Pathogenesis of Reactive Arthritis

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Reactive arthritis is a member of the spondyloarthropathy. Bacteria which cause reactive arthritis infect the mucosal surfaces. Either the whole bacteria or their fragments are subsequently carried to the joints inside which are induced a TH1 lymphocyte response in which oligoclonal T lymphocytes as well peptide-specific CD8+ T lymphocytes participate. Human lymphocyte antigen (HLA)-B27 is a predisposing gene. Besides being determinants for the CD8+ T lymphocyte response it can also modify the response of other cells to the invasive bacteria. This would lead to alteration of the fate of the bacteria as well as release of arthritis-causing cytokines. (Internal Medicine 38: 97-101, 1999)

Key words: HLA-B27, bacteria, Chlamydia, T lymphocytes, transgenic, spondyloarthropathy

Introduction

Before commencing on the review, it is necessary first to define the term “reactive arthritis”. In a broad sense, reactive arthritis is an arthritis induced by infections pathogens in which the pathogens cannot be recovered from the joints. However, this broad definition includes arthritis induced by many pathogens conventionally not included by investigators in the more narrow field of reactive arthritis. For the latter investigators, “reactive arthritis” is a member of the spondyloarthropathy family. The bacterial species which are commonly studied include Chlamydia trachomatis, Salmonella typhimurium or enteritidis, Shigella flexneri and Campylobacter jejuni. The reason why reactive arthritis induced by these bacteria is studied as a group is because they share common features not only among themselves but also with other members of the spondyloarthropathy family. Hence it is almost certain that they share common pathogenetic mechanisms with the more common members of the spondyloarthropathy family which are the undifferentiated spondyloarthropathy and ankylosing spondylitis (1, 2). This review will be restricted to the reactive arthritis in the family of spondyloarthropathy.

Are There Genetic Factors Causing Reactive Arthritis?

Reactive arthritis is a rare disease. It does not occur indiscriminately in all individuals who are infected with arthritis-causing bacteria. In epidemics caused by a single bacterial strains, only a small minority of the infected subjects develops arthritis. When studied in general surveys, there is a higher incidence of the human lymphocyte antigen (HLA)-B27 gene among patients compared to the general population. However, unlike ankylosing spondylitis, HLA-B27 is not essential. In some epidemics, there is no predilection for HLA-B27 (3). Because the incidence is too small, there is not sufficient data on family and twin analyses for precise estimation of the percent influence contributed by genetic factors. The question becomes: if there are genes which predispose to reactive arthritis, how can they be experimentally identified? The answer will have to wait for resolution in the case of ankylosing spondylitis. Ankylosing spondylitis is a much more common disease with an incidence of 0.5% in most Caucasian populations. This incidence has allowed multiple studies both in family members and in twin pairs (4). Such studies clearly show that ankylosing spondylitis is a largely genetic disease. In Caucasians, HLA-B27 is an essential gene so that 95% of the patients are HLA-B27 positive. However, the major histocompatibility complex (MHC) which includes the HLA-B27 contributes probably to only about 30% of the total genetic influence. Using positional cloning, at least seven other chromosomal regions have been identified to be candidates (5). Within
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tremely small copy numbers. Even among patients with well
DNA and RNA sequences in the synovial biopsies with high
development of the polymerase chain reaction (PCR) technol-
and Yersinia (12). In the case of Yersinia, bacterial fragments
in finding multiple microbes not usually associated with reac-
tive arthritis, and even detecting more than one species in a
After reports are confirmed by other investigators, it is commonly accepted that most of the
arthritis-causing bacteria are unlike Chlamydia in not being
in the joints as intact nucleotides.
Although the presence of nucleotides of bacteria other than
Chlamydia is very unusual, the presence of bacterial fragments
has been reported by several groups. These include Salmonella
and Yersinia (12). In the case of Yersinia, bacterial fragments
are also detectable in the phagocytes of the peripheral blood
Taking all the current information into account, it is rea-
sonable to conclude that the first stage in the development of
reactive arthritis is for the responsible bacteria to be delivered
from the mucosa to the joints via at least partly the circulatory
One can also conclude that the delivery of the bacteria
to the joints by itself is not necessarily sufficient to induce ar-
thritis. Lastly, to induce arthritis, it is not necessary for the
bacteria to survive and replicate inside the joints.
One question has not yet been addressed. This is whether
there are genetic factors which play an important role control-
ing the spread and survival of the arthritis-causing bacteria.
Such genes are important in experimental animals (14). Their
identification will be important in human diseases.

What is the Early Host Response to Infection by
Arthritis-Causing Bacteria?
Bacteria causing reactive arthritis share two common fea-
tures. They all infect mucosa and are capable of intracellular
survival and replication. Other than these two features, no single
molecular species or biological processes have yet been de-
scribed which are exclusive for the arthritis-causing bacteria.

