MATTERS ARISING

The Cambridge dilemma

A marked depletion of the CD8 T cell subset in the peripheral blood of patients with arteritis temporalis and polymyalgia rheumatica (AT/PMR) has now been reported from several centres[1–5] and has been found in 80–90% of patients with active, untreated disease.[6] How is it, then, that the group from Addenbrooke's Hospital in Cambridge in a report on this subject in the November 1993 issue of the *Annals*[7] concluded "that the case that CD8+ cells are lowered in patients with PMR/AT is not proven"?

We would like to comment on the paper in the light of our and other recent results. In the period 1984–91 we performed temporal artery biopsy and simultaneous determination of the CD8 T cell subset in 411 patients.[8] All patients were admitted to hospital; between 08:00 and 09:00 (before the subject undertook any exercise), blood specimens were drawn and separated within 30–60 minutes with the Ficoll-Hypaque method, followed by immediate incubation with monoclonal antibodies. We have previously described our inclusion criteria, the use of age and sex matched controls and our method,[6] which includes most of the analytical and biological variables important in evaluating T cell subsets—such as storage, exercise, age, diurnal variations, and treatment with prednisone. As shown in the figure, the depletion of percentage (and number, not shown) of CD8 cells was restricted to the 148 patients with active, untreated AT/PMR, with the exception of some patients with other vasculitides. CD8 T cell depletion is thus not a common feature of other rheumatic or medical diseases. It may also be used for screening purposes (table).

Other concerns of the Cambridge group have been about the density gradient separation method, in particular, use of Ficoll-Hypaque separation of blood cells, which they believe may decrease the CD8 T cell subset selectively. This is obviously not the case, as we found normal values of CD8 T cells in controls and in a large group of patients with a variety of other medical and rheumatological diseases. When freshly obtained blood is used, as we did, exactly the same values for CD3, CD4, and CD8 are obtained with the density gradient separation method and the whole blood lysis methods.

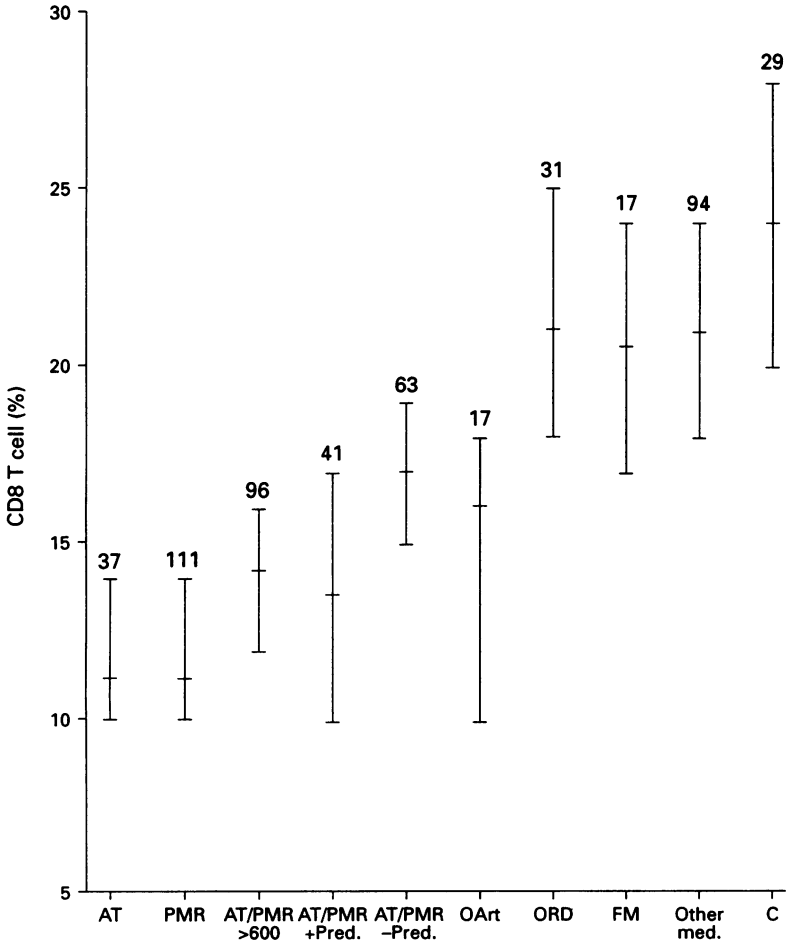
Another question posed by the Cambridge group was whether selective depletion of CD8 T cells may be caused by some intrinsic factor, specific to patients with AT/PMR, which affects the migration of lymphocytes on a density gradient used to separate the mononuclear cells from other blood cells. However, significant depletion of CD8 T cells has now been demonstrated by whole blood lysis methods also[5 9] and, although interesting, this viewpoint seems to be untenable.

