Indirect evidence of intra-articular immunoglobulin G synthesis in patients with *Chlamydia trachomatis* reactive arthritis

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Abstract

**Objectives.** To investigate whether B-cell stimulation occurs in joints of *Chlamydia trachomatis* reactive arthritis patients by comparing the immunoglobulin G (IgG) anti-*C. trachomatis* antibody responses in serum and synovial fluid (SF).

**Methods.** The number and spectrum of *C. trachomatis* antigens recognized by paired serum and SF samples from 16 patients with *C. trachomatis* reactive arthritis and 20 patients with other inflammatory arthropathies independent of this bacteria, were studied by immunoblotting. The responses to five different *Chlamydia* antigens were also determined in enzyme-linked immunosorbent assays.

**Results.** In *C. trachomatis* reactive arthritis patients, a higher number of *C. trachomatis* antigens was recognized by SF (17.6 ± 5.1) than by serum (11.1 ± 6.3) IgG and a higher intensity of SF IgG binding to the outer membrane protein 2 (OMP2) was observed.

**Conclusions.** These results suggest an intra-articular IgG production and a possible role of some *Chlamydia* antigens like OMP2 in the pathogenesis of *C. trachomatis* reactive arthritis.

**Key words:** *Chlamydia trachomatis*, Reactive arthritis, IgG, Joint, Synovial fluid, Immunoblotting, Outer membrane protein 2.

*Chlamydia trachomatis* is a common human pathogen which causes reactive arthritis in a low percentage of patients [1]. Persistence of chlamydial antigens [2] or repeated infections [3] might play a role in the pathogenesis of the disease. The possibility that this bacterium also plays a role in other arthropathies could also be considered, as the presence of *C. trachomatis* antigens, DNA and RNA in joints can also occur in patients with other diagnoses [4–9]. However, the articular presence of *Chlamydia*-derived antigens or nucleic acids does not prove its role in the pathogenesis, as bacteria could be mere bystanders [10, 11]. We therefore investigated whether intra-articular B-cell stimulation occurs in *C. trachomatis* sexually acquired reactive arthritis (SARA) but not in inflammatory arthropathies attributed to other causes, infectious or not.

In a previous study, with an enzyme-linked immunosorbent assay (ELISA) using purified elementary bodies of *Chlamydia* as the antigen, we have shown that intra-articular immunoglobulin A (IgA) production, in response to local chlamydial antigen, was likely to occur in patients with *C. trachomatis* SARA [12]. In the present study, intra-articular IgG production was investigated by comparing, in paired serum and synovial fluid (SF), the number and specificity of the responses to 27 different *C. trachomatis* antigens by immunoblotting and the binding intensity to five different *Chlamydia* antigens by ELISA. These antigens were three recombinant proteins [cysteine-rich protein outer membrane protein 2 (OMP2), heat shock protein (hsp) 60, polypeptide encoded by open reading frame 3 of plasmid (ppg3)], synthetic peptides [derived from species-specific epitopes in the variable domain IV of the major outer membrane protein (MOMP) (Labsystems, Finland)] and fragment of the total lipopolysaccharide (LPS) (Medac, Germany) [13].

Patients and methods

**Patients**

Diagnosis was taken from the chart at the time of sample collection. The patients were divided into the following groups:

*Chlamydia trachomatis* SARA (n = 16): asymmetrical mono/oligoarthritis with uveitis and evidence of urogenital *C. trachomatis* infection [two had a positive urethral/endorcervical *C. trachomatis* antigen detection by direct immunofluorescence, 12 had a positive urethral/endorcervical *C. trachomatis* culture, two had...
Fig. 1. Immunoblot evaluation of IgG responses to *C. trachomatis* antigens, obtained with serum (A) and the paired SF (B) from a *C. trachomatis* SARA patient and with serum (C) and the paired SF (D) from a *Salmonella enteritidis* reactive arthritis patient.

A positive urethral *C. trachomatis* DNA amplification with the Amplicor test of Roche Diagnostic Systems (Branchburg, NJ, USA); HLA-B27: eight positive, seven negative patients, one not determined; median age: 26 yr.

Inflammatory arthropathies independent of *C. trachomatis* (n = 20): rheumatoid factor positive (n = 5) or negative (n = 1) rheumatoid arthritis, gout (n = 4), Lyme arthritis (diagnosis confirmed by Western blot) (n = 5), non-specific post-dysenteric reactive arthritis (n = 2), *Salmonella enteritidis* reactive arthritis (n = 2) and septic arthritis (n = 1); median age: 43 yr.

