Rheumatology 2002;41:1017–1020

Importance of species-specific antigens in the serodiagnosis of *Chlamydia trachomatis* reactive arthritis

S. Bas, S. Genevay, M.-C. Schenkel and T. L. Vischer

Division of Rheumatology, Department of Internal Medicine, University Hospital, Geneva, Switzerland

Abstract

Objectives. To determine the most sensitive and specific method of anti-*Chlamydia* antibody measurement for the serodiagnosis of *Chlamydia trachomatis* reactive arthritis.

Methods. Immunoblotting, enzyme-linked immunosorbent assays using six synthetic peptides or recombinant antigens and a microimmunofluorescence test were used to determine the presence of IgG, IgM and IgA in serum samples from 17 patients with *C. trachomatis* reactive arthritis. Twenty patients with other inflammatory arthropathies without evidence of urogenital *C. trachomatis* infection were used as controls.

Results. The best association of sensitivity (76%) and specificity (85%) was obtained when IgG and/or IgA reactivity to two species-specific antigens was determined. These antigens were synthetic peptides, derived from species-specific epitopes in the variable domain IV of the major outer membrane protein (MOMP) (Labsystems, Finland) and recombinant polypeptide encoded by open reading frame 3 of the plasmid (pgp3).

Conclusions. IgG and/or IgA anti-MOMP-derived peptides and anti-pgp3 could be useful for the diagnosis of probable *C. trachomatis* reactive arthritis.

KEY WORDS: *Chlamydia trachomatis*, Sexually acquired reactive arthritis, Antibodies, MOMP, Pgp3.

Reactive arthritis due to *Chlamydia trachomatis* is not always associated with obvious urogenital symptoms. Moreover, evidence of *C. trachomatis* infection by positive urethral/endocervical culture of bacteria or positive antigen or DNA detection can be missing at the time of the arthritis, and the diagnosis of the triggering infection relies on serology. However, both the sensitivity and specificity of serological tests need to be improved [1].

In two previous studies, we compared serum anti-*Chlamydia* antibody responses, obtained for patients with well defined urethral/endocervical infection, with those obtained for healthy blood donors. The best sensitivity (86%) with a specificity of 81% was obtained for immunoblot results, when the number of individuals with $\geqslant 10$ IgG and/or $\geqslant 2$ IgM responses to the different *C. trachomatis* antigens was considered [2]. Concerning antibody responses to peptides or recombinant antigens, the best sensitivity (79%) associated with the best specificity (82%) was obtained when IgG responses to both synthetic peptides, derived from species-specific epitopes in the variable domain IV of the major outer membrane protein (MOMP) (Labsystems, Finland) and recombinant polypeptide encoded by open reading frame 3 of the plasmid (pgp3) were considered [3]. MOMP is a surface-exposed, integral membrane protein of approximately 40 kDa, found in both the extracellular infectious elementary bodies and the non-infectious intracellular reticulate bodies. Pgp3 is a protein of approximately 27 kDa, predominantly found in chlamydial outer membrane complex preparation [4].

Therefore, we evaluated whether these new assays can also increase sensitivity and specificity of the serological diagnosis of *C. trachomatis* reactive arthritis. The specificity was evaluated from samples of patients with inflammatory arthropathies without evidence of *C. trachomatis* infection, half of the cases being other bacterial arthropathies. Such controls are necessary in view of unspecific reactions possibly due to inflammation and/or presence of cross-reacting antibodies, due to other bacterial infections.

Patients and methods

Patients

Diagnosis was taken from the chart at the time of collection of the sera. They come from patients divided into the two following groups:

Submitted 17 September 2001; revised version accepted 13 March 2002.

Correspondence to: S. Bas, Research Laboratory, Division of Rheumatology, University Hospital, 1211 Geneva 14, Switzerland.

