Correspondence

Rheumatic manifestations of brucellosis

Sir, We read with interest the article by Norton on the rheumatic manifestations of brucellosis.¹ We reported our experience with 304 patients infected with Brucella melitensis in 1982.² Recently we have completed a prospective evaluation of 194 additional patients. Of these 498 patients, 180 (36%) presented with arthritis. In contrast to Norton’s findings, sacroiliitis and peripheral arthritis were the most common forms of joint involvement, accounting for 90% of all the arthritis cases. Spondylitis was seen in only 18 patients (10%). As reported by others³ ⁴ these patients were predominantly middle aged and older males with chronic brucellosis.

The discrepancy can be explained by the different age distribution of the populations studied. Only one of the 55 patients reported by Norton was younger than 15, while in our group 354 patients (71%) were younger than 35 and 125 (25%) younger than 15.

We agree with Norton in that immune abnormalities occur in human brucellosis, particularly during periods of active disease. Low titres of antinuclear antibodies were present in 25% and rheumatoid factor in 37-5% of a group of patients evaluated by us.⁵ Circulating immune complexes were detected by the Raji cell assay in 91.5%.

As in other infectious diseases⁶ ⁷ infectious and reactive arthritis occur in human brucellosis. In our series, using a selective culture medium for brucella,⁸ one third of the patients had sterile synovial fluids fulfilling the definition of reactive arthritis.⁹ Furthermore, the arthritis was non-destructive and resolved spontaneously in most of these patients, an event not commonly seen in infectious arthritis.¹⁰ Interestingly, in one of the patients described by Norton the arthritis resolved after an intra-articular injection of corticosteroids.¹¹ The immune abnormalities described above strongly suggest that an immune mechanism plays a part in the arthritis of these patients.

References
8 Ruiz-Castañeda M. Improved equipment for the isolation of brucella, salmonella, etc. by blood culture. Bol de Sanit Panam 1957; 42: 564-5.

Further observations upon HLA-B27, Yersinia enterocolitica, and ankylosing spondylitis

Sir, Some time ago we reported that the observations we had made, in our search for evidence for the molecular mimicry hypothesis of ankylosing spondylitis (AS), on serological cross reactions between Yersinia enterocolitica types 0:3, 0:9, and NCTC 10460 and lymphocytes from HLA B27 AS + subjects, with a haemagglutination technique, were in fact nothing more than a demonstration of an anti-yersinia factor present in normal human sera, quite distinct from a lymphocytotoxicity causing antibody.¹ We concluded that this did not rule out the possibility of the existence of some other undefined antigenic factor common to Y enterocolitica and HLA-B27. Subsequently we did detect such a factor in Y enterocolitica type 0:9, which was not present in type 0:3, and in lymphocytes from B27 AS + subjects as opposed to B27 AS - ones, by the use of an absorption/haemagglutination technique.² The numbers examined, however, were too small for us to draw any conclusions about the existence of any serological differences between the two groups of lymphocytes.

Later we found that the relevant class of antibody in these tests was IgG. We titrated erythrocytes coated with Y enterocolitica 0:9 against homologous antiserum, i.e.,
the system used in the previously mentioned absorption/haemagglutination technique,\(^2\) and added antirabbit IgA, IgM, and IgG to the wells beyond those in which haemagglutination had taken place. Haemagglutination occurred only in those wells containing anti-IgG. Having discovered this, we decided to investigate the \(Y\) enterocolitica HLA-B27 problem further by employing an absorption/enzyme linked immunosorbent assay (ELISA) technique using rabbit anti-\(Y\) enterocolitica 0:9 and antirabbit IgG enzyme conjugate.

\(Y\) enterocolitica serotype 0:9 (Institute Pasteur No 383) was used to raise antiserum in rabbits as previously described\(^3\) using formalised vaccine. Blood from AS patients was supplied by Dr Ebringer, and control blood samples, including five from HLA-B27 positive subjects, were taken from staff and students in this medical school and the General Hospital, Birmingham. For absorption equal volumes of lymphocytes (separated from blood collected in lithium heparin with Ficoll Trisol) and \(Y\) enterocolitica serum diluted 1/5 were mixed in 1.5 ml Eppendorf tubes fixed to a revolving drum (22 revolutions/hour) for two hours at 37°C. The absorbed sera were removed after centrifugation and stored at −20°C. For the ELISA a 24 h growth of \(Y\) enterocolitica was suspended in phosphate buffered saline (PBS) and steamed for 30 min. It was then washed with PBS three times and resuspended in carbonate buffer pH 9.6, washed twice, and resuspended in carbonate buffer at a concentration giving an optical density of 0.3 at 540 nm. Then 100 \(\mu\)l amounts were placed in each well of the microELISA plates, left overnight at 4°C, and then washed in PBS-Tween. Serum (0-1 ml) diluted 1 to 100 in PBS-Tween was added to each well of the plates, which were then left at room temperature for one and a half hours. After washing, 0-1 ml of the antirabbit IgG alkaline phosphatase conjugate (Sigma) was added and left to react for one and a half hours at room temperature. After further washing, 0-1 ml substrate (0-01% 4-nitrophenyl phosphate diluted in 10% diethanolamine buffer pH 9-8) was added and left to react for 30 min, after which time the reaction was stopped by the addition of 50 \(\mu\)l of 3 M sodium hydroxide. The amount of colour produced was measured with a Dynatech microELISA reader at 410 nm. Preimmunisation serum was included in each test as well as an anti-\(Y\) enterocolitica 0:9 serum absorbed with \(Y\) enterocolitica 0:9 itself, and the absorptions were worked out as percentages.

