Introduction

Splenic marginal zone lymphoma (SMZL) is a chronic B-cell disorder in which the splenic white pulp is infiltrated by an inner zone of small lymphocytes, frequently surrounding a residual germinal center, and an outer zone comprising larger cells with abundant pale cytoplasm and occasional blasts. Cases with blood involvement often include a proportion of cells with a characteristic villous appearance, and are diagnosed as splenic lymphoma with villous lymphocytes (SLVL).

The tumor cells are positive for surface immunoglobulin M (IgM) and IgD and express CD20, CD22, and CD79a, but not CD5, CD10, CD23, CD43, or cyclin D1. SMZL usually pursues an indolent clinical course, but transformation to a large-cell lymphoma may occur. The distinctive lymphocyte morphology, immunophenotype, and cytogenetic abnormalities distinguish SMZL from extranodal MZL and from the majority of cases of nodal MZL.

Analysis of mutated variable-region heavy chain \(\text{V}_H\) genes from a small number of cases with SMZL or SLVL has shown that most have undergone somatic mutation with or without intraclonal heterogeneity. Because tissue location may influence mutational activity, we analyzed matched spleen and blood tumor cells in 4 patients. One patient who subsequently developed a clonally related transformed tumor site in the chest wall allowed the comparison of tumor cells in 3 sites. The findings indicate no difference in mutational patterns between spleen and blood, but reveal the potential for initiating mutation within the tumor clone in the appropriate environment in vivo.

Results and discussion

Tumor \(\text{V}_H\) sequences were obtained from all 4 cases, with clear clonal identity between tumor cells from different tissues of each
In 3 of 4 patients, homology to the germ line sequence exceeded 98% and they were classified as unmutated. In these patients, \( V_H \) sequences from blood and splenic tumor cells were identical. Patient 3 showed a low but significant level of somatic mutation, with intraclonal heterogeneity, in tumor cells from both blood and spleen. Mutational patterns were similar in both tissues. Data from these patients confirm the mixed origins of tumor cells in SMZL. They are consistent with derivation from a heterogeneous population of normal SMZ B cells, including naive B cells poised for T-cell–independent type-2 responses against bacterial polysaccharide antigens, and memory B cells. Patient 3 represents those cases of SMZL with intraclonal heterogeneity, similar to that commonly observed in follicular lymphoma (FL). It illustrates that cells of SMZL may be responsive to factors known to be involved in inducing somatic mutation. These include stromal cells, CD4+ T cells, cytokines, and ligation of the B-cell receptor (BCR). A role for antigen may exist, although analysis of mutation distributions in V-genes is no longer considered adequate to reveal this role. The identity of mutation pattern between blood and splenic tumor cells is consistent with the movement of tumor cells between the 2 sites.

For patient 4, tumor samples were obtained from spleen, blood, and chest wall. The only phenotypic difference between the sites was the acquisition of bcl-6 expression in the chest wall biopsy. Immunohistochemical staining of this site showed no expression of CD10, but antibodies against CD21 and CD23 did reveal residual follicular structures among the diffuse infiltrate. The \( V_H \) gene amplified from the spleen, and from the blood 3 years later, contained no somatic mutations. The \( V_H \) sequences obtained from the DLBCL in the chest wall biopsy, obtained after a further 3 years, were clearly derived from the same tumor clone with an almost identical CDR3 sequence (Figure 1). However, there had been an accumulation of somatic mutations, with continuing mutational activity indicated by intraclonal heterogeneity (Figure 1). Some mutations were common to all sequences, but others existed only in subclones. The fact that repeated mutations were present in different clones, and that no mutations were detected in multiple clones from blood or splenic tissue, confirms that these changes

### Table 1. Patient data and summary of tumor \( V_H \) gene sequences

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>WBC ( \times 10^{-5} )</th>
<th>slg</th>
<th>( V_H ) donor</th>
<th>Homology (%)</th>
<th>Intraclonal variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>73</td>
<td>8.4</td>
<td>MD</td>
<td>V3-21</td>
<td>99.6 (B/S)</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>65</td>
<td>14.5</td>
<td>( \lambda )</td>
<td>V1-1</td>
<td>99.0 (B/S)</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>54</td>
<td>7.4</td>
<td>MD</td>
<td>V1-2</td>
<td>97.3* (B/S)</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>68</td>
<td>12.4</td>
<td>MD</td>
<td>V3-33</td>
<td>100 (B/S)</td>
<td>No</td>
</tr>
</tbody>
</table>

*Predominant clones.

The \( V_H \) sequences have been deposited in GenBank (Accession Nos. AF466106-42).

sIg indicates surface Ig; GL, germ line; B/S, sequences from blood and spleen; CW, sequences from chest wall.
were derived from mutation initiated within the tumor clone. This is likely to be associated with transformation of the tumor and location in the chest wall site. Interestingly, the replacement mutations found in all clones were present in, or near to, the CDRs (Figure 1). Replacement mutations accumulating in intraclonal variants, and verified by their presence in more than 1 clone, were also in these sites. The positions involved were as follows: 30 (AGT [Ser] to ACT [Thr]); 56 (AGT [Ser] to AAT [Asn]); 97 (GGA [Gly] to AGA/AAA [Arg/Lys]); 105 (AGT [Ser] to ACT [Thr]), with the last 2 in CDR3.

Induction of somatic mutation in vitro in normal B cells,14 and in most cases of neoplastic B cells,15,16 requires engagement of the BCR. This presumably reflects events occurring in the normal immune responses in vivo where antigen provides this stimulus. For gastric mucosa-associated lymphoid tissue (MALT) lymphomas, there is a clear role for somatic mutation of tumor growth, and possibly somatic mutation, by the presence of Helicobacter pyloris.17 In the case of FL, stimulation of the BCR could occur by direct interaction between glycosylated V-regions and stromal elements.18 This may free these tumors from a requirement for conventional antigen to maintain growth. It is unlikely that somatic mutation continues indefinitely in FL.19,21 SMZL is not considered to be a germinal center tumor, and glycosylation sites are not evident in published4 sequences or our own. The question of a role for interaction with antigen for stimulation of somatic mutation or for growth of tumor cells therefore remains open. The finding that a long-term in vivo tumor with unmutated V_H genes can apparently initiate somatic mutation and accumulate intraclonal heterogeneity with replacement mutations clustered in the CDRs is dramatic. It suggests that a factor in the environment, possibly able to bind to the BCR, has stimulated the cell. Alternatively, an event has occurred in the clone to activate somatic mutation independent of exogenous stimuli. Either change could be linked to the transformation to a rapidly dividing diffuse lymphoma, with karyotypic evolution. Transformation is an uncommon event in SMZL and we need to investigate whether this route is the exception or the rule.

References

$V_H$ gene analysis of splenic marginal zone lymphomas reveals diversity in mutational status and initiation of somatic mutation in vivo

Delin Zhu, Jennifer Orchard, David G. Oscier, Dennis H. Wright and Freda K. Stevenson