Finally, the Cambridge group found a significant depletion of numbers of CD8 T cells in 13 patients with "severe" AT/PMR (a rate of 36·1% which, incidentally, has a 99% confidence interval of 17·1 to 58·8%) and concluded that the CD8 T cell depletion was caused by an overall lymphopenia in these patients. As the term "severe" AT/PMR has not been characterised by the authors or been described in the literature, we cannot respond specifically to this finding. Signs of an overall lymphopenia in patients with active, untreated AT/PMR have not been reported previously, and we have re-examined our results with reference to this point. We found a median number of lymphocytes—counted on an automated haematological counter—of $1{\cdot}7 \times 10^9$/l (CI 95% 1·6 to 1·8), which is comparable to the figures for controls (CI 95% 1·3 to 3·5) and to published data (healthy persons, age 18–70 years: 25–75% percentiles 1·6 to $2{\cdot}4 \times 10^9$/l).[10] We have found no difference between patients with AT and PMR.

On this basis we still maintain that the percentage and number of CD8 T cells has proved to be a valuable diagnostic test which, in time, may be incorporated in the diagnostic armamentarium used for this disease.

Percentage sensitivity and specificity of various combinations of erythrocyte sedimentation rate (ESR) and CD8 T cells in a group of 104 patients with a low (39%) a priori probability of AT/PMR

Test	*Sensitivity (%)*	*Specificity (%)*
Test A ESR >30 mm/h	54	46
Test B CD8% <16	61	62
Test A + B	33	80
Test A or B	82	28



Percentage of CD8 T cells in peripheral blood of patients with various medical diseases including 148 patients with untreated, active arteritis temporalis and polymyalgia rheumatica. Number of patients in each group is shown on the top of bars. Median and 95% confidence interval. AT = Arteritis temporalis; PMR = polymyalgia rheumatica; >600 = treated >600 days; +/–Pred. = with/without prednisone; OArt = other arteritides; ORD = other rheumatic diseases; FM = fibromyositis; Other med. = other medical diseases; C = controls. (Reproduced, with permission, from Olsson A T et al.[8])

PREBEN ELLING
Department of Medicine, Randers Central Hospital, 8900 Randers, DK-Denmark

HANNE ELLING
A T OLSSON
Department of Rheumatology, 8800 Viborg DK-Denmark

Correspondence to: Dr P Elling.

1 Benlarache C, Segund P, Auquir J P. Decrease of OKT8 positive T cell subset in polymyalgia rheumatica. Lack of correlation to disease activity. *Arthritis Rheum* 1983; **26:** 1472–80.
2 Chelazzi G, Broggini M. Abnormalities of peripheral blood T lymphocyte subset in polymyalgia rheumatica. *Clin Exp Rheumatol* 1984; **2:** 333–6.
3 Elling H, Elling P. Decreased level of suppressor/cytotoxic T-cells (OKT8+) in polymyalgia rheumatica and arteritis temporalis: relation to disease activity. *J Rheumatol* 1985; **12:** 306–9.
4 Dasgupta B, Duke O, Timms A M, Pitzalis C, Panayi G S. Selective depletion and activation of the CD8 + lymphocytes from peripheral blood of patients with polymyalgia rheumatica and giant cell arteritis. *Ann Rheum Dis* 1989; **48:** 307–11.
5 Macchioni P, Boiardi L, Salvarani C, *et al.* Lymphocyte subpopulations analysis in peripheral blood in polymyalgia rheumatica/giant cell arteritis. *Br J Rheumatol* 1993; **32:** 666–70.
6 Elling P, Elling H. CD8 + T lymphocyte subset in giant cell arteritis and related disorders. *J Rheumatol* 1990; **17:** 225–7.