The results are shown in Fig. 1. The absorbing capability of the lymphocytes from 28 AS patients is shown. Five lymphocytes failed to absorb and the rest showed a range of absorbing ability. Apart from one, all patients were HLA-B27 positive. The lymphocytes from 28 B27 negative control subjects showed zero absorbing power, and of the lymphocytes from five B27+AS− controls, one showed 30% absorbing power, one 5%, and the rest zero.

This latter observation indicating that B27+AS− lymphocytes absorb yersinia antibodies is particularly interesting in view of the finding by Van Bohemen et al that the M1 epitope of HLA-B27 cross reacts with \(Y\) enterocolitica 0:9\(^4\) and by Kono et al that a monoclonal yersinia 0:3 antibody cross reacts with \(Y\) enterocolitica lymphoblastoid cell lines.\(^5\) It is probably also important to remember that \(Y\) enterocolitica is very prominent among the bacteria associated with HLA-B27 reactive arthritis,\(^6\) which is one of a whole spectrum of diseases in which there are complicated overlapping relationships, e.g., inflammatory bowel disease, psoriasis, uveitis, and in the present context, not least, ankylosing spondylitis.

At this stage, however, mainly because of the small number of HLA-B27 positive controls we have examined we feel that we cannot claim unequivocal support for either Ebringer’s belief in the existence of a direct cross reaction between bacterial antigen and HLA-B27 itself,\(^7\) or the belief of Geczy et al that a plasmid mediated antigen is able to attach itself specifically to the surface of HLA-B27 lymphocytes and also, in accordance with the Pease episome theory of autoimmune disease,\(^8\) that bacterial, possibly plasmid, DNA is capable of becoming incorporated into HLA-B27 DNA so that there is a continuous production of ‘bacterial’ antigen by these cells, which accounts for any cross reactivity.

![Graph showing percentage absorption of Yersinia enterocolitica 0:9 antiserum by lymphocytes from patients with ankylosing spondylitis and from controls as measured by an ELISA.](http://ard.bmj.com/)

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Correspondence, Book review, Notes

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References


Book review


In the Preface the editors of this 1000 page textbook describe it as 'a compilation of the basic and applied knowledge about rheumatoid arthritis, relevant to clinicians', and certainly every aspect is comprehensively covered, from minute details of cellular interactions to discussion of the 'rheumatoid personality'. Despite 75 contributors (all but three coming from North America) the style is reasonably consistent throughout, and the book is well produced with many good quality photographs, including a successful demonstration of the bulge sign, for detecting a small effusion of the knee! Much in the book will interest researchers, rheumatologists in training, and paramedical professionals, and its very large number of references (over 5000), a good proportion of which were written in the 1980s, make it an excellent source of material for further study and research. A few inconsistencies exist in the depth to which topics are discussed; aetiology and pathogenesis are extensively covered, whereas amyloidosis has relatively scant attention, and a whole chapter for apheresis is more space than it deserves. Further, the text is geared to a North American readership, with a fairly large section devoted to legislative and economic considerations (some of which are relevant to the UK), and a chapter on childhood arthritis follows the American rather than the British classification. My main criticism, however, concerns repetition. As each chapter has been designed to be read independently there is a great deal of overlap and redundant points. The editors feel that 'redundancy adds to the value of the text', but my feeling is that it adds to the weight of the book! With stricter editing and good cross referencing the text could have been reduced by a third, especially the clinical section. Although the price is reasonable considering the quality of the production, I suspect that sales in Europe will be limited.

Honorary Senior Registrar Fibrinolysis Research Group, John Radcliffe Hospital and Dept of Rheumatology, Nuffield Orthopaedic Centre, Oxford.  

MARGARET BYRON

Notes

Clinical metrology applied to rheumatic diseases

A course in clinical metrology applied to rheumatic diseases will be held at the Royal Bath Hospital, Harrogate, under the auspices of the University of Leeds from 1 to 12 September 1986. The course is designed to attract nurses, physiotherapists, occupational therapists, and basic scientists who wish to improve their skills in clinical aspects of rheumatology research. A Certificate of Attendance will be issued by the Arthritis and Rheumatism Council.

The course fee is £50. Support grants of up to £200 may be available from the ARC for those committed to rheumatology research who do not have access to alternative funds. Further information from Dr D A Bird, Royal Bath Hospital, Harrogate, North Yorkshire. HG1 2PS. Applications should be submitted by 30 June 1986.

European Rheumatology Research Workshop

The next workshop will be held on Friday–Sunday, 6–8 March 1987 at the Normandy Hotel, Renfrew, Scotland. Inquiries to Dr R D Sturrock, Centre for Rheumatic Diseases, University Department of Medicine, Royal Infirmary, 10 Alexandra Parade, Glasgow G31 2ER.
Further observations upon HLA-B27, Yersinia enterocolitica, and ankylosing spondylitis.

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