large joints of the lower extremity. Synovial fluid eosinophilia has been noted, but only infrequently.⁴ Although the arthritis is self-limited in most patients, it often recurs and may eventually become chronic. In contrast, patients with RA associated with bacterial infections, the majority of patients have no constitutional symptoms or other signs of filariasis. In this patient, the clinical picture was most consistent with ReA associated with filariasis. Although she did have other parasites in her stool, their association with the arthritis is mitigated against by the lack of gastrointestinal symptoms as well as the failure to respond to specific therapy.

Eosinophils are infrequently noted in the synovial fluid. When present, they typically constitute less than 2% of leucocytes.⁵ More pronounced synovial fluid eosinophilia has been reported in association with various rheumatological, infectious, allergic and malignant diseases. However, absolute synovial eosinophil counts above 10 000/mm³ are exceedingly rare, having been recorded only in the case of idiopathic transient synovitis,⁶ and two cases of Lyme disease.⁷

It might be unexpected for eosinophilia to be associated with ReA. The antigen specific T cells in the synovium of patients described to date have been predominantly of the T₄⁺ phenotype, which secretes primarily IL-2 and IFN-γ.⁸ On the other hand, eosinophilia is more commonly associated with activation of T cells of the T₃⁺ phenotype, which produce primarily IL-4 and IL-5. Because IL-5 promotes the differentiation as well as the survival of eosinophils, it may be critical to conditions associated with eosinophilia.⁹ This report demonstrates that ReA associated with filariasis may be associated with massive synovial eosinophilia. This association raises the hypothesis that in some instances, particularly those associated with ReA, ReA might be mediated by T₃⁺ cells of a T₄⁺ phenotype.

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GABAY et al. recently reported results of their study on the occurrence of antiperinuclear (APF), anti-keratin, and anti-RA33 antibodies in juvenile chronic arthritis (JCA). These data differ from ours, regarding APF and anti-RA33.¹ We reported that a third of patients with juvenile rheumatoid arthritis (JRA) were tested positive for APF in an undiluted serum, compared to only 1-6% in this study. Although several hypotheses may explain this difference, we believe that a major factor was the absence of antibodies used to define APF positivity. We have found that identifying five positive cells on a slide is sufficient to define positivity without decreasing the specificity of APF assay significantly in children with JRA. Reviewing our data, we found that using the criteria of Gabay et al. (that is, APF positivity only when 10% or more of cells are positive), 'APF positivity' was detected in only 8% of our patients. It is unclear whether this figure describes the 'true positivity' of APF in JRA. We believe that using less strict criteria would enable us to detect more APF-positive cases without lowering the specificity of the test in children with JRA.

The data by Gabay et al. and ours were also at variance regarding anti-RA33 occurrence. Although the rates were similar in patients with polyarticular disease, we found that 67% of pauciarticular JRA patients had anti-RA33, compared with only 2% in the study by Gabay et al. Again, many hypotheses may be entertained to explain this difference, such as the sensitivity of the method, differences in populations, etc. We believe it may reflect the differences in defining JCA and JRA, which is most noticeable in cases of pauciarticular-onset.¹⁰

In conclusion, these major differences in the occurrence of APF and anti-RA33 in children with chronic arthritis may be 'artificial'. It emphasises the need to form universal criteria regarding the definition of positivity of APF in children, and classification of chronic arthritis in childhood.

Author's Reply: Nesher et al suggest that the low prevalence of antiperinuclear and anti-RA 33 antibodies in our study was mainly related to the criteria used to define APF positivity and a subset of patients with pauciarticular onset juvenile chronic arthritis (JCA). Our assay for APF has already been validated in patients with rheumatoid arthritis. The prevalence of APF was in accordance with those published elsewhere, and the specificity was higher than 90%¹. We therefore think that the low prevalence of APF in our study does not reflect a lack of sensitivity of our assay. In addition, other investigators who did not use our methodology as ours, also found a very low prevalence of APF in a large cohort of JCA patients.² Finally, it should also be mentioned that the percentage of APF positive sera found by Nesher et al.¹ was not so impressive, as it only one third of their patients had a positive result with their assay. In addition, the occurrence of positive APF falls to 11% when sera were diluted 1:10.

The specificity of APF for the diagnosis of RA is well accepted; however, positive results have also been reported in other conditions.³ We and others⁴ have demonstrated that considering a positive result when 10% or more of the cells are positive increases the specificity without significantly decreasing the sensitivity of the test. Nesher et al compared their results in JCA with those found in sera from normal controls and found good specificity. They also reported positive results in up to 17% of the children with systemic lupus erythematosus (SLE). In our opinion their results in JCA should also be compared to those found in the patients with SLE to assess the specificity of their assay.

With the exception of a subset of patients with RF-positive polyarticular onset, we found that prevalence of anti-RA 33 antibodies was rather low in JCA patients. Again, we already validated our assay in previous studies.⁵ Nesher et al suggest that the low percentage of anti-RA 33 antibodies in the sera from our children with pauciarticular onset reflects the pauciarticular presentation of JCA in defining this subset of patients. We do not agree with them, because the criteria for pauciarticular onset JCA in the ACR or EULAR/WHO classification are almost the same. This subset of patients includes a group of young children—mainly girls—with four or less joints affected at onset, a high prevalence of positive antinuclear antibody (ANA) test and ocular complications. As described in our paper, the age of onset, articular features, sex ratio, and percentage of positive ANA test clearly show that our children fulfil both the European and American criteria for the classification of pauciarticular onset JCA (or JRA). The evolution of articular features may, however, vary in this subgroup of patients. Some have persistent pauciarticular involvement, whereas others convert to polyarticular disease. The high percentage of anti-RA 33 antibodies reported by Wilson et al.¹ could be related to the heterogeneity of this subset of JCA and/or to the few cases included in their study (12 patients).

Although anti-RA 33 antibodies have previously been reported to be highly specific for the diagnosis of RA, we also found this antibody in sera from our children with other conditions, such as mixed connective tissue disease and SLE.¹ Unfortunately, Wilson et al. could not answer the question of whether anti-RA 33 antibodies can be considered as a disease specific marker.
Cirulating T cell 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 and GCA, in which they failed to find a decrease of CD8+ T cells in correlation to our study.1,2

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 Broggi.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 sequestrene and dispatched by post for Ficoll 24 hours. We have checked this method and have found no difference in the absolute numbers or proportions of CD8+ T cells (paper in press). This supports already published work.5

4 Although in our first study,1 we used 'lymphoprep' separation which is known to decrease both CD8+ 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, what does it indicate? We believe it is the method of enumeration of T cell subsets. 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.

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AUTHORS’ REPLY: We thank Professor Panayi for his comments. There are certainly puzzling differences between studies of CD8+ cells and CD8− cells in PMR/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, due to the technical temperature 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 al5 does nothing to reassure us that storage conditions are unimportant. When specimens are transported by post the conditions must be at best unpredictable.

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 study6 but does not specify if the control samples 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 we know whether samples have been taken before or after the daily steroid dose, as the interval since the last dose affects the T cell subsets.

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 question marks regarding the 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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