(i) *Chlamydia trachomatis* sexually-acquired reactive arthritis (*C. trachomatis*-SARA) ($n=17$): asymmetrical mono/oligoarthritis with urethritis and evidence of *C. trachomatis* infection [three had a positive urethral/endocervical *C. trachomatis* antigen detection by direct immunofluorescence, 11 had a positive urethral/endocervical *C. trachomatis* culture, three had a positive urethral *C. trachomatis* DNA amplification with the Amplicor test of Roche Diagnostic Systems Inc. (Branchburg, NJ)]; median age, 26 yr (range 18–38), 29% of female patients, 62% of HLA-B27 positive patients.

(ii) Inflammatory arthropathies unrelated to *C. trachomatis* ($n=20$): gout ($n=6$), septic arthritis ($n=5$), Lyme arthritis (diagnosis confirmed by western blot) ($n=3$), rheumatoid factor positive rheumatoid arthritis ($n=3$), post-dysenteric arthritis ($n=2$) and oligoarthritis with positive *Salmonella enteritidis* serology ($n=1$); median age, 39 yr (range 17–73), 25% of female patients.

Immunoblot analyses of sera

They were performed as previously described [5].

Recombinant protein preparations and measurement of antibodies to hsp70, OMP2, hsp60 and pgp3 by enzyme-linked immunosorbent assays (ELISA), measurement of antibodies to C. pneumoniae, MOMP-derived peptides and LPS by commercially available ELISA and microimmunofluorescent (MIF) tests

Experimental conditions were previously described [2, 3].

Calculations

Sensitivity, specificity and agreement were calculated as described previously [6].

Statistical analysis

Where appropriate, results were analysed by the chi-square test.

Results

Diagnostic value of the different antibody determinations

A significantly higher number of *C. trachomatis* reactive arthritis patients were found to have IgG antibodies to MOMP-derived peptides, pgp3 and OMP2, and IgA antibodies to MOMP-derived peptides, when compared with the controls. The highest difference between both groups was observed when IgG response to both MOMP-derived peptides and pgp3 was examined ($P=0.0006$) (Table 1).

When positive responses to individual antigens were considered, the highest sensitivity was obtained for IgG and/or IgA anti-OMP2 (100%) but with a low specificity (45%) and the highest specificity for IgG and/or IgA anti-pgp3 (90%) with a sensitivity of 59%. When positive responses to a combination of two antigens were considered, the best diagnostic value was obtained for IgG and/or IgA responses to MOMP-derived peptides and pgp3 (sensitivity, 76%; specificity, 85%; agreement, 81%) (Table 2).

TABLE 1. Percentages of patients with serum antibodies to recombinant or synthetic *Chlamydia* antigens determined by ELISA

		Patients with		
		C. trachomatis reactive arthritis ($n=17$)	Inflammatory arthropathies independent of *C. trachomatis* ($n=20$)	Chi-test *P*
C. pneumoniae (Labsystems)	IgG	60[a]	42[b]	ns
	IgM	0[a]	0[b]	ns
	IgA	10[a]	26[b]	ns
Chlamydia LPS (Medac)	IgG	88	70	ns
	IgM	6	5	ns
	IgA	65	35	ns
C. trachomatis MOMP-derived peptides (Labsystems)	IgG	47	15	0.034
	IgA	29	5	0.045
Recombinant *C. trachomatis* proteins prepared in our laboratory				
hsp70	IgG	35	35	ns
	IgA	0	0	ns
hsp60	IgG	65	45	ns
	IgA	18	5	ns
pgp3	IgG	59	10	0.0016
	IgA	6	5	ns
OMP2	IgG	100[a]	55[c]	0.015
	IgA	20[a]	27[c]	ns
Best combination of antigens				
MOMP-derived peptides + pgp3	IgG	71	15	0.0006
	IgA	29	10	ns

[a] $n=10$; [b] $n=19$; [c] $n=11$.
ns, not significant.

A sensitivity of 69% with a specificity of 75% was obtained for immunoblot results, when the number of individuals with $\geqslant 10$ IgG and/or $\geqslant 2$ IgM responses to the different *C. trachomatis* antigens was considered (data not shown).

