CORRESPONDENCE

DISORDERS OF LARGE GRANULAR LYMPHOCYTES AND NATURAL KILLER-ASSOCIATED CELLS

To the Editor:

We read with interest the recent review of large granular lymphocyte (LGL) clonal proliferations by Loughran. This provides a timely synopsis of what still remains a rather ill-defined spectrum of disorders but we would like to comment on several aspects that we believe are of some importance to this issue. However, before dealing with these, it may facilitate any discussion of this subject by bringing together the morphologic definition of large granular lymphocytes (LGL) and the immunophenotypic definitions of natural killer-associated (NKa) cells. In most patients, an abnormal increase in LGL is apparent from conventional morphologic assessments of Romanowsky-stained preparations and, in such cases, morphologic observations are usually supported immunophenotypically by the demonstration of lymphocytes expressing one or more NKa markers (i.e., LGL+NKa+). However, relationships between the morphologic presence of lymphoid cells with features of LGL and the expression of NKa determinants is not invariable. For example, in some patients there may be considerable granule heterogeneity (ranging from very large prominent granules to fine azurophilic granules), and in some cases the proportion of lymphocytes with LGL morphology may be considerably lower than indicated from immunophenotypic estimates of NKa+ cells (designated LGL-NKa+). Additionally, LGL may occasionally not express NKa determinants (designated LGL+NKa-). In these circumstances, a more accurate cytomorphologic reflection of LGL may be obtained by benzylxoycarbonyl-L-lysine thiobenzyl (BLT) esterase cytochemistry. Additionally, LGL may occasionally not express NKa determinants (designated LGL+NKa-). In these circumstances, a more accurate cytomorphologic reflection of LGL may be obtained by benzylxoycarbonyl-L-lysine thiobenzyl (BLT) esterase cytochemistry.

The review by Loughran essentially provides an analysis of cellular and clinical features of clonal CD3+ LGL/NKa proliferations reported in previously published patient series. In addition, details are provided for a small number of patients with CD3- LGL/NKa proliferations, although these particular cases were predominantly of Japanese origin and comparisons with CD3+ LGL/NKa proliferations in Europe and the USA may not be entirely valid. We have also analyzed literature reports of LGL proliferations but, despite their value, such reviews are almost always affected by an inherent bias toward patients with clinical symptoms or a significant lymphocytosis. Furthermore, as it is possible that the specific selection of literature cases with clonality defined by TCR genotypic studies may also lead to additional bias, it is quite conceivable that the frequencies of clinical complications obtained from this type of metaanalysis will be overestimates. A prospective survey performed in the United Kingdom, by the Yorkshire Leukaemia Group (YLG), which commenced in 1989, has provided information that could more accurately reflect the incidence and cellular characteristics of clonal and nonclonal persistent LGL/NKa abnormalities.

In the first phase of this study, we examined 870 blood samples from different adult (>16 years of age) patients, initially submitted for routine blood counts, that were selected on the basis of either (1) an absolute lymphocytosis (>4.5 × 10^9/L), (2) an increased (>25%) proportion of peripheral blood lymphocytes with LGL morphology, or (3) neutropenia of unknown etiology. Patients who fulfilled one or more of these criteria but who had known lymphoprolifera-
tive disorders or a recent history of surgery were excluded. Of the 870 patients in total, 269 (31%) were found to have an increased proportion (>25%) or absolute numbers (>1.0 X 10^9/L) of LGL and/or immunophenotypically defined (CD16+, CD56+, or CD57+) NKa cells. Follow-up studies of 112 of these patients showed that LGL/NKa abnormalities were persistent (defined as a minimum of 6 months) in 82% (n = 92) of cases. Examining the data from a slightly different perspective showed that of the 498 cases with a lymphocytosis of >4.5 X 10^9/L, and after excluding 83 patients in which a diagnosis of chronic B- or T-cell malignancy was made, the primary abnormality in 243 (59%) was an increased LGL/NKa component. Although it is accepted that persistence of an abnormality over 6 months does not imply permanence, this is nevertheless a significantly higher frequency of LGL/NKa abnormalities than suggested by literature estimates.