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.
8 Olson A T, Elling P, Elling H. CD8 + T-lymfocytter. *Ugeskr Laeger* 1994; **156:** 1137–41. [Abstract in English]
9 Lamour A, Jouguan J, Mottier D, Le Goff P, Youinou P. Selective depletion of CD8 + S6F1 T-lymphocytes in giant cell arteritis. *Quatriemes Journees Bretonnes d'Autoimmunity* 1994: 174 [Abstract].
10 Hannet I, Erkeller-Yurksel F, Deneys V, Lydyard P, De Bruyere M. Lymphocyte populations as a function of age. *Immunol Today* 1992; **13:** 215–8.

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.[1]

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.[2] 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.[3] 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%,[4] 24·3%,[5] 26·6%,[6] 22·1%,[7] 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[8] and 28%,[1] and median values of 35%[9] and 33%[10] 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.[5] 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.[11] 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[8] showed a small but statistically significant reduction in CD8 cells, from 34·2 ± 7·7% in controls to 29·3 ± 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,[10] 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 ($0{\cdot}36 \times 10^9$/l compared with $0{\cdot}49 \times 10^9$/l in controls) and simply reflected a slight reduction in total lymphocyte count, from a median of $1{\cdot}67 \times 10^9$/l in controls to $1{\cdot}42 \times 10^9$/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.[7] 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.

G D POUNTAIN
B L HAZLEMAN
Rheumatology Unit, Addenbrooke's Hospital, Cambridge CB2 2QQ, United Kingdom

M T KEOGAN
D L BROWN
Department of Clinical Immunology

1 Pountain G D, Keogan M T, Brown D L, Hazleman B L. Circulating T cell subtypes in polymyalgia rheumatica and giant cell arteritis: variation in the percentage of CD8+ cells with prednisolone treatment. *Ann Rheum Dis* 1993; **52:** 730–3.
2 De Paoli P, Villalta D, Battistin S, Gasparollo A, Santini G. Selective loss of OKT8 lymphocytes on density gradient centrifugation separation of blood mononuclear cells. *J Immunol Methods* 1983; **61:** 259–60.
3 Renzi P, Ginns L C. Analysis of T cell subsets in normal adults. Comparison of whole blood lysis technique to Ficoll-Hypaque separation by flow cytometry. *J Immunol Methods* 1987; **98:** 53–6.
4 Elling P, Olsson A, Elling H. CD8+ T lymphocyte subset in giant cell arteritis and related disorders. *J Rheumatol* 1990; **17:** 225–7.
5 Dasgupta B, Duke O, Timms A M, Pitzalis C. Panayi G S. Selective depletion and activation of CD8+ lymphocytes from peripheral blood of patients with polymyalgia rheumatica and giant cell arteritis. *Ann Rheum Dis* 1989; **48:** 307–11.
6 Benlahrache C, Segond P, Auquier L, Bouvet J P. Decrease of the OKT8 positive T cell subset in polymyalgia rheumatica. *Arthritis Rheum* 1983; **26:** 1472–80.
7 Elling H, Elling P. Decreased level of suppressor/cytotoxic T cells (OKT8+) in polymyalgia rheumatica and temporal arteritis: relation to disease activity. *J Rheumatol* 1985; **12:** 306–9.
8 Macchioni P, Boiardi L, Salvarani C, *et al.* Lymphocyte subpopulations analysis in peripheral blood in polymyalgia rheumatica/giant cell arteritis. *Br J Rheumatol* 1993; **32:** 666–70.
9 Hannet I, Erkeller-Yuksel F, Lydlard P, Deneys V, DeBruyere M. Developmental and maturational changes in human blood lymphocyte subpopulations. *Immunol Today* 1992; **13:** 215–8.
10 Reichert T, DeBruyere M, Deneys V, *et al.* Lymphocyte subset reference ranges in adult caucasians. *Clin Immun Immunopathol* 1991; **60:** 190–208.
11 De Paoli P, Reitano M, Battistin S, Castiglia C, Santini G. Enumeration of human lymphocyte subsets by monoclonal antibodies and flow cytometry: a comparative study using whole blood or mononuclear cells separated by density gradient centrifugation. *J Immunol Methods* 1984; **72:** 349–53.

Silicon nephropathy and myeloperoxidase antibodies

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

In 1990[2] 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.[3] 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.[4] 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).