



## Role of bacteria and HLA-B27 in the pathogenesis of reactive arthritis

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Strictly speaking, “Reactive arthritis” (ReA) is a conventional term, with no study-verified definition. In this review, we will focus on the type of arthritis that is induced by the following species: *Chlamydia*, *Shigella*, *Salmonella*, *Yersinia*, and *Campylobacter*. The type of arthritis caused by these pathogens shares a clinical pattern, which is in common with the spondyloarthropathies (SpA), especially undifferentiated spondyloarthritis and Reiter’s syndrome. All these diseases including ankylosing spondylitis (AS) must also share major pathogenic pathways.

### ReA from the point of view of the pathogen

The number of ReA-causing pathogens is very few. For a particular ReA pathogen to become successful, first it has to be an obligate/facultative intracellular organism and then it needs to evolve so that it is capable of the following activities: (1) traveling to the joints from the mucosal surface; (2) adjusting its molecular activities to adapt to the joint environment; and (3) evading the host defense. With regard to all these activities, *Chlamydia trachomatis* is the best studied.

#### *How does Chlamydia travel to the joints?*

The primary site of infection of *C trachomatis* is the mucosa of the urogenital tract. At this site of entry, among the host cells that they enter and survive are the monocytes and/or dendritic cells. Thus, when these cells enter the bloodstream,

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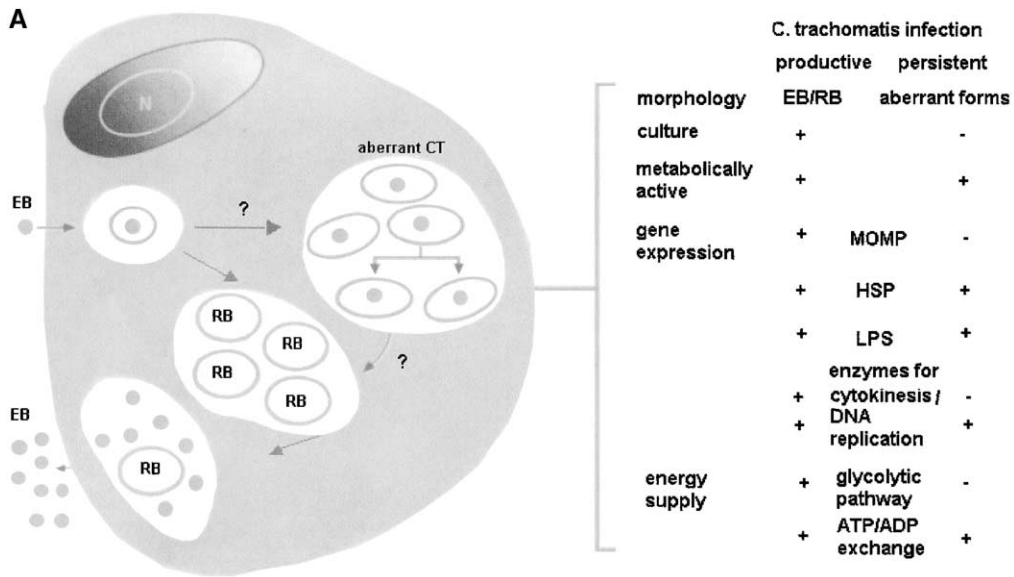


Fig. 1. (A) Schematic figures of the normal biphasic life cycle and the aberrant forms of persisting *Chlamydia*. *Abbreviations:* RB, reticulate body; EB, elementary body; aberrant CT, persisting *Chlamydia* with aberrant morphology; MOMP, major outer membrane protein; HSP, heat shock protein 60; LPS, lipopolysaccharide.