The MIF test was used to determine the presence of anti-*C. trachomatis* antibodies in seven samples from patients with *C. trachomatis* reactive arthritis. For IgG measurement, 14% were found to be positive whereas 71% of these samples had IgG antibodies to the MOMP-derived peptides and pgp3 ($P=0.031$). For IgA, no sample was found to be positive with MIF test whereas 29% had IgA antibodies to the MOMP-derived peptides and pgp3 (not significant) (data not shown).

Discussion

This study was performed to improve the serodiagnosis of *C. trachomatis* reactive arthritis. The MIF test, still considered as the gold standard for chlamydial serology, was used to determine the presence of anti-*C. trachomatis* antibodies in some samples from patients with *C. trachomatis* reactive arthritis. Very poor sensitivities were obtained: 14% for IgG and 0% for IgA whereas, when the MOMP-derived peptides and pgp3 were used as antigens, the sensitivities were 71 and 29%, respectively. These results are similar to those we obtained with samples from patients with acute *C. trachomatis* urogenital infection [2]. Several studies have also reported that MIF sensitivity and specificity were lower than generally appreciated [7–9] and MIF IgA has been found to be the least sensitive assay in a study comparing five different serological tests [10].

In a previous study, comparing acute *C. trachomatis* infected patients with healthy blood donors, we obtained the best results with immunoblots, when the number of individuals with $\geqslant 10$ IgG and/or $\geqslant 2$ IgM responses was considered (sensitivity, 86%; specificity, 81%) [2]. In the present study, using as controls, patients with inflammatory arthropathies unrelated to *C. trachomatis*, immunoblot results were found to be less sensitive (69%) and specific (75%). This lower sensitivity should be related to the observation we made earlier that significantly fewer *Chlamydia* antigens were recognized on the blots, by sera from patients with reactive arthritis than by those with uncomplicated *C. trachomatis* genito-urinary infection [5]. The lower specificity obtained in this study, with samples from patients with inflammatory arthropathies, compared with the one obtained with samples from healthy blood donors [2] suggests that some antibodies might be elicited by other micro-organisms, as 50% of these control patients had another bacterial infection. This hypothesis is also supported by low specificities obtained for IgG binding to other antigens tested in ELISA: LPS (30%), OMP2 (45%) and hsp60 (55%). As LPS and OMP2 are genus-specific antigens, low specificities obtained with them can be attributed to cross-reactivity with *C. pneumoniae* as 42% of these patients had IgG antibodies to it. If cross-reactivity to *C. pneumoniae* can also be involved for hsp60 recognition [3], cross-reactivity to other bacterial hsp60 is also possible because, for half of them, the arthropathy was dependent on *Borrelia burgdorferi*, *Brucella* or *Staphylococcus aureus*.

The most appropriate tests for the serodiagnosis of patients with *C. trachomatis* reactive arthritis were found to be IgG and/or IgA reactivity to MOMP-derived peptides + pgp3. Compared with a previous study [1], a lower sensitivity was obtained for MOMP-derived peptide antibodies, due to a modification of the cut-off calculation indicated by the manufacturer (Labsystems).

In conclusion, since *C. pneumoniae* infections are common and able to elicit cross-reacting antibodies, such as anti-LPS, -OMP2 or -hsp60 and since other bacterial infections, involved in inflammatory arthropathies, are also able to elicit cross-reacting antibodies such as anti-hsp60, only species-specific antigens, such as MOMP-derived peptides or pgp3, should be used.

TABLE 2. Best sensitivities, specificities and agreement obtained for the different anti-*Chlamydia* antibody assays

	Sensitivity (%)	Specificity (%)	Agreement (%)
IgG and/or IgA anti-LPS	94	20	54
IgG and/or IgA anti-MOMP-derived peptides	53	85	70
IgG and/or IgA anti-hsp60	71	50	59
IgG and/or IgA anti-pgp3	59	90	76
IgG and/or IgA anti-OMP2	100	45	71
IgG and/or IgA anti-LPS + MOMP-derived peptides	94	20	54
IgG and/or IgA anti-LPS + hsp60	94	20	54
IgG and/or IgA anti-LPS + pgp3	94	20	54
IgG and/or IgA anti-LPS + OMP2	100	18	57
IgG and/or IgA anti-MOMP-derived peptides + hsp60	82	50	65
IgG and/or IgA anti-MOMP-derived peptides + pgp3	76	85	81
IgG and/or IgA anti-MOMP-derived peptides + OMP2	100	45	71
IgG and/or IgA anti-hsp60 + pgp3	82	50	65
IgG and/or IgA anti-hsp60 + OMP2	100	36	67
IgG and/or IgA anti-pgp3 + OMP2	100	45	71

