

- 7 Pountain G D, Keogan M T, Brown D L, Hazleman B L. Circulating T cell subtypes in polymyalgia rheumatica and giant cell arteritis: variations in the percentage of CD8+ cells with prednisolone treatment. *Ann Rheum Dis* 1993; 52: 730-3.
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**AUTHORS' REPLY:** Our study of circulating T cell subtypes was designed to eliminate the known biological, technical and pharmacological factors which might distort the results. We did not find any difference between patients with polymyalgia rheumatica/giant cell arteritis (PMR/GCA) before the initiation of steroid therapy, and age and sex matched controls.<sup>1</sup>

Elling and colleagues take issue with our conclusion that the reported depletion of CD8 cells in patients with PMR/GCA remains to be proven. They also dismiss our concerns about the enumeration of lymphocyte subtypes after Ficoll-Hypaque separation of mononuclear cells.

Analysis of the results of Ficoll-Hypaque separation in normal individuals has shown that 13.3% of lymphocytes are lost from the interface to the bottom of the tube, and that this fraction contains an increased proportion of CD8 cells.<sup>2</sup> Direct comparison has shown that the percentage of CD8 cells measured after Ficoll-Hypaque separation may be significantly reduced compared with that after use of a whole blood technique, and this effect is more pronounced when samples are aged before processing.<sup>3</sup> It is also worth noting that the percentage of CD8 cells in control subjects in the studies cited by Elling and colleagues differs considerably, depending on the preparative technique used. After Ficoll-Hypaque separation, the median or mean values of CD8 cells in control subjects were 21%,<sup>4</sup> 24.3%,<sup>5</sup> 26.6%,<sup>6</sup> 22.1%,<sup>7</sup> and 24% in the figure in the accompanying letter. By comparison, the percentage of CD8 cells in control groups when lysed whole blood techniques were used were 34.2%<sup>8</sup> and 28%,<sup>1</sup> and median values of 35%<sup>9</sup> and 33%<sup>10</sup> have been reported in larger population studies using these techniques. Several factors may contribute to these differences; however, the consistent difference is the preparative technique used.

At present no data are available comparing the effects of different preparative techniques when lymphocyte subsets are enumerated in pathological conditions. However, it is possible that differences in activation, senescence, or functional heterogeneity may affect lymphocyte migration on a gradient. These and other biological variables would be expected to differ between control and study groups, and indeed increased numbers of activated CD8 cells in patients with active PMR/GCA have been documented.<sup>5</sup> Differences in CD8 cells recovered from the plasma-Ficoll interface and the bottom of the tube have not been examined to date, but significant phenotypic and functional differences have long been recognised between natural killer cells recovered from the interface and those which have migrated

through the gradient.<sup>11</sup> As the variables which will affect CD8 cell migration may well differ between control and study groups, we cannot accept Elling and colleagues' extrapolation of findings from the control group to the study group.

Elling and colleagues also cite two studies which used whole blood lysis and demonstrated a reduction in CD8 cells in PMR/GCA. One of these has been published only in abstract form in an unindexed journal which is unfortunately inaccessible to us. The title suggests that this deals with a distinct subset of CD8 cells rather than total CD8 cells; however, as we have not had the opportunity to evaluate this study in detail, we cannot comment further. The second study<sup>8</sup> showed a small but statistically significant reduction in CD8 cells, from  $34.2 \pm 7.7\%$  in controls to  $29.3 \pm 7.3\%$  in patients. The difference demonstrated in this study was considerably less than those reported in studies in which Ficoll-Hypaque separation was used. In addition the controls (normal blood donors) were probably considerably younger than the study group (mean age 70.2 years, range 54-85 years). As the percentage of CD8 cells tends to decrease with increasing age,<sup>10</sup> this particular factor makes this study difficult to evaluate.

We acknowledge that our subgroup analysis of patients with "severe PMR/GCA" is unconventional; however, our purpose was to examine the possibility that the absence of any CD8 depletion in our study was the result of milder disease in our study group. Even in this group, there was no difference in the percentage of CD8 cells. The reduction in CD8 cell count was small ( $0.36 \times 10^9/l$  compared with  $0.49 \times 10^9/l$  in controls) and simply reflected a slight reduction in total lymphocyte count, from a median of  $1.67 \times 10^9/l$  in controls to  $1.42 \times 10^9/l$  in patients (a change of 15%), which is comparable to the 13% reduction in total lymphocytes described previously by Elling and colleagues when untreated PMR patients were compared with controls.<sup>7</sup> We do not feel that this small change is of any biological significance, but the finding explains the slight reduction in the absolute CD8 count, in the absence of any change in T cell proportions.

In summary, we remain of the opinion that the reported depletion of CD8 cells remains to be proven. Studies after Ficoll-Hypaque separation of mononuclear cells are complicated by the possibility of differential migration of T cell subtypes in control and study groups. However, if such a difference proves to be the explanation for the discrepancy in the results of studies of T cell subtypes in PMR/GCA, characterisation of the basis for this observation may be of value.

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## Silicon nephropathy and myeloperoxidase antibodies

We read with interest the article by Sanchez-Roman and colleagues<sup>1</sup> describing a high prevalence of clinical and biological autoimmune manifestations in 50 workers after occupational exposure to silica.

In 1990<sup>2</sup> at the 3rd International Anti-Neutrophil Cytoplasmic Antibodies (ANCA) Workshop, we first described three silicotic patients with renal involvement, in a group of 28 ANCA positive patients. By contrast, no ANCA were found in seven silicotic patients without renal involvement, in one silicotic patient with lupus-like syndrome without renal abnormalities, and in another with lupus-like syndrome and focal and segmental glomerular sclerosis (FSGS). The three patients differed from those previously reported with silicon nephropathy, usually of the rapidly progressive glomerulonephritis (RPGN) type.<sup>3</sup> All three were slate workers and had a proven pulmonary silicosis. They did not fulfill the criteria for RPGN, either clinically (two had stable chronic renal failure) or histologically (no diffuse extracapillary proliferation). All had ANCAs with anti-myeloperoxidase (MPO) specificity, which are more frequent in RPGN.<sup>4</sup> Patient 1 (table) had focal and segmental hyalinosis with stable renal function over eight years. MPO-ANCA at the same titre were already present in a stored frozen serum obtained at the beginning of the renal disease. Patient 2 had FSGS with mild renal failure. MPO-ANCA were detected when he developed end stage renal failure with fatal pulmonary haemorrhage one year later. Patient 3 had a mild proteinuria with a stable advanced chronic renal failure of unknown aetiology (no biopsy).



## Authors' reply

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