Male sex predominance in *Chlamydia trachomatis* sexually acquired reactive arthritis: are women more protected by anti-chlamydia antibodies?

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Abstract

Objective—To determine whether the humoral anti-chlamydia antibody response might be related to the ineffective bacterial elimination seen in patients with *Chlamydia trachomatis* reactive arthritis, particularly in men, who have a higher prevalence of the disease than women.

Methods—The number and specificity of the antibody responses to 27 different *C trachomatis* antigens were determined by western blots in serum samples from patients with *C trachomatis* urogenital infection, with and without reactive arthritis, with a special regard to the sex of the patients.

Results—Patients with reactive arthritis had antibodies to significantly fewer chlamydia antigens than those with urethritis only. Antibodies from men recognised significantly fewer antigens than antibodies from women. The IgA class antibodies were slightly more relevant than those of the IgG class for differentiation of patients with reactive arthritis from those with uncomplicated genitourinary infection.

Conclusions—In patients with acute *C trachomatis* infection the development of reactive arthritis may be related, particularly in men, to a deficient humoral response, to antigens which perhaps play a part in the clearance of the bacteria. Men who cannot generate antibodies to a large number of antigens may be less able to contain the local infection, allowing a wide systemic dissemination of the organisms to the joints.

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of viable bacteria to the joints, as shown by the synovial presence of primary ribosomal RNA transcripts and messenger RNAs.⁹ In patients who do not develop arthritis, we can assume either that the bacteria remain in the urogenital mucosa or are less widely transported to the joints because the bacterium's ability to reach them is a determinant for arthritis induction.¹⁰ Is the humoral immune response playing a part in protection by preventing systemic bacterial dissemination? Is the humoral immune response comparable in patients developing, and in those not developing, reactive arthritis? What bacterial antigens are recognised by this response? In 1987, Inman *et al* compared humoral immune responses of men with complicated (Reiter's syndrome) and uncomplicated courses of non-specific urethritis.¹¹ An immunoblotting technique did not show any pattern of humoral immune response to *C trachomatis* characteristic for Reiter's syndrome. However, both groups of patients were not defined as *C trachomatis* infected, and other micro-organisms (*Ureaplasma urealyticum*, *Mycoplasma*, *Neisseria gonorrhoeae*) might have been present in some patients. We therefore chose to investigate the differences in the serological response between two groups of patients infected with *C trachomatis*, one with reactive arthritis and one with uncomplicated urogenital infection, and to determine whether this response was related to the sex of the patient. The number and specificity of the antibody responses to 27 different *C trachomatis* LGV2 antigens were studied after separation of proteins on polyacrylamide gel by immunoblotting techniques.

Patients and methods

PATIENTS

Serum samples came from our serum collection and had been kept for various lengths of time at -70°C. The diagnoses of the patients were taken from their medical charts at the time of collection of the serum samples. The study subjects were categorised in two groups. Group 1 patients (n=19) had acute *C trachomatis* SARA: first episode of asymmetrical mono/oligoarthritis with urethritis and evidence of *C trachomatis* infection (three had positive findings for urethral/endocervical *C trachomatis* antigen by direct immunofluorescence, 14 had positive urethral/endocervical *C trachomatis* cultures, two had positive urethral *C trachomatis* DNA amplification with the Amplicor test (Roche Diagnostic Systems, Branchburg, NJ)); Table 1 indicates the presence of the HLA-B27

In a small percentage of patients exposure to *Chlamydia trachomatis* leads to the subsequent development of reactive arthritis.^{1,2} This disease is more common in men than women,²⁻⁶ whereas the occurrence of post-enteric reactive arthritis is nearly equal in both sexes.^{7,8} The reasons for the male sex predominance are not known. There is little information on *C trachomatis* immunity in men. Most studies have concentrated on the pathogenesis and immunology of the infection in women because the major morbidity associated with chlamydial disease, in western countries, is the development of infertility resulting from salpingitis.

