ANTIBACTERIAL ANTIBODIES IN SERA OF PATIENTS WITH REACTIVE ARTHRITIS AND OTHER RHEUMATOID DISEASES

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Abstract. The presence of the specific antibodies for some enterobacteria - Yersinia, Salmonella and Shigella and Chlamydia, was investigated in patients with reactive arthritis and other rheumatoid diseases. The antibacterial antibodies in the diagnosis titers were found in 71 out of 489 patients: 54 of them were positive for enterobacteria and 17 for Chlamydia. From all positive patients 46 (64.7%) had clinical diagnosis of reactive arthritis, 20 (28.1%) of ankylosing spondylitis, each 2 (2.8%) of sacroilitis and Reiter’s syndrome and 1 (1.3%) with other disease. The most serology positive cases were of middle-age (31-50 years). There were no significant differences between sexes among serology positive cases.

Key words: antibacterial, antibody, Yersinia, Salmonella, Shigella, Chlamydia, rheumatoid disease

INTRODUCTION

Inflammatory joint disease can develop following an extra-articular infection. The term reactive arthritis (RA) was coined in order to differentiate this arthritis, which is often characterized by lack of cultivable organism in the joint, from septic arthritides (1-4).

The etiological diagnosis of RA is based on the demonstration of recent or ongoing infection with the causative bacterium. This may be done by serological demonstration of antibacterial antibodies, the presence of the causative microorganism at an extra-articular site by identification of bacterial nucleic acids or antigens in joint material from patients with aseptic arthritis. For clinical routine serological tests for bacterial specific antibodies (IgM and IgA class) are
often necessary to show recent or persistent infection with the triggering pathogen (3-6). In reactive arthritis following extra-articular infection with *Yersinia, Salmonella, Shigella, Campylobacter* or *Chlamydia*, one of the major shifts in perception of disease pathogenesis was the detection of bacterial determinants by immunological methods and polymerase chain reaction actually within the joint. In sexually acquired RA, the etiologic diagnosis should be based on the direct detection of the pathogen (mainly *C. trachomatis*) from the urogenital smear specimen. Antigen-specific proliferation of synovial fluid lymphocytes can confirm the clinical diagnosis of the patients with RA and Lyme disease, although unspecific proliferation to several bacteria can also be observed in reactive arthritis as well as in other arthritides (3-9).

The bacteria known to trigger RA as well many other organisms that have been postulated as potentially associated may be enteric pathogens such as *Shigella, Salmonella, Campylobacter, Yersinia, Clostridium difficile* and urogenital pathogens: *Chlamydia trachomatis* and *Ureaplasma urealyticum*. *Chlamydia trachomatis* is the only sexually transmitted infection that clearly triggers RA. There are other microorganisms which can be associated with rheumatic diseases: *Brucella, Borrelia burgdorferi, Leptospira, Mycobacterium, Streptococcus, Staphylococcus, C. psittaci, Neisseria gonnorhae* (1,4,6,8-12).

In order to evaluate the presence of specific antibodies against some enterobacteria and *Chlamydia*, patients with a recent inflammatory joint disease were investigated.

**PATIENTS AND METHODS**

489 patients treated for a recent inflammatory joint disease in the last three years (2000-2002) were investigated for the presence of the specific antibodies against *Yersinia, Salmonella* and *Shigella*. 93 of the patients were investigated also for the presence of specific antibodies against *Chlamydia*. Most of these patients have had an enteral prodrome of 4-20 days followed by articular pains.

Antibodies to *Y. enterocolitica* O:3, O:9, *Y. pseudotuberculosis* I, *Shigella flexneri* and *Salmonella* BO and DO groups were measured. The tube agglutination method has been used for *Yersinia* and *Shigella* antibodies. Sera were serially diluted from 1/10 to 1/1280 with a saline solution. As the diagnosis titer were accepted the 1/160 for *Y. enterocolitica* and *Shigella*, and 1/80 for *Y. pseudotuberculosis*. *Salmonella* antibodies were detected by Widal reaction and the titers of diagnosis were 1/250 for O antigen and 1/100 for H antigen.

The presence of anti- *Chlamydia* antibodies was determined using “Sero ELISA™ Chlamydia TRUE-IgM™ commercial kit (Savyon Diagnostics LTD). The technical procedure followed the general procedure required by the producer.