The classification of these disorders raises a number of difficulties. Not least in trying to differentiate between the primary or secondary nature of abnormally expanded LGL/NKa components. Using a nonclinical approach, the broad immunologic subdivision into clonal CD3+ and CD3- subtypes (designated T-LGL leukemia and NK-LGL leukemia, respectively, by Loughran) has some validity, although this does not understate their complexity. For example, clonal CD3+ cases in our series comprised TCRa/b'CD4'CD8dim or +, TCRa/b'CD4'CD8', TCRb'CD4'CD8- or dim+, and TCRa/b'CD4'CD8+ or dim subtypes, whereas CD3- cases were far more homogeneous with respect to CD4/CD8 expression. Leaving aside a further level of heterogeneity imposed by the differential expression of the three widely used NKa markers (CD16, CD56, and CD57), it is perhaps not surprising that empirical schemes such as those proposed by Chan et al and McDaniel et al have only limited relevance. In this context, we believe that the frequency of autoimmune disease and persistent neutropenia (detailed below) is an increased LGL/NKa component. Although it is accepted that persistence of an abnormality over 6 months does not imply permanence, this is nevertheless a significantly higher frequency of LGL/NKa abnormalities than suggested by literature estimates.

The clinical features of these patients were quite diverse although these disorders are undoubtedly associated with older age groups. Of our series of 92 persistent LGL/NKa abnormalities, the median age was 63 years with only 8% of patients being younger than 40 years. Additionally, there was a slight female predominance for both <60-year (0.88:1) and >60-years (0.85:1) age groups. All sera tested (75/92) were negative for antibodies to HTLV-I/II. Examination of clinical features for CD3+, CD3- and CD3' subgroups in the YLG series indicate a notably higher incidence of neutropenia for CD3+ cases (Table 1) whereas anemia, although more frequently associated with CD3- cases, is also seen in a significant minority of CD3- and CD3' patients. However, within these particular data there are two additional points of interest. Firstly, although there was a slight female predominance for all LGL/NKa abnormalities, when the CD3 subgroups were analyzed separately, the male:female ratio for CD3' cases was 1.3:1 whereas CD3- and CD3' cases showed a distinct female predominance (0.35:1 and 0.24:1, respectively). Secondly, of the 19 cases presenting with neutropenia 15 were persistent for a minimum study period of 6 months. Immunophenotypic and DNA genotypic analyses further showed that 12 of these 15 cases were CD3+ (none of which were of the CD4' subtype) and 10 of these 12 showed rearranged TCR genes.

It is evident that a proportion of patients with LGL/NKa proliferations have an aggressive form of the disease and treatment may be necessary, particularly for those patients with severe neutropenia. However, we have followed-up most of our cases over a 3-year period and our impression to date is that despite the persistence of cellular abnormalities, most patients have been clinically stable during this time. The differences between our conclusions and those of Loughran almost certainly reflect the way in which the cases were selected. In this respect, it is tempting to speculate that our systematic screening program has highlighted a high relative frequency of patients with those LGL/NKa disorders that have not been formally referred for hematologic assessment (ie, in contrast to those that are likely to predominate in Loughran's analysis).

If the phenotypic (CD4' and non-CD4') and genotypic (germline and rearranged TCR) subclassifications proposed above are viewed in conjunction with rheumatoid disease and persistent neutropenia (Table 2), which may well be the most important clinical factors in

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Table 1. Frequency of Anemia, Neutropenia, Thrombocytopenia, Rheumatoid Disease, and Rearranged TCR Genes in CD3', CD3-, and CD3' LGL/NKa Subtypes

<table>
<thead>
<tr>
<th>All Cases (%)</th>
<th>CD3' TCR-G (%)</th>
<th>TCR-R (%)</th>
<th>CD3' (%)</th>
<th>CD3+(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia (males, &lt;13.5 g/dL; females, &lt;11.5 g/dL)</td>
<td>42</td>
<td>26</td>
<td>52</td>
<td>26</td>
</tr>
<tr>
<td>Persistent neutropenia (&lt;2.0 X 10^9/L)</td>
<td>26</td>
<td>11</td>
<td>39</td>
<td>9</td>
</tr>
<tr>
<td>Thrombocytopenia (&lt;150 X 10^9/L)</td>
<td>7</td>
<td>5</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Rheumatoid disease (serologic and/or clinical evidence)</td>
<td>31</td>
<td>21</td>
<td>39</td>
<td>9</td>
</tr>
<tr>
<td>Rearranged TCR genes</td>
<td>59</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Data for CD3' LGL/NKa group also shown separately for rearranged and germline TCR cases. CD3' cases expressing membrane TCRb' chains are excluded from this analysis.
leukemia does, and the psychologic impact (as well as the financial/insurance ramifications) on patients, who in most cases will have a benign and nonprogressive disorder, cannot be justified. For the minor proportion of patients who do have clinical features that are indicative of 'true' malignancy (eg, lymphadenopathy, splenomegaly, tissue infiltration, progressive disease, etc), the term leukemia may be more acceptable. Such details need to be carefully considered before proposing an international scheme of nomenclature.

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