THE SEQUENTIAL ANALYSIS OF T LYMPHOCYTE SUBSETS AND INTERLEUKIN-6 IN POLYMYALGIA RHEUMATICA PATIENTS AS PREDICTORS OF DISEASE REMISSION AND STEROID WITHDRAWAL

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SUMMARY

CD4 and CD8 T lymphocyte subsets, the late T cell activation marker, HLA-DR, and serum interleukin-6 (IL-6) levels of 57 polymyalgia rheumatica (PMR) patients were followed over 2 yr to investigate whether they could be used to predict the safe withdrawal of steroid therapy. Cell phenotypes were studied by flow cytometry and IL-6 levels by ELISA. %CD8 cells were reduced below the normal range in PMR patients prior to steroid therapy. In 56% of patients, the %CD8 T lymphocytes failed to return to normal levels when quiescent disease allowed cessation of steroid therapy. Activated CD8 T cells, as detected by HLA-DR positivity, were above the normal range at the initiation of therapy and showed a negative correlation with %CD8 T cells. The serum concentration of IL-6 fluctuated over 24 months, and the correlation between IL-6 and erythrocyte sedimentation rate (ESR) seen prior to treatment was not seen at later intervals. The %CD8 T cell and serum IL-6 levels are not a good indicator of disease activity in PMR and are, therefore, unable to predict the safe withdrawal of steroids.

KEY WORDS: T-lymphocyte subsets, Interleukin-6, Polymyalgia rheumatica, Disease remission, Steroid withdrawal.

POLYMYALGIA rheumatica (PMR) is a disease of unknown aetiology, occurring in elderly patients and characterized by shoulder and pelvic muscle girdle stiffness with pain and general malaise. Once diagnosed, PMR may be readily managed by long-term, low-dose steroid therapy. However, the prolonged use of steroids may in itself be the cause of much morbidity and mortality [1]. The most common measures of disease activity are the acute-phase reactants, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) [2]. The acute-phase response is driven by the release of interleukin-6 (IL-6) from the inflammatory focus [3]. Since steroids rapidly normalize the acute-phase response [4] and clinical features, there is little guidance from clinical or laboratory parameters as to the underlying disease activity. A measure of the underlying disease activity, independent of the acute-phase response, is clearly needed for accurate monitoring which could tailor steroid usage to persistent disease.

Recently, we and others have shown that the measurement of %CD8 T cells in the peripheral blood prior to steroid therapy [5–9] may assist the clinician in identifying true PMR patients from those with the myalgic onset of other diseases such as rheumatoid arthritis [9]. We have also shown that CD8 T cells in PMR are activated since they are HLA-DR positive [6]. In this study, PMR patients were investigated over a 2 yr period to ascertain whether the monitoring of %CD8 T lymphocytes and/or IL-6 serum levels would give an indication of when the disease had gone into remission and, hence, steroids could be safely withdrawn.

MATERIAL AND METHODS

Patients

Patients were recruited from five hospitals within a 50 mile radius of London, and PMR was diagnosed according to the Jones and Hazleman criteria [10]. Patients were studied at presentation, prior to steroid therapy, and thereafter at 3, 6, 12 and 24 months. At each visit, clinical disease activity was assessed, using morning stiffness, muscle tenderness and visual analogue score for pain, the presence of synovitis noted and blood taken for the measurement of ESR, full blood count (FBC), serum IL-6 estimations and lymphocyte subset analysis. The ESR and FBC were performed at the referring hospital, while the serum and EDTA–sequestrene sample for immunofluorescence were mailed to the laboratory, where they arrived within 24 h. The validity of the postal delivery of the samples for fluorescence has been previously documented [9]. All patients were seen at the same time of day on each visit to reduce diurnal variation and blood was taken prior to daily or monthly steroid dosing.

Patients were randomized to receive treatment with oral prednisolone (P) or i.m. methylprednisolone acetate (MPA). Treatment with P was started at 15 mg daily. Those on MPA received 120 mg i.m. every 3 weeks for the first 12 weeks and then monthly. Steroids were reduced according to disease activity and ESR, and patients were considered in remission and withdrawn from steroid therapy as considered appropriate by the treating physician.

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Immunofluorescence

Blood samples were collected in EDTA–sequestrane tubes and double fluorescence on a whole blood sample was undertaken using the method described previously [9]. Briefly, 100 µl of blood were pipetted into a tube containing 5 µl of each directly conjugated antibody, either anti-CD3 or anti-HLA-DR conjugated with fluorescein isothiocyanate (FITC) and with either anti-CD4 or anti-CD8 conjugated with phycoerythrin (PE) [Becton Dickinson (BD), Oxford] and incubated on ice for 20 min. Negative controls were incubated with mouse IgG–FITC and mouse IgG–PE (BD). The erythrocytes were lysed with FACS lysing solution (BD) at room temperature for 10 min. The leucocytes were washed twice in phosphate-buffered saline with 0.2% bovine serum albumin and 0.01% sodium azide (Merck, Poole), and analysed on a FACScan using Lysis 2 software (BD). Each sample was individually gated for forward and side light scatter to isolate the lymphocyte population for accurate T cell subset analysis.

