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 to ReA 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 militated 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 leukocytes.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 reported to date in one case of idiopathic transient synovitis,⁶ and two cases of Lyme disease.⁵

It might be unexpected for eosinophilia to be associated with ReA. The antigen specific T cells in the synovium of ReA patients described to date have been predominantly of the T_H1 phenotype, which secrete primarily IL-2 and IFN- γ .² On the other hand, eosinophilia is more commonly associated with activation of T cells of the T_H2 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.⁷

This report demonstrates that ReA associated with filariasis may be associated with massive synovial eosinophilia. This association raises the hypothesis that in some instances, in particular those associated with eosinophilia, ReA might be mediated by T cells of a T_H2 phenotype.

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MATTERS ARISING

Antiperinuclear and anti-RA33 antibodies in juvenile chronic arthritis

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).¹ These data differ from ours, regarding APF and anti-RA33.^{2,3} 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 difference in criteria 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 assay, 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.⁴

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 arthritides in childhood.

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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 the same 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*³ was not so impressive, as 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.⁴ We and others⁵ have demonstrated that considering a positive result when 10% or more of the cells show typical staining 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 a 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.⁶ Nesher *et al* suggest that the low percentage of anti-RA 33 antibodies in the sera from our children with pauciarticular onset reflects the differences 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 classifications 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 higher prevalence of anti-RA 33 antibodies reported by Wilson *et al*⁷ 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 high percentages in other conditions, such as mixed connective tissue disease and SLE.⁸ Unfortunately, Wilson *et*

*al*⁷ did not show the results of their assay in patients with other rheumatic diseases. However, the fact that 2/5 of their control sera were also positive indicates that their assay is not specific. Finally, since ANA are found in a very large proportion of pauci-articular onset JCA patients (88% in our study), we do not think that the presence of anti-RA 33 antibodies in this particular subset of patients could add any useful information. In addition, anti-RA 33 antibody assay is clearly longer to perform and less widely available than ANA test.

In conclusion, we think that there is not enough evidence to support a wider use of APF and anti-RA 33 antibody assay in JCA.

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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 analysis within 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 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, what does? 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 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 al*² 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 al*³ similarly did not find any fall in %CD8, but when using the Ficoll Hypaque method they showed a marked reduction in both %CD4+ and %CD8+ cells on blood stored for 24 hours at 4°C. Unfortunately, storage at higher 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. When 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 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.¹

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 question marks hanging over 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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Circulating 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 contrast to our work.^{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 Broggin.⁴