**Immunoblot analysis of sera and SF**

For antigen detection, samples were diluted 1:100 (serum) or 1:50 (SF). Otherwise, the immunoblot analysis was performed as previously described [13, 14].

**Statistical analysis**

Where appropriate, the results were analysed by Student’s *t*-test and χ² test.

**Results**

**Number of *C. trachomatis* antigens recognized in immunoblotting, by paired serum and SF IgG antibodies from reactive arthritis and control patients**

Because the serum IgG concentration was about twice the SF concentration, immunoblotting was performed with SF diluted 1/50, whereas the sera were diluted 1/100. An example of immunoblot evaluations of IgG responses to *C. trachomatis* antigens, obtained with paired serum and SF from a *C. trachomatis* SARA patient and from a *Salmonella enteritidis* reactive arthritis patient is presented in Fig. 1.

In the *C. trachomatis* reactive arthritis group, three patients had serum and SF IgG recognizing an equal number of fractions, whereas the other 13 SF IgG recognized more fractions upon immunoblotting (Fig. 2A). The mean number of antigens bound by SF IgG was significantly (*P = 0.00034*) higher (17.6 ± 5.1)
than by serum IgG (11.1 ± 6.3), whereas no significant difference was observed for the control group (SF: 7.8 ± 6.4; serum: 7.8 ± 6.7). No relationship was observed between the increase in number of SF responses, the presence of HLA-B27, the disease duration or the number of active joints.

Chlamydia trachomatis antigens recognized more frequently in immunoblotting by SF than by serum IgG from reactive arthritis patients

The binding pattern varied from one sample to the other, in both groups of patients. However, in C. trachomatis SARA, three antigens of 64, 32 and 6 kDa were recognized significantly more often by SF than by serum IgG when high-intensity responses were considered. The 64-kDa antigen was the most frequently recognized (31% of serum and 75% of SF samples, $P = 0.013$).

OD responses obtained in ELISA for serum and SF IgG binding to OMP2, hsp60, pgp3, MOMP and LPS, for reactive arthritis and control patients

These responses were measured in the paired samples still available, i.e. 11 for the C. trachomatis reactive arthritis group and 13 for control patients, except IgG anti-OMP2 which was determined for five and 11 patients, respectively.

A significantly higher mean OD in SF (mean ± standard deviation: 1.14 ± 0.9) than in serum (0.65 ± 0.54) ($P = 0.05$) was only observed for IgG anti-OMP2 from C. trachomatis reactive patients and was mainly observed for three of these patients (Fig. 1C). No
significant difference was observed for IgG anti-OMP2 from control patients (serum: 0.48 ± 0.52 and SF: 0.24 ± 0.32) or for IgG binding to other antigens in both groups.

Discussion

The highest number of IgG reactions observed in SF from several patients with C. trachomatis SARA could reflect differences between systemic and intra-articular immune system activation. Because germinal centre-like structures have recently been found in the synovial membrane of a patient with post-Chlamydia reactive arthritis [15], a local B-cell activation and differentiation might be considered. As the persisting synovitis in reactive arthritis could be attributable to sequestered antigens, the 64-, 32- and 6-kDa antigens significantly more frequently recognized by SF than by serum IgG may play a role in immunopathology but these antigens were not identified. However, in ELISA, a significantly higher response to OMP2 was only observed in SF from patients with C. trachomatis SARA and not in controls. These results can be related to the antigens identified as targets for the synovial CD4+ T-cell response: hsp60, the 18-kDa histone-like protein (Hc1), an unidentified 30-kDa antigen, MOMP and OMP2 [11, 16–19].

In control samples with no apparent link to C. trachomatis, chlamydia antigens were also bound by IgG, both in serum and SF. This could correspond to cross-binding reactions with antigenic proteins from other micro-organisms. However, five patients could have previously encountered C. trachomatis as they had a high number (>10) of serum IgG responses [13]. As they did not have more SF than serum IgG responses, these SF antibodies could have been either plasma derived or locally produced if some B cells had been recruited non-specifically to the synovium. Therefore, no argument for a role of Chlamydia antigens in aggravation of the disease can be provided in these patients. Other factors such as the genetic background or the amount of microbial constituents present in the joint could also be crucial.

In conclusion, this study provides indirect evidence that specific IgG antibody production occurs in the joints of C. trachomatis SARA patients, as we and others have previously reported for IgA [12, 20, 21]. Using two different methods, immunoblotting and ELISA, some antigens were more often or more intensively recognized by IgG from SF than from serum. Thus, Chlamydia antigens of 64, 32 and 6 kDa and OMP2 might be immunodominant and play a role in immunopathology via T cell-mediated effector mechanisms or via antibodies forming immune complexes or by autoantigen recognition.

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References