

## IS UNDIFFERENTIATED SERONEGATIVE SPONDYLOARTHROPATHY A FORME FRUSTE OF REACTIVE ARTHRITIS?

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### SUMMARY

Undifferentiated spondyloarthropathy (USpa) may either represent a forme fruste of other spondyloarthropathies like reactive arthritis or be a different disease entity. To study the link between USpa and reactive arthritis, we studied the presence of IgA antibodies to *Yersinia enterocolitica*, *Salmonella typhimurium*, *Shigella flexneri*, *Campylobacter jejuni* and *Chlamydia trachomatis* in sera from 14 patients with USpa (European Spondyloarthropathy Study Group criteria) using ELISA. *Escherichia coli* was used as a control antigen. An OD value of more than the mean + 2 s.d. of 51 blood donors was considered positive. Five patients had elevated IgA antibodies to *S. flexneri*, while two patients each had elevated antibody levels to *S. typhimurium* and *Chlamydia*. No patient had elevated antibodies to *Y. enterocolitica*, *C. jejuni* and *E. coli*. Among 51 normals, 1, 4, 3, 2 and 3 had elevated antibodies to *S. flexneri*, *S. typhimurium*, *Y. enterocolitica*, *C. jejuni* and *E. coli*, respectively. Nine of 14 patients with USpa had antibodies to one of the bacteria implicated in reactive arthritis; of these, antibodies to *Shigella* were the most frequent. Thus, a proportion of patients with USpa may in fact have reactive arthritis.

KEY WORDS: Unclassifiable spondyloarthropathy, Enteric bacteria, Bacterial antibodies, *Chlamydia*.

THE term 'unclassifiable or undifferentiated spondyloarthropathy' (USpa) refers to a group of patients with inflammatory backache, oligoarticular asymmetrical arthritis, enthesitis with or without radiological evidence of sacroiliitis, but not fulfilling the diagnostic criteria for any of the currently established diseases such as ankylosing spondylitis, psoriatic arthritis, reactive arthritis or arthritis associated with chronic inflammatory bowel disease [1, 2]. In this context, 'undifferentiated' might mean an early stage of a definite spondyloarthropathy (Spa), a forme fruste of a definite Spa, an aetiologically as yet undefined subcategory of Spa [1].

Reactive arthritis is a form of Spa in which there is evidence of preceding genitourinary or enteric infection. These patients show heightened immune response to the triggering agent, as evidenced by an increase in IgG/IgA antibody level in sera [3, 4] and enhanced antigen-specific proliferation of synovial fluid lymphocytes [5]. In addition, bacterial components have been found in the synovial cells/tissue of these patients [6, 7]. Because of similarities in the clinical pictures of reactive arthritis and USpa, it is possible that the latter disease entity represents an incomplete form of the former in which the inciting infection is asymptomatic or clinically inapparent. In fact, in a recent study, 5/7 patients with USpa were

shown to have IgG antibodies against *Chlamydia trachomatis* [4]. As the triggering organism depends on the nature of infection in a community, we decided to look for evidence of an antibody response against implicated infectious agents in patients with USpa.

### PATIENTS AND METHODS

Patients with spondyloarthropathy as defined by 1991 European Spondyloarthropathy Study Group criteria [8] were included in the study. Patients with ankylosing spondylitis, inflammatory bowel disease, psoriasis and with a history of preceding infection were excluded. Sera were collected during the active phase and stored at  $-80^{\circ}\text{C}$  until analysis. Fifty-one healthy blood donors served as controls.

#### Antigen preparation

*Yersinia enterocolitica* O:3 strain, *Campylobacter jejuni*, *Salmonella typhimurium* (ATCC-13311), *Shigella flexneri* 2b (kind gift from P. Toivanen, Turku, Finland) and *Escherichia coli* (NCTC-10418) were grown overnight in nutrient broth at  $37^{\circ}\text{C}$ . The following morning, bacteria were pelleted and washed three times with phosphate-buffered saline (0.15 M, pH 7.2). The bacteria were lysed using a pulse sonicator. After centrifugation at 12 000 g for 30 min at  $4^{\circ}\text{C}$ , supernatant was collected, aliquoted and stored at  $-80^{\circ}\text{C}$ .

#### ELISA for IgA antibacterial antibodies

Ninety-six-well ELISA plates (Nunc, Germany) were coated with 50  $\mu\text{l}$  of the respective antigen (10  $\mu\text{g}/\text{ml}$ ) in carbonate–bicarbonate buffer (pH 9.6) and

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TABLE I  
Clinical profile of patients