Are Arthritis-Causing Bacteria Present in the Joints?
All the arthritis-causing bacteria listed in the previous sec-
tion initiate an infection at the mucosa, either at the gastrointes-
tinal or genitourinary tracts. The natural question becomes
whether in reactive arthritis these bacteria have been conveyed
to the joints to set up another focus of infections. In such a
case, the arthritis would be an infective arthritis rather than a
"reactive" arthritis. Except for rare reports, it is commonly
agreed that no replicable bacteria can be cultured from the joints
of these patients using standard techniques. This single feature
distinguishes reactive arthritis from infective arthritis. For more
precise study of the question whether the bacteria are localized
in the joints, Chlamydia trachomatis have been considered as
the prototype for investigators. That Chlamydia are present in
the joints has been suspected in 1988 based on electron mi-
croscopy of joint biopsies (6). However, such observations are
far from definitive. It became a major breakthrough when Keat
et al. first reported that using a monoclonal antibody, positive
reactivity could be observed by immunohistology with syn-
ovial biopsies of Chlamydia-induced arthritis (7). With the
development of the polymerase chain reaction (PCR) technol-
ology, investigators are able to search for Chlamydia-specific
data and RNA sequences in the synovial biopsies with high
specificity and sensitivity. Indeed, both DNA sequences and
RNA sequences from more than one Chlamydia gene have been
identified in the joints (8). Hence, at this point in time, it is
certain that Chlamydia trachomatis can be delivered from
the mucosa to the joints and “survive” in the joints with intact
DNA and RNA. Since in some of these patients, Chlamydia
DNA have also been detected in the peripheral blood cells, the
Chlamydia are probably carried by circulatory cells (9). If in-
tact Chlamydia are present in the joints, the questions are first
whether the arthritis is septic, and second whether it is of any
diagnostic significance. From the results reported, it is unlikely
that the arthritis is septic in not requiring an immune response.
This is because in the joints, the Chlamydia are present in ex-
tremely small copy numbers. Even among patients with well
defined Chlamydia-induced arthritis, they are not universally
detectable. The question of diagnostic specificity for reactive
arthritis is also important. Chlamydia is a common organism.
Even in the genital tract, their existence can be clinically si-
tent. There is a need to determine whether their residence in
the joints will necessarily lead to reactive arthritis. One way to
test this is to assess the specificity of presence of Chlamydia
from joints of patients who obviously do not have reactive ar-
thritis. Surprisingly Chlamydia are detected in a reasonably
significant percent of patients with rheumatoid arthritis as well
as osteoarthritis and even in a very small percent of apparently
normal individuals. A recent report describes detection in as

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Presence</th>
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<tbody>
<tr>
<td>Chlamydia</td>
<td>Present</td>
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<tr>
<td>Salmonella</td>
<td>Not detected</td>
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<td>Shigella</td>
<td>Not detected</td>
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<tr>
<td>Yersinia</td>
<td>Not detected</td>
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Table 2. Are Bacteria Present in the Joints?

Chlamydia trachomatis DNA/RNA and proteins are detectable in the
joints, but their presence are not totally specific for reactive arthritis.

Salmonella, Shigella and Yersinia fragments but not DNA have been
consistently reported.
Neither is it certain that among one species, e.g., the *Salmonella typhimurium*, whether arthritis-causing potential is restricted to a small number of virulent strains. Because of the low incidence of the disease, it is unlikely for these questions ever to be resolved. Instead, investigators will have to be satisfied on basing their research on the host response which are associated with infection with such species.

On contacting a host cell, arthritis-causing bacteria can invade into the cell and replicate intracellularly. Because HLA-B27 is a causative gene, considerable has been carried out to test if HLA-B27 modifies these events. Initial reports utilized mouse fibroblasts transfected with the HLA-B27 gene. The authors noticed that the presence of HLA-B27 led to decrease in the degree of invasion (15). Although this finding could be reproduced in the same laboratory, they have not been observed by other investigators even with human peripheral blood cells (16). Instead, it has been observed by another group of investigators that in a mouse fibroblast cell line and in a human promonocyte line, both *Salmonella* and *Yersinia* have a higher rate of intracellular survival in the host cells if the lines are also transfected with HLA-B27 (17). However, these findings are not applicable to all cell lines (18). Instead of focusing on invasion and intracellular survival, our group has studied the early molecular response of the host cells to bacterial invasion (19). We notice in the Hela cell line, during *Salmonella* invasion transfection of the HLA-B27 gene causes a heightened response in the immediate early response gene *c-fos* and the late response gene MCP-1. The validity of these findings is strengthened by finding of corresponding changes in transcription factors and in protein level using gel shift and enzyme-linked immunosorbent assay (ELISA) respectively. MCP-1 is a pro-inflammatory chemokine which is potentially arthritis-causing. A number of cytokines and chemokines have also been described to be arthritis-causing in human or in animal models. Such pro-inflammatory cytokines are kept in check by anti-inflammatory cytokines. Hence, the role of HLA-B27 in arthritis might perhaps be a modification of the balance between pro-inflammatory and anti-inflammatory host responses. A major problem in interpretation is that almost all these experiments have been carried out with long term cell lines. Although having the advantage of uniformity and easily manipulated by transfection, such cell lines are unlikely to contain all the genes residing in a patient with reactive arthritis. For a more meaningful study, use of cells derived from arthritis patients to define the molecular response of various cell types to bacteria invasion and residence will be very important.

