Role of infectious agents in systemic rheumatic diseases

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ABSTRACT
The relationship between infection and autoimmunity has been increasingly defined over the last twenty years or so. It is now quite clear that, in genetically susceptible individuals, environmental factors (mainly infections) play a critical role in the pathogenesis of autoimmune diseases. It is believed that infections contribute to the maturation of the immune system from the innate to adaptive phases, and that bacterial and viral infections are arthritogenic stimulants leading to various rheumatic conditions. A failure to isolate these microorganisms is probably due to the action of the immune system, but often casts doubt on their role in the pathogenesis of autoimmune diseases. Among bacteria, Helicobacter pylori has been associated with diseases such as autoimmune gastritis, Sjögren’s syndrome, atherosclerosis, immune thrombocytopenia purpura, inflammatory bowel diseases and autoimmune pancreatitis, in each of which it seems to play a pathogenic, but it has also been suggested that it may help to protect against the development of autoimmune gastritis, multiple sclerosis, systemic lupus erythematosus and inflammatory bowel diseases. Infectious agents may play a dual role in the etiopathogenesis of antiphospholipid syndrome (APS): they may be the initial trigger of the production of antibodies cross-reacting with beta 2 glycoprotein 1 (β2GPI) and infectious peptides, and also induce an inflammatory response. According to the two-hit theory, pathogenetic anti-β2GPI antibodies act as the first hit whereas inflammatory responses may represent the second hit. The slowly growing Propionibacterium acnes may be involved in the etiopathogenesis of SAPHO syndrome by triggering the non-specific activation of cell-mediated immunity. Its ability to persist in bone lesions in a form that is incompatible with culturing suggests the possibility an arthritis that is secondary to a “persistent” infection.

Introduction
Most rheumatic diseases are complex disorders for which pathogenetic mechanisms are poorly understood. Nonetheless, increasing evidence suggests that many of these illnesses result from one or more specific environmental exposures in genetically susceptible individuals (1, 2). These environmental factors are known to affect the immune system and may play a role as triggers of the rheumatic diseases. Bacterial, viral and parasitic infections are known to induce and exacerbate autoimmune diseases, mainly by the mechanism of molecular mimicry (3, 4). Although much progress has been made over the past few decades in advancing our knowledge of the genetics of rheumatic diseases, few studies have assessed infectious features and understanding of which exposures are important in pathogenesis remains limited (5, 6). In this article, we review the current state of knowledge of infectious risk factors is some rheumatic diseases and the possible mechanisms by which infectious exposures might induce pathologic processes.

Infectious aspects and the pathogenesis of rheumatoid arthritis
Rheumatoid arthritis (RA) is a chronic multisystem disease that primarily presents as inflammatory synovitis symmetrically involving the peripheral joints. It may also involve extra-articular organs and, if it remains untreated, may lead to significant morbidity and disability. As in the case of most connective tissue disorders, the etiology of RA is not clearly understood. Throughout the nineteenth century it was widely accepted among prominent physicians that it was the outcome of various infectious agents such Gonococcus, Mycobacterium tuberculosis or different streptococci species (7). Subsequently, the idea that infections play a direct role in the pathogenesis of RA was almost entirely
discarded, and it is now generally accepted that its etiology is multifactorial. It is known that patients with a given genetic background who are occasionally exposed to certain environmental hazards are at greater risk of developing RA: e.g., the siblings (particularly twins) of RA patients (8); a number of HLA alleles are particularly associated with RA, such as HLA-DRB1 and HLA-DRB4 (DRB1*0401), and smokers are at increased risk (interestingly, it has been found that the chemical constituents of smoke enhance the generation of anti-CCP antibodies) (9).

In addition to genetic susceptibility and environmental factors, infections have also been implicated in the induction of autoimmunity. One of the models our groups have used is antiphospholipid syndrome (APS): we have previously reported that Libman-Sacks non-bacterial endocarditis in patients with APS probably has an infectious origin, and that APS-like features are induced in mice transfused with immunoglobulins generated in mice immunised with Haemophilus influenzae, Neisseria gonorrhoeae or tetanus toxoid (10, 11).

Söderlin et al. (12) systematically screened patients for infections caused by Salmonella typhimurium and Salmonella enteritidis, Yersinia enterocolitica, Campylobacter jejuni, Borrelia burgdorferi, Chlamydia trachomatis, Chlamydia pneumoniae, and parovirus B19, and found that the history or laboratory test results of 45% indicated that a recent infection had preceded their arthritis. The fact that arthritis remitted in 69% of the patients with a preceding infection (against only 38% of those without) suggests that stimulation of the immune system by infectious agents affects the response to the agent itself and the autoimmune response. Other circumstantial evidence has associated the pathogenesis of RA with Mycoplasma pneumoniae (13), Epstein-Barr virus (EBV) (14), cytomegalovirus (CMV) (15), and parovirus and rubella (16). Ramirez et al. (13) observed an interesting relationship between RA antibodies against Mycoplasma pneumoniae and RA, and that patients with RA had higher antibody titres. Another clue supporting this association is the well-known and successful use of RA treatment with minocycline, which is also an effective antibiotic against mycoplasmal infections.

