

## Tissue typing in brucellosis

P T DAWES\*<sup>1</sup> AND SALIL K GHOSH<sup>2</sup>

From the <sup>1</sup>Department of Rheumatology, and the <sup>2</sup>Infectious Diseases Unit, Hemlington Hospital, Middlesbrough

**SUMMARY** The index case in this report was clinically, epidemiologically, and serologically proved to be suffering from acute brucellosis acquired in Britain and complicated by acute intractable reactive arthritis. HLA tissue typing was A1, A2, B7, and B27. As there is no information available about HLA typing in brucellosis in Britain, we examined further cases of human brucellosis retrospectively. Three further patients possessing B27 antigen were identified, only one of whom had had reactive arthritis. Eight of the 12 cases studied also carried A2, which is thus shown not to be protective against the development of brucellosis as had been suggested previously.

**Key words:** *Brucella abortus*, reactive arthritis, HLA antigens.

Reactive arthritis is associated with infection by various micro-organisms including salmonella, *Shigella flexneri*, yersinia, and campylobacter. An increased incidence of reactive arthritis in patients possessing HLA-B27 has been established.<sup>1</sup> In a report from Hungary Hodinka *et al.*<sup>2</sup> indicated a significantly increased frequency of spondylarthritis in chronic brucellosis associated with HLA-B27. However, a Peruvian study<sup>3</sup> did not confirm this finding; it did, however, note a significantly lower incidence of A2 in brucellosis. We report a case of acute brucellosis complicated by reactive polyarthritis in which tissue antigens included HLA-B27 and A2.

### Case report

A 24-year-old Caucasian male who had never been abroad was admitted in September 1980 to the Infectious Diseases Unit at Middlesbrough with a history of fever, sweating, and diarrhoea. He also complained of a painful right ankle, left little toe, and right wrist and of backache. He was an abattoir worker involved in slaughtering brucella infected cattle, the last exposure being two months before admission. He had received no antimicrobial treatment.

On examination no abnormality was detected

except for painful restriction of the symptomatic joints and also the left hip. Forty-eight hours after admission he developed synovitis of the left ankle and the fifth left metatarsophalangeal joint.

The following investigations were normal: haemoglobin, white cell count, and differential, plasma electrolytes, uric acid, urea, creatinine, bilirubin, calcium, inorganic phosphate, aspartate aminotransferase,  $\gamma$ -glutamyltransferase, and immunoglobulins (IgA, IgE, IgG, and IgM). Antinuclear factor, antismooth muscle antibody, anti-mitochondrial antibody, and Rose-Waaler were negative, and anti-DNA binding activity was normal. The erythrocyte sedimentation rate was slightly raised at 23 mm in the first hour (Westergren), and the globulin concentration was 37 g/l (normal 20-35 g/l). C3 was normal, but C4 was raised at 0.51 g/l (normal 0.10-0.35 g/l). Cultures of blood, urine, stool, and throat swab grew no bacterial pathogen, and no virus was isolated from the throat swab. Plain radiographs of the chest, left foot, and sacroiliac joints were normal. The tissue type was HLA-A1, A2, B7, and B27.

*Brucella* antibody titres on admission were 1/640 (agglutination) and 1/40 (complement fixation). Both were reduced to less than 1/20 nine months later.

The patient was treated with a standard regimen of 1 g streptomycin daily for one month, oral tetracycline 500 mg daily for six weeks, and with various non-steroidal anti-inflammatory drugs, as he continued to have fleeting arthralgia until July 1983.

Accepted for publication 4 March 1985.

Correspondence to Dr S K Ghosh, Hemlington Hospital, Hemlington, Middlesbrough TS8 9DS.

\*Present address: Staffordshire Rheumatology Centre, Stoke-on-Trent.

### Further studies

As no published British study of HLA antigens in brucellosis cases could be found, we decided to extend the study. The number of human brucellosis cases in Britain is falling, particularly those due to indigenously acquired infection, and the study was therefore carried out retrospectively. Four more cases of brucellosis were identified from the register of the Infectious Diseases Unit. All had suffered from acute brucellosis on epidemiological, clinical, and serological grounds. All had at least a fourfold alteration in antibody titres. *Brucella* culture was not specifically attempted in any of the cases. Seven further cases were traced from the Northern Regional Medical Statistics Department and the case notes so obtained were studied with permission of the medical practitioners in charge of each patient.

Heparinised blood samples from each patient were sent to the National Blood Transfusion Centre, Newcastle-upon-Tyne for HLA typing. The blood samples were treated with carbonyl iron and dextran. The resultant supernatant was layered over Ficoll-Isopaque solution, centrifuged, and the lymphocytes removed from the interface were washed into trometamol (TRIS)-buffered saline. A lymphocytotoxic test was then performed on each sample with a modification of the original technique suggested by Mittal and described in detail by Dewar.<sup>4</sup>

### Results

Details of the HLA tissue antigens of the 12 patients studied are given in Table 1. Two cases (16.7%) had reactive arthritis complicating acute brucellosis and were positive for B27. Two other patients also carried B27 but did not have this complication. Overall, four out of 12 patients (33%) were found to carry B27 and eight patients (67%) A2.

