LARGE GRANULAR LYMPHOCYTE EXPANSIONS IN FELTY’S SYNDROME HAVE AN UNUSUAL PHENOTYPE OF ACTIVATED CD45RA+ CELLS

S. J. BOWMAN, G. C. GEDDES, V. CORRIGALL, G. S. PANAYI and J. S. LANCHBURY

Molecular Immunogenetics and Rheumatology Units, Division of Medicine, UMDS, Guy’s Hospital, London SE1 9RT

SUMMARY

One-third of patients with Felty’s syndrome (FS) have significant clonal expansions of CD3+CD8+ large granular lymphocytes (LGLs) in their peripheral blood. The reasons for this are unclear, but one hypothesis is that they are activated antigen-specific cells of pathogenic relevance. Cytofluorographic analysis of activation markers demonstrated that the cell surface phenotype of these expansions was CD57+, HLA-DR+, IL-2R−, Leu-8+, CD69+, LFA-1+, ICAM-1+, VLA-4+, i.e. ‘activated’ T cells. However, they also expressed the phenotype CD45RA+, CD45RB+, CD45RO−, usually associated with ‘naive’ cells. This could result from aberrant activation, malignant transformation or from a ‘reversal’ of CD45 phenotype following chronic antigenic stimulation. In three patients with RA and non-clonal LGL expansions, a more variable phenotype was found. In one of these patients, the expanded population was identified in the peripheral blood, but not the synovial fluid. This may suggest that, at least in this individual, any pathogenic effect is exerted systemically.

KEY WORDS: Felty’s syndrome, Large granular lymphocyte, Activation markers, CD45.
RESULTS

The 10 RA patients with T-cell LGL expansions comprised six males and four females, with a mean age (± s.d.) of 70 ± 12 yr (range 49–83 yr). The mean duration of RA was 18 ± 15 yr (range 1–54 yr). All patients except one were rheumatoid factor positive, four were known to have splenomegaly and five were on prednisolone. Eight had FS with a mean duration (± s.d.) of 4.1 ± 4.8 yr (range 0.5–14 yr). The mean current lymphocyte count was 4.9 ± 3.7 x 10^9/l (range 1.3–11.2 x 10^9/l) and neutrophil count 1.7 ± 1.6 x 10^9/l (range 0.3–2.0 x 10^9/l, except for one patient whose neutrophil count had returned to normal at 5.4 x 10^9/l). Seven out of the eight with FS had evidence of clonal T-cell expansions by RFLP analysis, differed from this phenotype in unique ways. In patient 8 (with FS), the CD8+ cells were CD45RA-, CD45RB*, CD8+, HLA-DR+, ICAM-1/CD8, CD45RO-, Leu-8-, CD45RA+, CD45RB*bd, CD45RO-1, ICAM-1/Leu-8+/CD8+. The peripheral blood from the three patients with RA, but without evidence of clonal LGL expansions by RFLP analysis, differed from this phenotype in unique ways. In patient 8 (with FS), the CD8+ cells were CD45RA+, CD45RB*bd, CD45RO+. In patient 9, the phenotype was CD25+, Leu-8+, and both CD45RA+ and CD45RO+ were expressed at a high level, suggesting simultaneous expression on 52.3–72.9% of the cells (CD45RB was not analysed). In patient 10, the expanded cells did not express HLA-DR. Only two patients with clonal T-cell LGL expansions without RA were available for study. Although the data are limited, the initial results suggest that T-cell LGL expansions in such patients have a similar cell surface phenotype to the group with FS and clonal T-cell LGL expansions. Compared to CD8 T-cells from the peripheral blood of RA patients, the FS patients’ CD8 cells expressed higher levels of HLA-DR, CD45RA and ICAM-1, and lower levels of Leu-8 and CD45RO.

Table I shows the results of the cytofluorographic analysis of the peripheral blood from all the patients studied and the synovial fluid from patient 8. In the seven patients with FS and clonal LGL expansions, the phenotype of the expansions was CD3+, CD8+, CD57+, HLA-DR+, CD25-, CD45RA-, CD45RB*, CD45RO-. In patient 9, the phenotype was CD25-, Leu-8+, and both CD45RA+ and CD45RO+ were expressed at a high level, suggesting simultaneous expression on 52.3–72.9% of the cells (CD45RB was not analysed). In patient 10, the expanded cells did not express HLA-DR. Only two patients with clonal T-cell LGL expansions without RA were available for study. Although the data are limited, the initial results suggest that T-cell LGL expansions in such patients have a similar cell surface phenotype to the group with FS and clonal T-cell LGL expansions. Compared to CD8 T-cells from the peripheral blood of RA patients, the FS patients’ CD8 cells expressed higher levels of HLA-DR, CD45RA and ICAM-1, and lower levels of Leu-8 and CD45RO.