Patient	Low backache	Asymmetrical oligoarthritis	Positive family history	Buttock pain	Enthesitis	Radiological sacroiliitis
1	+	+	-	-	+	+
2	+	-	-	-	-	+
3	+	+	-	+	+	-
4	-	+	-	-	+	-
5	-	+	-	-	+	-
6	+	+	-	-	+	-
7	+	+	-	+	+	+
8	+	+	-	-	+	+
9	+	+	-	+	+	-
10	+	+	-	-	+	-
11	+	+	-	-	-	+
12	+	-	+	-	-	+
13	+	+	-	+	+	+
14	-	+	+	-	+	-

incubated at 4°C overnight. After blocking with 2% bovine serum albumin (BSA) for 2 h, diluted serum (1:100) was added and incubated for 2 h at 37°C. Following washing with phosphate-buffered saline (pH 7.2, 0.15 M) containing 0.05% Tween 20 (PBS-T), 50 µl of anti-human IgA conjugated with horseradish peroxidase (Dakopatts, Denmark, 1:5000) were added to each well and kept at 37°C for 1 h. Plates were washed three times with PBS-T. Colour was developed by addition of 50 µl of substrate solution [citrate phosphate buffer (pH 5.0) containing *o*-phenyldiamine (0.4 mg/ml) and 0.04% hydrogen peroxide]. The reaction was stopped by 2.5 N sulphuric acid and plates were read at 492 nm in an ELISA reader. OD values exceeding the mean + 2 S.D. of healthy controls were taken as positive.

#### ELISA for IgA anti-chlamydia antibodies

The ELISA was performed using a commercial kit (Eurogenetics, Belgium) according to the manufacturer's instructions. The samples with an absorbance value 15% higher than the mean absorbance of the cut-off control serum (provided in the kit) were classified as positive. Eighteen control sera from blood

bank donors were also tested. The antigen coated is cellular extract from the LV2 strain and it predominantly detects antibodies to *C. trachomatis*.

#### Statistical analysis

The  $\chi^2$  test was used for inter-group comparisons.

## RESULTS

There were 14 patients with USpa and four of these were females. The median age at presentation was 27 yr (16–45 yr) and the median duration of disease was 2.5 yr (0.6–15 yr). The main features were enthesitis and sacroiliitis along with inflammatory low backache and asymmetrical oligoarthritis (Table I). HLA B27 was present in three of the six patients tested.

The mean + S.D. (in parentheses) of absorbance in different antigen ELISAs among controls were as follows: *S. flexneri* (0.46 + 0.21), *S. typhimurium* (0.23 + 0.13), *C. jejuni* (0.21 + 0.11), *Y. enterocolitica* (0.22 + 0.14) and *E. coli* (0.20 + 0.10). Among 51 normals, 1, 4, 2, 3 and 3 had elevated antibodies to *S. flexneri*, *S. typhimurium*, *C. jejuni*, *Y. enterocolitica* and *E. coli*, respectively. The cut-off for

TABLE II  
Absorbance values with different antigens in 14 patients with USpa. Values in bold are above the mean + 2 S.D. of normals

Patient no.	<i>Yersinia</i>	<i>Salmonella</i>	<i>Shigella</i>	<i>C. jejuni</i>	<i>E. coli</i>	<i>Chlamydia</i>
1	0.1	0.12	0.83	0.1	0.1	<b>0.55</b>
2	0.1	0.06	<b>1</b>	0.12	0.06	0.41
3	0.1	<b>0.53</b>	0.78	0.12	0.08	0.37
4	0.37	0.12	<b>0.99</b>	0.12	0.06	0.15
5	0.25	0.08	0.45	0.13	0.08	0.50
6	0.31	<b>0.57</b>	0.19	0.18	0.24	0.45
7	0.25	0.26	0.56	0.23	0.27	0.31
8	0.1	0.34	<b>1.01</b>	0.31	0.32	0.35
9	0.38	0.16	<b>1.05</b>	0.15	0.14	0.32
10	0.18	0.35	0.25	0.29	0.21	0.29
11	0.13	0.48	<b>0.88</b>	0.33	0.35	0.17
12	0.18	0.11	0.34	0.16	0.1	0.15
13	0.18	0.19	0.1	0.2	0.17	<b>0.55</b>
14	0.1	0.27	0.36	0.2	0.25	0.42

*Chlamydia* was found to be 0.51 and none of 18 normals were above this value.

Five patients had elevated IgA antibodies to *Shigella*, while two each had elevated IgA levels to *Chlamydia* and *Salmonella* (Table II). No cross-reactivity was observed between different bacteria. The difference in the prevalence of antibodies to *Shigella* ( $P < 0.0005$ ) and *Chlamydia* ( $P < 0.05$ ) was significantly higher in the patient group, while there was no significant difference in the prevalence of antibodies to *Salmonella*.

#### DISCUSSION

We observed that nine of these 14 patients with USpa had a heightened serum IgA response to a single organism implicated in the pathogenesis of reactive arthritis, suggesting that 64% of patients might have an inapparent infection.

The presence of an IgA antibody response to a single bacterium and not to the whole panel of Enterobacteriaceae implies that the response is not directed against cross-reactive epitopes, but to species-specific antigens. Some of our patients, surprisingly, had an elevated IgA response even after several years of disease. In reactive arthritis, an IgA response may persist for years [3] and is most likely due to persistence of antigen either in the gut or synovium [9].

Most studies from the developed countries found direct or indirect evidence of *Chlamydia* infection in their group of patients with undifferentiated oligoarthritis [7, 10]. Among the enteric organisms, *Yersinia*-triggered arthritis accounts for most cases of sporadic enteric reactive arthritis [11]. On the other hand, our data suggest that *Shigella* and *Salmonella* are a quite common triggering factor, reflecting a higher incidence of gastrointestinal infections as well as the bacteriology of diarrhoea [12].