In patients with *C trachomatis* sexually acquired reactive arthritis (SARA), protective immunity is inefficient, allowing dissemination

Table 1 Characteristics of patients with *C trachomatis* sexually acquired reactive arthritis

Patient No	Sex, age	HLA-B27	Time since onset of effusion (weeks)	Number of active joints	Number of responses observed in immunoblot		
					IgG	IgM	IgA
18	M, 31	+	3	3	5	1	4
22	M, 23	—	19	1	8	0	3
24	M, 25	+	8	1	9	4	7
25	M, 32	—	4	3	10	5	6
26	M, 26	+	1	1	11	2	6
27	M, 25	+	2	1	7	1	4
28	M, 55	+	6	4	4	2	6
31	M, 18	+	9	2	9	1	3
36	M, 31	ND	8	2	8	3	4
37	M, 20	+	26	1	19	2	1
38	M, 23	+	9	4	4	0	3
39	M, 28	—	1	1	9	1	4
40	M, 23	—	11	5	21	1	7
20	F, 19	—	2	2	17	2	5
23	F, 27	+	1	2	10	0	8
32	F, 21	ND	7	2	11	2	6
33	F, 38	—	3	2	11	4	9
34	F, 35	+	1	3	23	1	20
35	F, 36	ND	1	1	21	4	14

antigen, time since onset of effusion, and the number of active joints for each patient. Group 2 patients (n=42) had acute *C trachomatis* urogenital infection: positive findings on urethral/endocervical *C trachomatis* DNA amplification with the Amplicor test. They were recruited from a sexually transmitted diseases clinic. They were examined by a general practitioner/urologist and were considered not to have arthritis because they did not complain about joint pain.

At the time of serum collection, patients from both groups were not being treated except for those who received non-steroidal anti-inflammatory drugs.

Table 2 gives the median ages (in years), age range, and percentages of female subjects, for each group.

MEASUREMENTS OF SERUM IgG, IgM, AND IgA BY AN ENZYME LINKED IMMUNOSORBENT ASSAY
Measurements of serum IgG, IgM, and IgA were performed as previously described.¹²

SDS-PAGE AND IMMUNOBLOTS

Sample preparation

Elementary body antigens of *C trachomatis* LGV2 strain 434 were purchased from Bio-design International, Kennebunk, ME. These antigens were suspended (0.72 mg protein/ml) (bicinchoninic acid protein assay) in sample buffer (0.0625 M Tris-HCl, pH 6.8, 0.0005 M phenylmethylsulphonylfluoride, 10% (vol/vol) glycerol, 2% (wt/vol) sodium dodecyl sulphate (SDS), 0.0025% (wt/vol) bromophenol blue, 2.5% (vol/vol) 2-mercaptoethanol), heated to 100°C for five minutes, and centrifuged at 10 000 g for five minutes.

Antigen electrophoresis

Precast Tris-glycine polyacrylamide gels (10–27% with a 4% stacking gel; 2D well, 8 cm × 8 cm × 1 mm) were purchased from Novex, San Diego, CA. Two hundred microlitres of antigen sample (145 µg of protein) were loaded in the 2D well. Two microlitres of Novex prestained, multicoloured protein standard were loaded in the small well. Electrophoresis was performed for 120 minutes at a constant voltage of 125 V in an Xcell II vertical slab gel system (Novex). The running buffer consisted of 0.025 M Tris, 0.192 M glycine, 0.1% (wt/vol) SDS, pH 8.3.

Immunoblotting

C trachomatis antigens were transferred to a Hybond C-extra nitrocellulose membrane (7 cm × 8 cm) purchased from Amersham, England. The transfer was performed for one hour at a constant voltage of 40 V by semi-dry blotting in an Xcell II blot module (Novex) at room temperature. The transfer buffer consisted of 0.012 M Tris, 0.096 M glycine, 20% (vol/vol) methanol, pH 8.3. Efficient and homogeneous protein transfer was checked by silver staining.

