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SYNOVIAL FLUID AND SERUM ANTIBODIES AGAINST *CHLAMYDIA* IN DIFFERENT FORMS OF ARTHRITIS: INTRA-ARTICULAR IgA PRODUCTION IN *CHLAMYDIA* SEXUALLY ACQUIRED REACTIVE ARTHRITIS

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SUMMARY

Since the presence of *Chlamydia* has been shown in synovial fluid (SF) from some patients with *Chlamydia* reactive arthritis, we investigated whether anti-*Chlamydia* antibodies present in the joint are derived from the circulation or are locally produced. We compared titres of IgG, IgM and IgA antibodies against *Chlamydia*, and against a control antigen (tetanus toxoid), by an enzyme-linked immunosorbent assay (ELISA), in paired samples of serum and SF from *Chlamydia trachomatis* sexually acquired reactive arthritis (CT-SARA) patients and from patients with other forms of arthritis. The ratio of serum/SF IgA anti-*Chlamydia* antibodies was significantly decreased in CT-SARA patients. It is concluded that, in our experimental conditions, we found evidence for intra-articular production of IgA anti-*Chlamydia* antibodies.

KEY WORDS: *Chlamydia* antibodies, Synovial fluid, Enzyme-linked immunoassay.

CERTAIN infections of the urogenital tract, such as those caused by *Chlamydia trachomatis*, are sometimes followed by the development of reactive arthritis [1]. The diagnosis of the triggering infection relies on clinical symptoms, isolation of *Chlamydia* from the urogenital tract and antibody determination. The role of serological testing in the diagnosis remains controversial because serum antibodies against *Chlamydia* are frequent in the general population. Therefore, it could be important to investigate joint production of anti-*Chlamydia* antibodies in *C. trachomatis* sexually acquired reactive arthritis (CT-SARA) patients and to examine whether measuring specific isotypes of anti-*Chlamydia* antibodies in synovial fluid (SF) can improve the diagnosis of *Chlamydia* reactive arthritis. The presence of chlamydial antigens with a strongly suggestive appearance of elementary and reticulate bodies has been demonstrated in articular material of patients with arthritis following chlamydial infection [2–7]. Whole organisms could be present in some cases since the presence of chlamydial RNA and DNA was shown in SF and in synovium [8–11]. Thus, stimulation of B cells by *Chlamydia* antigens in the joints of CT-SARA patients could be expected with consequent higher antibody concentration in SF than in serum, depending on the equilibrium between both compartments. Two studies have suggested that specific antibody production occurs in the joint. Inman *et al.* [12] found that the concentration of total polymeric IgA was higher in SF than in serum in patients with Reiter's disease and reactive arthritis, and Hughes *et al.* [13] found higher titres of SF chlamydial IgG antibodies in some patients with SARA and seronegative oligoarthritis. However, no significant differences were found by Sieper *et al.* [14] in the antibody levels against CT between serum and SF samples from patients with undifferentiated oligoarthritis and reactive arthritis.

In order to determine whether *Chlamydia* antibodies present in the SF are derived from the circulation or are locally produced, and whether their isotype concentrations could be helpful for reactive arthritis diagnosis, we compared titres of IgG, IgM and IgA antibodies against *Chlamydia* and against a control antigen [tetanus toxoid (TT)] by an enzyme-linked immunosorbent assay (ELISA) method, in paired samples of serum and SF from CT-SARA patients and from patients with other forms of arthritis. Since there are less immunoglobulins in SF than in serum [15, 16], we calculated the results both in units/ml of serum or SF and in units/mg of immunoglobulins of the corresponding isotype.

The prevalence of patients and healthy blood donors with serum anti-*Chlamydia* antibodies is discussed in the accompanying paper.

PATIENTS AND METHODS

Only points specific to this paper are mentioned, the rest of this section is explained in the accompanying paper.

Patients

SF samples came from our collection. They were centrifuged (1600 *g* for 10 min) and aliquots of the supernatants were kept for various times at −70°C.

Patients were divided into the following groups. 1. CT-SARA ($n = 17$): three had a positive urethral/endocervical *Chlamydia* antigen detection by direct immunofluorescence (IF), 13 had a positive urethral/

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TABLE I

Chlamydia-specific antibodies expressed in units/mg of immunoglobulins of the same isotype [mean ± S.D. (% of patients with antibody concentration greater than the mean serum values of controls + 3 S.D.)*]

