Pathogenesis of Reactive Arthritis

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Current Rheumatology Reports 2001, **3:**412–418
Current Science Inc. ISSN 1523–3774

There is good evidence that bacteria persist in vivo in patients with reactive arthritis (ReA). While *Chlamydia* seem to hide inside the joint, other areas such as gut mucosa or lymph nodes seem to be more likely places for *Salmonella* and *Yersinia*. T-helper (Th) 1 cells secreting cytokines such as IFNγ and TNFα are crucial for an effective elimination of these bacteria. An inhibited Th1-response could be demonstrated in ReA, probably contributing to bacterial persistence. While HLA-B27 is found in only approximately 50% of patients with acute ReA, HLA-B27 seems to be crucial for the development of features typical with chronic spondyloarthropathy, such as sacroiliitis. Among several hypotheses to explain the interaction of bacteria with HLA-B27, the most likely seems to be that until now unknown bacterial or self-antigens were presented by HLA-B27 to CD8+ T-cells. An important site where the immunopathology takes place seems to be at the insertion of tendons and ligaments at bone. Because antibiotics have failed so far in the treatment of ReA immunomodulatory therapies, based on a better understanding of the pathogenesis, alone or in combination with antibiotics might be an option for the future.

Introduction

Reactive arthritis (ReA) can be regarded as a subgroup among the infection-associated arthritides. It occurs after a preceding infection of the urogenital tract with *Chlamydia trachomatis* or of the gut with enterobacteriae such as *Yersinia*, *Salmonella*, *Shigella*, and *Campylobacter jejuni*. Because of the HLA-B27 association, its pattern of joint involvement (asymmetrical, predominantly of the lower limbs), and the possible affection of the spine, it is part of the spondyloarthropathies (SpA). Because the bacterial trigger is known, SpA-related research has concentrated on the pathogenesis of ReA and the interaction between bacteria and HLA-B27, hoping also to solve the riddle about the pathogenesis of the whole group of SpA.

Epidemiology of Reactive Arthritis As a Clue to Pathogenesis

Reactive arthritis occurs in approximately 2% to 4% after previous infections mainly of the urogenital tract with *Chlamydia trachomatis* (Keat) or of the gut with enterobacteriae. However, the frequency of ReA after gut infections with *Salmonella*, *Shigella*, *Campylobacter*, or *Yersinia* can range between 0% and 15% in epidemic patients [1,2]. There may be several explanations for these differences, such as variations in the genetic background and the local environmental factors, differences in the arthritogenicity of bacteria, and differences in the study designs. In any case, this variation in the frequency of ReA after previous infections demonstrates that there are differences in the prevalence of ReA and differences in the relative contribution of the single bacteria locally.

HLA-B27 is positive in 50% to 80% of patients with ReA. Most of the earlier studies reporting an HLA-B27 association of 80% were hospital based and thus most likely included only the more severe cases. Analyzing more recent reports, mainly based on epidemic patients, it seems that the HLA-B27 frequency is not higher than 50% for *Salmonella*-, *Campylobacter*-, and *Chlamydia*-induced ReA [2]. For *Yersinia*, the data are not that clear because there have been only a few epidemics, but an HLA-B27 frequency of approximately 80% has been reported. In *Shigella*-induced ReA, older studies suggest that the frequency in epidemics is approximately 80% [1]. Future studies based on community investigations or epidemics should determine whether there are real differences in the HLA-B27 association for the different bacteria.

Spinal manifestations, especially chronic sacroiliitis, occur mainly in HLA-B27-positive patients with ReA. In hospital-based studies, patients with ReA (the majority of whom were HLA-B27 positive) were followed up during the next 10 to 20 years. In ReA due to *Yersinia*, *Salmonella*, *Shigella*, or *C. trachomatis*, 20%, 14%, 32%, and 49% of these patients, respectively, had radiologic evidence of sacroiliitis and 15%, 12%, 14%, and 26% of these patients, respectively, developed ankylosing spondylitis with symmetrical sacroiliitis resembling primary AS. All patients with AS were positive for HLA-B27 [3]. Thus, the interaction between bacteria and HLA-B27 seems to be crucial in understanding the pathogenesis of ReA and of the whole group of SpA.