Antigen detection

The blotted nitrocellulose membrane was cut into strips 4 mm in width, which were incubated overnight at 4°C in phosphate buffered saline (PBS) with 5% (wt/vol) bovine serum albumin (BSA) and 0.0003 M NaN₃ to block unspecific binding sites. The strips were washed four times for five minutes each time in PBS with 0.05% (vol/vol) Tween 20 (PBS-Tween) and incubated for two hours at 37°C in sera diluted 1:100 in PBS-Tween. The strips

Table 2 Number of humoral immune responses to *Chlamydia trachomatis* LGV2 antigens in infected patients with and without reactive arthritis

	Age (years) *	Number of responses observed in immunoblot		
		IgG mean (SD)	IgM mean (SD)	IgA mean (SD)
1 <i>Chlamydia trachomatis</i> sexually acquired reactive arthritis (n=19) No (%) of female patients	26 (18–55) 6 (32)	11.4 (5.9)	1.9 (1.5)	6.3 (4.4)
2 <i>Chlamydia trachomatis</i> urogenital infection (n=42) No (%) of female patients	27 (17–55) 15 (36)	17.4 (6.2)	2.8 (1.9)	11.0 (5.5)
Student's <i>t</i> test between groups 1 and 2		p<0.001	NS	p<0.002

*Median (range).

Figure 1 Example of immunoblot analysis. Each immunoblot was evaluated independently with a GS-700 imaging densitometer and the Molecular Analyst software, version 2.1 (Bio-Rad Laboratories, Hercules, CA). The apparent molecular weight (mol wt) of all distinguishable bands was determined by interpolation from the lanes obtained with a commercially available mol wt marker (range 250 to 4 kDa). A quadratic regression method was used to calculate a standard curve: the y axis displayed the relative mobility (Rf) values of the mol wt standards and the x axis displayed the corresponding mol wt.

were then washed 4 × 5 minutes and incubated for two hours at 37°C with the alkaline phosphatase conjugated F(ab')₂ fragment of polyclonal goat IgG antihuman IgG (Fc specific), IgM (μ chain specific), or IgA (α chain specific) diluted with PBS-Tween containing 5% (wt/vol) BSA. The strips were rinsed twice and washed 4 × 5 minutes in PBS-Tween and then rinsed 2 × 2 minutes in the predetection buffer (0.1 M Tris HCl, pH 8.8, 0.1 M NaCl, 0.005 M MgCl₂). The colour was developed by adding the predetection buffer containing 0.35 mM BCIP (5-bromo-4-chloro-3-indoyl phosphate) and 0.046 mM

NBT (nitroblue tetrazolium) in the predetection buffer. Colour development was stopped by washing the membrane with water after 5–10 minutes.

Analysis of immunoblots

To avoid biases due to limitations of reproducibility, at least two immunoblots were done with each serum sample and evaluated independently with a GS-700 imaging densitometer and the Molecular Analyst software, version 2.1 (Bio-Rad Laboratories, Hercules, CA). The apparent molecular weight (mol wt) of all distinguishable bands was determined by interpolation from the lanes obtained with a commercially available mol wt marker (range 250 to 4 kDa). A quadratic regression method was used to calculate a standard curve: the y axis displayed the relative mobility (Rf) values of the mol wt standards and the x axis displayed the corresponding mol wt. Only the 27 most frequently recognised antigens, with a mol wt range of 117 to 5 kDa, were considered. Each antigen was represented by its most commonly observed mol wt (± 1–2 kDa) (fig 1). The percentage of patients with serum antibodies against each antigen was calculated.

STATISTICAL ANALYSIS

Where appropriate, results were analysed by Student's *t* test and the χ^2 test.

Results

CHARACTERISTICS OF PATIENTS WITH C TRACHOMATIS SEXUALLY ACQUIRED REACTIVE ARTHRITIS

Although the mean time since onset of effusion had a tendency to be shorter for women, no significant difference was seen between male (mean (SD) 8.2 (7.3) weeks) and female (2.5 (2.3)) disease duration and no correlation was found between disease duration and the number of active joints or the number of responses observed in immunoblot. In the same way, no correlation was found between the presence of HLA-B27 antigen and the number of active joints or the number of responses seen in immunoblot, and no correlation was found between the number of active joints and the number of responses observed in immunoblot (table 1).

