Bacterial triggers and autoimmune rheumatic diseases

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ABSTRACT

Autoimmune rheumatic diseases are generally considered as a multifactorial aetiology, mainly genetic susceptibility combined with environmental triggers of which bacteria are considered one of the most prominent. Among the rheumatic diseases where bacterial agents are more clearly involved as triggers are: reactive arthritis (ReA), rheumatic fever (RF) and Lyme disease. The role of bacterial infections in inducing other seronegative spondyloarthritis and antiphospholipid antibody syndrome has been hypothesized but is still not proven. The classic form of ReA is associated with the presence of HLA-B27 and is triggered by the urethritis or enteritis causing pathogens Chlamydia trachomatis *and the enterobacteria* Salmonella, Shigella, *and* Yersinia, *respectively. But several other pathogens such as* Brucella, Leptospira, Mycobacteria, Neisseria, Staphylococcus *and* Streptococcus *have also been reported to cause ReA. RF is due to an autoimmune reaction triggered by an untreated throat infection by* Streptococcus pyogenes *in susceptible individuals. Carditis is the most serious manifestation of RF and HLA-DR7 is predominantly observed in the development of valvular lesions. Lyme disease is a tick-transmitted disease caused by the spirochete* Borrelia burgdorferi. *Knowledge is limited about how this spirochete interacts with human tissues and cells. Some data report that* Borrelia burgdorferi *can manipulate resident cells towards a pro- but also anti-inflammatory reaction and persist over a long period of time inside the human body or even inside human cells.*

Introduction

Until the second half of the 20th century, infectious diseases, especially of bacterial etiology, were the first cause of mortality in the world population. The discovery of antimicrobial drugs changed this state, but a lower lethality has not led to a lower morbidity, in fact some bacterial infections have been shown to cause a number of rheumatic and non-rheumatic diseases which were previously considered of unknown origin. A possible mechanism to explain how a pathogen may induce these diseases could be molecular mimicry (1) in which host immune responses are inadvertently directed against endogenous host tissues by virtue of antigenic cross-reactivity between host and microbial determinants (2, 3). Another possible explanation is that the persistence of bacterial agents could give a long-lasting over-stimulation of the immune system characterized by a robust production of cytokines and consequently tissue damage. Also, during normal immune response, the presence of particular polymorphisms of immune related genes (*i.e.*, human leukocyte antigens, cytokines) could produce an abnormal reaction towards autologous tissues (4). In this review, we discuss some rheumatic diseases in which bacteria are considered the most prominent triggers.

Reactive arthritis

The current definition of reactive arthritis (ReA) was introduced in 1969 by Ahvonen (5) referring to a joint inflammation which is triggered by a preceding bacterial infection of an extrarticular site, most commonly urethritis, enteritis, and respiratory infections. As opposed to septic arthritis, ReA is sterile and thus the causative microbial agent can not be cultured from the synovial compartment of the joint. There are several predisposing factors in ReA of which the best studied one is HLA-B27; in fact, 30-80% of ReA patients carry this antigen and they are more likely to experience a chronic course or an axial manifestation. Some studies suggest correlations with sequence variants of the interleukin-10 gene. The

estimated prevalence is 30-40 cases per 100,000 adults; the annual incidence is about 4-5/100.000 for *Chlamydia*- and *Enterobacteria*- induced arthritis (6, 7). About 1-3% and 6-30% of patients with chlamydial and enterobacterial infections, respectively, go on to develop reactive arthritis. A minimal interval between preceding symptoms and the arthritis is proposed to be between 1-7 days and 4 weeks. The typical clinical picture is usually oligoarticular and affects most frequently the lower extremity joints. Prognosis of ReA is generally good. Most patients recover within months. However, a considerable percentage suffers from long-lasting joint symptoms and 10-20% of patients experience an ongoing disease even two years after the first symptoms. Sacroiliitis can develop in up to 30% of patients.