Table 1 *HLA tissue typing of 12 brucellosis cases*

Patient No	Diagnosis	HLA tissue type
1 (index case)	Acute brucellosis + reactive arthritis	A1, A2, B7, B27
2	Acute brucellosis + reactive arthritis	A2, B18, B27
3	Chronic brucellosis	A2, A3, Bw44, B5
4	Acute brucellosis	A1, B15, B40
5	Chronic brucellosis	A1, A2, B13, B17
6	Acute brucellosis	A2, A3, B15, Bw44
7	Acute brucellosis	A1, B8
8	Acute brucellosis	A10, A11, Bw16, B40
9	Acute brucellosis	A1, Aw19, B17, Bw44
10	Acute brucellosis	A2, Aw24, B8, B27
11	Acute brucellosis	A1, A2, Bw39, Bw44
12	Acute brucellosis	A2, A9, B27, Bw44

### Discussion

The major histocompatibility complex, including HLA genes, is linked to the immune response. A disproportionate number of patients who develop reactive arthritis<sup>1</sup> after infection by a variety of micro-organisms, including salmonella, *Shigella flexneri*, yersinia, and campylobacter, possess B27.

Human brucella infection occurring in Britain has steadily declined from 101 cases in 1976 to 22 cases in 1980.<sup>5</sup> *Brucella melitensis* was isolated in only five cases in 1980, and these were almost certainly contracted abroad. In England, Wales, and Scotland since 1981 more than 99% of herds have been certified as brucellosis-free, and by 1983 only 10 human cases were reported in England and Wales.<sup>6</sup> The incidence of joint involvement is about 19% in patients with *B. melitensis* and 10% in those with *B. abortus* infection.

In 1978 Hodinka *et al.*<sup>2</sup> reported a positive relationship between possession of HLA-B27 and spondylarthritis in chronic brucellosis caused by *B. abortus*. 44% of 27 patients with spondylarthritis had B27, whereas 41 patients without spondylarthritis had an incidence of B27 of 12% ( $p < 0.01$ ), which was similar to the frequency in healthy Hungarians. However, Alarcon *et al.*<sup>3</sup> found no increased incidence of any HLA type in 16 patients with acute brucella-induced reactive arthritis compared with 16 cases of brucellosis without arthritis in a Peruvian population. *B. melitensis* is the prevalent strain in Peru. They did note a low frequency of HLA-A2 in the disease group as a whole compared with the general population ( $p < 0.01$ ) and postulated that possession of A2 might protect against brucellosis.

Our patients were infected in north east England with *B. abortus*. Two cases (17%) had reactive arthritis associated with acute brucellosis; both possessed HLA-B27. However, two others possessing B27 had no musculoskeletal complication. It is interesting to note that four patients (33%) had the B27 antigen (half of whom had reactive arthritis) compared with the prevalence of B27 in the general population in England of 9%. The equal distribution of B27 between those with and those without reactive arthritis is in accord with the findings of Alarcon *et al.*<sup>3</sup>

Eight patients (67%) possessed HLA-A2, whereas the general population frequency is 51% in England. This finding contrasts with the Peruvian finding, and though this discrepancy could be explained by the antigenic difference between the brucella strains<sup>7</sup> it is clear that possession of HLA-A2 is not a genetically protective factor against development of all types of brucellosis.

We thank the physicians who allowed their cases to be studied; the National Blood Transfusion Service, Newcastle-upon-Tyne for the determination of HLA and helpful suggestions; and Dr I Haslock for his help with the manuscript.

**References**

- 1 Ebringer R W. HLA-B27 and the link with rheumatic diseases: recent developments. *Clin Sci* 1980; **59**: 405–10.
- 2 Hodinka L, Gömör B, Meréty K, *et al.* HLA-B27 associated with spondyloarthritis in chronic brucellosis. *Lancet* 1978; **i**: 499.
- 3 Alarcon G S, Bocanegra T S, Gotuzzo E, *et al.* Reactive arthritis associated with brucellosis: HLA studies. *J Rheumatol* 1981; **4**: 621–5.
- 4 Dewar P J. HLA antigens. *Clin Rheum Dis* 1983; **9**: 93–116.
- 5 Epidemiology, brucellosis: England and Wales. *Br Med J* 1981; **283**: 1477.
- 6 Public Health Laboratory Service. Brucellosis: England and Wales. *Communicable Disease Report* 1984; **84/19**: 3.
- 7 Joshi D V, Prakash O, Talwar G P. Antigenic analysis of different strains of brucella. *Indian J Med Res* 1971; **59**: 1225–30.