MEASUREMENTS OF SERUM IgG, IgM, AND IgA

There were no significant differences between patients with and without reactive arthritis in serum IgG (means 14.0 mg/ml and 12.2

Table 3 Different number of humoral immune responses to *Chlamydia trachomatis* LGV2 antigens between infected men and women with or without reactive arthritis

Patients without reactive arthritis				Patients with reactive arthritis			
		Number of responses observed in immunoblot				Number of responses observed in immunoblot	
Age (years) *		IgG mean (SD)	IgA mean (SD)	Age (years) *		IgG mean (SD)	IgA mean (SD)
Men (n=27)	25 (17–55)	15.3 (4.2)	9.9 (4.3)	Men (n=13)	25 (18–55)	9.5 (5.1)	4.5 (1.8)
Student's <i>t</i> test between patients without and with arthritis				p<0.001			
Women (n=15)	29 (17–54)	21.3 (7.5)	12.8 (7)	Women (n=6)	31 (19–38)	15.5 (5.6)	10.3 (5.7)
Student's <i>t</i> test between patients without and with arthritis				NS			
Student's <i>t</i> test between men and women		p=0.0018	NS			0.036	0.003

*Median (range).

Figure 2 Immunoblot evaluation of IgG responses to *C trachomatis* antigens, obtained with serum from a man with *C trachomatis* sexually acquired reactive arthritis (A) and from a non-arthritic man infected with *C trachomatis* (B).

mg/ml, respectively), IgM (0.9 mg/ml for both groups), and IgA (2.4 and 2.3 mg/ml) concentrations.

NUMBER OF ANTIBODY RESPONSES TO *C TRACHOMATIS* LGV2 ANTIGENS IN INFECTED PATIENTS WITH AND WITHOUT REACTIVE ARTHRITIS

The mean (SD) number of IgG (11.4 (5.9)) and IgA (6.3 (4.4)) responses found by western blot for patients with *C trachomatis* SARA were significantly lower than for patients with non-arthritic *C trachomatis* infection (17.4 (6.2) and 11.0 (5.5), respectively) but the number of IgM responses were not significantly different between the groups (table 2). When the results were expressed according to

the sex of the patients, the number of IgG (9.5 (5.1)) and IgA (4.5 (1.8)) responses seen for men with *C trachomatis* SARA was significantly lower than for women (15.5 (5.6) and 10.3 (5.7), respectively), and the number of IgG responses (15.3 (4.2)) seen for non-arthritic men infected with *C trachomatis* was significantly lower than for women (21.3 (7.5)). The differences in the number of responses between men with *C trachomatis* SARA and non-arthritic men were significant; for women the differences did not reach significance (table 3).

NUMBER OF PATIENTS WITH SERUM ANTIBODY RESPONSES TO THE DIFFERENT LGV2 *C TRACHOMATIS* ANTIGENS

Different immunoblot patterns were obtained according to the type of sample tested (fig 2). Among the 27 bacterial antigens considered, 13 were recognised significantly more often by antibodies of non-arthritic patients infected with *C trachomatis* than by those with reactive arthritis, especially by IgA. Seven of them (40, 36, 21, 18, 13, 10, and 6 kDa antigens) were recognised more frequently by both IgG and IgA and nine of them (71, 40, 36, 32, 27, 18, 13, 10, and 6 kDa antigens) were recognised significantly more frequently by IgA than by IgG. On the contrary, no antigen elicited a significantly more frequent response in patients with *C trachomatis* SARA than in non-arthritic patients (table 4).

No pattern of humoral immune response to *C trachomatis* was found to be specific for patients with *C trachomatis* SARA.

Discussion

Our purpose was to analyse carefully the humoral anti-chlamydia antibody response in order to determine whether a deficiency might be involved in the inefficient bacterial elimination seen in patients with reactive arthritis. Indeed, a recent study, using antibody deficient gene knockout mice, showed that both antibody and cell mediated immunity are probably required for resolution of *C trachomatis* infection.¹³

When the number of antibody responses to 27 different *C trachomatis* antigens was compared in two populations of infected patients, with and without reactive arthritis, we found that arthritic patients had antibodies to fewer chlamydia antigens than patients with urogenital infection without joint involvement. These deficiencies were more significant for IgA antibodies than for the other isotypes. As IgA is the primary humoral response on the mucosae and *C trachomatis* infection is mucosa associated, these IgA shortcomings may have a role in the disease development. Indeed, it has been shown that IgA may be the first line of defence against a chlamydial infection,¹⁴⁻¹⁶ mediating protection from reinfection in animal models.¹⁷

