

## Evidence of *Chlamydia trachomatis* infection in sexually acquired reactive arthritis

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**SUMMARY** Thirty male patients with sexually acquired reactive arthritis (SARA) have been studied at the time of their initial presentation and thereafter. *Chlamydia trachomatis* was isolated from the urethral exudate of 9 (36.0%) of the 25 patients from whom urethral specimens were taken, and elevated titres of IgM antibody to *C. trachomatis* were detected in 11 (36.6%) of the 30 initial sera. Thirteen (43.3%) of the patients had a positive urethral culture and/or elevated titre of IgM antibody, and it is therefore suggested that 43.3% of these patients suffered an acute chlamydial infection at or near the time of the onset of their joint disease. The demonstration of 4-fold or greater rises and/or falls in IgM antibody titre (8 patients) and IgG antibody titre (6 patients) in a group of 15 men studied throughout the course of their disease strongly supports this conclusion. A positive urethral culture and/or raised titre of IgM serum antibody was also detected in 25 (50%) of 50 men with uncomplicated nongonococcal urethritis (NGU), suggesting that the prevalence of chlamydial infections in the 2 conditions is similar. Titres of IgG serum antibody to *C. trachomatis* were, however, significantly higher in patients with SARA than in those with NGU or other rheumatic diseases, and in healthy controls. The geometric mean titres (GMT) of IgG serum antibody in patients with SARA, NGU, rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus, and in healthy controls were 1:47.5, 1:8.6, 1:2.2, 1:2.2, 1:3.5, and 1:1.4, respectively. These findings suggest that an exaggerated antibody response to acute infection by *C. trachomatis* may be an important factor in the development of SARA in some but not all patients.

Reactive arthritis, which may be defined as acute sterile synovitis associated with a localised infection elsewhere in the body,<sup>1</sup> is a well-recognised complication of sexually transmitted nongonococcal urethritis (NGU). This condition has been documented and referred to as sexually acquired reactive arthritis (SARA),<sup>2,3</sup> and although the incidence is difficult to assess it is probably the commonest cause of acute arthritis among young adults in Great Britain. In only 25% of cases of SARA is the triad of features of Reiter's syndrome present. A genetic predisposition to the development of this condition, marked by the presence of the histocompatibility

determinant HLA B27 in 66% of patients, is widely recognised, as in other forms of reactive arthritis, but the cause of NGU and the exact nature of the relationship between NGU and SARA are uncertain. It is, however, a plausible hypothesis that antigens derived from the agents causing the urethritis are responsible for initiating inflammatory musculo-skeletal and other lesions in genetically predisposed individuals.

Strong evidence has now accumulated from both human and animal studies that infection by *Chlamydia trachomatis* causes a substantial proportion—perhaps about 50%—of cases of NGU,<sup>4-6</sup> and we have therefore sought evidence that infection by this micro-organism may be important in the development of SARA. We have previously reported the isolation of *C. trachomatis* from the urethra of 5 of 14 patients presenting with SARA.<sup>7</sup> However, it is

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unlikely that isolation of the micro-organism alone can either reflect the true rate of infection, as a detectable antibody response to *C. trachomatis* occurs in some patients with NGU from whom an isolate cannot be obtained,<sup>8,9</sup> or indicate whether or not this infection is of special significance in the initiation of SARA. Moreover, since it is likely that specific immune responses and aspects of antigen handling play a central role in the development of this condition, we consider that an assessment of the immune response to *C. trachomatis* in patients with SARA may provide a crucial first step to the understanding of its pathogenesis.