**RESULTS AND DISCUSSION**

Antibacterial antibodies were detected in 71 (14.5%) of 489 patients with
rheumatoid diseases. 54 (11.0%) out of 71 positive patients presented antibodies for enterobacteria. Increased antibodies levels to \textit{Y. enterocolitica} O:3 and \textit{Y. pseudotuberculosis} were observed in the sera from 32 (59.2%) of the 54 patients. The positive antibodies levels found were against \textit{Y. enterocolitica} in 17 patients and for \textit{Y. pseudotuberculosis} in 15 patients. Specific antibodies for \textit{Shigella} were present in 20 patients and for \textit{Salmonella} in 2 cases. The results are presented in table 1. 17 out of the 93 tested patients (18.2%) presented anti-\textit{Chlamydia} IgM antibodies (table 1 and fig. 1).

Table 1. Distribution by age and bacterial entities of positive serology cases

<table>
<thead>
<tr>
<th>Age of group (y)</th>
<th>Y. enterocolitica</th>
<th>Y. pseudotuberculosis</th>
<th>Shigella</th>
<th>Salmonella</th>
<th>Chlamydia</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-19</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2.8</td>
</tr>
<tr>
<td>20-29</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>8</td>
<td>11.5</td>
</tr>
<tr>
<td>30-39</td>
<td>7</td>
<td>4</td>
<td>6</td>
<td>0</td>
<td>4</td>
<td>21</td>
<td>29.6</td>
</tr>
<tr>
<td>40-49</td>
<td>5</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>9</td>
<td>23</td>
<td>32.3</td>
</tr>
<tr>
<td>50-59</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>14.0</td>
</tr>
<tr>
<td>60-69</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>8.4</td>
</tr>
<tr>
<td>&gt; 70</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td>Total no %</td>
<td>17</td>
<td>15</td>
<td>20</td>
<td>2</td>
<td>17</td>
<td>71</td>
<td>100</td>
</tr>
</tbody>
</table>

Fig. 1 The distribution on the bacterial entities of the positive serology cases

Antibodies for:
- \textit{Enterobacteriaceae}
- \textit{Chlamydia}
From all 71 positive patients 46 (64.7%) had clinical diagnostic of reactive arthritis, 20 (28.1%) of ankylosing spondylitis, 2 (2.8%) of sacroilitis, 2 (2.8%) of Reiter’s syndrome and 1 (1.3%) of intervertebral disc protrusion (fig. 2). In our study 35 (49.2%) of the serology positive patients were male and 36 (50.1%) were female (fig. 3). In the group of enterobacteria positive patients 53.1% were female and 46.2% were male. 41.2% of Chlamydia positive patients were female and 58.8% were male. Generally, it is known that enteric reactive arthritis is equally distributed between the sexes but sexually acquired reactive arthritis is reported 20 times more common in males than females (6,11). In our study no statistical significant difference on the sex distribution of the serology positive cases, was found. The ages of the patients were of 12-72 years (table 1). 61.9% of the cases were middle-aged (31-50 years of age) people (fig. 4). The arthritis, as resulted from our investigations as well as from the studies published by other authors were located both in the inferior and in the superior limbs (8,9,12-15). The arthritic symptomatology could manifest itself as long as up to 12-22 months. The arthritic symptoms changed in time and tended to develop chronic arthritis in the absence of the rheumatoid factor (6,9,15).
Fig. 3 Sex distribution of patients with positive serology by rheumatic disease

Fig. 4 The distribution of positive serology cases by age groups
Whereas *Chlamydia* can be demonstrated in urogenital specimens in at least one-third of patients with *Chlamydia* induced acute RA, the triggering bacteria are usually no longer detectable in post-enteric reactive arthritis (1-4,11,15).

Sometimes stool culture may reveal infection with one of the causative enteric organisms even in a patient without bowel symptoms. Lozada et al pointed out that about 10% of patients do not have a preceding symptomatic infection (4).

Reactive arthritis is a clinical diagnosis and there are few diagnostic laboratory tests. However, detecting a current or prior infection with specific causative organisms is strong supportive evidence for the disease. Since culturing *Chlamydia* is difficult in a routine clinical setting, non-culture techniques are more readily used. Cervical and urethral swabs analyzed by direct fluorescent antibody and enzyme immunoassay provided 90% sensitivity and 98% specificity for *Chlamydia* infection (11,12,14-16,17-19). Newer, DNA probes to *Chlamydia* major outer membrane protein (MOMP) gene are highly sensitive and specific (9,11,12). Convincing “proof” of RA causation by *Chlamydia* is the discovery of synovial chlamydial antigens and genetic material by immunoperoxidase, direct immuno-fluorescent and polymerase chain reaction (PCR) techniques (6-12).