A study comparing the number of antibody responses to 14 different *C trachomatis* antigens, in Reiter's syndrome and patients with non-specific urethritis has already been reported by Inman *et al.*¹¹ When we applied a χ^2 test to their results, we found that the 78 kDa antigen was recognised significantly more often

Table 4 *Chlamydia trachomatis* LGV2 antigens differently recognised by infected patients according to the presence or absence of reactive arthritis

Antigens (kDa)	Percentage of patients with serum antibodies to <i>Chlamydia trachomatis</i> LGV2 antigens					
	IgG			IgA		
	Reactive arthritis (n=19)	Urogenital infection (n=42)	p*	Reactive arthritis (n=19)	Urogenital infection (n=42)	p*
99	32	67	0.011	21	42	NS
96	16	49	0.011	16	26	NS
72	16	42	0.039	5	21	NS
71	47	65	NS	21	56	0.0088
40	21	49	0.033	5	42	0.0033
36	42	79	0.005	21	65	0.0018
32	68	86	NS	21	65	0.0018
27	37	63	NS	16	51	0.011
21	42	81	0.0024	26	63	0.01
18	53	79	0.04	26	74	0.0005
13	47	86	0.0016	5	77	<0.0001
10	37	67	0.029	16	42	0.0039
6	32	65	0.018	0	33	0.0041

* χ^2 test between *C trachomatis* infected patients with or without reactive arthritis.

by IgG and the 32 kDa antigen by IgA from patients with non-specific urethritis, but the 59 and 57 kDa antigens were recognised significantly more often by IgG and IgA from patients with Reiter's syndrome. In the present study we did not find any antigen of about 60 kDa recognised significantly more often by serum antibodies of patients with *C trachomatis* SARA (IgG 68%; IgA 60%) than by those of non-arthritic infected patients (IgG 84%; IgA 88%). To further examine this point we also determined the IgG responses to a recombinant *C trachomatis* heat shock protein 60 and found no significant difference in prevalence between patients with and without reactive arthritis (data not shown). This discrepancy between both studies could be explained if some patients with non-specific urethritis were in fact infected by other micro-organisms (*Ureaplasma urealyticum*, *Mycoplasma*, *Neisseria gonorrhoeae*) or if patients with Reiter's syndrome had more chronic disease than our patients with *C trachomatis* SARA, at the time of serum collection.

To compare validly the humoral immune responses between *C trachomatis* infected patients with or without arthritis, the time period between *C trachomatis* infection and serum collection has also to be considered and must not significantly differ between the two groups. Actually, it is impossible to know the infection time, it is only possible to speculate about it. Patients with urethritis only provided serum samples at the time of the acute infection. Published reports show that the incubation period for symptomatic chlamydial urethritis may range from two to 35 days, but it usually lasts from seven to 14 days.¹⁸ Patients with *C trachomatis* SARA provided serum samples at the time of the acute arthritis (for the 19 patients, the median duration of disease was four weeks (range 1–26 weeks). Because the minimum interval between the onset of arthritis and the primary infection is thought to be a week and the maximum four to six weeks,^{19, 20} it is likely that at least some patients with *C trachomatis* SARA were infected for a longer time than patients without arthritis at the time the serum was drawn. However, if samples from patients with *C trachomatis* SARA were

collected in the declining phase of antibody production a lower number of responses seen in immunoblot should be found in those patients with the longest disease durations (Nos 37, 22, and 40), and this was not the case. In the same way, if samples from patients with urethritis only were provided very early after infection, they would be in the latent phase of antibody production and no specific IgG response would be expected. As no significant difference was found in the number of IgM responses, both groups of patients were more likely to have been in the exponential or in the steady state phase of antibody production.

When the responses to chlamydia antigens were examined according to the sex of the patients, a higher humoral response was generally seen for women than for men, and this difference was more pronounced for the group of patients with *C trachomatis* SARA. A higher prevalence of antibodies to *C trachomatis* in infected women compared with men has already been reported.^{21–23} Although, in the female genital tract, the contribution of antibodies to the protection against ascending *C trachomatis* infection is under debate. Some studies have shown their potential to reduce the risk and severity of pelvic inflammatory disease^{24, 25} and, another, their inefficiency.²⁶ Nevertheless, it is unlikely that the local immunity is identical in the male and female urogenital tracts. The more frequent immune responses seen in women compared with men has been partly attributed to the involvement of a larger area of epithelium.²⁷ It is also known that women's immune responses are stronger than those of men.^{28, 29} They have higher immunoglobulin levels and better responses to a variety of antigens.^{30, 31} Women generally resist a variety of bacterial infections more successfully than men.^{32, 33} This sex dimorphism is considered to be a result of the effects of sex hormones on the immune system. Androgens exert an inhibitory influence on both humoral and cell mediated immune responses, whereas oestrogens have a dual role—suppressing cell mediated, but enhancing humoral, immune responses.³⁴ The androgen effect on immunity may be more marked in HLA-B27 positive subjects (host factor reported to be present in