Group	Age†	IgG	IgM	IgA	
Chlamydia trachomatis sexually acquired reactive arthritis	25 (12%)	Serum	449 ± 728 (24%)	3500 ± 3762 (24%)	310 ± 196 (35%)
(n = 17)	18–58	SF	503 ± 943 (29%)	4486 ± 4285 (41%)	427 ± 260 (53%)
Sexually acquired reactive arthritis	39 (12%)	Serum	381 ± 689 (12%)	3968 ± 9111 (12%)	568 ± 773 (41%)
(n = 17)	22–62	SF	325 ± 455 (18%)	1976 ± 1431 (12%)	592 ± 666 (41%)
Undifferentiated seronegative mono/oligoarthritis	42 (46%)	Serum	227 ± 343 (12%)	1675 ± 1778 (15%)	258 ± 323 (23%)
(n = 26)	16–59	SF	368 ± 832 (15%)	1874 ± 2161 (15%)	533 ± 885 (35%)
Rheumatoid arthritis (n = 6)	59 (83%)	Serum	83 ± 109 (0%)	1547 ± 1373 (0%)	237 ± 182 (17%)
	45–75	SF	123 ± 140 (0%)	1155 ± 1083 (0%)	343 ± 284 (33%)
Crystal-induced arthritis (n = 13)	51 (31%)	Serum	120 ± 122 (0%)	1656 ± 1869 (8%)	240 ± 226 (15%)
	33–83	SF	153 ± 154 (0%)	1824 ± 1430 (8%)	168 ± 113 (0%)
Mechanical arthropathies (n = 9)	63 (33%)	Serum	177 ± 131 (0%)	2299 ± 2114 (22%)	218 ± 180 (11%)
	22–80	SF	204 ± 189 (0%)	2191 ± 2270 (22%)	347 ± 244 (44%)
Controls (blood donors) (n = 100)	44 (37%)	Serum	118 ± 147 (2%)	1139 ± 1094 (2%)	98 ± 110 (1%)
	22–68				

*IgG anti-*Chlamydia*: 559, IgM: 4421, IgA: 428.
†Median (% of female patients) range.

endocervical *Chlamydia* culture and had been included in a study on reactive arthritis [17], one had a positive urethral *Chlamydia* DNA amplification with the Amplicor test of Roche Diagnostic Systems Inc., Branchburg; HLA-B27: eight positive, eight negative patients, one not determined. 2. SARA (n = 17): 12 had a negative urethral/endocervical *Chlamydia* detection, for five patients the detection was not performed; HLA-B27: five positive, seven negative patients, five not determined. 3. Undifferentiated rheumatoid factor-negative mono/oligoarthritis (n = 26): HLA-B27: three positive, 13 negative patients, 10 not determined. 4. Rheumatoid factor-positive rheumatoid arthritis (RA) (n = 6). 5. Crystal-induced arthritis (n = 13): gout (7), chondrocalcinosis (6). 6. Mechanical arthropathies (n = 9): osteoarthritis (3), post-traumatic arthropathies (3), meniscus lesion (2), femoropatellar chondropathies (1).

Materials

Standard human serum was purchased from Behringwerke AG, Marburg, Germany, and TT from Institut Sérothérapique et Vaccinal Suisse, Berne.

Measurements of IgG, IgM and IgA by ELISA

Microtitre plates were 'coated' with the $F(ab')_2$ fragment of polyclonal goat IgG anti-human IgG or IgM or IgA (10 μg/ml). A commercial serum was used