We have therefore attempted to assess both the prevalence of *C. trachomatis* infection and also quantitative aspects of the humoral immune response to this micro-organism in SARA compared with uncomplicated urethritis, using a modification of the microimmunofluorescence method of Wang and Grayston,<sup>10</sup> which provides a sensitive, specific, and reproducible method for detecting and quantifying the different immunoglobulin classes of antibody to chlamydiae in man.<sup>11,12</sup>

#### Patients, materials and methods

Thirty male patients with sexually acquired reactive arthritis (SARA) were studied at the time of their presentation to venereology or rheumatology departments of the Charing Cross and West London Hospitals, London, as part of a prospective study. The diagnosis of SARA was based on the presence of sterile synovitis, with or without additional features, occurring in association with definite, sexually acquired NGU, according to a previous clinical study<sup>2</sup>; in 23 of the patients rheumatic symptoms had been present for less than 3 weeks. NGU was diagnosed if 10 or more polymorphonuclear leucocytes were seen per high-power field ( $\times 100$  objective) of a urethral smear, in association with negative tests for gonococci.<sup>13</sup>

A serum sample, defibrinated or citrated blood for HLA typing, and an endourethral swab for *C. trachomatis* culture were obtained from patients at presentation. Radiographs and other blood tests were performed as clinically indicated. A total of 34 synovial fluid samples (with corresponding sera) were obtained from 20 patients. All were cultured on routine bacteriological media with negative results and were shown to be free of crystals. Flexible and sometimes informal arrangements were made to follow up patients; blood samples were obtained at convenient intervals and the clinical features throughout the course of the rheumatic disease were documented. The severity and extent of disease features have been represented (see Fig. 3)

on a simple arbitrary 0 to 5 scale. Fifteen patients with SARA who were first seen within 3 weeks of the onset of joint symptoms (mean 10 days) have been followed up throughout the course of their illness and for a variable period after cessation of all signs of disease activity. The mean duration of active rheumatic disease in these 15 patients was 13.6 weeks and the mean surveillance period was 36.5 weeks.

During the time of this study 555 men presenting to the venereology clinic with uncomplicated NGU were also studied. All were HLA typed for the presence or absence of HLA B27 by a modification of the microlymphocytotoxicity technique of Terasaki and McClelland,<sup>14</sup> and satisfactory specimens of urethral exudate were obtained from 452 men for culture of *C. trachomatis*. Sera from 50 of these patients, of whom 14 yielded isolates of *C. trachomatis*, were randomly selected for chlamydial serology. All patients in both SARA and NGU groups received a standard course of antibiotic therapy (usually oxytetracycline), and when necessary this was supplemented by other antibiotics in order to achieve eradication of signs of urethritis.

Urethral material was cultured for *C. trachomatis* in 5-iodo-2-deoxyuridine-treated McCoy cells,<sup>15</sup> and antibodies to *C. trachomatis* of IgG and IgM classes were sought by a quantitative microimmunofluorescence technique using immunotypes B, D, E, F, G, H, I, and J.<sup>16</sup> In accordance with data previously collected in our laboratory serum IgG titres of 1: <16 were considered to be of doubtful significance. Changes in IgG titres between 1: <2 and 1:8 are therefore not shown in Fig. 2a. IgM antibody titres of 1:  $\geq 2$  were regarded as signifying recent or current chlamydial infection.

Data on antibodies to *C. trachomatis* in other rheumatic disorders and in healthy individuals were obtained by testing groups of 30 sera withdrawn from storage ( $-70^{\circ}\text{C}$ ) from patients with active rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and ankylosing spondylitis (AS) and from asymptomatic males of similar age distribution to the patients with SARA.

To assess whether serological responses were exclusive to *C. trachomatis* in the SARA patients antibodies to measles and rubella viruses were measured using standard complement-fixation tests and to influenza A/Victoria/3/75 virus by a standard haemagglutination-inhibition assay. This latter assay was also used to assess the relative concentrations of antibody to influenza virus in paired sera and synovial fluid samples. Immunoglobulin estimations were performed by a single radial-immunodiffusion technique with Behringwerke Tri-Partigen plates. In all tests in which levels or titres in serum and

synovial fluid were compared both members of the pair were analysed simultaneously.

Statistical analyses of data were carried out as indicated in the text.