What has also been relatively neglected is how the phagocytes carrying arthritis-causing bacteria enter the joints. Presumably, homing molecules are induced which promote these cells to traverse the endothelial cells to enter the joint compartment. Again whether there are any genetic predispositions to these events in reactive arthritis patients will require study.

### How Does the Host Immune Response Control These Infections?

Much of the data reviewed in the previous section suggest that reactive arthritis is partly due to a failure of the host to completely eliminate an infection. Hence, analyses of host immune factors which normally suppress infections will be critical. The CD8+ T lymphocyte events are especially of interest because they play a role in host defense against intracellular pathogens. An additional reason why CD8+ T lymphocytes are considered as strong candidates for mediating reactive arthritis is because the predisposing gene HLA-B27 is an HLA class I allele. HLA-A and -B alleles are traditionally regarded as a restrictive element for the T cell receptors of CD8+ T lymphocytes. At least two questions concerning T lymphocyte activities in reactive arthritis have been experimentally addressed. The first question is whether the global T lymphocyte response is of the TH1 or TH2 type. The second question is whether there is a restricted number of antigens observed in reactive arthritis patients. Regarding the first question, TH1 cells are distinguished by their release of interferon (IFN)γ, TH2 cells of interleukin (IL)-4 and IL-10. In experimental animals, defense against intracellular pathogens is mediated by the TH1 response. Synovial membranes from patients with reactive arthritis have been compared to those of rheumatoid arthritis using RT-PCR and *in-situ* hybridization. IFNγ is found in both diseases, while IL-4 is found more often in reactive arthritis. The conclusion is that the T lymphocyte response in reactive arthritis is predominantly of the TH2 type. Based on these findings, the authors postulate that this aberrant response contributes to reactive arthritis because the released IL-4 protects the pathogens from control by IFNγ (20). Although the results of these studies on reactive arthritis are not very precise, they are potentially very important. Identification of critical cytokines might provide targets for therapeutic intervention. The striking efficacy of TNFα-directed modalities in the treatment of rheumatoid arthritis is a precedence. Hence drugs which can modulate cytokine responses are potentially useful for reactive arthritis.

A great deal of research has focused on whether HLA-B27 presents bacteria-derived peptides to activate CD8+ T lymphocytes. That there are CD8+ T lymphocytes in the arthritis joints specific for bacterial infected target cells appear to be beyond question (21). Immunodominant peptides have also been identified which are derived from 13 kDa and 60 kDa hsp and 19 kDa urease protein of *Yersinia*, and the 75 kDa hsp of *Chlamydia* (22). That there is a CD8+ T lymphocyte response and that there are immunodominant peptides for each bacterial species

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**Table 3. Can HLA-B27 Modify Host Reactivity to Invasive Bacteria?**

| Possible decreases degree of invasion. |
| Increases the survival of bacteria inside the cells. |
| Modifies expression of immediate early genes and chemokine response. |
is expected even in infections without any sequelae or in which the patients do not carry HLA-B27. They are probably an integral part of the immune response against bacterial antigens in the joints. What is needed is an experimental approach to test the roles of these antigens/peptides in arthritis. One possibility is to assume that the arthritis-causing T lymphocytes are oligoclonal, and that these T lymphocyte clones recognize these peptides and are the mediators of the arthritis processes. Indeed striking oligoclonality has been recently described (23). The fact that there is oligoclonality indicates that the T lymphocyte response is directed toward small numbers of discrete antigens and not at random. Identifying their antigen/peptide specificities would be very important. If this can be achieved, one approach to assess the arthritis-causing potential of these T lymphocyte clones would be to define their cytokine/chemokine profiles. Arthritis-causing T lymphocytes should be synthesizing pro-inflammatory and not anti-inflammatory molecules.

Several other possibilities regarding how HLA-B27 modulates lymphocyte reactivity either of CD4+or CD8+ T lymphocytes or even NK cells also exist. They are not yet supported by much experimental evidences and will not be reviewed here.