about 45% of patients with *C trachomatis* reactive arthritis³⁵), because this antigen is associated with high testosterone concentrations in men.³⁶

The reduced production of antibodies, found particularly in male arthritic patients, may also indicate a polarisation towards a proinflammatory, Th1-type response, with a decrease of immunoglobulin inducing mechanisms. As, during the extracellular phase, elementary bodies are vulnerable to antibody mediated effector mechanisms, a Th2-type response may be crucial in preventing the systemic dissemination of chlamydiae and reducing the number of organisms reaching the joint. Ghaem-Maghami *et al* have also suggested that, in trachoma, a humoral response may protect against colonisation and limit immunopathological events because chlamydia-specific antibodies were present in children with no evidence of ocular disease, whereas those with the most intense ocular inflammation had almost no antibody production of all isotypes and to all chlamydial antigens considered.³⁷

In this study we identified, by their apparent mol wt, 13 bacterial antigens recognised significantly more often by antibodies of non-arthritic patients, but as we do not know their identity, we can only speculate about them. To play a part in antibody mediated effector mechanisms, protective antigens should be immunoaccessible on native elementary bodies. Therefore, the components of the chlamydial outer membrane complex—the lipopolysaccharide (LPS), the major outer membrane protein (MOMP; 40 kDa), the cysteine-rich outer membrane proteins OMP2 (60/62 kDa) and OMP3 (12.5 kDa), and the polymorphic membrane proteins (98 kDa)³⁸—may be interesting antigens. However, the anti-LPS antibodies are known to be unable to protect against infection.³⁹ The main candidate antigen identified as target for neutralisation is the MOMP.⁴⁰ Two other have also been described—the macrophage inhibitory protein, a 27 kDa lipoprotein,²⁵ and the heat shock protein 70.⁴¹ Whether some of these 13 antigens, with appropriate apparent mol wt, might be one of them is only speculative.

From the present study, the humoral immune response in *C trachomatis* reactive arthritis does not appear to be similar to the response seen in yersinia reactive arthritis. Indeed, in yersinia infected patients, the immunoblotting analyses were similar in non-arthritic and arthritic patients,^{42,43} but when humoral immune responses to *Yersinia enterocolitica* O:3⁴⁴ or to the released protein of mol wt 36 kDa⁴⁵ were analysed, IgA antibody responses were found to be higher in arthritic than in non-arthritic patients. These different results may be related to a different pathophysiology (higher prevalence in men for *C trachomatis* SARA, presence of live chlamydia in the joint when enterobacteria seem not to be present in the joint, different responses to antibiotic treatment).⁴⁶

In conclusion, the results presented in this study provide some evidence that patients with *C trachomatis* SARA may lack protection

through humoral immunity because their serum antibodies recognised a lower number of target antigens than those from non-arthritic patients. They may be deficient in mounting an effective immune response, directed to the right components, and thus unable to clear *C trachomatis* rapidly and without sequelae. Thus chlamydia antigens can be deposited in the joint where they remain as a long term stimulus for cell mediated immune responses. The observation that the amount of chlamydial antigen in the joints of B cell deficient mice is markedly greater than in control animals lends credence to this explanation.⁴⁷ The striking predominance of men in this disease,²⁻⁶ not seen in post-dysenteric reactive arthritis, may be related to the effect of sex hormones on the local immune response in the early phase of infection. The degree of protection due to antibodies, higher in women than in men, may prevent wide dissemination of the bacteria through the circulation and may protect them from reactive arthritis. Even if women develop the disease, it has a somewhat milder clinical expression.⁴⁸

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