**Results**

**PREVALENCE OF CHLAMYDIA TRACHOMATIS INFECTION**

Urethral exudate from 25 of the 30 patients with SARA and from 452 unselected men with uncomplicated NGU was cultured for *C. trachomatis*. Isolates were obtained from 9 (36.0%) and 165 (36.6%) individuals respectively (Table 1), the isolation rates being almost identical. Elevated titres of IgM antibody (1:≥2) were detected in initial sera from 11 (36.6%) of 30 patients with SARA and 21 (42%) of 50 with NGU alone (Table 1). Evidence of current or recent *C. trachomatis* infection as indicated by positive urethral cultures or the presence of IgM serum antibody was found in 13 (43%) of 30 patients with SARA and 25 (50%) of

50 patients with uncomplicated NGU. Thus the prevalence of *C. trachomatis* infection is apparently similar in SARA and NGU.

**IgG AND IgM CLASSES OF ANTIBODY TO C. TRACHOMATIS**

*Titres at presentation*

In spite of the prevalence of *C. trachomatis* infection being similar in SARA and NGU the magnitude of the IgG antibody titres in the 2 groups differed significantly (P=0.01; Wilcoxon's sum of ranks test; Fig. 1a). Twenty-two of 30 (73%) SARA patients and 26 of 50 (52%) NGU patients had elevated titres of IgG antibody at presentation, the geometric mean titre (GMT) in each entire group being 1:47.5 and 1:8.6 respectively. Furthermore, whereas all 9 of the isolate-positive patients with SARA had very high titres of IgG antibody (1:≥128), only 1 of the 14 NGU patients from whom chlamydiae were isolated had such a titre. The GMT of antibody in each of these isolate-positive groups was 1:188 and 1:15.2 respectively.

Twelve patients with SARA and 11 of those with NGU gave a history of 1 or more previous episodes of NGU. It is therefore unlikely that the different antibody titres in the 2 groups of patients can be attributed to previous sensitisation to chlamydial antigens.

Although very high IgM antibody titres were detected in sera from 2 patients with SARA, the distribution of titres in the 2 groups of patients did not differ significantly (Wilcoxon's sum of ranks test; Fig. 1b).

Table 1 Incidence of urethral isolates of *C. trachomatis* and of elevated titres of IgM chlamydial antibody in SARA and NGU (at presentation)

	Culture		Serology		Combined data
	No. of patients	% +ve	No. of patients	% IgM 1:≥2	
SARA	25	36.0	30	36.6	43
NGU	452	36.6	50	42.0	50

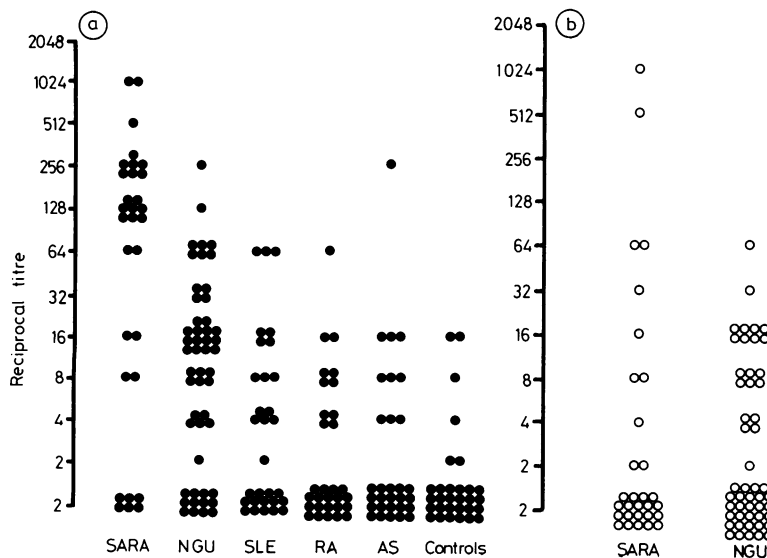


Fig. 1 Titres of antibody to *C. trachomatis* in groups of 30 patients with SARA, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), ankylosing spondylitis (AS), in 30 healthy male controls and in 50 patients with uncomplicated NGU. Fig. 1a: IgG antibody; Fig. 1b: IgM antibody (SARA and NGU only).

