A restricted *IGHV* gene repertoire in splenic marginal zone lymphoma is associated with autoimmune disorders

Some lymphoma subtypes, particularly marginal zone lymphoma (MZL), are associated with autoimmune diseases or chronic infections.[1,2] Mucosa-associated lymphoid tissue (MALT) lymphomas derive from an acquired lymphoid tissue in the context of a chronic inflammation induced either by a bacterium[1] (e.g. *Helicobacter pylori* (HP) in the stomach) or by an autoimmune reaction.[2] Evidence supporting a strong link between Sjögren sialadenitis or Hashimoto thyroiditis and the occurrence of MALT lymphomas has been reported.[2]

Autoimmune disorders have been less frequently described in splenic MZL (SMZL) than in MALT lymphomas. Based on multiple patients series, 3-20% of SMZL patients display autoimmune manifestations,[3] mainly autoimmune hemolytic anemia (AIHA) and immune thrombocytopenia (ITP). Other autoimmune conditions have also been described concomitantly with SMZL, such as cold agglutinin disease, cryoglobulinemia, antiphospholipid anticoagulant syndrome, acquired Von Willebrand disease, acquired angioedema, Still disease, Biermer disease, lupus erythematosus, Sjögren syndrome and rheumatoid arthritis (RA). These manifestations can occur years before the diagnosis of lymphoma or during the course of the disease, and can occasionally reveal a lymphoma.

To better delineate the role of the autoimmune component in SMZL, we analyzed the mutational pattern of the immunoglobulin heavy variable (*IGHV*) gene in a group of well-characterized SMZL patients presenting with autoimmune manifestations, and compared this profile to cases without autoimmune disorders. In SMZL patients, three *IGHV* genes account for more than 40% of cases,[4-6] with the *IGHV1-2*04* allele being found in almost 30% of cases;[4-6] this enrichment suggests the presence of antigen selection.

Cases were obtained from the Hematopathology Department of the Hospital Lyon Sud, France, and classified according to the WHO 2008 lymphoma classification criteria. *IGHV* DNA sequences were obtained from frozen tissue by PCR amplification and direct sequencing as described previously.[5] Some of the cases described in the present study have already been reported.[4,5,7] To compare the *IGHV* gene utilization frequency between the two groups, we used Fisher's exact test, and $P<0.05$ was considered statistically significant.

Among 85 SMZL patients with *IGHV* mutational patterns, 59 had complete detailed medical histories available, and 20 showed autoimmune disorders; 13 patients had AIHA, 3 had ITP, 3 had mixed cryoglobulinemia (MC), and one had RA. In 6 patients (3 with MC, 2 with AIHA and one with RA), the autoimmune disorder preceded the diagnosis of lymphoma; the times from autoimmune to lymphoma diagnosis ranged from 3 months to 20 years. In 7 patients (6 with AIHA and one with ITP), the diagnosis of the autoimmune disorder revealed the presence of the lymphoma, while in 5 others AIHA was identified during the diagnostic workup. In 2 other cases, the date of onset of the autoimmune disease remained unclear. Nine SMZL patients presenting with a positive direct antiglobulin test (*Online Supplementary Table S1*) but without AIHA at the time of diagnosis of SMZL could not be included as controls and were removed from the analysis. The 30 remaining cases with no characterized autoimmune manifestation were used as a control group (*Online Supplementary Table S1*).

The *IGHV1-2*04* allele was found to be over-represented in the group of patients presenting with autoimmune manifestations (11 of 20, 55% of patients; $P=0.03$) and especially in the patients with AIHA (10 of 13 patients; $P=0.0018$) when compared to the control group (7 of 30, 23% of patients) (Table 1). Interestingly, the 3 patients that presented with MC expressed the *IGHV1-69* gene, which was not

Table 1. Distribution of *IGHV* genes in SMZL patients with or without autoimmune disorders.

	SMZL with autoimmune disease		SMZL without auto-immune disease (n=30)
	All patients (n=20)	Patients with AIHA (n=13)	
*IGHV1-2*04*	11 (55%)	10 (77%)	7 (23%)
IGHV1-69	3 (15%)	0 (0%)	0 (0%)
Other *IGHV*	6 (30%)	3 (23%)	23 (77%)

SMZL: splenic marginal zone lymphoma; AIHA: auto-immune hemolytic anemia; IGHV: immunoglobulin heavy variable gene; SMZL: splenic marginal zone lymphoma; IGHV: immunoglobulin heavy variable gene; AIHA: autoimmune hemolytic anemia; n: number of cases.