Recent infection with *Chlamydia*, *Shigella*, *Salmonella*, *Yersinia* and *Campylobacter* can be confirmed by positive organism specific antibodies serology (positive IgM, fourfold rise in IgG titer, or IgG titer greater than 1:128) (1,2,8,9,14,20).

Recently, the application of immunoenzymatic techniques allowing objective quantification of IgM, IgG and IgA class antibodies many studies of specific humoral immune responses in R.A. have become possible. Thus, in patients with RA following *Yersinia* infection, the anti-*Yersinia* antibodies response (especially of the IgA class) is notably vigorous (4,6,11,20-23).

The attempts to culture bacteria from the synovial fluids had negative results but there is a strong evidence for the presence of bacterial antigens in the affected joints of patients with RA. Using enzyme immunoassay unit, it was demonstrated the presence of immune complexes consisting of *Yersinia* antigen and specific antibodies in synovial fluid samples of patients with *Yersinia*-triggered RA (24). Granfors et al, 1989 observed positive staining in synovial fluid cells from 12 of 15 patients with reactive arthritis after *Y. enterocolitica* infection. Bacterial antigens were visualized by immuno-fluorescence or Western blot, using rabbit antiserum to the corresponding bacteria and monoclonal antibody to the P-polysaccharide chain of the same bacteria (8,10).

In our previous paper we presented some data concerning *Yersinia*- triggering arthritis (13). Another authors found positive serological results for *Yersinia* in 6-7% to 48.1% of the investigated cases (12-24).

Belen’kii et al found percentage of 11.1 of the patients with high level of
specific antibodies and 8.3% with coproculture positive for *Yersinia*. They concluded that microbiological tests are more successful in patients with the symptoms of concomitant enterocolitis (18). Wakefield et al. in a group of 22 patients with ankylosing spondylitis and Reiter’s syndrome, investigated by a sensitive ELISA assay found 29% positive *Yersinia* serology, all patients HLA B27-positive and four with a history of diarrhea preceding the onset of their disease (21). Serology for *Yersinia* was positive in 39 of 81 SNA patients. Locht et al. found high frequency of reactive joint symptoms after an outbreak of *Salmonella enteritidis* (19). We found only 2 (0.4%) of the cases with specific antibodies for *S. typhimurium* and *S. enteritidis* at titers >1/250 for BO and DO antigens and titers >1/100 for iH and g, mH antigens.

Our study also represents a serological survey of the chlamydial antibodies in a population’s group. In our country there are a few data concerning the presence of *Chlamydia* antibodies in the different categories of population. Botez et al. pointed out that the cases with Reiter’s syndrome were confirmed by isolation of the *Chlamydia* and by positive serological tests (25).

About the serological tests in *Chlamydia* induced reactive arthritis Bas S et al. showed that these patients have a pattern of reactivity that is compatible with infection by several serotypes to this bacterium might be involved in the development of reactive arthritis. It was also investigated whether determination of serum or synovial fluid IgG and IgA anti-*Chlamydia* antibodies could be clinically helpful in detecting possible *Chlamydia trachomatis* infection. The synovial fluid IgG antibody against the major outer membrane protein (MOMP) was the most appropriate determination with sensitivity and a specificity equal to or close to 80% (11,26).

In a study about clinical findings of 242 patients with *Chlamydia* diagnosis and complement fixation titers, were found the joint symptoms, mainly in the form of arthralgias and reactive arthritides in 31 (13%) cases (12). The mechanism by which specific intestinal or urogenital infections can trigger an HLA-B27 predisposed individual to develop reactive arthritis is not precisely understood. HLA-B27 is present in approximately 80% of RA patients as compared to 7% of the general population. The arthritogenic peptide theory proposes that, on infection, a bacterial peptide that is antigenically similar to a self-peptide (a cartilage component) is preferentially recognized by HLA-B27 antigen presenting cells. Following antigen presentation, CD8+ CTL response cross-reacts with the structurally similar self-peptide resulting in disease. Since the HLA-B27 antigen is found on all nucleated cells throughout the body this theory does not explain the organ specificity seen in RA.

Synovial fluid cultures are negative for enteric organisms or *Chlamydia* species. However, a systemic and
intrasyovial immune response to the organisms had been found with intra-articular antibody and bacterial reactive T cells. Furthermore, bacterial antigen has been found in the joints. Thus the elements exist for an immune-mediated synovitis.