*Serial studies*

Eight of 15 patients with SARA studied serially had elevated titres of IgG antibody at presentation (Fig. 2a). Three of these had an 8-fold or greater rise in titre during the period of active joint disease and subsequently 6 patients, including these 3, had a 4-fold or greater fall in titre. The 2 remaining patients with elevated initial titres had no significant change during the period of surveillance. In contrast, no IgG antibody could be detected at any time during the course of disease in the sera of the remaining 7 patients (not shown). Six of the 8 chlamydial antibody-positive patients were also culture-positive while none of those without antibody were culture-positive.

Seven of the 15 SARA patients who were studied serially had IgM antibody in the initial serum (Fig. 2b), and 1 of these subsequently had a 4-fold rise in titre; 1 patient without detectable IgM antibody in the initial serum developed a peak titre of 1:16, and subsequently a significant reduction in titre was demonstrated in all 8, in 5 cases to  $1:\leq 2$ . These data strongly suggest an acute immune response to chlamydial antigens. Seven patients (not shown) who also lacked IgG antibody did not develop detectable IgM antibody at any time during the course of their disease.

## CORRELATION BETWEEN ANTIBODY TITRES AND CLINICAL DISEASE FEATURES

The peak of the IgM antibody response occurred early in the course of disease, the highest titres being noted at presentation in almost all patients, but elevated titres of this antibody were short-lived

(mean 24 weeks). The duration of this response bore no relationship to the presence of active joint disease. In contrast titres of IgG antibody, though often raised at presentation, reached a peak at 1 to 10 weeks (mean 6 weeks) from the onset of arthritis. In spite of a gradual downward trend thereafter in most patients, in none did the titre of IgG serum antibody fall below 1:16 during the period of observation and in 4 patients elevated titres persisted for more than 1 year after cessation of all symptoms. The changes in titre of IgG and IgM antibody during the course of SARA are illustrated by results from a single patient (Fig. 3).

The musculoskeletal features of the 22 chlamydial antibody-positive patients did not differ in any way from those of the 8 others.

## RELATIONSHIP BETWEEN CHLAMYDIAL ANTIBODIES AND HLA PHENOTYPES

Twenty-nine of the patients with SARA were HLA typed, and, of these, 14 (46.6%) possessed the antigen HLA B27. In contrast only 32 of 555 (5.7%) men with uncomplicated NGU, and 20 of 280 (7.1%) healthy controls possessed this antigen. Initial titres of IgG and IgM antibody in the sera of SARA patients with and without the HLA B27 antigen are shown in Fig. 4. It is apparent that antibody titres of both classes are similarly distributed in the B27-positive and -negative groups.

## SYNOVIAL FLUID STUDIES

Thirty-four corresponding pairs of serum and synovial fluid obtained from 20 patients were examined for the presence of IgG antibody to *C. trachomatis*.

