CLL as a neoplasm of this separate lineage of CD5+ B cells, equivalent to the Ly-1 B cells of mice, it has been shown that strains of mice prone to autoimmune disorders as (NZBxNZW) F1 develop an expanded Ly-1 B cell pool [8]. Borche et al. [9] in their study with hybridomas generated from CD5+ B-CLL lymphocytes with non-secreting murine myeloma X-63 cells argued that B-CLL lymphocytes are frequently committed to the production of natural autoantibodies. In our laboratory, Marcus and co-workers [10] demonstrated the production of human red cell antibodies in the sera of chimeric mice transplanted with CLL cells from patients with AIHA. Another study conducted by our group [5] found that the anti-red cell antibodies in CLL display identical light-chain isotypes as the tumor cells. Other investigators [6,11] studying sera of CLL patients with AIHA found the same light-chain type as that of the surface immunoglobulin in only half of the cases. Thus, the autoantibodies may not necessarily be derived from the tumor and could be produced by the residual B cells. Dighiero [12], working with murine models, demonstrated that the number of CD5+ B cells was not increased in mice prone to autoimmunity, while strains such as Xid mice, which do not express CD5 marker at all on B cells, have a similar incidence of autoimmunity as other strains. It was also possible to up-regulate CD5 on B cells activated by phorbol ester, demonstrating that the marker is not lineage-specific. The potential role of T cell defects in inducing autoimmune complications in B-CLL has been emphasized in recent publications [1,2,13]. The increased
incidence of AIHA in patients treated with purine analogues has been associated with the loss of T cell regulatory control of autoreactive T cells. These drugs induce severe and long-lasting depletion of the CD4 subset and to a lesser inhibition of the CD8 subset.

The first two cases of AIHA in CLL patients treated with purine analogs were reported in 1992. Since then, other reports have added evidence to this strong association [13]. In our series, six patients developed AIHA following fludarabine treatment. Fludarabine combined with cyclophosphamide may be a less effective trigger of AIHA than fludarabine alone [14]. The other purine analogues, cladribine and pentostatin, may also induce hemolysis [15]. Recent findings from the CLL4 study showed, unexpectedly, that chlorambucil induced more AIHA than the combination of fludarabine and cyclophosphamide [16]. The hemolysis is severe and often difficult to treat [13]. Rituximab could be of benefit, as shown in one of our cases [17].

For many years CLL was considered a progressive accumulation in blood, bone marrow and lymphoid tissue of functionally incompetent and long-lived lymphocytes. It was also believed that B cell chronic lymphocytic leukemia cells accumulate in vivo in the G0/G1 phase of the cell cycle, suggesting that their malignant expansion is due, at least in part, to a delay in cell death [18]. This was in line with the demonstration that B-CLL cells are not an effective antigen-presenting cell [19]. The stimulation of B-CLL cells via CD40 induced a strong up-regulation of co-stimulatory adhesion molecules and turned the B-CLL cells into efficient antigen-presenting cells; CD40 activation of B-CLL cells might reverse T cell anergy against the neoplastic cell clone [20]. The variable region of immunoglobulins bears mutation in at least 50–75% of CLL patients [21]. This situation is related to the antigen-driven lymphocyte activation. The cells from all B-CLL patients, including those lacking significant numbers of V gene mutations, bear the phenotype of activated B cells based on the over-expression of the activation markers CD23, CD25, CD69 and CD71 and the under-expression of CD69 and CD71 and the under-expression of CD38 and FMC7 that are down-regulated on the over-expression of the activation markers CD23, CD25, CD69 and CD71 and the under-expression of CD69 and CD71 and the under-expression of CD38 and FMC7 that are down-regulated.

In summary, the recent advance in our understanding of CLL biology has proved that the B lymphocyte is in an activated state and can be induced to differentiate.

Interestingly, several parameters from the data we presented may reflect B cell activation. CD38, which is first expressed by lymphocytes at an early stage of their differentiation, is down-regulated in resting normal B cells and highly re-expressed by plasma cells [26]. FMC7 is clearly related to diseases of activated B cells. These include prolymphocytic leukemia, Waldenstrom's macroglobulinemia, hairy cell leukemia, and splenic lymphoma with villous lymphocytes [27]. Low immunoglobulin levels are a prominent feature in CLL, but its normal or high levels in patients with autoimmunity may be a consequence of B cell activation. Finally, we previously reported higher B2 microglobulin levels in CLL with prolymphocyte transformation and prolymphocytic leukemia than in typical CLL [28].

Conclusions

Our data showing activated lymphocyte morphology, high levels of IgG and beta-2-microglobulin and increased expression of CD38 and FMC7 in a significant number of cases suggest that a state of activation of B cells may be associated with the occurrence of autoimmune complications in B cell CLL.

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Hematology

Capsule

Chondromodulin-I maintains cardiac valvular function by preventing angiogenesis

The avascularity of cardiac valves is abrogated in several
valvular heart diseases (VHDs). A study by Yoshika et al.
investigated the molecular mechanisms underlying valvular
avascularity and its correlation with VHD. Chondromodu-
lin-I, an anti-angiogenic factor isolated from cartilage, is
abundantly expressed in cardiac valves. Gene targeting of
chondromodulin-I resulted in enhanced Vegf-A expression,
angiogenesis, lipid deposition and calcification in the cardiac
valves of aged mice. Echocardiography showed aortic valve
thickening, calcification and turbulent flow, indicative of early
changes in aortic stenosis. Conditioned medium obtained
from cultured valvular interstitial cells strongly inhibited
tube formation and mobilization of endothelial cells and
induced their apoptosis; these effects were partially inhibited
by chondromodulin-I small interfering RNA. In human VHD,
including cases associated with infective endocarditis, rheu-
"mative heart disease and atherosclerosis, VEGF-A expression,
neovascularization and calcification were observed in areas
of chondromodulin-I down-regulation. These findings provide
evidence that chondromodulin-I has a pivotal role in main-
taining valvular normal function by preventing angiogenesis
that may lead to VHD.

Eitan Israeli

Capsule

Fighting cancer stem cells

Acute and chronic myeloid leukemia (AML and CML, respec-
tively) are maintained by populations of rare cancer stem cells
that divide infrequently and therefore escape the effects of
conventional chemotherapies that are designed to destroy
rapidly dividing cells. Defining the properties of cancer stem
cells and, in particular, identifying which of these might render
the cells selectively vulnerable to therapy, are topics of great
current interest. The normal stem cells that repopulate the
hematopoietic system depend on interactions with stromal
elements in their bone marrow microenvironment (niche) for
survival and function. To explore whether cancer stem cells
are similarly dependent, Jin et al. treated mice carrying human
AML cells with an antibody to CD44, an adhesion molecule
that is overexpressed on AML cells and that is involved in
cell trafficking. Disruption of CD44 function markedly reduced
the growth of leukemic cells in the mice, in part because the
cells failed to migrate effectively to their bone marrow niche.
Independent analysis of human CML stem cells by Krause
et al. revealed a similar dependence on CD44. Importantly,
in both studies, CD44 blockade had a preferential effect on
cancer stem cells versus normal hematopoietic stem cells.
These results suggest that certain leukemias may respond to
treatments that disrupt the interaction of cancer stem cells
with their supportive surroundings.

Eitan Israeli