IL-6 estimation

Quantikine kits (R & D Systems, Abingdon) were used for the estimation of the serum concentration of IL-6 at different time intervals during the study. Sera were separated on arrival in the laboratory, aliquoted and stored at –70°C.

Statistics

Descriptive statistics are shown either as median and quartiles or mean and s.d. Sequential data were analysed by Freidman’s ANOVA, paired Student’s t-test and Spearman rank correlation test.

RESULTS

Sequential analysis of percentage CD4 and CD8 lymphocyte subsets

Since there was no difference between the patients treated with P and MPA, the results have been pooled. Lymphocyte subsets over 24 months are shown in Table I. The low %CD8 T cells prior to treatment in PMR was confirmed in this study. However, following 3 months of steroid therapy, the %CD8 T cell population showed a small but significant increase (P = 0.004) when compared with that at presentation. This increase persisted for 12 months, but the %CD8 T cells returned to pre-treatment levels by 24 months. An increase in %CD8 cells at 12 months showed no correlation with the likelihood of the patients entering remission by 24 months, with

<table>
<thead>
<tr>
<th>Time (months)</th>
<th>No. of patients</th>
<th>ESR</th>
<th>%CD8</th>
<th>CD8 number (10⁹/l)</th>
<th>%CD8 HLA-DR</th>
<th>%CD4</th>
<th>CD4 number (10⁹/l)</th>
<th>Lymphocyte number (10⁹/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>54</td>
<td>61.5 (43–75)</td>
<td>20.8 (12–32)</td>
<td>0.3 (0.2–0.5)</td>
<td>7.9 (3.0–7.0)</td>
<td>69.3 (54–79)</td>
<td>1.06 (0.8–1.4)</td>
<td>1.9 (1.4–2.3)</td>
</tr>
<tr>
<td>3</td>
<td>52</td>
<td>14 (6–24)</td>
<td>24.2 (17–36)*</td>
<td>0.3 (0.2–0.4)</td>
<td>9.9 (4–16)</td>
<td>67.4 (55–77)</td>
<td>0.88 (0.4–1.23)</td>
<td>1.5 (1.0–2.0)</td>
</tr>
<tr>
<td>6</td>
<td>48</td>
<td>15 (7–25)</td>
<td>23.4 (16–31)*</td>
<td>0.4 (0.3–0.5)</td>
<td>6.7 (4–17)</td>
<td>70.8 (57–78)</td>
<td>1.04 (0.7–1.4)</td>
<td>1.7 (1.1–2.0)</td>
</tr>
<tr>
<td>12</td>
<td>45</td>
<td>15 (10–26)</td>
<td>23.5 (15–32)*</td>
<td>0.4 (0.3–0.5)</td>
<td>9.9 (3–15)</td>
<td>69.1 (58–75)</td>
<td>1.1 (0.6–1.4)</td>
<td>1.5 (1.0–1.9)</td>
</tr>
<tr>
<td>24</td>
<td>33</td>
<td>27 (12–36)</td>
<td>20.7 (15–35)</td>
<td>0.4 (0.2–0.6)</td>
<td>11.3 (3–27)</td>
<td>70.0 (62–78)</td>
<td>ND</td>
<td>1.5 (1.2–2.3)</td>
</tr>
<tr>
<td>Normal range</td>
<td></td>
<td>0–12</td>
<td>26–42</td>
<td>0.55–0.96</td>
<td>1–10</td>
<td>42–68</td>
<td>0.68–1.25</td>
<td>1.5–3.5</td>
</tr>
</tbody>
</table>

Change in T-lymphocyte subsets over 24 months expressed both as a percentage and as absolute cell numbers. Total lymphocyte number and ESR are shown for all patients included in the study. Results are expressed as median and quartiles.

*P < 0.004 for %CD8 T cells when compared with %CD8 T cells at presentation (time 0).

FIG. 1.—%CD8 T cells in patients at presentation and at 24 months either continuing on steroid therapy (A) or off steroid therapy (B) at 24 months.
the chance of withdrawal from steroids. No significant change in \%CD4 T lymphocytes was observed during the study. There was no significant difference between the pre-steroid \%CD8 T cell levels and those after 24 months whether the patients remained on steroids or not (Fig. 1A and B). The CD4/CD8 ratio prior to treatment was 4.2 (median; range 10.8–1.7) and after 2 yr was similar at 4.0 (median; range 10.0–2.3).

Sequential analysis of the absolute number of CD8 and CD4 lymphocytes

Although there was a significant fluctuation of \%CD8 populations during the 24 month period, this was not reflected in the absolute numbers of CD8 cells. The CD8 T cell number remained low compared with the defined normal range for this subset (0.55–0.96 x 10^9/l) (Table I). No significant change in the total lymphocyte count or in the absolute number of CD4 T cells was seen (Table I). There was no difference between those patients treated with P and MPA.