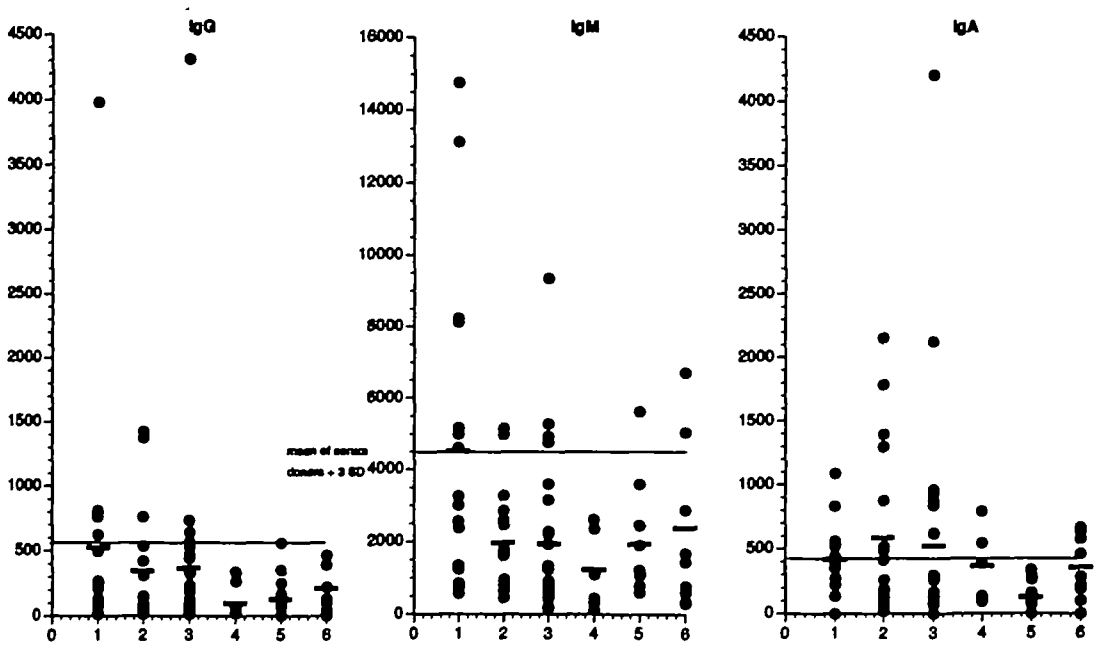

FIG. 1.—Distribution of synovial fluid anti-*Chlamydia* antibody concentrations expressed in units/mg of immunoglobulins of the same isotype. 1. *Chlamydia trachomatis* sexually acquired reactive arthritis (CT-SARA), n = 17; 2. SARA (negative or not performed urethral/endocervical *Chlamydia* detection), n = 17; 3. undifferentiated seronegative mono/oligoarthritis, n = 26; 4. rheumatoid factor-positive rheumatoid arthritis, n = 6; 5. crystal-induced arthritis, n = 13; 6. mechanical arthropathies, n = 9.

TABLE II
Ratios of serum/SF *Chlamydia*- and tetanus toxoid-specific antibody concentrations expressed in units/mg of immunoglobulins of the same isotype (mean ± S.D.)

Group	IgG anti-*Chlamydia*	IgG anti-TT	IgM anti-*Chlamydia*	IgM anti-TT	IgA anti-*Chlamydia*	IgA anti-TT
Patients from group 1	0.96 ± 0.38	0.83 ± 0.43	0.87 ± 0.38	1.01 ± 0.39	0.72 ± 0.25	1.05 ± 0.57
$n = 16$: *Chlamydia trachomatis* sexually acquired reactive arthritis						
t-test between *Chlamydia* and TT results	ns		ns		$P = 0.05$	
Patients from groups 5 and 6	0.96 ± 0.61	1.47 ± 1.09	0.92 ± 0.31	1.58 ± 1.29	1.26 ± 0.82	2.42 ± 3.02
$n = 15$: 5 chondrocalcinosis, 5 gout, 2 post-traumatic arthropathies, 2 meniscus lesion, 1 osteoarthritis						
t-test between *Chlamydia* and TT results	ns		ns		ns	

as standard. The other conditions were similar to those described in the accompanying paper.

Measurements of anti-TT antibodies by ELISA

Microtitre plates were 'coated' with TT (3300 Lf/ml, 1.41 mg of protein/ml) diluted at 2×10^{-4} and the other conditions were similar to those described in the accompanying paper.

Statistical analysis

Results are expressed as the mean ± S.D. Ratios of serum to SF *Chlamydia*- and TT-specific antibody concentrations, expressed in units/mg of immunoglobulins of the same isotype, were compared using paired Student's *t*-test.

RESULTS

Serum and SF Chlamydia-specific antibody concentrations

The results, expressed in units/mg of immunoglobulins of the corresponding isotype, are presented in Table I. The CT-SARA patients had the highest means of IgG antibodies against *Chlamydia* in serum and SF (but less than the mean of serum control values + 3 S.D.) and the highest mean of IgM in SF (greater than the mean of serum control values + 3 S.D.). The SARA patients had the highest mean of IgM in serum (but less than the mean of serum control values + 3 S.D.) and the highest means of IgA both in serum and SF (greater than the mean of serum control values + 3 S.D.). The means of antibodies in SF tended to be higher than in serum, in all groups tested.

The prevalence of patients with positive results for chlamydial antibodies was highest in the group of patients with CT-SARA both in serum and SF, except for IgA antibodies in serum where the prevalence was highest in the group of SARA patients.

No difference was observed between the HLA-B27-positive and -negative patients, either for the mean antibody concentration of the different isotypes or for the prevalence of positive values.

SF concentrations of antibodies against Chlamydia

The distributions obtained for IgG, IgM and IgA anti-*Chlamydia* antibodies, expressed in units/mg of immunoglobulins of the same isotype, in the six diagnostic groups of patients are given in Fig. 1. Patients with an IgG value higher than the mean + 3 S.D. of blood donor serum values, considered positive for *Chlamydia* antibodies, were observed only in groups 1, 2 and 3 (CT-SARA, SARA and undifferentiated seronegative mono/oligoarthritis). Patients with IgM and IgA values higher than the mean + 3 S.D. of blood donor serum values were observed in all groups tested, except group 4 (RA) for IgM and group 5 (crystal-induced arthritis) for IgA.

