**Correspondence**

Rabbit antisera to cross-reactive organisms—where's the problem?

SIR, The ability of rabbit antisera to certain enteric bacteria to recognise an HLA-B27 associated marker on the cells of the majority of HLA-B27 positive patients with ankylosing spondylitis (B27+AS+) has been extensively documented by our group during the past six years, but despite two confirmatory reports (one in preparation) the phenomenon remains controversial. The reasons for the inability of several other groups to confirm our observations have not yet been fully identified, though factors such as the use of ill-defined bacterial strains and failure to adhere to published experimental protocol may explain many of the 'negative' reports. Another explanation for our success, advanced privately by some investigators, is that the colony of rabbits used for the production of antisera is somehow unique and that we have been fortunate in eliciting antibodies to 'cross-reactive' bacterial antigens, i.e., antigens, antibodies to which lyse B27+AS+ cells. We feel this explanation is unlikely as we have successfully raised 'cross-reactive' antisera not only in 29 outbred rabbits from several different colonies but also in nine guinea pigs and 10 BALB/c mice (Table 1). Antisera have been successfully raised against (a) formalin-killed whole bacteria and sonicated bacteria (administered with either complete or incomplete Freund's adjuvant); (b) bacterial culture filtrates (unfractionated or partially purified); and (c) culture supernatant from B27+AS+ Epstein-Barr virus-transformed lymphoblastoid cell lines (Sullivan and Geczy, unpublished observations). In our initial studies the first inoculum was given intravenously (10⁶ to 5×10⁷ organisms) and subsequent injections were either subcutaneous or intramuscular, or both (10⁶ to 10⁹ organisms). More recently we have abandoned the intravenous route and all immunisations are now given subcutaneously or intramuscularly, or both (10⁶ to 10⁹ organisms). With the exception of one rabbit which died 24 hours after an intravenous injection, all animals survived for at least six months, despite regular boosts at about monthly intervals, and all immunisation schemes were equally effective in eliciting specific 'cross-reactive' antibodies. These antibodies, in the presence of prescreened complement from a number of different commercial sources (e.g., Pel-Freeze, Behring Institut), reacted specifically with B27+AS+ cells. All antisera were frozen in aliquots of 2-3 ml and thawed once only.

During the past seven years over 50 rabbits have been immunised with either 'cross-reactive' or 'non-cross-reactive' bacteria and only three animals have been encountered whose naturally occurring serum cytotoxins gave unacceptable cytotoxicity values (> 10% ³⁵Cr release) when tested on a panel of human lymphocytes.

<table>
<thead>
<tr>
<th>Animals</th>
<th>No of animals in which antisera could be raised</th>
<th>No of animals tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbis</td>
<td>29</td>
<td>33*</td>
</tr>
<tr>
<td>Guinea pigs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outbred</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Inbred strain 13</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Mice (BALB/c)</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

*One rabbit died shortly after an intravenous injection of formalin-killed bacteria, while the serum of three animals had high (>10% ³⁵Cr release) levels of naturally occurring cytotoxins.

These three animals were, of course, not used for the production of antibacterial sera.

Our ability to raise antisera in a number of different laboratory rodents by a variety of immunisation schemes and antigenic preparations is quite imposing, but it leaves unexplained why other laboratories have not met with more success.

This work was supported in part by grants from the National Health and Medical Research Council of Australia and from the Philip J Benjamin Memorial Research Grant, New South Wales, Australia.

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References

Sclerodactyly, CREST syndrome, proximal scleroderma

Sir, In their interesting paper Furst et al. define CREST as patients with sclerodactyly and PSS as those with 'proximal scleroderma'.

It is surprising that the authors found differences in the internal organs of the two subsets only in the case of lung involvement. It is reasonably well known that patients with limited skin sclerosis (so-called CREST syndrome) have a less severe prognosis compared with patients with 'diffuse scleroderma'. One possible explanation of this unexpected finding could be that the PSS subset with proximal scleroderma constitutes too broad a group of patients, including also cases in whom skin sclerosis is confined to a few areas of the skin (face, neck). Such cases could be closer to sclerodactyly than to diffuse scleroderma.

Furthermore, the clinical findings in Table 1 of the paper by Furst et al. show that not all cases of CREST syndrome presented all five features of CREST, and that other patients with proximal scleroderma also had the features of CREST. What in effect distinguishes the two groups is that the so-called CREST group presents skin sclerosis confined to the fingers, whereas the other group has skin sclerosis also in other areas.

This being so, would it not be better to speak of patients with sclerodactyly rather than CREST?

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References

Sir, Professor Giordano's letter points out a source of misunderstanding that has existed for some time in the rheumatic disease literature. For some groups, the diagnosis of CREST has separated patients specifically from those with PSS. In most cases, however, patients with criteria for CREST syndrome can also have PSS. Thus the presence of CREST criteria per se, does not separate patients in terms of prognosis.

Those few patients who have CREST alone (without criteria for PSS) may, in fact, have a different prognosis than those with the 'usual' diagnosis of CREST syndrome. However, the patients with CREST alone are relatively infrequent. As stated in our paper, of over 150 prospectively followed-up patients with systemic sclerosis, we only found five CREST patients who did not meet criteria for PSS. Thus all of our CREST patients had skin involvement confined to the fingers, and we referred specifically to that difference in our paper. On the other hand, we had five CREST patients who did not have PSS by any criteria, and they did not appear different in any way from the 12 CREST patients who met the minor criteria for PSS (also as mentioned in our paper).

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Anti-Ro positive rheumatoid arthritis

Sir, The high incidence of side effects in patients with anti-Ro positive rheumatoid arthritis treated with d-penicillamine noted by Moutsopoulos will be of interest to rheumatologists. A 61% incidence of side effects compared with only 8-5% in the anti-Ro negative group would question the use of d-penicillamine in anti-Ro positive patients.

Our own observations on a small group of patients with rheumatoid arthritis treated with d-penicillamine have not confirmed this striking difference. A group of 17 rheumatoid arthritis patients, most of whom had developed serious side effects, was studied retrospectively. The results are shown in Table 1. Of the 13 who developed side effects, four had anti-Ro antibodies. A further nine patients had their anti-Ro status determined before commencing d-penicillamine. Two of these had developed side effects, and both were anti-Ro negative. The two patients who were anti-Ro positive had not developed side effects after more than six-months' treatment.

Of the 15 patients with significant side effects, only four...