**Lessons from Animal Models of Arthritis**

A major problem in studying reactive arthritis is that reactive arthritis is a relatively uncommon disease. Identification of susceptible subjects before they develop arthritis is almost impossible. Serial studies during the course of infection are limited to a very small number of patients. Pinpointing critical molecules by creating knockout of the target genes or molecule-targeted therapies are impossible. Much of these difficulties can be overcome in experimental animal models.

Early animal models of reactive arthritis consist of administering live bacteria to rats. Aseptic arthritis is observed when a *Yersinia enterocolitica* 0:8 strain is injected intravenously into Lewis rats (24). Arthritis is not observed in the Fischer and BN rats. Serial study of ten cytokines suggests that there is a higher level of expression of anti-inflammatory cytokines in the arthritis-resistant rats (25). However, the differences in cytokine profiles do not appear to be totally clear-cut.

Much more precise analyses are offered by the creation of transgenic HLA-B27 animals. The first of such animal models is the HLA-B27 transgenic rat (1). This was followed after some years by the HLA-B27 transgenic mice (26). In both animal models, transgenic human β2-microglobulin was also introduced into the animals to generate arthritis. In the case of the rats, no control with no human β2-microglobulin transgene has been generated. In the case of mice, arthritis is not observed in animals in which only endogenous mouse β2-microglobulin is expressed. Surprisingly, HLA-B27 transgenic mice develop arthritis even in absence of endogenous mouse β2-microglobulin.

In both HLA-B27 transgenic rats and mice, the development of arthritis requires environmental flora. In this sense, they appear to be animal models of reactive arthritis. Rats do not develop arthritis in a germ-free, and mice in a pathogen-free environment. However, such animals develop arthritis when they are switched to a conventional environment. The responsible bacterial species do not appear to be conventional pathogens (27).

T lymphocytes appear to be critical for arthritis in both rats and mice. In the case of rats, arthritis does not develop in athymic nude rats. Transfer of T lymphocytes from euthymic to athymic rats induces arthritis. As for the subtype of T lymphocytes, transfer of CD4+ T lymphocytes clearly induces arthritis, while the role of CD8+ T lymphocytes is questionable. Regardless of the subtype of T lymphocytes, presentation of peptides by HLA-B27 appears to be important in the rat model. This is because generation of a transgene encoding an HLA-B27 peptide derived from the influenza virus prevents development of arthritis. The peptide generated by this transgene monopolizes the peptide-binding groove of most of the HLA-B27 molecules. Presumably the arthritis-causing peptides are being displaced. One hope in creating these animal models is more precise identification of the critical factor responsible for arthritis. This is traditionally achieved in animal models by transfer experiments as described above or by knockout of discrete genetic elements. In the rats, results of transfer of bone marrow cells suggest that the culprit is the antigen-presenting cell. However, the conclusion is an indirect one. In the case of transgenic mice, knockout of CD4 molecules prevents the appearance of arthritis. By itself, it would indicate that the responsible T lymphocytes are CD4+ T lymphocytes which normally recognize MHC class II antigens. However, in the case

**Table 4. Is There a Disease-Associated CD8+ T Lymphocyte Response in the Joints?**

CD8+ T lymphocytes with specificity towards bacteria or bacterial peptides can be detected.

Some of the T lymphocytes are also autoreactive.

There is an oligoclonality in response suggesting a restricted number of antigens.

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of the HLA-B27 mice, the mechanism of arthritis is not that of an MHC class II mediated CD4+ T lymphocyte response. This is because knockout of the MHC class II molecules does not abrogate appearance of arthritis. The authors propose that arthritis is caused by presentation by peptides by the free heavy chains of HLA-B27 molecules. No peptides have been isolated from such free heavy chains to provide direct evidence of this hypothesis (28).

While these results with HLA-B27 transgenic animals are extremely provocative, they have so far not been able to contribute to testing in the human patients. It is possible that when more information is obtained from the human disease, the animal models can serve the purpose of testing hypotheses not feasible in human experiments.

**Future Directions**

Many of the projects outlined above are ongoing. It is possible with the resolution of questions such as what antigens activate oligoclonal T lymphocytes, how HLA-B27 modifies cell response, what is the biology of bacteria inside the joints, the cause of reactive arthritis will become clear. Also very likely is that certain of the cellular processes causing reactive arthritis are shared with those of ankylosing spondylitis. If we can identify the genes and the molecular processes mediating ankylosing spondylitis, the cause of reactive arthritis will also become clear. Multiple groups are launching genome-wide screening for susceptibility genes in ankylosing. Such data will probably prove to the most important in this field of investigation.

**References**