Sequential analysis of \%CD8 HLA-DR T cells

HLA-DR expression on the T cell surface is an activation marker. Two interesting observations were made. First, there was a significant negative correlation between \%CD8 T cells and \%CD8 HLA-DR cells ($r = 0.35$, $P = 0.009$). Second, there was a positive correlation between \%CD8 HLA-DR cells and ESR ($r = 0.399$, $P = 0.007$) prior to steroid therapy.

**Serum IL-6 concentration**

PMR patients showed a higher than normal serum concentration of IL-6 prior to treatment (50.3 ± 13.8 pg/ml vs 3 ± 1.0 pg/ml in controls; $P < 0.001$). There was a significant correlation between ESR and IL-6 concentration ($r = 0.537$, $P = 0.036$) only at presentation. Following steroid therapy, there was a significant fall in serum IL-6 concentrations by 3 months post-treatment (50.3 ± 13.8 pg/ml vs 8.9 ± 2.7 pg/ml; $P = 0.014$), but these then fluctuated over the subsequent 24 months despite control of clinical symptoms (Fig. 2).

**DISCUSSION**

The concept that PMR is an inflammatory vascular disease is supported by its association with giant cell arteritis (GCA), the finding of increased serum concentrations of von Willebrand factor [11] and an increased mortality rate in patients showing vascular involvement [12]. Of the many immunological investigations in PMR, decreased \%CD8 T cells [5–9] and increased IL-6 [13, 14] serum levels are two of the most consistently reported.

Interleukin-6 is markedly elevated in PMR sera and its concentration correlates with ESR [14]. The fact that IL-1β (unpublished results) and tumour necrosis factor α (TNF-α) [14] serum levels are not raised would suggest that the source of the IL-6 may be activated endothelial cells rather than macrophages/monocytes from which concomitant release of TNF-α and IL-1β would be expected. After the initial sharp fall in IL-6, which occurred following the introduction of steroids, there is a slight fluctuation of IL-6 levels during the course of the disease. At this stage, there was no correlation with the ESR. Thus, IL-6 serum levels are significantly altered by corticosteroids and cannot be used to monitor the underlying activity of PMR after the start of therapy.

The cause of the reduced CD8 T cells is unknown. There is little evidence from immunohistological studies for the accumulation of CD8 T cells at any site of inflammation which might explain their reduction in the circulation. In polymyositis, the decrease in \%CD8 cells is associated with an influx of these cells into the muscles [15]. During this long-term study, the \%CD8 T cell levels showed a slight but significant increase for 3–12 months following initial steroid therapy, but thereafter fell to their initial levels. This is usually described as being an effect of prolonged steroid treatment, although in a short-term study Pountain et al. [16] have shown that steroids can have diverse effects on lymphocyte populations within hours. The final results show that there was no significant difference between \%CD8 T cells prior to therapy or at 2 yr whether the patients remained on or had been withdrawn from therapy at this time.

Recently, Amadori et al. [17] have shown that the range [females 2.79 median (1.86–3.65 quartiles) and...
mALES 2.54 MEDIAN (1.21–3.38 quartiles, > 60 YR) of normal values for the CD4:CD8 ratio and for CD4 and CD8 absolute numbers in the circulation is much larger than previously reported, and that part of this variation may be under genetic control. Our results show a mean CD4:CD8 ratio of 4.2 (range 1.7–10.8) at presentation and 4.0 after 24 months, which is a great deal higher than the accepted ratio of 1.2 in this age group [18]. Similarly, the decreased %CD8 T cells in GCA may have a hereditary characteristic as the decrease was also seen in the non-diseased first-degree relatives [19]. It is not surprising, therefore, that low CD8 T cells cannot be used to monitor disease status. These findings collectively suggest that patients with PMR may have two genetic predispositions towards the development of their disease, namely, a low CD8 T cell count and HLA-DR4 [20, 21].

In addition, there was evidence for activation of the CD8 population. Some investigators have not found an increase in HLA-DR CD8 T cells, but this may be a technical failure as they did not look at HLA-DR expression of CD8 T cells, but rather that of all CD3-positive T cells [8]. There was a negative correlation between %CD8 T cells and %CD8 HLA-DR T cells, which suggests an association between increased activation of CD8 T cells and reduction in numbers. The cause of this activation is presently unknown. Salvarani et al. [22] have shown increased serum levels of soluble IL-2R in patients with PMR; this may be taken as evidence for immune activation. Since PMR is linked to HLA-DR4 [19], it may be speculated that activation of T cells by unknown antigens drives the endothelial inflammation [11, 12, 23].

In conclusion, the measurement of %CD8 T cells in PMR may be a useful clinical parameter at presentation when PMR patients with low %CD8 T cells can be distinguished from myalgic presentation of rheumatoid arthritis [9]. The problem of when to wean the patients off steroids remains unclear and cannot be predicted by either %CD8 T cell levels or serum IL-6 concentration.

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