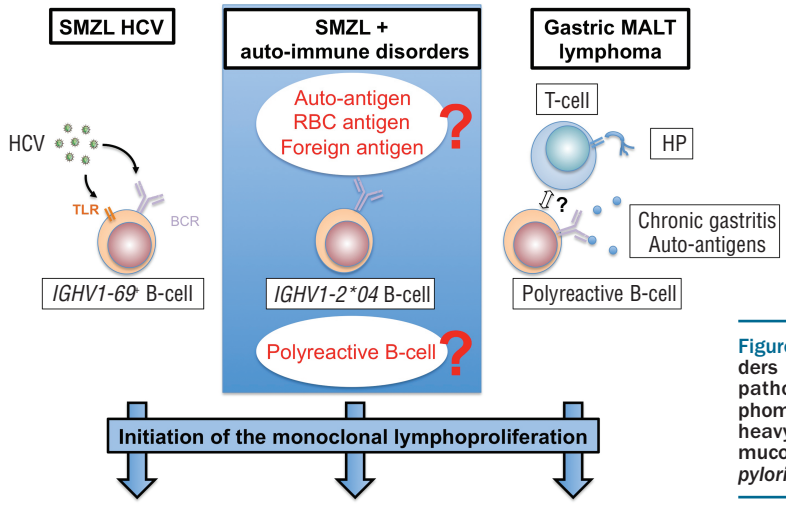

Figure 1. Possible implication of auto-immune disorders in the initial steps of marginal zone lymphoma pathogenesis. SMZL: splenic marginal zone lymphoma; HCV: hepatitis C virus; *IGHV*: immunoglobulin heavy variable gene, RBC: red blood cell, MALT: mucosa-associated lymphatic tissue; HP: *Helicobacter pylori*.

found in the control group (Table 1). However, because of the small number of cases and the retrospective nature of our data, we cannot rule out a selection bias accounting for such differences.

Various immune-mediated microenvironment signals may be involved in the early steps of marginal zone lymphomagenesis (Figure 1). This has already been reported in a subset of SMZL patients with Hepatitis C virus (HCV) infection.[8] MC occurring over the course of HCV infection is associated with the clonal expansion of B cells that recurrently express *IGHV1-69*.[9] It has been suggested that SMZL in HCV-infected patients arises by *IGHV1-69* B-cell clones[1] recognizing a specific viral glycoprotein;[10] this model demonstrates the antigen-driven nature of this type of lymphoma. Among the 3 *IGHV1-69*-expressing patients in our series, one patient had a chronic HCV infection, while another patient had a chronic hepatitis B virus infection.

The overrepresentation of the *IGHV1-2*04* allele in those SMZL cases suggest that particular immune-driven signals may select those clones, hence contributing to marginal zone lymphoproliferation associated with autoimmunity. Recently, Warsame *et al*. produced recombinant antibodies from the rearranged immunoglobulin genes of 5 SMZL patients expressing the *IGHV1-2*04* allele.[11] They found that 4 of the 5 obtained antibodies had features that were consistent with polyreactivity, indicating that those antibodies were able to bind various foreign and autoantigens.[11] These findings raise the possibility that malignant SMZL clones could produce antibodies that recognize red blood cell antigens or alternatively act as aberrant antigen-presenting cells, as was recently described in chronic lymphocytic leukemia.[12]

In HP-associated gastric MALT lymphomagenesis,[1] tumor B cells appear to require two signals for proliferation: 1) a signal from tumor-infiltrating T cells that enhances B-cell proliferation;[13] and 2) a signal through the polyreactive B-cell receptor (BCR), which is able to bind multiple foreign and self antigens.[14] These data emphasize the importance of the microenvironment in providing various signals that support polyreactive marginal zone B-cell proliferation.

The initial mechanisms of antigen-driven MZL proliferation appear to be heterogeneous and may involve signals through the BCR and other pathways (Figure 1). Our data suggest that in HCV-negative SMZL, various antigens and different microenvironmental signals may be involved in lymphoproliferation. The heterogeneity that is observed at the molecular level in those patients likely reflects this variability. This hypothesis is strengthened by the differences observed in *IGHV1-2* gene frequencies among different groups of SMZL patients. To our knowledge, this is the first description of a link between the *IGHV1-2*04* allele and autoimmune disorders in SMZL. Given the efficacy of antimicrobial agents in HCV-positive SMZL and HP-associated gastric MALT lymphomas, determining the mechanisms of chronic stimulation that underlie B-cell proliferation in SMZL may help to develop specific targeted therapies.

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