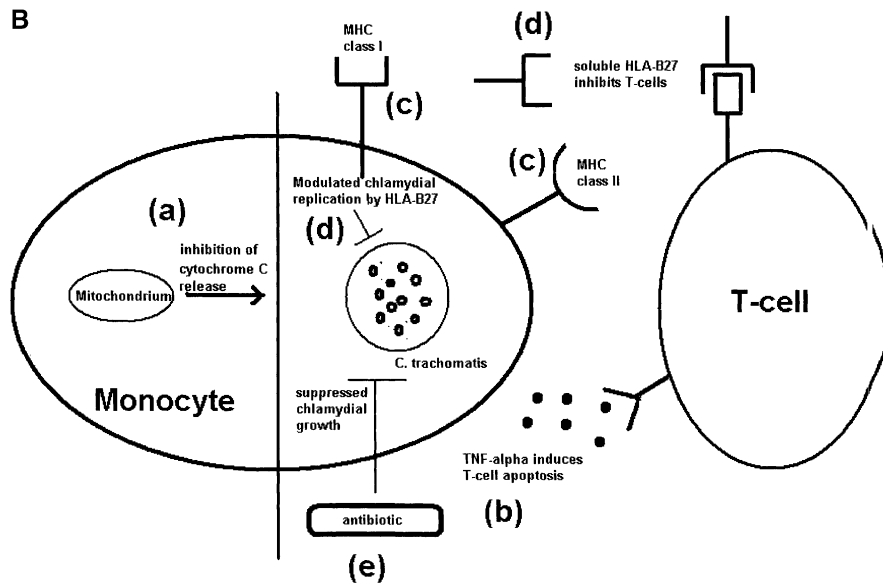


Fig. 1. (B) Schematic representation of mechanisms of chlamydial persistence. Letters in parentheses denote the various activities that can be modified by the presence of *Chlamydia*.

they become vehicles to disseminate the bacteria, one destination being the joints [1]. This form of dissemination is probably universal for all ReA pathogens including *Yersinia* [2].

*How does the unique life cycle behavior of Chlamydia make it a perfect ReA pathogen?*

One of the reasons for the success of *Chlamydia* in being a ReA pathogen is its unique life cycling potential (Fig. 1A). *Chlamydia* infects host cells in the form of metabolically silent but infectious elementary bodies (EB). These EB first bind to the host cell surfaces and are then engulfed by endocytosis. Within a few hours the EB transform themselves into larger and metabolically active reticulate bodies (RB). The RB then multiply by binary fission within a vacuole, the so-called “inclusion body.” Ultimately, these RB once more reorganize themselves into EB, to be released by either cell lysis as well as exocytosis. This cycle takes about 48 hours. The released chlamydial EB are powerful pathogens in their ability to infect further host cells.

Inside the ReA joint, the ideal host cells for *Chlamydia* are also the monocytes [3]. Monocytes are ideal for *Chlamydia* because they are long-living cells, so the *Chlamydia* can survive persistently. We know that to become persistent, the *Chlamydia* themselves also have to modify their normal biphasic life cycle. This is because the *Chlamydia* cannot be cultured from the joint anymore but yet they are viable and metabolically active, as demonstrated by the expression of very short-lived chlamydial rRNA transcripts and mRNA in addition to the normal RNA and DNA [4]. On a molecular level, the important characteristics of this persistent infection are the altered regulation of the expression of several *Chlamydial* antigens. The synthesis of the chlamydial major outer membrane protein (MOMP) is reduced. On the other hand, the 57-kDa heat-shock protein (HSP60) and lipopolysaccharide are highly augmented. These changes in structure would explain the electron microscopy finding of an aberrant morphology of persisting *Chlamydia* [3,4]. In addition, the genes involved in energy pathways of *Chlamydia* are also altered during persistent infection. In early replicative infection, energy is derived from the glycolytic and pentose phosphate pathways. Once persistence has become established, the primary source of ATP is switched to that of the host. Transcripts for glycolytic and pentose phosphate pathway enzymes can no longer be detected [5].

In summary, there are certain features that are unique to the *Chlamydia* in the ReA joints. These *Chlamydia* cannot be cultured by traditional techniques. Their morphology appears aberrant. Although they are still metabolically active, they have modified their gene expression profile to a considerable extent.

The life cycle behavior described above cannot be extended to ReA-causing enterobacteria. Enterobacteria are not obligate intracellular pathogens. For such bacteria, it is still an enigma whether those inside the ReA joints are viable and metabolically active. Early polymerase chain reaction (PCR) reports are not

supportive. More recently, investigators appear to have become successful in amplifying *Yersinia* and *Salmonella* DNA from the joints [6,7]. If this is a general rule, it would mean that enterobacteria are also able to survive persistently in ReA joints.

