ELSEVIER

Institut Pasteur

Microbes and Infection xx (2015) 1−5

www.elsevier.com/locate/micinf

Do IgA antibodies to *Chlamydia trachomatis* have protective role in humoral immunity: a study in reactive arthritis patients

Praveen Kumar [a], Darshan Singh Bhakuni [b], Sangita Rastogi [a,*]

[a] *Microbiology Laboratory, National Institute of Pathology (ICMR), Sriramachari Bhawan, Post Box No. 4909, Safdarjung Hospital Campus, Ring Road, New Delhi 110029, India*

[b] *Department of Rheumatology and Clinical Immunology, Army Hospital (Research & Referral), Dhaula Kuan, Delhi Cantt 110010, India*

Received 19 June 2015; revised 24 September 2015; accepted 28 September 2015

Available online ■ ■ ■

Abstract

Chlamydia trachomatis-induced genitourinary Reactive Arthritis (ReA) can serve as good model for host−pathogen interaction. However, due to poor antigen presentation, cell-mediated immunity does not contribute as anticipated. Present study aims to evaluate protective role of anti-*C. trachomatis* antibodies *vis-a-vis* inflammatory chlamydial Major Outer Membrane Protein (MOMP). Prospective study was undertaken in 30 patients with genitourinary ReA. 30 Rheumatoid Arthritis (RA) and 30 osteoarthritis patients constituted controls. Subjects found to be PCR-positive for *C. trachomatis* were investigated for presence of MOMP in Synovial Fluid (SF) by fluorescence assay while anti-*C. trachomatis* IgA/IgM antibodies were estimated in SF/venous blood by ELISA. *C. trachomatis* MOMP was evident by the presence of elementary bodies in SF of 9 ReA PCR-positive patients (30%; $p < 0.05$ *versus* controls). Local secretory IgA antibodies were detected in 12 (40%) patients with ReA ($p < 0.0001$ *versus* controls); among 12 patients with anti-chlamydial IgA antibodies, 9 showed the presence of both MOMP and IgA antibodies in SF. 58.3% ReA patients (7/12) with secretory IgA antibodies were also positive for circulatory IgA antibodies ($p < 0.01$ *versus* controls). Serum IgM antibodies were present in 4 ReA (13.3%) and in 1 RA (3.3%) patient, respectively. In conclusion, the present study suggests that in ReA patients with chronic, persistent *C. trachomatis* infection in synovium, the chlamydial MOMP is triggering factor for generating a protective immune response by inducing anti-*C. trachomatis* IgA antibodies in the SF of large number of patients.

Keywords: *Chlamydia trachomatis*; Reactive arthritis; Humoral immunity; Major outer membrane protein; Antibodies

1. Introduction

The importance of anti-*Chlamydia trachomatis* antibodies in serum or other body fluids has been investigated several times in terms of its diagnostic utility or marker of active infection, however, its role in protective immunity against *C. trachomatis* infection is unclear. Immunological duality exists wherein immunity to intracellular pathogens is dependent on cell-mediated responses, while antibody-mediated responses confer immunity to extracellular pathogens [1]. Several studies clearly show that antibody does *provide* a level of protective immunity to intracellular pathogens [2,3]. Also, monoclonal antibodies raised to surface-exposed chlamydial antigens, such as Major Outer Membrane Protein (MOMP), and convalescent serum neutralize the infectivity of *C. trachomatis* and *C. muridarum* in *in vitro* cell infection assays [4,5]. It has been observed that in *C. trachomatis*-induced Reactive Arthritis (ReA), females are more protected to anti-*C. trachomatis* antibody responses, hence, this might be the reason that males are predominantly reported in many studies [6−8]. In *C. trachomatis*, MOMP is surface-exposed and is capable of eliciting protective antibodies in infected hosts. It, therefore, has potential as candidate vaccine to prevent infection with this significant human pathogen [9].

* Corresponding author. Tel.: +91 (011) 26198402.
E-mail address: rastogi_sangita@rediffmail.com (S. Rastogi).

http://dx.doi.org/10.1016/j.micinf.2015.09.028

Immunoglobulin A (IgA) is the most abundant antibody isotype found in the body and has an important role in the immune responses elicited at mucosal surfaces. It mediates its effector function through multiple mechanisms [10], including interactions with mucosal epithelial cells, binding to a receptor, and high- and low-affinity antigen binding. IgA clearly has a multi-faceted role in mucosal immunity but whether IgA alone is critical to immunity against pathogens such as *C. trachomatis* that invade and cause disease at mucosal surfaces remains unresolved. We aimed to evaluate the role of anti-*C. trachomatis* antibodies in conferring immune protection against *C. trachomatis* MOMP in ReA patients.