The classic concept of ReA is based on a persistent bacterial infection which is different to the early, productive infection and originates from the primary site of infection or the joint. This requires an ongoing synthesis of bacterial antigens which maintain the synovial inflammation. Persistence is a long-term interaction of the pathogen and its host in which the latter one is not able to eliminate the bacteria. The molecular processes involved lead to modifications of gene and protein expression patterns of both the pathogen and the infected host cells (8, 9). Following the current hypothesis on this concept: a) the triggering pathogen is an obligate or at least a facultative intracellular organism, in addition, b) the pathogen and/or its antigen is delivered from the primary site of the infection to the joint; c) the pathogen escapes the immune response mounted by the host; d) the pathogen is capable of surviving and persisting within the joint (10). In this regard, *Chlamydia* provide one of the best studied examples. They exhibit a modified metabolism, an aberrant morphology, and a reversible arrest of development explaining the fact that they can not be cultured from the joint material. However, for *Enterobacteria*-induced reactive arthritis intraarticular persistence of viable organisms is not definitely established but one study has reported *Yersinia enterocolitica* antigens in the joint and the circulation suggesting bacterial persistence at the site of entry or within the joint. Apart from *Chlamydia (C. trachomatis* as well as *C. pneumoniae*) and *Yersinia*, intraarticular bacterial DNA has also been demonstrated for *Salmonella* and *Campylobacter*. Recently, also a non-classical ReA form initiated by a variety of additional bacteria has been established. Among them are *Borrelia, Brucella, Haemophilus, Hafnia, Leptospira, Mycobacteria, Neisseria, Staphylococcus, Streptococcus, Ureaplasma*, and *Vibrio*. This form is not associated with HLA-B27 and shows a more diverse clinical pattern affecting more peripheral joints and the upper extremity (polyarthritis) with less extraarticular features. Some authors suggest terming this condition as postinfectious or infection-related arthritis thereby excluding it from the spondyloarthritis group (11). From that perspective, reactive arthritis is definitely an infectious disease and might rather be seen as a "slow-bacterial" infection instead of a mere post-infectious, *i.e.*, "reactive" disease. The following pathogenic mechanisms are currently under scrutiny: a) the innate immunity derived pathogen associated molecular patterns (PAMPS) such as chlamydial LPS binding to Toll-like receptors (TLRs); b) bacterial effector proteins being secreted into the host cell cytosol acting as virulence factors, and c) adaptive immune response mechanisms induced by MHC class I and II restricted $CD4^+$ and $CD8^+$ T cell responses to bacterial antigens. These specific T-cell responses to reactive arthritis triggering bacteria do also support the hypothesis of an intraarticular persistence of viable microbial pathogens or bacterial antigens. There are conflicting data in regard to pro- and antiinflammatory cytokines, *i.e.*, a potential Th1/Th2 imbalance generated by ReA derived T lymphocytes (12). In synovial fluid mononuclear cells, a Th2 cytokine response as one ReA mechanism was reported whereas other studies in synovial fluid derived T cell clones have shown a predominance of Th1 cytokines (13-16).

Spondyloarthritis

The role of infection in the pathogenesis of spondyloarthritis (SpA) is best displayed in reactive arthritis. Epidemiological and clinical studies have shown a close association between infection with certain arthritogenic bacteria (*Yersinia, Campylobacter, Salmonella*, and *Chlamydia*) and the development of arthritis. The role of bacteria in other seronegative spondyloarthropathies, such as ankylosing spondylitis (AS), is less clear. However, given the clinical and genetic similarities between reactive arthritis and AS, research continues to explore the role of bacteria in the pathogenesis of AS. In humans, research continues to explore the role of gut microflora in the pathogenesis of AS. This association follows the findings that 15% of patients with AS show clinical manifestations of gut inflammation and 60% show subclinical manifestations. The recent finding that IL23R polymorphisms represent susceptibility factors for both Crohn's disease and AS keeps the interest high in the role of the GI tract in SpA. With respect to infectious triggers in AS, most interest is focused on Klebsiella pneumoniae, following earlier reports of increased recovery of Klebsiella from stool cultures of patients with active AS. A recent re-evaluation of this phenomenon did not confirm earlier studies (17). It is of interest to note that on clinical grounds, acute infection with K. pneumoniae is not followed by reactive arthritis, unlike Salmonella or Yersinia infections. There seems to be no intrinsic tropism for joints on these grounds. Furthermore, PCR approaches to identifying persisting pathogens in ReA following infections with other gram-negative bacteria have been negative, so possible roles for Klebsiella in chronic arthritis remain obscure. It should also be pointed out that arguments for causality based solely on serological grounds depend critically on the prevalence of the pathogen in that local population. A clear definition of control groups continues to be a key issue in this area.

There have been several strong links between infection and SpA, and this is most clearly seen in reactive arthritis.