#### *How does Chlamydia evade the host defense?*

*Chlamydia* evades the host defense with several strategies (Fig. 1B):

##### *Chlamydia inhibits apoptosis of host cell*

*Chlamydia* requires its host cells to be alive for their own survival. Apoptosis of host cells would lead to death of the bacteria. *Chlamydia* inhibits this host cell apoptosis by inhibiting release of mitochondrial cytochrome C and also by directly engaging the death domains of the tumor necrosis factor (TNF) receptor family [8].

##### *Chlamydia induces apoptosis of T lymphocytes*

*C Trachomatis*-infected monocytes induce apoptosis of autologous T lymphocytes through the release of TNF- $\alpha$  [9,10].

##### *Chlamydia downregulates the expression of antigen presentation molecules*

During persistent infections, *Chlamydia* downregulates the synthesis of immunodominant antigens such as MOMP. They also inhibit interferon (IFN)- $\gamma$ -induced major histocompatibility class (MHC) class II, as well as constitutive and IFN- $\gamma$ -inducible MHC class I expression. They secrete protease- or proteasome-like activity factor (CPAF) into the host cell cytosol to degrade host transcription factors RFX5 and upstream stimulation factor 1, which are crucial for MHC class I and II expression. The downregulation of both potentially chlamydial antigens and antigen-presenting molecules enable chlamydial evasion from an effective immune response.

##### *HLA-B27-mediated mechanisms of chlamydial persistence*

In an in vitro infection model, we demonstrate that HLA-B27 suppresses chlamydial replication [11]. Also, soluble HLA-B27, which is induced by reactive arthritis-triggering bacteria, is able to inhibit HLA-B27-restricted T cells and thus may also promote chlamydial persistence [12].

##### *Antibiotic treatment converts productive chlamydial infection from a productive to a persistent mode*

*Chlamydia* persists in ReA hosts despite prolonged antibiotic treatment [3]. As a matter of fact, antibiotic treatment not only fails to eliminate *Chlamydia*, it might even convert them from a productive phase to a persistent phase [13].

### **Pathogenesis of ReA from the point of view of the host: theoretical possibilities**

In the subsequent review, we will first outline the major discoveries in several areas of reactive arthritis and then attempt to integrate the seemingly diverse findings into one working hypothesis.

Investigators have addressed the following theoretical mechanisms of ReA:

1. A CD8<sup>+</sup> T lymphocyte antibacterial response subverted to become auto-immune
2. A subverted CD4<sup>+</sup> T lymphocyte antibacterial response
3. A defect in those cytokines and innate immunity, which are responsible for defense against arthritis causing bacteria
4. Abnormal permissiveness of host cells to bacterial invasion, replication, or pro-inflammatory signaling.

#### *CD8<sup>+</sup> T lymphocytes and HLA-B27 in ReA*

The CD8<sup>+</sup> T lymphocytes have always been regarded as the primary culprit in ReA. Discovering how they induce arthritis was at one time regarded as the ultimate objective of ReA investigators. This is because CD8<sup>+</sup> lymphocytes, until recently, have been regarded as the only cells carrying high specificity for HLA-B27, which is the essential gene for AS, a particular form of SpA. HLA-B27 is an allele of the HLA class I molecules, a major function of which is to

