Correspondence

Expanded Fc receptor bearing 'third population' lymphocytes in rheumatoid arthritis: analysis by cell partitioning in two-polymer aqueous phase systems

Sir, Goulding and coworkers have demonstrated increased numbers of Fc receptor bearing lymphocytes in rheumatoid arthritis, especially in patients with Felty's syndrome. A presumably related expansion of large granular lymphocytes (LGLs) also occurs in rheumatoid arthritis, frequently associated with neutropenia. The large, granular morphology is a phenotypic characteristic of lymphocytes with killer activity, especially natural killer cells. This population of lymphocytes has been of particular interest to us because of its distinctive partitioning behaviour in charge sensitive, two-polymer aqueous phase systems that presumably subfractionate cells on the basis of charge associated surface properties. In an appropriately selected phase system 8-33% of lymphocytes from normal individuals are found in a discrete peak, having a partition ratio that consists of cells with high affinity for the upper, positively charged phase. This subpopulation contains virtually all the LGLs of the unfragmented lymphocytes, and is also enriched with natural killer and antibody dependent cellular cytotoxic activity. Further confirming this enrichment is our finding that cells with the natural killer antigens Leu 7 and HNK-1 are found almost exclusively in the peak with a high partition ratio (including those HNK-1+ lymphocytes that coexpress the suppressor/cytotoxic T cell antigen, CD8).

To examine the partitioning of lymphocytes from subjects with rheumatoid arthritis we studied 13 patients with this disease and compared them with 32 control subjects. Most of the patients were men from a Veterans Administration Hospital clinic who had relatively severe disease. Peripheral blood mononuclear cells were isolated on Ficoll-Hypaque cushions and depleted of monocytes by velocity sedimentation at unit gravity on a Bont apparatus. The lymphocytes were then subfractionated in a charge sensitive dextran-polyethylene glycol aqueous phase system by countercurrent distribution (a multistep extraction procedure) as previously described. The phase system used contained 5% (w/v) dextran, 4% (w/v) polyethylene glycol, 130 mM NaCl, 150 mM sodium phosphate buffer pH 7.4, and 5% (w/v) heat inactivated fetal bovine serum. The distribution of lymphocytes was plotted as the number of cells in different cavities along the extraction train. Two peaks were obtained and the relative percentage of cells in the two peaks was determined by plotting curves on heavy bond paper, cutting out the two peaks, and weighing them.

When countercurrent distribution curves of lymphocytes from patients with rheumatoid arthritis were compared with those from normal individuals we found that the patients had a modest but statistically significant increase in the peak with a high partition ratio. In the 13 patients with rheumatoid arthritis (RA) 18.6 (0.8-0%) (mean (SD)) of lymphocytes were found in this peak compared with only 15.2 (4.8%) in the 32 controls (p<0.05). Although the patients with RA and normal subjects had comparable percentages of cells with LGL morphology in this peak 53.4 (8.9%) vs 49.7 (3.1%) respectively; p>0.15), there was a marked difference in the percentage of these cells that expressed Fc receptors. Only 31.8 (11.3%) of these cells from nine normal individuals formed rosettes with antibody sensitised sheep erythrocytes compared with 54.0 (7.0%) of cells from five patients with RA (p<0.001).

Our results were consistent with those of Goulding and colleagues and suggest both a quantitative and qualitative alteration of non-B, non-T, ‘third population’ lymphocytes in rheumatoid arthritis. There was a modest but significant increase of peripheral blood lymphocytes having a high partition ratio and LGL morphology. Most striking, however, was the marked increase in the proportion of lymphocytes in the peak with the high partition ratio which had readily demonstrable Fc receptors. This latter finding could represent either a specific increase in a subset of LGL bearing Fc receptors or, alternatively, a secondary activation of these lymphocytes in the patients that results in an increased expression of IgG binding surface molecules.

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References

Table 1 Facb-R+ cell characteristics

<table>
<thead>
<tr>
<th>Facb-R+ cells (%) in:</th>
<th>Patients with RA (n=8)</th>
<th>Healthy controls (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DR+ Density &lt;1·062 g/ml</td>
<td>71 (19)*</td>
<td>44 (19)</td>
</tr>
<tr>
<td>Density &lt;1·062 g/ml</td>
<td>75 (12)</td>
<td>19 (17)</td>
</tr>
</tbody>
</table>

*Results are presented as mean (SD). Statistical analysis by Mann-Whitney U test.

References

Pulse steroid therapy in rheumatoid arthritis

Sir, After a decade of the use of intravenous 'pulse' megadose corticosteroids in rheumatoid arthritis we now have a controlled study that shows there is no difference in effect from a similar 'pulse' given orally. Although the authors pointed out that this obviates the need for hospital admission and thus makes fewer demands on medical and

Sir, Thank you for the opportunity to reply to the letter by Dr Michalski and colleagues. We are uncertain whether the Fc receptor bearing cells isolated from the upper phase of their partition system are the same as those detected in our Facb rosette assay. We have shown previously that Facb-R+ cells share some surface characteristics with monocytes and appear not to show natural killer or antibody dependent cytotoxic activity. Thus enhanced expression of Fcy receptors on rheumatoid mononuclear cells probably reflects changes in a number of distinct subpopulations. Our own recent evidence would support Dr Michalski's final comment that these changes could be associated with cell activation. We have found that Facb-R+ cells from rheumatoid patients express significantly more II major histocompatibility complex antigen by immunofluorescence and are of lower density on Percoll gradient centrifugation than equivalent cells from healthy control subjects (Table 1). Thus rheumatoid Facb-R+ cells appear to be identical to similar cells isolated from healthy individuals three days after secondary challenge with specific antigen. The maintenance of increased numbers of activated Facb-R+ cells in RA over long periods is probably due to repeated stimulation of the immune system in these patients.
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