Ratios of serum to SF Chlamydia- and TT-specific antibody concentrations expressed in units/mg of immunoglobulins of the same isotype

These ratios were only determined for patient groups 1, 5 and 6 when sufficient material was still available. The results obtained for CT-SARA patients (group 1) compared to those obtained for crystal-induced arthritis and mechanical arthropathies (group 5 and 6) are presented in Table II.

The means of the ratios were not significantly different when we compared results obtained for the same patients with *Chlamydia* or TT as antigen, except for IgA of CT-SARA patients where the ratio calculated for the *Chlamydia* antigen was lower than that calculated for TT.

DISCUSSION

From the present study, we observed that the groups of patients with CT-SARA and SARA had the highest means of serum and SF antibodies against *Chlamydia*. The existence of an intra-articular specific B-cell response in CT-SARA patients and its possible diagnostic importance could be documented by comparing the chlamydial antibody concentrations in both serum and SF. The ratios of serum to SF chlamydial antibody concentrations expressed in units/ml of sample were always higher than one (the value representing antibody concentration equality between serum and SF) (data not shown). However, it is known that the Ig concentration is lower in SF than in serum: in normal joint fluid, the IgG concentration is ~25–30% of the serum IgG concentration [15, 16], indicating that some degree of a blood–joint barrier exists. In the RA inflammatory joint, permeability increases over 40 times for macroglobulins [18]. Consequently, as the degree of joint inflammation varies between the different patients, an appropriate

comparison between both compartments has to be made by expressing the results in units/mg of immunoglobulins of the same isotype, present in the same sample. When our results are expressed in units/mg of the corresponding immunoglobulins, chlamydial antibody concentrations tended to be higher in SF than in serum, in all groups tested. The increase in antibody concentrations in SF could be explained by local antibody production. If intra-articular stimulation by antigen could be possible for patients with CT-SARA, SARA or undifferentiated seronegative mono/oligoarthritis, it is unlikely for the other patients and could be due to non-specific phenomena, such as the extensive transudation of proteins through the inflamed synovial membrane. Indeed, activation of B cells could occur inside the joint or outside with subsequent transudation of antibody into the joint fluid, or vice versa. In order to differentiate between transudation and local production, we examined the B-cell response directed to TT, a common antigen, without a known link to arthritis and assumed to be not compartmentalized to the joint. In all groups of patients studied, samples with *Chlamydia* and TT antibody level higher in SF than in serum were found, which is likely to be due to antigen-independent reactions. During inflammation, B cells could be present in the joint due to non-specific recruitment. It is possible that upregulation of adhesion molecules on the surfaces of endothelial cells and B cells is sufficient to increase their entry into the joint without regard to antigen specificity. Non-specific recruitment of the memory cells can also occur [19]. A general stimulation of B cells locally present due to different factors of inflammation or to more efficient T-cell help or to differences in the antigen-presenting cell population within the joint as compared to peripheral blood might be involved [20]. However, for IgA of the CT-SARA patients, the mean of the ratios of serum to SF antibody concentrations calculated for the *Chlamydia* antigen was significantly lower than that calculated for TT. From our results, an intra-articular IgA production in response to local microbial antigen is therefore likely. Our results agree with those of Inman *et al.* [21] who observed an increase in *Chlamydia*-specific IgA in the SF during the disease process in a patient with Reiter's syndrome. This observation is in line with a report of intra-articular production of *Salmonella* antibodies of the IgA2 subclass [22]. Whether intra-articular stimulation exists only for this isotype is unknown. Even if the differences between CT and TT are not significant for IgG and IgM antibodies, the SF may have contained locally produced antibodies and some of these SF antibodies could have been bound to local *Chlamydia* antigens and remained undetectable.

To explain the higher amount of antibodies in the SF, two mechanisms could co-exist: an antigen-driven stimulation only detectable with IgA in our study and non-specific phenomena. In order to know whether IgG or IgM *Chlamydia* antibodies present in the SF are derived from the circulation or are locally produced, other investigations could be carried out, such as the use of other antigens with *Chlamydia* epitopes more closely involved in reactive arthritis, the comparative analysis of antibody specificities in both compartments or the demonstration of a relative accumulation of antibody-secreting cells in joint fluid.

To summarize, in our experimental conditions, the measurement of specific isotypes of anti-*Chlamydia* antibodies in SF cannot improve the diagnosis of *Chlamydia* reactive arthritis. However, an intra-articular production of IgA anti-*Chlamydia* antibodies was observed.

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