A substantial proportion of primary AS and uSpA may also be caused by ReA-associated bacteria. Infections with *C. trachomatis* and *Y. enterocolitica* often go along with zero or only a few clinical symptoms. *C. trachomatis* has been detected in synovial fluid or synovial membrane of patients with so-called undifferentiated oligoarthritis (without clear clinical or laboratory evidence of a preceding infection) in up to 30% [4–6]. Earlier reports indicated that gut infections with *Yersinia* are often asymptomatic or associated only with minor abdominal symptoms in patients developing *Yersinia*-induced ReA.

Reactive Arthritis-associated Bacteria: Similarities and Differences

Understanding the pathogenesis of reactive arthritis includes determining how ReA-associated bacteria differ in their biology and their antibacterial immune response and in what they have in common.

Yersinia-arthritis in rats can be induced with the *Yersinia* 0:8 strain but not with the human pathogenic strain 0.3. It occurs in Lewis but not in Fisher rats, although they have an identical MHC background pointing to other relevant genetic factors in this animal model. Arthritis can be induced after intravenous inoculation of *Yersinia*. Live *Yersinia* or *Yersinia*-specific DNA could not be detected in synovial tissue, but six of eight lymph nodes were still positive for *Yersinia* after 8 months. The rats have a long persistence of an antibody response; therefore, lymph nodes seem to act as a reservoir [7]. This situation is similar to that of ReA in humans. *Yersinia* are difficult to detect in the joint by polymerase chain reaction (PCR), with only one positive result in a patient reported in each of the two studies [8,9]. IgA-antibodies are found for months in the serum of patients with ReA [10]. *Yersinia* antigens were detectable in peripheral blood cells for years in patients with *Yersinia*-induced ReA [11••]. These results suggest that *Yersinia* live outside the joint for a long time, possibly (as in the animal model) in lymph nodes or mucosa, that bacterial antigen is transported to the joint, and that if *Yersinia* are alive inside the joint, it is for only a short period.

Less data are available on *Salmonella*. Lymph nodes and intestinal mucosa are likely places where *Salmonella* can persist because *Salmonella* DNA, similar to *Yersinia*, is difficult to detect in the joint [12]. In early phases of *Salmonella* infections, *Salmonella* could be identified in peripheral blood monocytes [13] thus are probably transported by monocytes.

This is different for *Shigella*. Major insights in the behavior of *Shigella* is hampered by the lack of an animal model because man is the only host. Epithelial cells are the major target of *Shigella* infections; transportation by monocytes is highly unlikely because *Shigella* kills monocytes through apoptosis. *Shigella* DNA has not been demonstrated convincingly in the joint of patients with ReA; therefore, it is more likely that pieces of bacteria rather than live bacteria are transported into the joint [14]. It has been reported that reactive arthritis occurs only or mainly after infections with *S. flexneri* but not with *Shigella sonnei* and that the pHS-2 plasmid must be present in *S. flexneri* [15]. To follow up on this hypothesis, we investigated different *Shigella* strains for the presence of this plasmid. Of 99 different serovars for *S. sonnei* none was positive for the pHS-2 plasmid. For *S. dysenteriae*, which has also been implicated in reactive arthritis, two of two from serovar 1 but none of the other serovars were positive for pHS-2. In addition, *S. boydii* was negative. Interestingly, 34 of 34 *S. flexneri* serovar 1–4 but zero of 11 from serovar 6 were positive. These findings further support the hypothesis that pHS-2 may be relevant in *Shigella*-induced arthritis. The pHS-2 is important to determine the invasiveness of *Shigella* and to determine the intracellular and intercellular movement. Thus, this plasmid may be important in the route of infection.