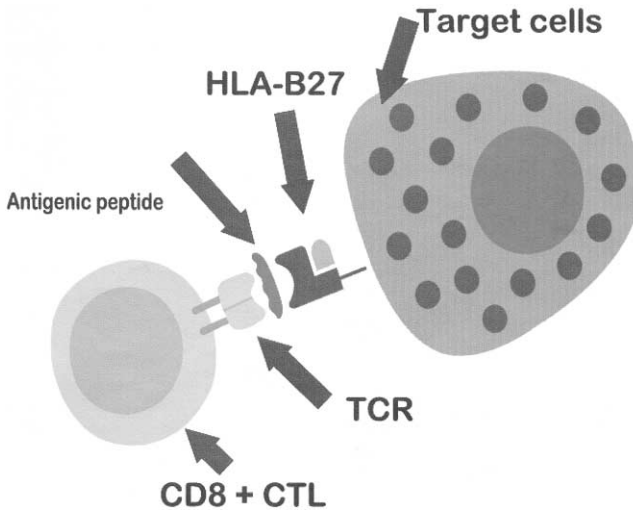


Fig. 2. A major physiological function of HLA class I molecules, such as HLA-B27, is to present antigenic peptides to the T cell receptors (TCR) of CD8<sup>+</sup> T lymphocytes (CTL). Because in *in vitro* experiments, this can lead to lysis of the HLA-B27 cells, the peptide-presenting cells are conventionally designated as target cells.

present peptides to CD8<sup>+</sup> T lymphocytes (Fig. 2). The motif of peptides carried by each HLA allele is allele specific. The motif for HLA-B27 and its subtypes have been well defined. Naturally, the identification of HLA-B27-restricted bacterial peptides has been a concern for major investigators. In the first phase of their study, investigators would first select certain promising candidate bacterial proteins and then synthesize peptides corresponding to the sequences of those proteins for testing. Several peptides have been identified [14–16]. Since such candidate bacterial proteins comprised only a tiny percentage of the total bacterial repertoire, more comprehensive approaches are needed. Sieper and his group recently have extended this to the extreme by synthesizing and testing 199 peptides derived from the entire genome sequence of *Chlamydia trachomatis*. When tested against the synovial fluid, CD8<sup>+</sup> T lymphocytes of three patients with acute *Chlamydia*-induced arthritis, 11 reactive peptides are identified. Notably, 4 of the 11 peptides are derived from hypothetical proteins derived from the *Chlamydia* genome sequence [17]. How do we know if any of these bacterial peptides identified so far are relevant to ReA? In theory, the chance for a particular peptide to be important to ReA would be high if it is also immunodominant and therefore reactive with most ReA patients. One of the 11 peptides identified from the *Chlamydia* genome is indeed reactive with T lymphocytes from all three patients tested in the study. This peptide is derived from clp protease-ATPase and carries the sequence NRAKQVIKL. At this point in time, this is probably the most promising bacterial peptide identified so far. The screening described above, however, although enormous in scale, is far from comprehensive. In that screening test, only those T lymphocytes producing IFN- $\gamma$  in response to the peptides are identified. It is very likely that there are many other HLA-B27 chlamydial peptides that also react with CD8<sup>+</sup> T lymphocytes but do not induce IFN- $\gamma$ . Nevertheless, this study is the most comprehensive screening up to date. At the minimum, this and previous studies demonstrate very clearly that there is an active CD8<sup>+</sup> antipathogen activity that can be observed in the joints.

An active T lymphocyte activity would certainly explain why there is an acute arthritis after mucosal infections. What is just as challenging to investigators is why the acute arthritis would become chronic in some patients, especially if they are HLA-B27 positive. The natural hypothesis is that in chronic ReA, the acute antibacterial response is followed by a self-perpetuating autoimmune response against an autologous peptide (Fig. 3). This hypothesis can be tested if such autoantigens can be identified. Fiorillo and her group approach this question by searching for autologous peptides that are homologous to those of an Epstein-Barr virus (EBV) protein [18]. The particular EBV protein they focus on is one that has previously been found to provide an immunogenic peptide for HLA-B27 subjects. The autologous peptide they identified is derived from 400 to 408 residues of the vasoactive intestinal peptide receptor 1 (VIP-1R). The sequence is RRKWRRWHL. Supporting its role in SpA, this VIP-1R peptide reacts with CD8<sup>+</sup> T lymphocytes from AS patients in the context of the arthritis-predisposing B\*2705 subtype but not with the arthritis-sparing B\*2709 subtype. This