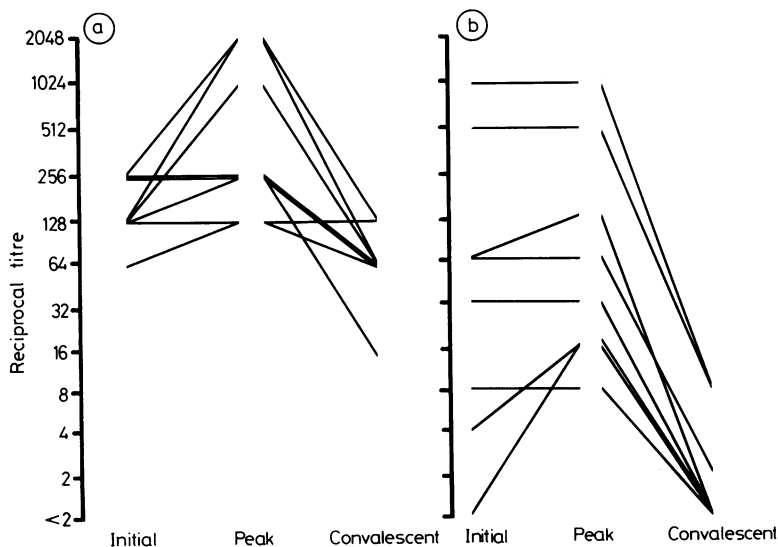


Fig. 2 Titres of IgG (Fig. 2a) and IgM (Fig. 2b) antibody to *C. trachomatis* at presentation (initial) and after resolution of the disease (convalescent), showing the maximum titre (peak) reached for each patient during this period.

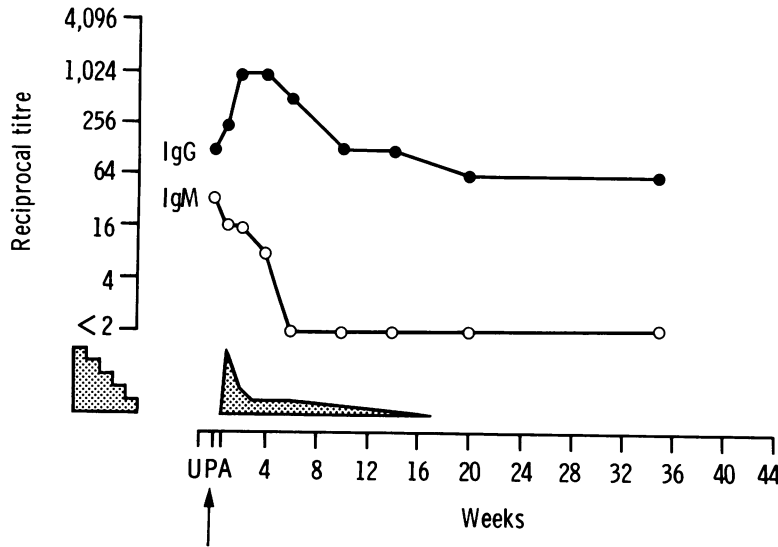
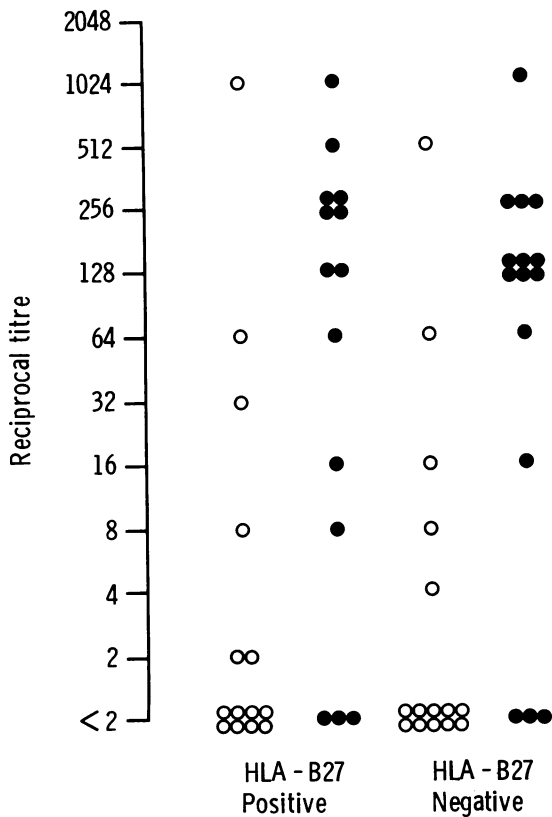


Fig. 3 Changes in titre of IgG and IgM antibody to *C. trachomatis* during the course of SARA in one patient. (Hatched area indicates clinical disease activity on a 0 to 5 scale.) U=Onset of urethritis. P=Presentation to hospital. A=Onset of arthritis.