Whereas the enterobacteria have a high grade of homology, *Chlamydia* is deeply separated from other bacteria because of their developmental cycle and their different antigenicity. It is unclear whether inflammation is the consequence of repeated or persistent infections. Although there are no clear data, both events may be necessary: reinfection may stimulate the immune response against persistent *Chlamydia* leading to hypersensitivity and immunopathology. *Chlamydia* persist for a long time in the joint. DNA and RNA can be detected by PCR [4–6,16], indicating that *Chlamydia* are alive. However, a humoral immune response against different serovars of CT in patients with ReA compared with patients with only urethritis has been reported, indicating repeated infection [17•]. This would argue for a role of reinfection and persistence of *Chlamydia* in the pathogenesis of reactive arthritis.

There is evidence for a differential expression of chlamydial antigens in the joint: LPS and hsp60 is upregulated, whereas the major outer membrane protein (MOMP) is down-regulated [17•]. However, *Chlamydia trachomatis* is a common pathogen that is detected by PCR in the joint of patients with ReA and in other arthritides. PCR-positive results were obtained in 65% of patients with Reiter's syndrome, 42% of patients with other reactive arthritides, but also in rheumatoid arthritis and osteoarthritis patients [2]. *C. pneumoniae* is present in joint material but less frequently so than *C. trachomatis* [18]. Schumacher *et al.* [19•] reported recently on the investigation of synovial biopsies in 30 healthy volunteers, of whom two were positive for *C. trachomatis* by PCR. Although there is no doubt that *C. trachomatis* plays a causative role in reactive arthritis and Reiter's syndrome, these results limit the specificity of a positive PCR result and stresses the point that such a result can be interpreted only in the clinical context. Furthermore, this questions whether *Chlamydia* are just innocent bystanders in RA or OA or whether they can contribute to the immunopathology. A few recent studies could show that many different bacteria can be found in the joint of patients with RA and other arthritides

[8,20,21]. However, these bacteria are unlikely to be the cause of the joint inflammation. Thus, the more that sensitive techniques such as PCR are used the more likely it is that microbes are detected in the joint.

Monocytes seem to be an obvious candidate for the transportation of *Chlamydia* from the site of entry to the joint [2]. However, *Chlamydia* seems to be in the blood only transiently. Investigating peripheral blood monocytes by PCR for the presence of *Chlamydia*, Kuipers *et al.* [22] detected *Chlamydia* only in two of 28 patients who were *Chlamydia* positive in synovial fluid.

There seems to be different ways how and in which stage ReA-associated bacteria access the joint. *Chlamydia* reach the joint and stay alive, whereas *Yersinia* and *Salmonella* reach the joint and are alive, if at all, only for a short time. There seems to be another reservoir where they survive in vivo, most likely in the lymph nodes or the intestinal mucosa. For all these bacteria, monocytes seem to be likely candidates for transportation to the joint. It is unlikely that *Shigella* are transported in monocytes or that they survive somewhere in vivo for a longer time. Rather, pieces of bacteria seem to be sufficient for the induction and maintenance of inflammation. That such different bacteria can induce arthritis but that some subtypes (such as *Yersinia* 0:8 or *Shigella sonnei*) in the single species do not, argues that the possibility of the bacteria to get access to the joint or to special cell types, rather than antigenicity, is crucial for the induction of arthritis.

Role of HLA-B27

The only known function of MHC molecules is the presentation of antigen. Therefore, the arthritogenic hypothesis stating that a microbial or self-antigen is presented to CD8+ T cells has received some attention. However, additional models have been put forward to explain the association of HLA-B27 with disease [23,24].

In general, there is a good correlation between the prevalence of spondyloarthropathies and HLA-B27. HLA-B27 is a serologic specificity that encompasses 20 different natural variants (subtypes) that have been given the designations B*2701 to B*2720 [25]. The most widespread subtype in the world is B*2705. Epidemiologic studies show that the common subtypes B*2705, B*2702, and B*2704 are clearly associated with SpA. Some of the rare or not-so-well-studied subtypes (B*2701, B*2703, B*2707, and B*2708) have also been observed in one or more patients with SpA. A lack of association has been reported for B*2706 and B*2709. B*2706 is antigenically one of the most distant subtypes from B*2705; it evolved from B*2704 by substitutions at residues 114 (His-to-Asp) and 116 (Asp-to-Tyr). B*2709 is a rare subtype present among Italians, primarily among those residing on the island of Sardinia, that is not associated with AS. This subtype differs from B*2705 by a single amino acid substitution (His-to-Asp) at position 116, which is located at the bottom of the peptide binding groove, suggesting that an arthritogenic peptide can by bound by the susceptible subtypes but not by the others.