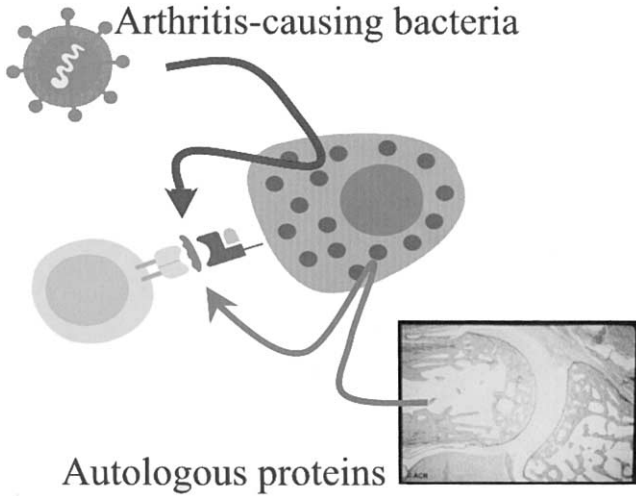


Fig. 3. The arthritogenic peptide model of reactive arthritis. HLA-B27 presents certain antigenic peptides derived from the arthritis-causing bacteria. These peptides are cross-reactive with certain autologous peptides derived from proteins in the joint tissues. The cross-reactive immunological reactions lead to an autoimmune response.

discovery is certainly a major event. The next major breakthrough will be to identify autologous peptides that can react with CD8+ T lymphocytes from a larger panel of HLA-B27–positive SpA patients.

### **Supportive evidence for role of T lymphocytes in ReA: oligoclonality of ReA T lymphocytes in the joints**

The existence of reactive bacterial and autologous peptides do not necessary mean that they play an important role in SpA. They would be important if they are the major T lymphocyte antigens in the arthritis joints. The likelihood of this can be assessed from the degree of oligoclonality in T cell receptors (TCR) displayed by ReA T lymphocytes. A high degree of oligoclonality would indicate that there is T lymphocyte reactivity against only a very small number of antigens. A polyclonal reactivity would be nonspecific. The combined effort of several groups has provided a positive answer. By pooling their data, they discover that there is a conservation of the TCR  $\beta$ -chain complementarity-determining region 3 (CDR3) of T cell clones derived from patients with early ReA, and that there are CDR3 sequences that are even shared between patients [19,20]. If one takes into account the data on the peptides described above as well as and these TCR data, one would be tempted to speculate that identifying the particular peptides responsible for the oligoTCR clones would provide the answer for what initiates ReA. The technical barrier is so far enormous.

### **There is more than one side to the HLA-B27 story: some puzzling messages from HLA-B27 transgenic animals**

CD8+ T lymphocytes would be indisputably accepted as the mediator of ReA and other SpA if the above results are the only experimental data available. However, experimental data have been accumulating from alternative approaches that point at other possibilities. One major source of data is from experimental arthritis, which develops in mice and rats when they carry the HLA-B27 transgene. Somewhat similar to human ReA, spontaneous arthritis will not develop in these animals if they are kept in a germ- or pathogen-free environment, but would appear when such animals are brought into a regular environment [21,22]. Most remarkably, mice carrying the HLA-B27 transgene, but knockout of the  $\beta$ 2-microglobulin ( $\beta$ 2m) gene, would still develop arthritis [23]. Because  $\beta$ 2m is essential for the quaternary structure of HLA-B27, it would be impossible for such cells to present those candidate arthritogenic peptides so far identified in man. In these animal models, no single cell type has yet been demonstrated to be the sole mediator of arthritis. In summary, although these animal studies do not provide us with a mechanism of arthritis in man, they do question whether HLA-B27-related ReA is mediated by conventional peptide presentation by HLA class I molecules.

#### *CD4+ T lymphocyte activities in ReA*

What about CD4+ T lymphocytes in human ReA? Investigators have so far been focusing only on identification of the CD4+ T lymphocyte-activating antigens. The major difficulty has been to identify a small number of antigens out of an enormous number of candidates expressed by a bacterial species. In the early phase of their studies, individual bacterial proteins were isolated by SDS-PAGE. In *Chlamydia*, three antigens have been identified: Hc1, hsp60, and omp-2. The same group of authors has recently proceeded to an even more comprehensive approach by screening a gene expression library. They identify three more chlamydial antigens: enolase, pmpD, and CT579. CD4+ T lymphocyte reactivities toward these proteins are observed in the joints of ReA patients [24]. Whether they play an indispensable role in human ReA is far from clear. In transgenic mice, knockout of the CD4 gene will prevent the mice from developing arthritis. Surprisingly, knockout of MHC class II genes in the B27 mice will not prevent arthritis. Hence, the murine arthritis-causing CD4+ T cells are not those mediating disease via the MHC class II molecules [21]. They are different from those so far examined in human ReA.