In this case, an antecedent infectious trigger in the gastrointestinal or genitourinary tract initiates a non-septic arthritis (18). Several hypotheses have been developed. One possibility is that ReA represents an example of molecular mimicry, in which host immune responses are inadvertently directed against endogenous host tissues by virtue of antigenic cross-reactivity between host and microbial determinants. *In vitro* studies have provided some evidence of an intimate relationship between antichlamydial T cell responses and anti-B27 autoimmune responses (19). The role of anti-B27 cytotoxic T lymphocytes in SpA pathogenesis remains unknown. The primary mechanism underlying ReA has not been definitively resolved, but there is evidence of microbial antigens and possibly transiently viable organisms depositing in the joint. There are numerous points at which genetic alteration in host defense might contribute to these events. From animal models of reactive arthritis, there is evidence of a genetically programmed cytokine signature in the joint, which dictates local clearance of the pathogen from joint tissues (20). In this experimental model, resistant rats (*e.g.*, Brown Norway rats) are characterized by a robust production of TNF and IFN in the microenvironment of the joint, with an effective clearance profile of the Chlamydia resulting. Susceptible strains of animals (*e.g.*, Lewis rats) have a more intense inflammatory response in the joint, and have delayed clearance of the pathogen. This is temporally related to a relative impairment in pro-inflammatory cytokine in the synovial tissues. This intrinsic host defense profile can be modified by non-infectious environmental factors. The experimental models have also implicated innate immune responses in defining the initial stages of reactive arthritis, with both Toll-like receptors –2 and –4 playing a role in this process (21). From recent studies involving epidemic enteric pathogens, there is evidence that polymorphisms in TLR-2 represent a genetic susceptibility factor for acute reactive arthritis. In AS there is recent evidence for a role for TLR-4 as a susceptibility factor (22-25). Innate immune responses not only define the first line of host defense against arthritogenic pathogens, but also serve as a bridge to adaptive immunity and the long-term clinical course of reactive arthritis.

Rheumatic fever

Rheumatic fever (RF) is the most convincing example of molecular mimicry in human pathological autoimmunity. The disease is triggered by *Streptococcus pyogenes* (Group A streptococcus) and affects susceptible untreated children. In the 1950's, Jones established the major criteria for diagnosing initial attacks of RF, which comprised polyarthritis, carditis, chorea, erythema marginatum and/or subcutaneous nodules. These criteria remain useful to date, despite small periodic changes (26). Carditis, the most serious manifestation of the disease, occurs a few weeks after throat GAS infection and frequently leads to chronic rheumatic heart disease (RHD) (27).

The *Streptococcus pyogenes*, cell wall is composed of carbohydrates as N-acetyl β D-glucosamine linked to a polymeric rhamnose backbone. Group A streptococci contain M, T and R surface proteins and lipoteichoic acid (LTA), involved in bacterial adherence to throat epithelial cells. The M protein, which extends from the cell wall, is composed of two polypeptide chains with approximately 450 amino acid residues, in an alpha-helical coiled-coil configuration. The M protein is the most important antigenic structure of the bacteria and shares structural homology with alpha-helical coiled-coil human proteins like cardiac myosin, tropomyosin, keratin, laminin, vimentin and valvular proteins (28). As only 1 to 5% of untreated children develop RF/RHD, genetic predisposition is considered plausible. Several polymorphic systems were studied in order to define some alleles associated with the disease. The search for genetic markers revealed that HLA class II genes or other genes located in the same chromosome as TNF alpha genes probably in linkage disequilibrium were potentially involved with the development of RF/RHD. Several HLA class II genes (DRB1, DQB and DQA) were found in association with RF and RHD in different populations. HLA-DR7 allele is most consistently associated with RHD. The association of DR7 with some DQ-B or DQ-A alleles seems to be associated with the development of multiple valvular lesions in RHD patients (29). The polymorphism at the promoter region of the TNFA-308G/A was found to be associated with the susceptibility to RHD in patients from Mexico, Turkey and Brazil (19). Recently, genetic variations in the exon 1 of MBL (mannose binding lectin) gene were found in Brazilian RHD patients (29). Autoimmune reactions are mediated by the molecular mimicry mechanism by which streptococci antigens and human proteins that exhibited some degree of homology are recognized by T and B lymphocytes. The presence of cross-reactive antibodies against M protein and N-acetylglucosamine as well as human proteins, mainly heart-tissue proteins have been described since 1945 (30, 31). Only recently, the potential role in the development of RHD of the cross reactive antibodies has been demonstrated by showing that they are able to bind to the valvular endothelial surface leading to the upregulation of the adhesion molecule VCAM-1 that facilitates inflammation and T cell infiltration (29, 31). $CD4^+$ T cells are predominant in the heart tissue (32) and the first evidence of molecular mimicry between streptococci and heart tissue was shown through an analysis of the T cell repertoire leading to local damage in RHD (33). Three immunodominant regions of the M protein (residues 1-25, 81-103 and 163-177) and heart-tissue proteins were recognized by cross reactivity by intralesional T cell clones (27). These auto reactive T lymphocytes were in oligoclonal expansions (29). Both myocardium and valvular infiltrating mononuclear cells secrete IFN-γ and TNF-α which are inflammatory cytokines. Mononuclear cells secreting IL-10 and IL-4 (regulatory cytokines) were also found in the myocardium tissue, however, in the valvular tissue only a few cells secrete IL-4 suggesting that these low numbers of IL4-producing cells might contribute to the progression of valvular RHD lesions (34, 35). All the