### Table I

<table>
<thead>
<tr>
<th>Markers (%)</th>
<th>7 FS patients with clonal expansions</th>
<th>Patient 8 PB</th>
<th>Patient 8 SF</th>
<th>Patient 9</th>
<th>Patient 10</th>
<th>Patient 11</th>
<th>Patient 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3/lymphocytes</td>
<td>89.2 ± 6.9</td>
<td>88.5</td>
<td>89.2</td>
<td>72.7</td>
<td>91.0</td>
<td>83.0</td>
<td>77.8</td>
</tr>
<tr>
<td>CD4/CD8</td>
<td>10.2 ± 5.7</td>
<td>26.9</td>
<td>34.9</td>
<td>23.1</td>
<td>14.5</td>
<td>3.6</td>
<td>15.1</td>
</tr>
<tr>
<td>CD8/CD8</td>
<td>85.2 ± 8.1</td>
<td>72.3</td>
<td>63.8</td>
<td>66.1</td>
<td>81.9</td>
<td>94.0</td>
<td>83.2</td>
</tr>
<tr>
<td>CD57/CD8</td>
<td>60.3 ± 20.8</td>
<td>69.8</td>
<td>56.7</td>
<td>77.1</td>
<td>56.1</td>
<td>88.4</td>
<td>33.5</td>
</tr>
<tr>
<td>HLA-DR/CD8</td>
<td>74.8 ± 8.2**</td>
<td>ND</td>
<td>ND</td>
<td>10.9</td>
<td>91.3</td>
<td>(96.4*)</td>
<td>87.7</td>
</tr>
<tr>
<td>CD25/CD8</td>
<td>0.7 ± 0.2**</td>
<td>ND</td>
<td>ND</td>
<td>0.44</td>
<td>47.9</td>
<td>(0.3*)</td>
<td>0.7</td>
</tr>
<tr>
<td>CD69/CD8</td>
<td>43.9 ± 24.2*</td>
<td>ND</td>
<td>ND</td>
<td>1.01</td>
<td>2.51</td>
<td>23.3</td>
<td>51.5</td>
</tr>
<tr>
<td>Leu-8/CD8</td>
<td>4.2 ± 2.4**</td>
<td>14.1</td>
<td>ND</td>
<td>5.06</td>
<td>52.9</td>
<td>7.0</td>
<td>22.4</td>
</tr>
<tr>
<td>CD45RA/CD8</td>
<td>89.4 ± 9.1</td>
<td>7.7</td>
<td>2.4</td>
<td>74.8</td>
<td>79.4</td>
<td>77.0</td>
<td>70.4</td>
</tr>
<tr>
<td>CD45RB**/CD8</td>
<td>95.4 ± 6.4**</td>
<td>99.3</td>
<td>90.3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CD45RO/CD8</td>
<td>71.7 ± 7.1*</td>
<td>41.3</td>
<td>89.2</td>
<td>14.3</td>
<td>72.9</td>
<td>14.4</td>
<td>26.3</td>
</tr>
<tr>
<td>ICAM-1/CD8</td>
<td>94.8 ± 6.2***</td>
<td>99.2</td>
<td>ND</td>
<td>86.8</td>
<td>79.0</td>
<td>48.3</td>
<td>32.6</td>
</tr>
<tr>
<td>LFA-1/CD8</td>
<td>95.8 ± 6.5***</td>
<td>99.5</td>
<td>ND</td>
<td>99.5</td>
<td>77.7</td>
<td>77.2</td>
<td>ND</td>
</tr>
<tr>
<td>VLA-4/CD8</td>
<td>93.6 ± 7.4***</td>
<td>ND</td>
<td>ND</td>
<td>97.6</td>
<td>79.4</td>
<td>81.8</td>
<td>ND</td>
</tr>
</tbody>
</table>

Results are expressed as the percentage of expression of the first marker on cells positive for the second. Results that differ from the phenotype in patients with clonal expansions are underlined.

*CD57/CD8 = 90.1%, Vβ5.3/CD8 = 32.3%, CD45RO/Vβ5.3 = 85.7%.
*CD57/CD8 = 71.7%, Vβ5.3/CD8 = 7.9%, CD45RO/Vβ5.3 = 96.8%.
*Data represent the percentage expression on CD57+ cells not CD8+ cells.
*Vβ8/CD3 = 54.8%.
*CD16/CD1 = 54.2%.
*CD57/CD8 = 34.4 ± 14.5% (range 13.1–45%).
*Monoclonal antibodies 15/2 (anti-ICAM-1) and m38 (anti-LFA-1) were kindly provided by Dr C. Buckley (see Patients and methods).
*Ten months previously, Vβ5.3/CD3 was 23.1% in PB, and 2.9% in SF, suggesting a stable phenomenon.