### **ReA arthritis from the point of view of a microbiologist: invasion of bacteria into a cell and subsequent survival**

Arthritis-causing species of bacteria share one common property: they are all facultative intracellular pathogens. A traditional microbiologist would ask

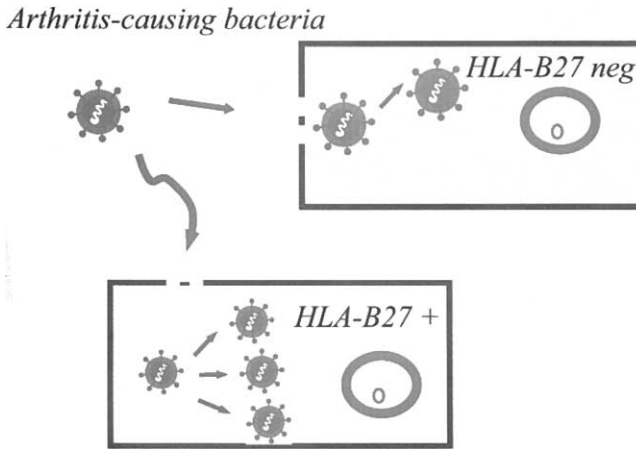


Fig. 4. HLA-B27 modifies the ability of cells to accommodate replication of arthritis-causing bacteria. Arthritis-causing bacteria are able to survive in higher numbers in HLA-B27-positive cells compared to HLA-B27-negative cells.

whether HLA-B27 subjects are more susceptible to ReA because their cells are more permissive to bacterial invasion and intracellular replication. Several groups of investigators have addressed these questions using experimental cell lines as well as cells from the peripheral blood of arthritis subjects. In the case of cell lines, there is no consensus of results from the various groups. One group has consistently reported that using several different cell lines, bacterial invasion is not different between HLA-B27-positive or -negative cells. Cells transfected with HLA-B27, however, are observed to be more permissive for bacterial survival (Fig. 4) [25]. The problem is whether this conclusion can be extended from *in vitro* short-term study to events in the hosts. If the projection is valid, one would conclude that arthritis-susceptible subjects harbor bacteria for a longer period of time compared to control subjects. This question has been addressed in a study of 198 *Salmonella*-infected subjects [26]. The results show that HLA-B27 does not modify susceptibility to infection, duration of infectious symptoms, or duration of excretion of bacteria. This would question the relevance of the *in vitro* bacterial survival findings. Nevertheless, the results of these *in vitro* experiments are very important. At the minimum, they indicate that HLA-B27 can modify cell behavior without involving an interacting immune network. They have led to the signaling theory to be described in the next section.

### **HLA-B27 causes arthritis by modifying cell signaling: a renegade hypothesis**

A completely different hypothesis appeared when it was reported in 1998 that HeLa cells transfected with HLA-B27 respond to *in vitro* bacterial invasion with signals not observed in control cells [27]. This is an interesting observation because it would explain why HLA-B27 cells accommodate bacterial survival

differently from control cells. It was puzzling, however, why HLA-B27 would be different from other HLA alleles in signal transduction. The intracytoplasmic domain of HLA-B27 is very similar in sequence to those of other HLA-B alleles. The explanation did not come until 1999, when it was observed that HLA-B27 is unlike some other HLA alleles in that when maturing inside the endoplasmic reticulum, the protein is folded at a significantly slower rate [28]. In theory, this slow folding might lead to intracellular signaling via what has been termed the endoplasmic reticulum “unfolded protein response” and “overloaded protein response” [29]. The latter can activate the proinflammatory NF $\kappa$ B pathway and has been incriminated in cystic fibrosis, Alzheimer disease, and a form of  $\alpha$ 1-antitrypsin deficiency. This concept that HLA-B27 causes arthritis because it is folded more slowly is appealing in other aspects. HLA class I molecules, which are folded slowly, might actually become expressed on the cell surface as free heavy chains [30]. This would explain why mice that carry the HLA-B27 heavy chain transgene but do not express any light chain could develop arthritis.