findings described in this review contributed to a better understanding of the pathogenesis of RF/RHD considered as a model of autoimmune disease caused by infection. The association of the disease with HLA class II alleles reflects the role of HLA class II molecules in the presentation of streptococci and self antigens. The description of new polymorphisms in TNF alpha and MBL genes contributed to the definition of new genetic markers. Both T and B lymphocyte reactions against streptococci and self antigens contribute to the development of RF/RHD lesions. The progression and maintenance of valve lesions in RHD patients probably is due to the low numbers of IL-4, a regulatory cytokine.

Lyme disease

Even though the first descriptions of the clinical disease caused by borrelia can be dated back to the late 19th century, a variety of pathophysiological aspects of this infectious disease are still unknown. Certainly, early stages of skin disease and neurological disease can successfully be treated with antibiotics. Long-term sequels, however, are still a challenge with regard to diagnosis and treatment. Chronic borreliosis manifests itself as a multisystem disease affecting skin, joint, the nervous system, the heart and the eyes. *Borrelia burgdorferi* uses vectors from the ixodes species which transmit the bacterium to predominantly mammals. The natural reservoir is rodents and deer. The transmission from the "low temperature" vector ixodes towards the "high temperature" mammal organism goes along with a profound change in the expression of genes mainly transcribing surface proteins of the pathogen. The outer surface lipoproteins A and C seem to be of major relevance in this regard. Especially outer surface lipoprotein C (OspC) is predominantly expressed in the human host. Further outer surface proteins like OspE, OspF are of relevance for the interaction with the host. Several borrelia-related pathogenic factors have been described which can lead to a prolonged infection. One of them is called antigenic variation: *Borrelia burgdorferi* can change the genomic sequences at various loci finally deteriorating the borrelia-specific immune response of the host against proteins derived from these genes (36-44). Involved are the genes *ospC* and variable major protein like sequence *vls*. Especially the *vlsE* locus is extremely variable by modes of gene recombination. Another pathogenic factor is the expression of the ERP- and CRASP (complement acquiring surface protein) protein families (41). These proteins are able to bind complement regulatory proteins, factor H and FHL-1, present in the host serum to their own surface. Subsequently, complement components C3b, c and d are bound to factor H and FHL-1 and are inactivated. Impaired activation of the complement cascade in the alternative pathway represents an important mechanism in spirochete transmission and propagation of the infection (44).

Persistence of *Borrelia burgdorferi* in bradytrophic tissues, deep invaginations of the outer cell membrane or even in the cytosol of local joint cells, might contribute to chemotherapeutic resistance and interference with immune clearance (36). In this regard we were able to show that *Borrelia burgdorferi* can persist inside human synovial cells for months (38). Subsequently, the spirochete modulates the gene expression capacity of these infected synovial cells by down regulating, for instance, cell adhesion molecule ICAM-1 on these cells (39). In addition, major histocompatibility antigen expression is not altered and especially not stimulated by this infection (39). Obviously, one major goal of the spirochete is to avoid the attack of the immune system leading to a long-term establishment of the infection (37).

Nevertheless, the human innate and adaptive immune system tries to detect and eliminate *Borrelia burgdoferi*. Since the pathogen lacks lipopolysaccharide on its surface, lipoproteins are major determinants of spirochete-host interaction. These lipoproteins, like OspC, are detected by the human Toll-like receptor system, here especially Toll-like receptor 2 (44). By this interaction, human resident tissue cells become activated and potentially subsequent immune recognition or immune activation can take place. However, we have discovered particular immuno-suppressive capacities of the living pathogen in comparison with the isolated outer surface proteins A and C alone (42, 46). By using *Borrelia burgdorferi* as a whole cell stimulant, we were able to demonstrate a suppression of IL-8 chemokine expression and others in synvoial fibroblast cells. Overall this reaction can be described as a stealth phenomenon. The infected and/or stimulated synovial cell is no longer as recognisable to the immune system as it had been before. This finding might be a major step for the spirochete in establishing a long-term infection and overcoming the attack of the immune system. Even though the host finally does mount a strong immune reaction against the pathogen, this reaction does no longer seem to be of relevance with regard to pathogen elimination. Chronic immuno-stimulatory features might have primed the organism towards an uncontrolled auto-immune reaction (35, 41, 44, 45, 47). *Borrelia burgdorferi* has a strong potential for acclimatisation in the invertebrate as well as in the vertebrate host. During this process it adopts different molecular strategies needed for survival in these different environments. The spirochete *Borrelia burgdorferi* challenges the human host immune system through a broad variety of molecular mechanisms which include a complex differential gene expression. Strategies for the treatment of chronic, antibiotic-resistant Lyme disease, which is probable of auto-immune and auto-inflammatory nature, might evolve based on the described findings and future research in the pathogenesis of Lyme disease.