Both members of each pair were tested in the same assay. Twenty-six of the sera had antibody titres of 1:16 or greater, as did 25 of the corresponding fluids, and in 31 of the pairs there was agreement between serum and synovial fluid antibody titres to within one 2-fold dilution. The GMT of antibody in the 34 sera was 1:59 and in the corresponding synovial fluids was 1:64. All patients had detectable antibody to influenza virus; the GMT of antibody in the 34 sera and in the corresponding synovial fluids was 1:9.4 and 1:8.6 respectively. The concentration of total IgG protein was also measured and the mean synovial fluid level (6.46 g/l) was found to be approximately half that of the mean serum level (13.14 g/l).

CONTROL STUDIES

IgG antibody to *C. trachomatis* at a titre of 1:16 or greater was present in sera from 3 of 30 (10%) patients with rheumatoid arthritis, 4 of 30 (13%) with ankylosing spondylitis, 7 of 30 (23%) with systemic lupus erythematosus, and 2 of 30 (7%) sera from healthy male controls (Fig. 1a). The GMT of serum antibody in these groups was only 1:2.2, 1:2.2, 1:3.5, and 1:1.4 respectively, in marked contrast to that of 1:47.5 in the SARA group (Table 2). It is apparent from Fig. 1a that the distributions of antibody titres in these control groups are essentially similar but that they differ markedly from that in the SARA group ( $P < 0.001$ ; Wilcoxon's sum of ranks test), antibody titres in NGU occupying an intermediate position.

Titres of antibody to measles, rubella, and influenza viruses were measured in single sera drawn

Fig. 4 Titres of IgG (closed circles) and IgM antibody (open circles) to *C. trachomatis* in HLA B27-positive and negative patients with SARA.

Table 2 Frequency of elevated titres and geometric mean titres of IgG antibody to *C. trachomatis* in SARA, NGU and control groups

Population	No. examined	Titre		GMT
		No.	%	
Rheumatoid arthritis	30	3	10	1:2.2
Ankylosing spondylitis	30	4	13	1:2.2
Systemic lupus erythematosus	30	7	23	1:3.5
Healthy males	30	2	7	1:1.4
NGU	50	26	52	1:8.6
SARA	30	22	73	1:47.5

during the period of active synovitis: all were found to be within ranges normally found in adults currently uninfected by these viruses.

### Discussion

The importance of this study lies in its clear demonstration of an exaggerated humoral immune response to chlamydial antigens which appears to characterise a high proportion of, though not all, patients with SARA. Since the concept of reactive arthritis implies that tissue damage in affected areas is brought about by the action of antibodies, immune complexes, sensitised cells and/or other inflammatory mediators arising from the focus of infection, which in the case of SARA lies within the genital tract, this finding suggests that the organism *C. trachomatis* or antigens derived from it may play a central role in the initiation of SARA.

The isolation of *C. trachomatis* from the urethra of 36% of men with SARA is consistent with the earlier findings of Vaughan-Jackson *et al.*,<sup>17</sup> who isolated the organism from 3 of 10 patients with Reiter's syndrome, but differs substantially from the 69% isolation rate found by Kousa *et al.*<sup>18</sup> in patients with Reiter's syndrome and active urethritis. Our isolation rate of 36.5% in men with uncomplicated NGU is consistent with other published data.<sup>4-6</sup>