Even if HLA-B27 causes arthritis via an internal cell signaling, this signaling event cannot be occurring on all HLA-B27 subjects in all cell types at the same time. The affected cell type was discovered by J. Gu when she screened for differentially expressed genes using the microarray technique. Screening in peripheral blood mononuclear cells provided only marginal results [31]. When synovial fluid mononuclear cells of SpA are compared to RA with a 1200-gene microarray, however, both types of arthritis show a considerable number of genes being more highly expressed compared to the peripheral blood cells of normal individuals. Some of these encode potentially proinflammatory molecules. Surprisingly, there is no significance difference between SpA and RA in the level of expression of these genes. This would suggest that the immediate mediators of the two types of arthritis are very similar. However, one small cluster of genes appears to be differentially expressed in the synovial fluid cells of SpA. One of these genes encodes the proteasome subunit C2. Because expression of this gene is linked to an endoplasmic reticulum unfolded protein response, we proceed to test the presence of such a response by assaying for expression of the BiP gene. The level of expression of BiP is commonly accepted as a measurement of the unfolded protein response. Our results show that it is indeed expressed at a higher level in SpA. In preliminary experiments, we also show that the high expression is restricted to the macrophage fraction of the synovial fluid cells [32]. Hence, there is a possibility that the unfolded protein response is indeed active in the joint compartment of SpA patients, and the macrophage fractions are the ones being most affected. In the field of SpA, this is the first clue concerning a possible central role of the macrophages.

### **Antibacterial cytokines and innate immunity in ReA**

For more than two decades, HLA-B27 has been the focus of attention in ReA and other SpA. HLA-B27 occurs in 95% of Caucasian patients with AS, for

which it is an essential gene. How much it contributes to ReA is an important question. The most systematic evaluation has been carried out using 198 consecutive patients infected with *Salmonella*, comparing the data to 100 healthy controls. HLA-B27 is not more frequent in those with joint symptoms. In contrast, of the eight patients with physician-confirmed reactive arthritis, 75% are HLA-B27 positive. This is strong verification that HLA-B27 is a major player in ReA; however, unlike AS, it is not essential [26]. Indeed, the incidences of HLA-B27 in ReA patients identified among outbreaks of infections are rather variable. The numbers of HLA-B27-positive individuals identified in three outbreaks of *Salmonella*, for example, are only 4 out of 11 total, 4 out of 13 total, and 10 out of 22 total, respectively [33]. Hence, other factors must play major roles in some and perhaps many ReA patients.

At least three cytokines are commonly regarded as crucial in defense against intracellular bacteria: TNF- $\alpha$ , IFN- $\gamma$ , and interleukin IL-10. Of the three, direct demonstration of its role in arthritis has been demonstrated only for TNF- $\alpha$ : mice that are knockouts of the p55 receptor are more susceptible for infection by *Yersinia*. These TNFR p55 - / - mice also develop more severe arthritis [34]. Evidence in human arthritis is necessarily indirect. Nevertheless, a low production of TNF- $\alpha$  by peripheral blood T lymphocytes has been demonstrated at onset of ReA. In addition, the lower the level of TNF- $\alpha$  production is in a patient, the more likely is his potential to develop a chronic course afterwards [35]. Why is TNF- $\alpha$  production impaired? HLA-B27 might be one reason for this defect, as it can also be observed with healthy HLA-B27-positive individuals. The other reason might be because ReA is associated with low-producing TNF- $\alpha$  alleles. Only one study has addressed this. In that study, the microsatellites of TNF-a, -b, and -c of 59 Finnish patients are compared to 285 controls. An HLA-B27-independent increase in the low-producing allele TNF c1 is observed with ReA [36]. This result cannot be universally accepted until it has been extended to other populations. Multiple studies have been reported on TNF- $\alpha$  polymorphism in AS; however, there is no agreement of findings among different ethnic groups [37].