Numbers of patients analysed were 6*, 5**, 4***, 3****.
In patient 8, we were able to compare synovial fluid mononuclear cells from the left knee with those of peripheral blood (Table I). This patient has an expansion of CD3+ CD8+ CD57+ cells in the peripheral blood with 90% of the CD8 cells expressing CD57 (normal range <30%) (data not shown). T-cell clonality could not be demonstrated using RFLP analysis, although this does not fully exclude this possibility [3]. A total of 32.3% of peripheral blood CD8 cells expressed Vβ5.3 (normal range in our laboratory 0.4-1.8%, data not shown). This patient's peripheral blood was also examined using seven other anti-Vβ antibodies without finding any further expansions [3]. These Vβ5.3+ cells were shown to be mostly CD45RA-, CD45RB 

-------

The starting point for this study was the hypothesis that clonal LGL expansions in FS reflected chronic antigenic stimulation and, hence, would have the phenotype of activated 'memory' cells. This was obviously not the case in terms of CD45 isoform expression in that the commonest phenotype was of CD45RA-, CD45RB 

-------

The expression of these adhesion molecules and of HLA-DR is, therefore, in keeping with the original hypothesis that these expansions are chronically activated cells. The CD45R isoform expression is more difficult to interpret according to the above scheme. This scheme may, however, be more complex in CD8 T cells in that high levels of LFA-1, an adhesion molecule that is upregulated following T-cell activation, have been shown to be expressed on the surface of a proportion of CD45RA+, RO-, CD8+ T cells [14]. This expression increased with age, ranging from 8% in a 14 yr old to 94% in a 79 yr old [14]. Furthermore, in vitro studies have demonstrated reversal of CD45 isoform expression from CD45RA to CD45RA in long-term CD8 T-cell lines [15] comparable to data from CD4 cells [16]. Hence, CD45R isoform expression may not distinguish 'memory' from 'naive' T-cell populations in all circumstances and the CD45R phenotype of the T-cell LGL expansions in FS may still be compatible with the hypothesis of an antigen-driven process.

In normal individuals, increased numbers of CD8+, CD57+ T cells have been shown to correlate with previous exposure to cytomegalovirus [17]. In that study (see also reference [2]), over 40% of those cells co-expressed CD45RA and CD45RO (as did patient 9 in this study) and had suppressive properties [17]. In RA patients, CD3+ CD57+ T cells have been shown to have the phenotype CD45RA+, CD45RO-, CD25-, i.e. of conventionally 'naive' T cells [18]. In the current study, a proportion of CD8 T cells from the peripheral blood of four RA patients showed evidence for activation in terms of CD45R isoform and HLA-DR expression. All were LFA-1+, compatible with data from reference [14] discussed above. These activation phenotypes differ from that of the clonal expansions among the FS patients. Nevertheless, the possibility that cytomegalovirus or a related virus could be involved in driving these expansions remains an obvious hypothesis. Whether a similar or different antigen could be involved in driving T-cell LGL expansions in patients without RA has not yet been directly addressed, although from the limited data in this study the activation phenotype was similar.

It has been shown in both acute and chronic leukemias that CD45RA+ expression is common, while CD45RO expression is more variable, with uncoupling of the normal relationships between these isoforms and activation markers, as seen in this study [19, 20]. A transformed phenotype may thus be more likely to express CD45RA and may not reflect the normal differentiation status of untransformed cells. The finding that the seven FS patients with clonal expansions had a uniform phenotype, while the three RA patients with LGL expansions that were non-clonal by RFLP analysis had more variable results, as well as our previous work [21], support this proposal. Such a transformation event could still occur on a background of chronic antigenic stimulation [21]. A third possibility is that this unusual phenotype represents the activation of cells in a non-antigen-specific manner through ligands other than the T-cell receptor. T-cell activation by this mechanism has been proposed to occur in RA [13].
Evidence exists for a selective increase in CD8+ CD57+ morphologic LGLs in RA synovial fluid [22]. This raises the issue of whether these cells are locally expanded Vβ3.3+ LGLs in the peripheral blood, but patients had an effusion during the period of the study. One previous report describes a patient with a peripheral blood CD8+ Vβ3 expansion that was also not seen in the synovial fluid. It was clear that the expanded population was not seen in the synovial fluid. If these results are confirmed when data from more patients become available, it suggests that if LGLs are pathogenic in RA/FS, they exert their effects systemically, perhaps due to the effects of secreted cytokines.

ACKNOWLEDGEMENTS

We thank all our colleagues and their patients who assisted with this study. We are also grateful to Professor A. W. Boylston (St James University Hospital, Leeds), Dr Chris Buckley (Institute for Molecular Medicine, Oxford), Dr David Mason (The John Radcliffe Hospital, Oxford) and Dr Costantino Pitzalis (Rheumatology Unit, UMDS) who provided antibodies used in this work. SB was supported by an MRC Training Fellowship. We gratefully acknowledge support for the laboratory from the Arthritis and Rheumatism Council.

REFERENCES