Patients in our studies, whether single or multi-centre, had blood taken at the same time on each occasion.

In our multi-centre studies blood was collected into sequestrine and dispatched by post for 24 hours. We have checked this method and have found no difference in the absolute numbers and proportions of CD8+ T cells (paper in press). This supports already published work.

4 Although in our first study, we used 'lymphoprep' separation which is known to decrease both CD4+ and CD8+ T cells, in our subsequent work we have used a whole blood method which shows decreased CD8+ T cells in untreated patients with PMR (unpublished data).

Thus if steroid treatment, diurnal variation, storage conditions and leucocyte separation cannot account for the different results obtained by the Cambridge group and ourselves (and others),

the only difference that we can ascertain is that whereas they use the Simulset software to gate for lymphocytes, we do this manually.

The final answer must surely come from the exchange of samples and direct comparison of the two techniques.

CIRCULATING T SUBTYPES IN POLYMYALGIA RHEUMATICA AND GIANT CELL ARTERITIS: VARIATION IN THE PERCENTAGE OF CD8+ CELLS WITH PREDNISOLONE TREATMENT

We would like to respond to the paper by Pountain et al on circulating T cell subsets in PMR/GCA, in which they failed to find a decrease of CD8+ T cells in comparison to the control group.1,2

1 Our published studies have been done on patients before treatment with corticosteroids. This is also true for the studies by Elling et al,3 Dasgupta et al,1 and Chelazzi and Brogini.4

2 Patients in our studies, whether single or multi-centre, had blood taken at the same time on each occasion.

3 In our multi-centre studies blood was collected into sequestrine and dispatched by post for 24 hours. We have checked this method and have found no difference in the absolute numbers and proportions of CD8+ T cells (paper in press). This supports already published work.

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AUTHORS' REPLY: We thank Professor Panayi for his comments. There are certainly puzzling differences between studies of CD8+ cells in PMR and GCA. Corticosteroid treatment does alter T cell subsets as we have shown in healthy volunteers,1 but if initial blood samples from PMR/GCA patients have been obtained before any corticosteroid treatment, the chief sources of variation are likely to be transport and storage of specimens, mismatch of control samples, and technical methods of T cell enumeration. We cannot comment on the three unpublished studies referred to by Professor Panayi, so we confine ourselves here to discussing the published work.

1 Ekong et al2 did not find any fall in %CD8+ cells after storage of cells at a range of temperatures when using the whole blood lysis technique. When using this technique Ashmore et al3 similarly did not find any fall in %CD8+, but when using the Ficoll Hypaque method they showed a marked reduction in both %CD8+ and %CD8+ cells on blood stored for 24 hours at 4°C. Unfortunately the storage temperatures was not investigated, but it is clear from this work that the data on blood storage cannot be extrapolated from the whole blood lysis technique to the Ficoll Hypaque method.

As most of the studies before ours had used the Ficoll Hypaque method (including the Guy's study4) the data from Ekong et al does nothing to reassure us that storage conditions are unimportant. Whether specimens are transported by post the conditions must be at best unpredictable.

2 In our paper we referred to the importance of matching controls for age (which has usually been done in the published work) and for the time of day of blood sampling. Professor Panayi's letter refers to the constant timing of patient samples in the Guy's multicentre study4 but does not specify if the controls were matched for time of day. This, in addition to the storage differences between control and patient samples, could introduce variation. In following patients on corticosteroids, the T cell data cannot be interpreted unless all patients samples have been taken before or after the daily steroid dose, as the interval since the last dose affects the T cell subsets.

3 The whole blood lysis method has largely superseded the Ficoll Hypaque method for separation of mononuclear cells. Hence it is desirable that further studies in PMR/GCA be published using this method. The other possible source of variation referred to by Professor Panayi is the setting of the lymphocyte gate. Although we use the Simulset software for the subsequent analysis, the lymphocyte gate is set manually, which the Guy's group also do.

In summary, there are still several questions marking the ongoing role of CD8+ cells in PMR/GCA, therefore at present we cannot recommend CD8 enumeration as helpful in assessing this disease.

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Circulating T cell subtypes in polymyalgia rheumatica and giant cell arteritis: variation in the percentage of CD8+ cells with prednisolone treatment.

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