HLA-B27-transgenic animal models have been used to clarify the role of HLA-B27. HLA-B27 transgenic rats develop gut inflammation and arthritis/spondylitis, a disorder resembling human SpA, but not when they are born and kept in a germ-free environment. Assessment of the caecal microflora of HLA-B27 transgenic rats that spontaneously develop chronic gut inflammation first and then develop SpA, shows an increase in the number of *Escherichia coli* and *Enterococcus* species in the gut that correspond to the presence and severity of gut inflammation [26]. It was recently shown that bacteroides are necessary in this model [27]. These data support a central role for bacterial triggers in SpA. CD4+ and CD8+ T cells seem to be relevant in disease acquisition. However, it was impossible when using the B27-transgenic rat model to find an HLA-B27-restricted CTL response against *Yersinia* after infection with *Yersinia* [28]. The same group also presented data recently on the immune response to *Yersinia* in non–B27-transgenic rats [29]. For the induction of cytotoxicity the *Yersinia* protein invasin is essential; the CTL response was directed against the Yad A molecule from *Yersinia*.

In the model of HLA-B27-transgenic/murine β2-negative mice, the mice were knocked out for different molecules, which made it clear that MHC class II and TAP (transporter-associated protein; necessary for the transportation of intracellular peptides to intracellular MHC class I molecules) are unnecessary to acquire the disease, but CD4 and CD8 molecules are essential for disease development [30,31]. Disease can be prevented in this model if a strong HLA-B27-binding molecule (GRID-phe-LK) is given, suggesting an antigen-presenting role of HLA-B27. Thus, HLA-B27 seems to act as an antigen-presenting molecule rather than a donor for peptides presented by MHC class II. One of the explanations for these findings could be the formation of single heavy chain (HC) B27-presenting peptides [32]. Arthritis does not occur in this model if mice are raised in a germ-free environment.

Role of Cytokines

Given that the interactions of bacteria/bacterial antigens with the HLA-B27 molecule seem to be crucial for the pathogenesis and clinical manifestation of reactive arthritis and other spondyloarthropathies, it is obvious to question what other factors of the immune response are relevant. Cytokines, derived mainly from T cells and monocytes (macrophages), and their different patterns are important in the outcome of bacterial infections. In addition, they are relevant in the suppression or modulation of immune responses against self-antigens.

There is good evidence from animal models, especially for the ReA-associated bacteria *Yersinia* [33] and *Chlamydia* [34], that T helper (Th)1 cytokines such as IL-12, IFNγ, and TNFα are crucial in the elimination of these bacteria. Evidence also indicates that a lack of these cytokines and an elevated production of Th2/Th3-cytokines (especially

IL-10) inhibit an effective clearance of these pathogens and can lead to persistence. Because bacteria persist at different places for a long time in vivo, as discussed earlier, an obvious question is whether a "wrong" cytokine pattern contributes to the pathogenesis of ReA. From our results, it is clear that the ratio of IFNγ/IL-4 positive cells in synovial fluid and synovial membrane is lower than in RA, another inflammatory arthritis [35]. In another prospective study, we observed 51 patients with early ReA [36••]. In this study, the cytokine pattern was determined in peripheral blood mononuclear cells and correlated with the outcome. Surprisingly, the clearest results were found for TNFα. TNFα was significantly lower at the beginning of the disease than it was in 30 patients with early ReA. A low TNFα level correlated well with a chronic course.

All these results were obtained with no or only mitogenic T-cell stimulation. When synovial mononuclear cells from patients with ReA were again stimulated with the (whole) triggering bacterium, a low TNFα/IL-10 ratio was found compared with T cells from patients with Lyme arthritis stimulated with *Borrelia burgdorferi* [37]. Therefore, there is a relatively low production of Th1-cytokines in ReA, which may partly explain bacterial persistence. The relative contribution of TNFα and IL-10 should be explored in more detail in future studies.