Positive patients were 10–17 patients with *C. trachomatis* reactive arthritis and negative patients were 11–20 patients with inflammatory arthropathies independent of *C. trachomatis*.

The improvement of sensitivity and specificity obtained with recombinant pgp3 prepared under native conditions (in order to retain an antigenic structure able to detect antibodies directed to conformational epitopes) is shown for the first time in the serodiagnosis of this disease. Finally, if the MIF test is the most documented serological method and is commonly accepted as the reference assay, we did not find it as the best test. ELISA using MOMP-derived peptides or pgp3 are rapid methods, completely objective, giving reproducible results, easier to perform than the MIF test and appeared to be more helpful in the serodiagnosis of *C. trachomatis* reactive arthritis. However, due to the difficulty of having well characterized patients with this uncommon disease, the number of samples tested was small and the results obtained with these tests should be confirmed in bigger studies.

Acknowledgements

The technical assistance of Yvette Froment, Ursula Spenato and Monique Stucker is gratefully acknowledged. This study was supported by grant nos 32-47299.96 and 3200-061500.00 from the Fonds National Suisse de la Recherche Scientifique.

References

1. Bas S, Vischer TL. Chlamydia trachomatis antibody detection and diagnosis of reactive arthritis. Br J Rheumatol 1998;37:1054–9.
2. Bas S, Muzzin P, Ninet B, Bornand JE, Scieux C, Vischer TL. Chlamydial serology: comparative diagnostic value of immunoblotting, microimmunofluorescence test, and immunoassays using different recombinant proteins as antigens. J Clin Microbiol 2001;39:1368–77.
3. Bas S, Muzzin P, Vischer TL. *Chlamydia trachomatis* serology: diagnostic value of outer membrane protein 2 compared with that of other antigens. J Clin Microbiol 2001;39:4082–5.
4. Comanducci M, Cevenini R, Moroni A *et al*. Expression of a plasmid gene of *Chlamydia trachomatis* encoding a novel 28 kDa antigen. J Gen Microbiol 1993;139:1083–92.
5. Bas S, Scieux C, Vischer TL. Male sex predominance in *Chlamydia trachomatis* sexually acquired reactive arthritis: are women more protected by anti-chlamydia antibodies? Ann Rheum Dis 2001;60:605–11.
6. Bas S, Cunningham T, Kvien TK, Glennas A, Melby K, Vischer TL. The value of isotype determination of serum antibodies against *Chlamydia* for the diagnosis of *Chlamydia* reactive arthritis. Br J Rheumatol 1996;35:542–7.
7. Moss TR, Darougar S, Woodland RM, Nathan M, Dines RJ, Cathrine V. Antibodies to *Chlamydia* species in patients attending a genitourinary clinic and the impact of antibodies to *C. pneumoniae* and *C. psittaci* on the sensitivity and the specificity of *C. trachomatis* serology tests. Sex Transm Dis 1993;20:61–5.
8. Biendo M, Eb F, Lefebvre JF, Orfila J. Limits of the microimmunofluorescence test and advantages of immunoblotting in the diagnosis of chlamydiosis. Clin Diagn Lab Immunol 1996;3:706–9.
9. Wagenvoort JH, Koumans D, van de Cruijs M. How useful is the *Chlamydia* micro-immunofluorescence (MIF) test for the gynaecologist? Eur J Obstet Gynecol Reprod Biol 1999;84:13–5.
10. Clad A, Freidank HM, Kunze M *et al*. Detection of seroconversion and persistence of *Chlamydia trachomatis* antibodies in five different serological tests. Eur J Clin Microbiol Infect Dis 2000;19:932–7.