The Cambridge dilemma

A marked depletion of the CD8 T cell subset in the peripheral blood of patients with arthritis temporalis and polymyalgia rheumatica (AT/PMR) has now been reported from several centres and has been found in 80-90% of patients with active, untreated disease. 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 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. 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 Ficol-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, which includes most of the analytical and biological variables important in evaluating T cell subset responses 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 same 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 and, although intriguing, 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 30-1% which, incidentally, has a 95% confidence interval of 17-1 to 58-9%) 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 we 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 1.7 × 10^11 (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 2.4 × 10^11). 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 proved to be a valuable diagnostic test which, in time, may be incorporated in the diagnostic armamentarium used for this disease.

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Matters arising


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 prednisolone therapy, and age and sex matched controls.1

Ehling and colleagues take issue with our conclusion that the reported depletion of CD8+ T 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.

Although 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 dose-dependent.3

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As the variables which will affect CD8 cell migration may well differ between control and study groups, we cannot accept Ehling and colleagues' extrapolation of findings from the control group to the study group.

Ehling and colleagues also cite two studies which used whole blood lysis and demonstrated a reduction of CD8+ T 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 other suggests that this deals with a different 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 study12 showed a small but statistically significant reduction in CD8 cells, from 34±2–7%±3% in controls to 29±3–7%±3% in patients. The difference demonstrated in this study was considerably less than those reported in Ehling and colleagues 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 54–65 years). As the percentage of CD8+ T cells tends to increase with increasing age,13 this particular factor makes this study difficult to evaluate.

We acknowledge that our subgroup analysis of patients after 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 (036–10X17 compared with 0.49–10X17 in controls) and simply reflected a slight expansion in total lymphocyte count, from a median of 167–10X17 in controls to 142–10X17 in patients (a change of 15%), which is comparable to the 13% reduction in total lymphocytes described previously by Ehling and colleagues in unpretreated PMR patients were compared with controls.14

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 colleagues1 describing a high prevalence of clinical and biological autoimmune manifestations in 50 workers after occupational exposure to silica. In 1990 at the 3rd International Anti-Myeloperoxidase Cytotoxic Antibody (ANCA) Workshop, we 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 with renal involvement. We also described a 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.2 All three were slate workers and had a proven pulmonary silicosis. They did not fulfil the criteria for RPGN, either clinically (two had stable chronic renal failure) or histologically (no diffuse extracapillary proliferation). All had ANCAs with myeloperoxidase (MPO) specificity, which are more frequent in RPGN.3 Patient 1 (table) had focal and segmental hyalinosis with stable renal function over eight years. MPNCA present at the same time 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).

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