An elevated amount of IL-10 (together with IFNγ) was found in the joints of patients with early *Chlamydia*-induced arthritis compared with patients in another group with undifferentiated oligoarthritis [38]. We introduced the new technique of measuring antigen-specific cytokine by flow cytometry. This allows for the possibility of analyzing antigen-specific T cells after short-term in vitro stimulation directly ex vivo. In patients with *Chlamydia*-induced ReA, we found a response of peripheral blood and synovial fluid CD4+ T cells to the *Chlamydia*-specific recombinant proteins MOMP (major outer membrane protein) and heat shock protein 60 (hsp60) and to the *Yersinia*-specific recombinant proteins 19kd (the β_2-subunit of the urease) and to the *Yersinia*-hsp60. The T-cell frequency was approximately 1% in synovial fluid, which was approximately five to 10 times higher compared with peripheral blood. We could also detect IL-10-secreting CD4+ T cells in approximately 0.2% of the CD4 T cells [39•]. The good T-cell response to the MOMP-antigen in *Chlamydia*-induced ReA contrasts the reported down-regulation in the joint of MOMP mRNA compared with the chlamydial hsp60 [16].

In addition to having environmental influences on the cytokine pattern, such as different patterns of infections or atopic diseases, genetic polymorphisms may explain the differences in the cytokine pattern. Some data are available for TNFα. Investigating TNFα microsatellites, an association of ReA with a TNFa6-allele was described [40], an allele that has been associated with low TNFα secretion. Two promotor polymorphisms of the TNFα gene at positions -308 (308.1 and 308.2) and -238 (238.1 and 238.2) have been investigated in ankylosing spondylitis, a disease related to ReA. The 308.2 and the 238.1 genotypes were found significantly less frequently in AS [41]. Thus there is some evidence that TNFα genotypes that seem to be associated with a low TNFα production are present in a higher percentage in ReA- and AS-patients. We can show that TNFα secretion by CD4 T cells was lowest in patients with AS but significantly lower in B27-positive healthy controls compared with B27-negative controls. Interestingly, in B27-positive controls, 308.2 was associated with high and 308.1 with low TNFα production independent of the disease state. No such difference was seen among B27-negative individuals [42]. If high TNFα production is protective against AS, the 308.2 genotype that appears to be associated with high TNFα production in B27-positive individuals could represent a protective genotype against AS.

T-cell Epitopes in Reactive Arthritis

In ReA, antigen-specific CD4 and CD8 T cells have been detected in synovial fluid and synovial membrane, although CD4 T cells seem to be predominant. In addition, the antigen-specific CD4 T-cell response has been better investigated. An important step in clarifying the pathogenesis of ReA is to identify the target for the synovial T-cell response. In *Chlamydia*-induced arthritis, the chlamydial hsp60, the Hc1-protein (a histone), and the omp2 (an outer membrane protein) were recognized by CD4+ T cells [43]. However, several T-cell clones did not respond to any of the tested candidate proteins, although whole *Chlamydia* elicited an immune response, suggesting that there are other possible relevant antigens. T cells specific for the chlamydial hsp60 did not recognize the human counterpart.

In *Yersinia*-induced arthritis, the strongest CD4 response was found against the β-subunit of the urease (the 19kd protein) and the hsp60 [44]. Similar to *Chlamydia*-induced arthritis, the hsp60-specific T cells did not cross-react with the human hsp60. The IL-10 secretion after antigen (peptide)-specific stimulation showed a wide variation among the T-cell clones, suggesting that high and low IL-10 secretion can occur in vivo after specific stimulation with *Yersinia*-antigens. An immunodominant epitope for the CD4 response could be mapped to sequence 322–333 [45] overlapping with a CD8 epitope [46]. Therefore, T-cell responses directed against bacterial hsp60 seem to be relevant in ReA. However, because there is zero or little cross-reactivity among different bacteria and among bacterial and self-hsp60, these results suggest that hsp60 is the least searched antigen. Using the new "affinity matrix technology," we separated live antigen-specific T cells according to their IFNγ-secretion and used this short-term T-cell line for the fine mapping of T-cell epitopes in patients with *Yersinia*-induced ReA. Again one peptide in the whole *Yersinia* hsp60 molecule could be identified to be immunodominant for the CD4 T-cell response. However, the immunodominant peptide was different in different patients (Thiel, Wu, Radbruch, Sieper: manuscript submitted).

