reduced diffusion capacity had more often higher values of DNA antibody binding capacity, antinuclear antibodies, and rheumatoid factor, but these differences were not significant. Similar results are reported by Turner-Warwick.⁷ Immune complex deposition in lung capillaries, detected with immunofluorescence methods, are described in only a few cases.⁸⁻¹⁰ Despite this fact it can be suggested that immunological reaction, for example the deposition of immune complexes, is one of the initial steps in the development of pulmonary manifestations.

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HLA B27, *Yersinia enterocolitica*, and ankylosing spondylitis

Sir,

Klebsiella aerogenes and *Yersinia enterocolitica* are two organisms commonly mentioned as having a possible role in the pathogenesis of ankylosing spondylitis,^{1,2} which is associated with the histocompatibility antigen HLA B27.^{3,4} It has been suggested that a possible mechanism of pathogenesis is the inability of the host to distinguish the organisms from 'self' because of its antigenic resemblance to HLA B27 itself,⁵ to a cell-membrane structure associated with B27, or to genetically modified HLA B27,^{6,7} and so is unable to react adequately against them. Evidence for antigenic cross-

reactivity with HLA B27 already exists in the case of *K. aerogenes*. Ebringer *et al.*⁸ showed cross-reactions between B27-positive (B27+) lymphocytes and *K. aerogenes* by means of haemagglutination and gel-diffusion tests, and other workers have demonstrated cross-reactions by observation of diminished T-cell proliferative responses to *K. aerogenes* in the lymphocytes of B27+ AS+ individuals as compared with those from B27+ AS- and B27- individuals.⁹ Lymphocytotoxic activity in klebsiella antiserum to B27+ AS+ cells, again as compared with B27+ AS- and B27- ones^{6,9} has also been shown. However, there is less evidence for a cross-reaction with *Y. enterocolitica*. Welsh *et al.*¹⁰ reported a cross-reaction between serum from rabbits immunised with B27+ lymphocytes and *Y. enterocolitica* serotype 3 in immunodiffusion experiments, but, whereas Nikbin *et al.*¹¹ originally found a diminished T-cell response to *Y. enterocolitica* in vitro, they were subsequently unable to confirm their findings,¹² and Seager *et al.*⁶ indicated that in preliminary experiments antiserum to *Y. enterocolitica* (American Type Collection no. 10598, serotype unspecified) failed to lyse lymphocytes from AS+ B27+ as well as those from normal individuals.

In view of these varied findings we thought it would be worthwhile to acquaint readers with the results of our own experiments on the possible antigenic relationship between *Y. enterocolitica* and HLA B27. We raised antisera in rabbits to 3 strains of *Y. enterocolitica* (serotypes 3 and 9, and NCTC 10460) each grown at 37°C and at room temperature using techniques as described previously¹³ and tested each for lymphocytotoxic activity using the Standard National Institute of Health complement dependent microlymphocytotoxic test.¹⁴ In summary rabbit antisera were first screened against a panel of 10 normal human peripheral lymphocyte samples to test for 'nonspecific' or antispecies activity. They were then tested against lymphocytes from healthy B27+ and B27- donors and also from B27+ patients with ankylosing spondylitis and acute anterior uveitis. The cells from these B27+ and B27- individuals, which were first of all selected after routine HLA typing using up to 6 different anti-B27 sera, were tested against 2 B27 antisera, one from a commercial source (Biotest) and the other a local pregnancy antibody (Chantrell), before and after absorption. All tests were carried out with neat serum and 1:1 dilutions in PBS. For absorption of the B27 antisera with bacterial antigens bacteria were scraped off a 24 hours 37°C agar (Columbia Oxoid Blood Agar Base) culture suspended in 10 ml of saline and heated at 60°C for 30 minutes or steamed for 1 hour. They were then washed 3 times in saline. 20 µl of packed cells were mixed with 20 µl of B27 antiserum and left for 24 hours at 4°C.

No cytotoxic activity against lymphocytes of B27+ AS+ B27+ AS- or B27- individuals was shown by any of the *Y. enterocolitica* antisera, nor was the activity (100% kills with B27+ cells) of the B27 antisera absorbed out by any of the yersinia antigens.

We also used haemagglutination tests. For these, bacteria from a 24 hours 37°C agar culture were suspended in 10 ml PBS and autoclaved at 15 lb (6.8 kg) for 1 hour. After centrifugation the supernatant was

Table 1 Haemagglutinating and lymphocytotoxic activity of antibodies to *Y. enterocolitica* and HLA B27 against yersinia antigens and B27+ lymphocytes

Antiserum	Absorption material	Haemagglutination					Lymphocytotoxic activity B27+ AS+
		3	9	Antigens 10460	CF	UnCO	
<i>Y. enterocolitica</i>							
3	0	1280	40	2560	2560	0	0
9	0	40	640	80	80	0	0
10460	0	640	40	1280	640	0	0
CF	0	640	40	256	320	0	0
B27							
Biotest	0	40	40	40	10	0	+
	3	0	0	0	0	0	+
	9	0	0	0	0	0	+
Chantrell	0	40	80	20	20	0	+
	3	0	0	0	ND	0	+
	9	0	0	0	ND	0	+
Normal							
1	0	80	10	40	80	0	0
2	0	80	40	40	40	0	0
3	0	20	10	20	10	0	0
4	0	40	20	20	10	0	0

removed to use as the antigen in the test. Group O human erythrocytes were washed 3 times in PBS and then made up to a 10% suspension. For sensitisation equal volumes of erythrocyte suspension and antigen were mixed and incubated at 37°C for 2 hours. These erythrocytes were then washed 3 times with PBS solution and for use in the test made up to a 1% suspension. Then, starting with 1:10 dilution of serum, doubling dilutions of this serum were made leaving 10 µl in each well of microhaemagglutination tray. 10 µl of sensitised erythrocyte suspension was added to each dilution and the mixtures were incubated at 37°C for 2 hours in a humidified incubator. Absorption with antigen-coated erythrocytes was carried out by mixing 20 µl of these packed with 20 µl of serum and leaving these at 4°C overnight.

The results of these tests are shown in Table 1. There was a positive reaction between the B27 antisera and all 3 strains of yersinia. However, a similar cross-reaction occurred with non-B27 sera, and absorption with antigen-coated cells, while absorbing out haemagglutinating activity from B27, failed to remove lymphocytotoxic activity to B27+ lymphocytes. Thus it appears that the haemagglutinating activity of B27 antiserum for *Y. enterocolitica*-coated cells is not specific for B27 but is an anti-yersinia factor occurring in a range of human sera including the 'specific' B27 antisera used in these experiments.

The possibility still remains that there exists some hitherto undefined specific antigen factor other than the lymphocytotoxic factor common to *Y. enterocolitica* and HLA B27, but it is evident that more work needs to be done before such a connection can be regarded as proved.

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