*Shigella* is the commonest cause of diarrhoea in North India, as reflected in a study from our centre [13]. It is generally believed that inapparent infection with *Shigella* is uncommon and this bacterium does not persist in the body. Contrary to this belief, inapparent infection was quite common in an outbreak of *S. flexneri* [14]. This, in turn, may lead to spondyloarthropathy in a susceptible host. In addition, a long-term *Shigella* carrier state is also known [15] and bacterial lipopolysaccharides have been found in synovial cells of patients with reactive arthritis [6]. Further, *Shigella*-induced reactive arthritis can have a chronic course which may simulate USpa if the infection was asymptomatic.

Involvement of *Chlamydia* as the triggering factor in two patients supports the observation of others [7, 10, 11]. *Chlamydia* was found to be the most important inciting agent in patients with asymmetrical oligoarthritis without any preceding history of infection [10]. Further IgA antibodies to *Chlamydia* were indicative of infection. A lack of antibodies to enteric organisms in their study may be due to differences in

the prevalence of these infections in their community [10].

Thus, our study suggests that ~64% of patients with USpa may indeed be cases of reactive arthritis where either the infection was asymptomatic or was too trivial to be remembered by the patient. Our data have to be viewed in light of the fact that a humoral response is still indirect evidence for a cause and effect relationship. The diagnosis of enteric infection based on serology has its own limitations. Therefore, further studies on synovial fluid lymphocytes, such as antigen-induced proliferation or cloning of antigen-specific T cells from this group of patients, along with a search for antigen or bacterial DNA and RNA in the synovium, may provide the final proof.

#### REFERENCES

1. Zeidler H, Mau W, Khan MA. Undifferentiated spondyloarthropathies. *Rheum Dis Clin North Am* 1992;18:187–202.
2. Prakash S, Mehra NK, Bhargava S *et al.* HLA-B27 related unclassifiable seronegative spondyloarthropathies. *Ann Rheum Dis* 1983;42:640–3.
3. Toivanen A, Lahesmaa-Rantala R, Vuneto R, Granfors K. Association of persisting IgA response with yersinia triggered reactive arthritis: A study in 104 patients. *Ann Rheum Dis* 1987;46:898–901.
4. Silveria LH, Gutierrez F, Scopelitis E, Cuellar ML, Citera G, Espinoza LR. Chlamydia induced reactive arthritis. *Rheum Dis Clin* 1993;19:351–62.
5. Gasten JSH, Life PI, Granfors K *et al.* Synovial T lymphocyte recognition of organisms that trigger reactive arthritis. *Clin Exp Immunol* 1989;76:348–53.
6. Granfors K, Jalkanen S, Toivanen A, Koski J, Lindberg AA. Bacterial lipopolysaccharide in synovial cells in shigella triggered reactive arthritis. *J Rheumatol* 1990;19:500–4.
7. Bas S, Griffais R, Kvein TK, Glennas A, Melby K, Vischer TL. Amplification of plasmid and chromosomal chlamydial DNA on synovial fluid of patients with reactive arthritis and undifferentiated seronegative oligoarthritis. *Arthritis Rheum* 1995;38:1005–13.
8. Dougados M, vander Linden, Juhlin R *et al.* The European Spondyloarthropathy Study Group preliminary criteria for the classification of spondyloarthropathy. *Arthritis Rheum* 1991;34:1218–27.
9. Sieper J, Braun J. Pathogenesis of spondyloarthropathies—Persistent bacterial antigens, autoimmunity or both? *Arthritis Rheum* 1995;11:1547–54.
10. Erlacher L, Wintersberger W, Menschik M, Benckstudnieko A, Machold M, Stanek G *et al.* Reactive arthritis: Urogenital swab culture is the only useful method for the detection of the arthritogenic infection in extra-articularly asymptomatic patients with undifferentiated oligoarthritis. *Br J Rheumatol* 1995;34:838–42.
11. Sieper J, Braun J, Brandt J *et al.* Pathogenetic role of Chlamydia, Yersinia and Borrelia in undifferentiated oligoarthritis. *J Rheumatol* 1992;19:1236–42.
12. Guerrant RL, Hughes JM, Lima NL, Crane J. Diarrhea in developed and developing countries: magnitude, special setting and etiology. *Rev Infect Dis* 1990;12(suppl. 1):41–50.
13. Prasad K, Anuppurba S, Dhole TN. Enterotoxigenic

- Campylobacter jejuni* & *E. coli* in the etiology of diarrhea in northern India. Indian J Med Res [A] 1991;93:81-6.
14. Cohen D, Green MS, Block C, Rouch T, Ofek I. Serum antibodies to lipopolysaccharide and natural immunity to shigellosis in an Israeli military population. J Infect Dis 1988;57:1068-71.
15. Levine MM, DuPont HL, Khodabandelou M, Hornick RB. Long term shigella-carrier state. N Engl J Med 1973;288:1169-71.