Antiphospholipid syndrome

Antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized by numerous manifestations and mainly by thrombosis and recurrent pregnancy loss in the presence of elevated titers of circulating antiphospholipid antibodies (48). Like many other autoimmune diseases, APS is generally considered as having a multifactorial etiology, mainly genetic susceptibility

combined with environmental triggers, of which infectious agents are considered most prominent. Beta-2-glycoprotein-I (β2GPI) is considered the autoantigen in APS. Following β2GPI attachment to phospholipids, both molecules undergo conformational changes that result in the exposure of cryptic epitopes from within the β2GPI structure, allowing the subsequent binding of anticardiolipin antibodies (aCL) (49). Using a phage display library three hexapeptides have been identified to be specifically recognized by pathogenic anti-β2GPI antibodies eluted from APS patients (50). In a study of 295 APS patients (49) heterogeneous activity of anti-β2GPI antibodies directed against various epitopes of β2GPI has been demonstrated. These target peptides have been found to have similarities with peptides within common infectious pathogens (51-52).

Circulating anti-β2GPI antibodies have been identified in the sera of patients having different infectious conditions, and have been associated with various clinical manifestations of APS (53). One example would be *H. pylori*. A screen of 50 patients with *H. pylori* infection has revealed 33.3% prevalence of anti-β2GPI antibodies. Circulating anti-β2GPI antibodies have been found in patients infected with many other bacteria, viruses or yeasts which have also been found to share structural homologies with the β2GPI molecule (54). The involvement of molecular mimicry in the induction of APS has been demonstrated in an experimental animal model (55). In this study, naive BALB/c mice were immunized with microbial pathogens which share structural homology with the TLRVYK peptide, a peptide previously shown to be specifically recognized by the pathogenic H-3 anti-β2GPI antibody, and proven to induce endothelial cell activation and APS in experimental models (50, 56). Mouse antibodies were then purified from the sera of the previously immunized mice and infused intravenously into naive mice at day 0 of pregnancy (55). The mice infused with anti-TLRVYK antibodies developed experimental APS, namely thrombocytopenia, prolonged aPTT and increased fetal loss, similar to a control group of mice immunized with pathogenic anti-β2GPI antibodies. In other studies by Gharavi *et al*. (57) the production of anti-β2GPI and antiphospholipid antibodies was induced by the immunization of NIH/Swiss mice with synthetic peptides which share structural similarity with the putative phospholipid-binding region of the β2GPI molecule. These peptides were also shown to bear homology to various infectious agents such as cytomegalovirus, human adenovirus type 2 and *Bacillus subtilis* (57). These studies thus provide evidence supporting epitope mimicry as a possible mechanism for APS development. Those peptides of APS-inducing potential, sharing structural homology with infectious pathogens, can inhibit the binding and biological effect of antiphospholipid antibodies (50, 55-64). This has important implications in designing new targeted therapies. Unraveling the epitope specificity of antiphospholipid antibodies may allow design of therapeutic agents to act as toleragens for specific B cells that secrete these autoantibodies (50).

The above-mentioned studies are encouraging in terms of rationale for the search for prevalence and clinical associations of various infectious agents in APS. Identification of the exact prevalence of various infectious agents in APS compared to control subjects or subjects with other autoimmune diseases can help discriminate between those infectious agents which are associated with APS and those that are not or even might be protective. In addition, a comparison of infectious agent prevalence between APS and related conditions such as systemic lupus erythematosus can help define which infectious agents are more important in any of these syndromes, and whether a single infectious agent can increase the chance of a patient having one disease develop another. Finally, within patients having APS which is also considered a systemic syndrome, the presence of specific infectious agents might also be associated with a given clinical manifestation, hence suggesting that these antibodies could also be of predictive or therapeutic importance (64).

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