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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.

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*

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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).

## ANCA and ANA in eight of 145 (5.3%) ANCA-positive silica exposed patients

Patient	Silica status	Renal diseases	ANCA		PR-3	MPO	ANA		ENA	RF
			St.	Titre			St.	Titre		
1	Lung disease	FSH	p	200	-	+	H	200	NI	+
2	Lung disease	FSGS	c	160	-	+	-	-	SS-A	-
3	Lung disease	CRF	p	20	-	+	S	50	-	-
4	Exposure	MGN	p	320	ND	+	S	50	SS-A	-
5	Exposure	NCGN	p	640	-	+	S	100	-	-
6	Exposure	FSGS	p	160	-	+	-	-	-	-
7	Exposure	NCGN	c	100	-	+	H	50	-	-
8	Exposure	NCGN	p	320	-	+	H	1000	-	-

FSH = Focal and segmental hyalinosis; FSGS = focal and segmental glomerulosclerosis; CRF = chronic renal failure; MGN = membranous glomerulonephritis; NCGN = necrotising and crescentic glomerulonephritis; St. = staining pattern; p = perinuclear; c = cytoplasmic; PR-3 = proteinase-3; ND = not done; H = homogeneous; S = speckled; ENA = antibodies against extractable nuclear antigens; RF = rheumatoid factor; NI = not identified.

Since then, two additional patients with silicon nephropathy and MPO-ANCA have been found: one had a membranous glomerulonephritis and the other a necrotising crescentic glomerulonephritis (NCGN). For the latter (No 5 in the table), MPO-ANCA were already present in a 10 year old frozen serum sample. In addition, three other patients with renal disease (one with FSGS and two with NCGN) having MPO-ANCA were exposed to silica without documented lung disease. Indeed, the overall incidence of patients with silica exposure and renal involvement among our ANCA-positive patients is 5.5% (eight of 145). All had MPO-ANCA whatever the staining pattern; it is now well known that some MPO-ANCA may have a c-ANCA staining pattern.<sup>5</sup>

In view of our observations, we would like to know more about the renal status (haematuria, proteinuria, glomerular filtration rate) of the 32 affected individuals of Sanchez-Roman's group. It could be of great interest to screen for ANCA in this silicotic population.

Silica is well known to trigger autoimmune processes, probably by macrophage stimulation and subsequent increased interleukin-1 production.<sup>6</sup> Only a few such patients develop silicon nephropathy and this almost always presents as RPGN.<sup>3</sup>

Antinuclear antibodies (ANA) positivity in sera of such patients is well documented.<sup>7</sup> In our group all but one patient had ANA: three had antibodies against extractable nuclear antigens (two anti-SS-A and one unidentified), and only one had rheumatoid factor. No patient had anti-native DNA. Finding of ANCA may be just another marker of polyclonal activation. Silica could also have direct tissue toxic effects to explain the nephropathy.

Conversely, MPO-ANCA may be of importance in the pathogenesis of the associated disease, in association with lysosomal enzymes and release of oxygen metabolites as suggested by recent studies in an animal model.<sup>8</sup> Nevertheless two of our patients (Nos 1 and 5) had stable chronic renal failure for eight to 10 years with persistent ANCA positivity, confirming the observations of a previous report.<sup>9</sup>

In our area, which has numerous slate mines, a prospective study is in progress to determine the relevance of this new association that has also been observed in another country and was reported to the 4th International ANCA Workshop.<sup>10</sup>

A CHEVAILLER  
F CARRERE  
G RENIER  
D HUREZ

Laboratoire d'Immunopathologie, CHU 49033  
Angers Cédex 01, France

J F SUBRA  
P REBOUL  
P RIBERI  
Service de Néphrologie

C MASSON  
Service de Rhumatologie

Correspondence to: A Chevailler.

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**AUTHOR'S REPLY:** We read carefully the letter by Dr Chevailler and colleagues. Their findings are very interesting in relation to the pathology we are studying. We wish to make the following comments:

- The methods of selection of Chevailler's patients and ours were different: our selection criterion was an occupational exposure to silica, while Chevailler's subjects were selected for positivity for ANCAs.
- Renal disease was not present in any of the 50 individuals of our study: creatinine clearance and urine sediment were normal; proteinuria was absent in all of them. In addition, we have information about three

other subjects that worked at the same factory but were not included in our study. Two suffered from renal insufficiency and died before our study began; their diagnosis was an extracapillary glomerulonephritis. The third, still living, has been a dialysis patient for 14 years; her biopsy showed extracapillary glomerulopathy. A serum from 1985 and a recent one were negative for ANCAs by immunofluorescence and gave negative results in an antimyeloperoxidase enzyme linked immunosorbent assay (Ferring/Diagnostica). This patient is hyperimmunised against HLA antigens, with a negative autocrossmatch. At present, antinuclear antibodies are negative.

Our present study has now included 150 individuals, two of whom have renal disease: one has a nephrotic syndrome and has refused further studies. The other patient has SLE with a renal disease of slow evolution; her biopsy is pending. On neutrophil substrate her serum shows a perinuclear pattern at 1/10.

3) We did not study ANCAs in our first 50 patients, but it would be interesting to perform this test in our patients exposed to silica.

4) Further, we think it could be very interesting to compare the frequency of ANCAs in these patients with the frequency in specified connective tissue diseases (the antimyeloperoxidase antibodies are very rare in systemic sclerosis, but frequent in SLE).<sup>1,2</sup> In the light of Chevailler's data from patients exposed to silica, myeloperoxidase antibodies would not be markers for systemic disease but, rather, predictors or markers for renal sequelae of slow evolution.

ANTONIO NUÑEZ-ROLDAN  
JULIO SANCHEZ-ROMAN  
INGEBORG WICHMANN  
Servicio de Inmunología, Hospital Universitario Virgen del Rocío, Seville, Spain

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## Rheumatoid arthritis in black Americans

Recently, MacGregor and colleagues<sup>1</sup> have reported a low prevalence of rheumatoid arthritis (RA) in black Caribbeans compared with white patients in inner city Manchester. Although clinical features of RA were similar in both groups, the small number of subjects precluded detailed comparison. Similar studies in black Americans are scanty. López-Méndez *et al*<sup>2</sup> observed no significant clinical differences between black and white Americans with regard to frequency of extra-articular manifestations (EAM), disability, fatality rate, and functional outcome.

We undertook a cross sectional study of 47 black American outpatients with RA (American Rheumatism Association classification<sup>3</sup>) who were consecutively examined and interviewed using a questionnaire that sought information on their demographic and clinical status. The following data were recorded: formal educational level, marital status, and employment (during more than