In summary, we agree with the findings of Font et al that significant renal pathology in lupus patients without clinical renal abnormalities is rare, but we do not advocate renal biopsy in this group of patients.

Department of Internal Medicine, J R O'Dell
Section of Rheumatology and Immunology,
University of Nebraska Medical Centre,
Omaha, NE 68105, USA

References

Ankylosing spondylitis, HLA-B27, and klebsiella

Sir, With particular reference to our studies1 2 and those of Ebringer and his associates,3 4 the article by Singh et al (p. 190, this issue), attempts to address the controversial issue of cross reactivity between certain strains of klebsiella and the lymphocytes of HLA-B27 positive patients with ankylosing spondylitis (B27'AS'). Controversy is a healthy byproduct of scientific investigation, and it is essential that our findings and those of other workers in the field be subjected to the closest scrutiny and evaluation. Those entering this debate, however, should do so with a sense of responsibility; presentation of data which are based on questionable or inadequately controlled techniques must be considered scientifically misleading. I refer specifically to the 51Cr release technique used by Singh et al that attempts to reproduce the specific cytotoxicity of anti-klebsiella sera for B27'AS' cells. There are several important differences between their technique and ours, and their departure from our published methods passes without comment or justification. Firstly, the 51Cr labelled cells are incubated simultaneously with antiserum and diluted complement for one hour at 37°C. The more conventional method and the one favoured by our group is to incubate the radiolabelled cells with antiserum for 30 min at room temperature (20-24°C) before adding neat complement for a further 60 min at the same temperature. Secondly, Singh et al complete their assay by adding to each tube 1-5 ml of cold 0-9% NaCl. The significance of this cold saline step is not entirely clear. We have recently tested five B27'AS' cells (which were positive with our 51Cr release technique) according to the Singh method and we were unable to demonstrate cross reactivity between klebsiella K43 BTS1 and other enteric bacteria and the patients' lymphocytes. Furthermore, the failure of the Singh method in our hands is due largely to the simultaneous, rather than sequential, addition of antiserum and complement. Singh et al justify their choice of this unconventional cytotoxicity technique by pointing out that . . . 'high levels of cytotoxicity were obtained against the lymphocytes in control tests with antilymphocytic serum'. However, most antilymphocytic sera are active in the absence of complement and since details on the production and source of this reagent are not given in the paper one assumes that the success of their assay technique rests largely on the activity of an alloantiserum, anti-HLA-B27.

We have been concerned by the non-confirmatory reports of this work and in an attempt to identify some of the factors which might contribute to the failure of others to confirm our findings we have recently completed a double blind trial involving B27'AS' cells from a New Zealand population.3 This successful study, together with two previous confirmatory reports (in preparation),6 suggests that the phenomenon of 'cross reactivity' between enteric bacteria and B27'AS' cells is not simply an Antipodean curiosity. Although many aspects of this controversy remain to be resolved, it is worth noting that of the non-confirmatory reports only Beaulieu et al have adhered to our published methods, while other workers found it necessary to modify one or more parameters of the 51Cr release assay.

Blood Transfusion Centre, A F Ge czy
Australian Red Cross Society,
153 Clarence Street,
Sydney,
NSW 2000,
Australia

References
Correspondence

Sir, Dr Gecey 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 Gecey 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 Gecey and his colleagues. Workers in other laboratories (Kinsella personal communication), Georgopoulos et al,3 Struthers et al,3 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 Gecey and his coworkers is dependent on simple procedural differences as suggested by Gecey, no other laboratory has as yet reported a body of data similar to that of the Sydney workers six years after their first report.3 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.

University Dept of Medicine, B SINGH
Royal Liverpool Hospital, J D MILTON
PO Box 147, J C WOODROW
Liverpool L69 3BX

References

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 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

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