SIR, Dr Geczy suggests that differences in the details of the techniques used can account for our failure to find any clear evidence of cytotoxic activity of anti-klebsiella sera against B27⁺ AS⁺ lymphocytes. It is suggested in particular that our procedure for the cytotoxicity test is unconventional; in fact it has been in common use for at least 20 years.^{1 2}

Dealing with two minor points raised, firstly, saline was added, as is common practice, at the end of the assay merely to stop the reaction and to facilitate transfer of the radioactive supernate for measurement in the gammacounter. Secondly, the antilymphocytic serum used was obtained by bleeding a NZ white rabbit three weeks after a single intravenous injection of pooled human lymphocytes. It was only 'active' in the absence of complement up to a dilution of 1:4 with a ⁵¹Cr release assay, and thus the cytotoxicity of this serum when used in high dilution was a check on the activity of the complement.

In regard to the major point as to whether simultaneous or sequential addition of complement is important, we did in fact carry out experiments following his method in all respects and using one of the antisera supplied by him and two local anti-K43 sera, and this made no difference to the results obtained. Moreover, when the sequential method as described by Geczy was used in a microcytotoxicity assay negative results were also obtained. Conversely, when our method was used in the case of anti-B27 alloantisera high levels of cytotoxicity were obtained comparable with those described by Geczy and his colleagues. Workers in other laboratories (Kinsella (personal communication), Georgopoulos *et al*,³ Struthers⁴) have also failed to obtain consistent results similar to those of the Sydney group in spite of the fact that they have used exactly his technique for the cytotoxicity assay.

We find it difficult to understand why, if the difference between success and failure in attempts to reproduce the results of Geczy and his coworkers is dependent on simple procedural differences as suggested by Geczy, no other laboratory has as yet reported a body of data similar to that of the Sydney workers six years after their first report.⁵ It would be very interesting if, with the same antisera and target cells, it could be shown that the differences between the techniques are critical in determining the outcome of the cytotoxicity assay.

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Serology and bacteriology in reactive arthritis

SIR, Valtonen *et al* recently gave a valuable account of 'triggering infections in reactive arthritis' (ReA).¹ In the context of this paper we have over the past two years encountered four cases of ReA, which were attributed to *Campylobacter jejuni*. The interest of these cases was that, despite positive stool cultures, the four acute phase sera were all negative for *C jejuni*, but titres were exceptionally high for *Yersinia enterocolitica* 3 in three instances and for *Salmonella typhimurium* in one. An example of the antibody response found in one patient is given in Fig. 1. As raised serum titres to *C jejuni* are normally detectable during enteritis³ it may be that the above cases of ReA were not due to *C jejuni*, but to *Y enterocolitica* or *S typhimurium* or other enterobacteria (partially) cross reactive with these. Alternatively, it could be that not all

Fig. 1 Antibodies to *Salmonella typhimurium* and not *Campylobacter jejuni* in a patient thought to have *C jejuni* reactive arthritis. Three other patients displayed reactivities comparable with *Yersinia enterocolitica* 3. Symbols A = *Y enterocolitica* 3; B = *Y enterocolitica* 9; C = *S typhimurium*; D = *Shigella flexneri* 2a; E = *Klebsiella pneumoniae* K43; F = *C jejuni*. The gel containing the above bacteria was incubated in acute phase serum as described previously.²

Table 1 Number of positive* serological tests to 11 enterobacteria in acute phase sera of patients with idiopathic reactive arthritis

N	YE3	YE9	KP21	KP43	KA	ST	SP	SM	CJ	SF	SS
72	13	9	4	19	28	20	21	13	4	8	16

*IgM, IgG, or IgA titres at least twice that of controls (n=35).

YE=*Yersinia enterocolitica*; KP=*Klebsiella pneumoniae*; KA=*Klebsiella aerogenes*; ST=*Salmonella typhimurium* (group B); SP=*Salmonella panama* (group D); SM=*Salmonella montevideo* (group C); CJ=*Campylobacter jejuni*; SF=*Shigella flexneri*; SS=*Shigella sonnei*.

Serum titres were determined by ELISA to whole bacteria according to standard procedures as described elsewhere.⁴

enteritis patients show a serological response to certain infectants and that one is therefore detecting persistent titres to previous infections. Also, the number of cases studied hitherto is too small to determine the frequency of the above phenomenon. Nevertheless, the point we wish to make is that these cases emphasise the need for combined serological and bacteriological analyses in order that a confident identification of the 'triggering infection' in ReA (and enteritis) may be made.

In this connection we determined IgM, IgG, and IgA serum titres to 11 enterobacteria in 72 patients with idiopathic ReA. The results are shown in Table 1. Statistically raised titres to at least one of the bacteria in the panel were found in 53 cases (74%), i.e., IgM, IgG, or IgA enzyme linked immunosorbent assay (ELISA) titres were much higher (two to eight, four to 10, or four to 18 times respectively) than the average value of titres for 35 controls (hospital patients without known arthropathies or enteritis). Like Valtonen *et al*¹ we too found that serum titres to *Y enterocolitica* are often raised. Also, we found increased titres to *Salmonella* in several cases, thus indicating that this might also be an important organism in ReA. It is interesting that raised titres to *Klebsiella* were often encountered; this genus is possibly involved in the aetiology of ankylosing spondylitis.⁵ Antibodies to *Chlamydia trachomatis* were not determined; these are likely to predominate in the patients not displaying positive serologies to the panel given in Table 1 (25%), as implied by the findings of Valtonen *et al*.¹

The above results represent a serological impression relating to the involvement of enterobacteria in ReA. They suggest that stool cultures should be complemented by serological studies. Perhaps routine testing could be refined by using certain selected antigens and guarding against cross reactions among the various species. These cross reactions are likely to account for the positive serologies to *Shigella sonnei*, as the latter is thought to be a non-arthritisgenic species⁶ and perhaps also those to *Klebsiella*. It was interesting that one patient displayed high titres to the cell envelope antigens of an enteropathogenic *Escherichia coli* strain (0127), detected by the same method as shown in Fig. 1 and to none of the other enterobacteria noted in this letter. This indicates that a

range of enterobacteria, possibly exceeding those incriminated hitherto, might be involved in ReA.

Finally, even if the clinical picture of ReA is not influenced by the identity of the triggering agent we agree with Valtonen *et al*¹ that prophylactic measures can only be undertaken after the mode of pathogenesis of ReA is understood. This in turn will only be possible when a correct inventory is available of triggering infectants, clinical pictures, and immunogenetic constitutions of the patients.⁴ To achieve this one should combine clinical, serological, and bacteriological observations.

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