Molecular evidence of bacterial DNA using PCR in synovial fluids has been found only in *Chlamydia*–related reactive arthritis and one placebo controlled trial of a tetracycline derivative (Lymecycline) showed a reduction in the duration of acute *Chlamydia*-related but not enteric-related to RA. This suggests the persistent infection may play a role, at least in some cases of *Chlamydia* reactive arthritis.

Serological evidence for *Chlamydia* was studied also with micro-immunofluorescence- IgM –class antibodies. Using this method and endourethral or endocervical cultures in 35 individuals 30% of seronegative arthritis cases were positive for *Chlamydia* infection (17). It has been speculated that the microbial antigen could be transported to the joint by monocytes and macrophages that take up the microbes intracellularly in the gut or genitourinary mucosa. In demonstration of Chlamydial DNA and RNA in inflamed joints indicate that, at least in the early phase of *Chlamydia trachomatis* infection, intact micro-organisms have persist in dormant, unculturable form (9,12).

In addition to those “conventional” microorganisms, several other infectious agents, like *Borrelia burgdorferi*, ß-hemolytic streptococcus, *Clostridium difficile*, *Neisseria*, some viruses have been suggested to trigger RA. There is also evidence that *Ureaplasma urealyticum*, another common agent in nongonococcal urethritis, cervicitis, may induce RA (1-3,8,9,11-15,18-27).

Pavlica et al, has isolated *Chlamydia trachomatis* from synovial fluid in 4 (22.2%) patients with Reiter’s syndrome while *Ureaplasma urealyticum* was isolated in 7 (38.9%) of cases (28). Sieper J. et al examined 4 groups of patients for the presence of antibodies against to *Chlamydia* (CT), *Mycoplasma urethritidis* (MU), *Y.enterococolitica* (YE) and *Borelia burgdorferi* (BB). CT IgA (20%,31%,16%) and IgG (40%,51%,34%), YE (7%,6%,0%) and BB (17%,2%,10%) were found. The positive cultures were found for CT (28%,29%) and MU (14%,17%) (15). The ability to induce arthritis has a great variance even among the different *Yersinia*, *Salmonella*, *Shigella* and *Campylobacter* species and serogroups.

Infections due to *S. typhimurium*, *S. enteritidis*, *Shigella flexneri* and *Y. enterocolitica* O:3 are often associated as a cause of human arthritis. In contrast, *Salmonella* carrying O antigens other than 1,4,5,9,12, *Shigella sonnei*, *Y. enterocolitica* O:8 are rarely associated (1-3,7,8,12).

Most of the microbes’ associated arthritis had common features. Lipopolysaccharide (LPS) is an important antigenic and functional component. Another shared structural feature is that they contain a virulence plasmid. Further, many of these microbes can enter in the host cells and survive here (1-10,12,14).
Through new sophisticated techniques, studies of humoral immune responses and studies demonstrating microbial antigens in the joint of patients with R.A. triggered by various microbes will be undertaken. This will inevitably enhance the understanding of the pathogenesis of R.A. Microbiological diagnosis for rheumatic diseases is increasingly used as a part of the diagnostic work-up in rheumatological practice due to growing of the knowledge about bacteria-induced rheumatic diseases (2,3,6,10).

CONCLUSIONS
- The antibacterial antibodies were present in 71 (14.5%) patients with different rheumatoid diseases.
- The presence of the specific Yersinia antibodies was detected in 32 patients (6.3%); 17 patients with Y. enterocolitica O:3 and 15 patients with Y. pseudotuberculosis antibodies in significant titers were recorded.
- 20 patients presented specific antibodies for Shigella and 2 positive for S. enteritidis and S. typhimurium.
- Antibodies anti – Chlamydia IgM have been detected in 18.2% of rheumatic patients.
- Clinical diagnosis of the positive cases was of reactive arthritis in 64.7%, ankylosing spondylitis in 28.1%, 2.8% of sacroilitis, 2.8% of Reiter’s syndrome and one case with other rheumatic disease.
- The age of the patients ranged from 12 to 71 years; 61.9% of them were middle-age people (30-49 y).
- It is not significant difference on the sex distribution of the positive cases.
- The serological investigation, to detect the specific antibodies in patients with reactive arthritis, to identify and to obtain a better knowledge about the germs responsible for the sero-negative arthritis, appears as necessary.

REFERENCES
ANTIBACTERIAL ANTIBODIES IN SERA OF PATIENTS WITH REACTIVE ARTHRITIS