HLA-B27-restricted CD8 responses to bacteria have been described in humans. A few years earlier, Hermann *et al.* [47] demonstrated that synovial T cells had a CD8+ HLA-B27-restricted cytotoxic immune response against whole *Yersinia*. Later, an hsp60-derived peptide from *Yersinia* was identified that was presented by HLA-B27 to CD8-positive T cells in several patients with *Yersinia*-induced ReA. Regarding the CD8-response, peptides derived only from the hsp60 and not any other *Yersinia*-proteins were recognized by synovial fluid T-cell lines [46].

A central role for CD8+ T cells is also supported by the finding of an oligoclonal expansion among CD8+ T cells in the synovial fluid of HLA-B27-positive patients with ReA [48–50]. A high homology of T-cell receptors and an even identity was found in different patients with ReA triggered by different bacteria in these studies, suggesting that similar yet unidentified antigens are seen by these CD8+ T cells.

Localization of Immunopathology

Clarifying the reasons why specific sites such as the sacroiliac joints and entheses are preferentially affected in patients with ReA and other spondyloarthropathies (mainly in HLA-B27-positive individuals) may help us understand the pathogenesis. Extensive soft tissue and bone marrow edema at enthesitis lesions is frequently observed on magnetic resonance imaging (MRI). In addition, synovial joint inflammation is frequently associated with clinically unrecognized enthesitis [51,52•]. This was the main, and rather surprising, result of an MRI study in patients with gonarthritis comparing 10 patients with spondyloarthropathy (including four with ReA) with patients with RA [52•]. Thus, even in peripheral arthritis, there are SpA-typical sites of inflammation. The recognition of the primary role of enthesitis and the distinctive inflammatory changes in the adjacent bone marrow suggest that this is the primary site to study for investigating the relationship between HLA-B27 and the triggering microbes in human and experimental forms of SpA. The nature of the T-cell response in the bone adjacent to the sites of enthesitis has yet to be determined. Comparing MRI results with histology of the sacroiliac joint in the same patients with spondyloarthropathies, we could show that (especially in early acute cases of sacroiliitis) T cells are present and infiltrate the cartilage [53••]. Therefore, the cartilage may be a primary target of the immune response in SpA, including ReA, possibly because of the presence of bacterial antigen in these structures or because of an autoimmune response to cartilage-derived antigens [54]. This must be further addressed in future studies. Synovitis could be a secondary event, at least in some joints, possibly resulting from liberation of pro-inflammatory mediators from the enthesitis-associated primary lesion, as discussed recently by McGonagle *et al.* [55].

Lessons Learned about the Pathogenesis of Reactive Arthritis

Regarding the pathogenesis of ReA, different studies on 3 months of antibiotic treatment could not show that the antibiotics are superior to placebo. This is especially true for ReA after preceding infections with enterobacteriae, but not for *Chlamydia*-induced ReA [2]. However, because of the small number of patients in these studies, the observed trend favoring the antibiotics was unconvincing in *Chlamydia*-induced ReA. Perhaps antibiotic treatment must be combined with an immunomodulating approach. It is impossible to predict whether anti-TNFα therapy or a cytokine-directed treatment stimulating a Th1-response to eliminate bacteria will provide the best approach for treating ReA. It remains to be determined whether the anti-TNFα therapy can exacerbate the infection or suppress the inflammation without stimulating bacterial growth. Our data suggest that anti-TNFα therapy is highly effective in patients with ankylosing spondylitis, a disease related to ReA [56].

Conclusions

Important progress has been made in the attempt to clarify the pathogenesis of ReA. Similar pathomechanisms may be relevant in other spondyloarthropathies, especially ankylosing spondylitis. This will hopefully lead to a new and effective treatment in chronic forms of spondyloarthropathies in the near future.

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