IFN- $\gamma$  is another potent antibacterial cytokine. Normally, IFN- $\gamma$  is activated when bacterial antigens activate TH1 lymphocytes. Hence, we should expect a high level of IFN- $\gamma$  in patients with ReA. Contrary to expectation, compared to RA, the amount of IFN- $\gamma$  is lower in SpA joints. This would reflect a lower ratio of TH1/TH2 activities in SpA compared to RA [38,39]. The reason for this inappropriate modulation of the TH1/TH2 ratio in SpA is not clear. It is at least an indicator of disease activity, as the ratio becomes reversed when patients are induced into remission by treatment with anti-TNF- $\alpha$  antibody.

The role of IL-10 is quite unlike that of IFN- $\gamma$ . IL-10 inhibits the antibacterial activities of macrophages. Its role in ReA can only be deduced from the results of a study on Finnish patients. In those patients, IL-10 G8 is associated with the development of chronic arthritis. On the other hand, the IL-10 G12 and G10 microsatellites are associated with protection [40]. The effect of these markers,

however, is indirect because they are not by themselves related to the level of IL-10 production.

Although there is a vast array of surface molecules developed for innate bacterial defense, only the class A scavenger receptors (SR-A) have been reported in ReA. These are the SR-AI, SR-AII, and MARCO (macrophage receptor with collagenous structures). All three are surface macrophage proteins. The level of expression of MARCO is lower in SpA joints compared to those RA [41]. Although this is the first ReA study of innate defense surface molecules, it will soon become an exploding field. Their possible role in ReA will be discussed in the final section.

In summary, the above studies with the four factors would suggest that ReA-susceptible subjects are those who are unable to mount an effective anti-bacterial response in the joints.

### A working hypothesis

So far, we have outlined the research on the pathogenesis of ReA according to the areas being focused on by each major group of investigators. One can see that each group of investigators has anchored its approach on one special type of experimental technique. Interestingly, each provides a different clue to the ReA puzzle. A comprehensive model will be needed to integrate these different ideas. This model will be useful if it also indicates what additional information is needed and how to design for future therapies. One possibility is represented in Fig. 5. In this model, a T CD8<sup>+</sup> T lymphocyte is placed in the central position. Its TCR is specific for foreign and self-peptides carried by HLA-B27. This event is

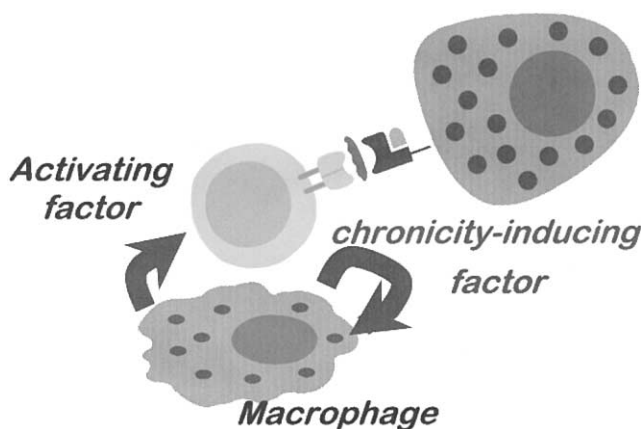


Fig. 5. An updated hypothesis: macrophages are activated, perhaps by an unfolded protein response or overloaded endoplasmic reticulum response. These activated macrophages in turn present antigenic peptides via HLA-B27 to CD8<sup>+</sup> T lymphocytes. The activities of these cells are amplified by certain chronicity-inducing factors, perhaps focusing once more on the macrophages.

probably very important, at least during the initial phase of ReA. We postulate that what drives the T lymphocytes are cells of the monocyte lineage, and that these cells are abnormal because their reactivities are modified by the HLA-B27–induced endoplasmic reticulum overloaded protein response. Supportive evidences for each of the steps have been outlined in this review. What has not yet been experimentally explored is the mechanism that maintains this activity and perhaps drives the T cell reactivity from an oligoclonal to a polyclonal one. An amplifying process probably exists and will become the focus of future research.

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