It is clear from other studies in which the micro-immunofluorescence test or a modification of it has been used that raised titres of IgG antibody to *C. trachomatis* are found in 70-100% of isolate-positive men with NGU and that similar titres also occur in 21-44% of those in whom cultures are negative.<sup>12, 19, 20</sup> Our data on both the SARA and NGU groups of patients are therefore comparable in this aspect, but in view of the duration of persistence of raised titres of IgG antibody after resolution of other signs of infection the significance of raised IgG titres as an indicator of infection is uncertain. In contrast, the short duration of the IgM antibody response suggests that detection of elevated titres of this class of antibody provides reasonable evidence of a recent or current chlamydial

infection. We have therefore, regarded only raised titres of IgM antibody and/or isolation of *C. trachomatis* from the urethra as indicators of chlamydial infection around the time of onset of arthritis. Thus, while a higher proportion of patients with SARA had raised titres of IgG antibody (73%) than those with NGU (52%), our evidence indicates that approximately 40-50% of patients in both the SARA and NGU groups suffered an acute *C. trachomatis* infection at or near the time of onset of their disease. The demonstration of 4-fold or greater rises and falls in IgG titres (6 patients) and similar falls in IgM titres (8 patients) in approximately half of 15 patients studied serially strongly supports this conclusion. It is of interest that 7 (27%) of the patients with SARA had negative urethral cultures and neither class of antibody detectable in their serum, indicating that in these individuals, at least, *C. trachomatis* infection is unlikely to be relevant to the pathogenesis of their disease.

In spite of the apparent similarity in rate of chlamydial infection in SARA and NGU our finding of high titres of IgG antibody to *C. trachomatis* in initial sera from the majority of patients with SARA (GMT 1:47.5), as compared with those with NGU alone (GMT 1:8.6), confirms the data of Kousa and colleagues.<sup>18</sup> The fact that the humoral immune response to this micro-organism was profoundly different in the 2 groups of patients is further emphasised by the demonstration of serial changes in antibody titre in the SARA patients, a finding which has been seen less convincingly in the case of NGU. Thus, although some authors have reported seroconversion from the absence of detectable antibody to titres of 1:>8 in some patients with NGU,<sup>19, 20</sup> other workers have been unable to demonstrate this phenomenon at all.<sup>8, 21</sup> Serial studies were not performed on our patients with uncomplicated NGU owing to difficulties in maintaining long-term follow-up. No firm data are available on the duration of antibody persistence after NGU, but it is clear from our study that in SARA, whereas IgM antibody is usually short-lived, IgG may remain in the circulation for periods in excess of 1 year after all cessation of apparent disease.

It may be suggested that these changes in antibody titre are not peculiar to SARA. We therefore examined sera from patients with other rheumatic diseases, including ankylosing spondylitis, which resembles SARA in predominantly affecting HLA B27-positive individuals. In these patients, however, the antibody response to chlamydiae was comparable to that of healthy controls. We are aware, however, that groups of patients with chronic disease, whose sera were not examined at a similar early stage, may not constitute optimal controls. The possibility

that these high titres of antibody found in SARA have been produced as part of a polyclonal immune response remains, though the absence of abnormally high titres of antibody to common viruses tends to militate against this.

It has previously been reported that the presence of the HLA B27 antigen does not influence susceptibility to *C. trachomatis* infection *per se*,<sup>22</sup> and it is now clear from the present study that no direct relationship exists between the presence of B27 and titres of IgG or IgM chlamydial antibody. However, since high titres of antibody are associated with disease, and therefore epidemiologically with B27, it may be that they are influenced by HLA-linked immune response genes. Since such genes may lie in the HLA D region, an analysis of HLA DW and DRW antigens may provide information to support or refute this possibility.

The presence of antibody in synovial fluid raises the possibility of local production, which may in turn indicate the presence of chlamydial antigens within the joint. In the absence of information on local antibody production in human arthritis in which micro-organisms are known to be present in the joint these data are impossible to interpret with certainty. However, the fact that antibody to influenza virus was distributed almost equally between serum and synovial fluid suggests that both types of antibody are derived from the circulation rather than from local intra-articular sources.

Thus it appears that *C. trachomatis* infection could be an initiating factor in approximately 50% of patients with SARA. The exaggerated antibody response to this organism in these patients may have very important pathogenetic implications, and this phenomenon certainly requires further study. Other trigger factors, however, remain to be identified.

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