2. Materials and methods

2.1. Enrollment of patients and sample collection

Age-matched (age ranging from 18 to 45 years) arthritic patients were enrolled in the study in consultation with the rheumatologist at Department of Rheumatology and Clinical Immunology, Army Hospital (Research and Referral), New Delhi, India. A total of 90 patients were enrolled in the study (30 ReA and controls, *viz.*: 30 Rheumatoid Arthritis (RA) and 30 Osteoarthritis (OA) patients). ReA and RA patients were enrolled following ESSG [11] and ACR criteria [12], respectively while OA patients underwent radiological diagnosis. The study was approved by local hospital's ethics committee and all participants gave written informed consent. Those with defined arthropathy/preceding enteric/any other infection were excluded. Detailed history and clinical data were collected from each patient. Neat Synovial Fluid (SF) and 2.0–5.0 ml non-heparinized blood (for serum collection) were collected and stored at −80 °C and −20 °C, respectively in sterile vials till assayed.

2.2. Screening of Chlamydia trachomatis MOMP and IgM/IgA antibodies

SF samples were screened by Direct Fluorescence Assay (DFA) for detecting the presence of chlamydial Elementary Bodies (EBs) using a commercial kit (MicroTrak *C. trachomatis* Direct Specimen Test; Trinity Biotech, USA) as per the manufacturer's guidelines and as per the criteria standardized earlier in our laboratory for the diagnosis in SF [8]. Briefly, after centrifugation (Shandon, Astmoor Run, UK) at 600 × g for 10 min, a total of four slides were prepared for each patient. Methanol-fixed cells were incubated with fluorescein isothiocyanate-conjugated monoclonal antibody to *C. trachomatis*-MOMP. Evans blue dye was used as control for autofluorescence while fluorescein isothiocyanate-conjugated monoclonal antibody to herpes simplex was used as another control antibody. Each slide was read by two independent observers after blinding. Each observer counted EBs in five fields per slide using 100× oil objective and calculated the average number of EBs. Brightly shining fluorescent green EBs were counted in the SF and samples were reported as *C. trachomatis*-positive when a minimum number of 7 EBs was present [8].

Detection of both circulatory and localized IgM and IgA antibodies to *C. trachomatis* was done in SF and sera of arthritic patients by commercially available Elisa kits (IBL International, Germany and Savyon Diagnostics, Israel, respectively) following the manufacturer's guidelines. Each sample was run in duplicate. SF and serum were diluted with the sample diluents. Cut-off indices were calculated according to the manufacturer's guidelines. As per the kit manual, IgM antibodies were calculated in units, and samples containing more than 11 units were considered to be positive, while for IgA, the absorbance value (A 450) of >1.1 was considered to be positive.

PCR for the detection of *C. trachomatis* was performed in the DNA of SF as described earlier [7]. Nested/semi-nested PCR assays were performed in 25 μl reaction mixture by using SF DNA (200 ng). In the first step, primer sequences which amplify plasmid (201 bp) and MOMP gene (537 bp) were applied while in the second step, a second set of primers which amplify the internal region of the first step PCR product was used. The PCR was subjected to 35 cycles. Final PCR products for plasmid (141 bp) and MOMP (380 bp) genes were viewed in gel doc.

2.3. Statistical analysis

Statistical analysis was performed with GraphPad Prism software (version 5.0; GraphPad Software Inc., San Diego, CA, USA). Fisher's exact test was performed for comparison between groups. Mean, median, inter quartile range and standard deviation were derived for numerical data using Column statistics. 'p' value < 0.05 was considered to be statistically significant.

3. Results

3.1. Clinical features

The age range, disease duration and male: female ratio of ReA patients was 18–44 years, 3–36 months and 7: 5, respectively. Majority of ReA patients presented with an oligoarthritic profile while all RA patients had polyarthritic pattern in their joints. The average number of joints involved was 2–5 in *C. trachomatis* IgA antibodies-positive ReA patients. Moderate to severe effusion pattern was observed in these patients. Overall, 10% patients had family history of arthritis. Among anti-*C. trachomatis* IgA-positive ReA patients (n- 12), 9 patients were confirmed for either endogenous plasmid/MOMP gene. The optical density for anti-*C. trachomatis* IgA antibodies was significantly higher ('p' < 0.05) in SF as compared to serum in ReA patients. HLA B27 gene was present in 42% (5/12) ReA patients with *C. trachomatis* IgA antibodies while only 33% (6/18) ReA patients without *C. trachomatis* IgA antibodies had HLA B27 gene (Tables 1 and 2).

Please cite this article in press as: Kumar P, et al., Do IgA antibodies to *Chlamydia trachomatis* have protective role in humoral immunity: a study in reactive arthritis patients, Microbes and Infection (2015), http://dx.doi.org/10.1016/j.micinf.2015.09.028