The association between Helicobacter pylori (HP) and RA has not been entirely determined. Most investigators have referred to a link between RA and HP by associating the impact of HP eradication on RA disease activity. However, the conclusions are controversial as some studies have shown that patients benefit from HP eradication and others that they do not, thus suggesting that HP plays a defensive role possible through mechanisms involving oral tolerance (17).

CMV has also been mentioned in the pathogenesis of RA, as CMV antibodies have been recorded in RA patients and viral DNA has been identified in synovial tissue using DNA in situ hybridisation and PCR. Furthermore, it has been shown that CMV seropositivity is associated with the expansion of CD4+CD28− and CD8+CD28− T cells in patients with RA (14).

Another herpes virus that has been thought to play a significant role in the pathogenesis of RA is EBV but, although there is some circumstantial data supporting this hypothesis, there is a lack of solid evidence. EBV is a widespread virus that is easily recognised by antibodies but hardly ever eliminated, and ideal for triggering a chronic immune complex disease. Viral DNA has been identified in the synovial tissue of RA, and a number of mechanisms have been suggested as potentially explaining its relationship to the disease, including the recognition of EBV antigens by anti-citrullinated protein antibodies, and impaired cytotoxic T cell function in RA patients infected by EBV, a finding attributed to abnormal signalling of the lymphocytic-activation molecule (SLAM)-associated protein (SAP) (14). These data support previous reports indicating that infections play a role in autoimmunity in general, and in RA in particular, and it would be worth re-analysing data in order to clarify whether certain immune responses against specific infectious agents may predict a certain clinical behaviour and outcome.

**Helicobacter pylori in autoimmune diseases**

*Helicobacter pylori* is a gram negative, spiral-shaped bacterium which, according to data from the Centers for Disease Control and Prevention (CDC), lives in the acidic stomachs of 50% of the people living in the Western world and up to 90% of those living in developing countries. It is an infamous cause of chronic gastritis and gastrointestinal ulcers, associated with the development of mucosa-associated lymphoid tissue (MALT) and gastric cancers. It has also been linked to many autoimmune diseases although it is still largely unclear whether such links are epiphenomenal or really reflect a role in their pathogenesis. Furthermore, some recent data (which will be discussed below) has shown that it may actually protect against autoimmunity.

**H. pylori as an inducer of autoimmune diseases**

If *H. pylori* induces autoimmune disease, how does it do so? There are various mechanisms by which an infecting agent may induce autoimmunity, including molecular mimicry, polyclonal activation, epitope spread, bystander activation and superantigens. One of the autoimmune diseases with which it has been associated is autoimmune gastritis (AIG). Amedei et al. (17) found that, by molecularly mimicking H+, K+-adenosine triphosphatase, *H. pylori* antigens and can activate cross-reactive T cells, trigger or accelerate the development of AIG, and ultimately lead to gastric atrophy. Yamanishi et al. (18) have demonstrated the activation of B-1 cells by *H. pylori* components (urease in particular), leading to the creation of autoreactive antibodies such as IgM rheumatoid factor, anti-single-stranded DNA antibodies and anti- phosphatidyl choline antibodies. It is also believed that heat-shock protein 60 (HSP60) plays a role in the pathogenesis of both primary and secondary Sjögren’s syndrome, and it has been shown that the local and systemic immune response elicited as a result of the homology between *H. pylori* and human HSP60 may play a role in generating the disease (19). This, together
with studies showing seropositivity for \textit{H. pylori} in patients with Sjögren’s syndrome, suggests that it plays a role in triggering this autoimmune state. Heat shock protein 60/65 has similarly been described as a possible activator of coronary atherosclerosis (20), thus raising the possibility that exposure to \textit{H. pylori} leads to an increased risk of coronary artery disease due to an autoimmune response. Furthermore, on the grounds of this convincing link, it has been suggested that appropriate treatment with antibiotics or anti-HSP60 antibodies may reduce the progression of atherosclerosis.

Most of the other associations between \textit{H. pylori} and autoimmune diseases are still controversial. It is known that immune thrombocytopenia purpura (ITP) is primary or secondary to lymphoproliferative infectious or autoimmune diseases, and eradicating \textit{H. pylori} is becoming increasingly widely used in standard ITP treatment because it has been shown to be effective in increasing platelet counts in 50% of cases. However, the mechanisms underlying this association have not yet been sufficiently clarified (21). Similarly, \textit{H. pylori} has been associated with rheumatoid arthritis and autoimmune pancreatitis, but the data are still insufficient.

\textit{H. pylori as a protector from autoimmune diseases}

Inflammatory bowel disease (IBD), Crohn’s disease and ulcerative colitis have also been described as being possibly activated by \textit{H. pylori} infection. However, in the case of IBD, the association is not only controversial, but some data even show seronegative results, thus leading to the possibility that \textit{H. pylori} may actually protect infected patients from developing IBD (22). The prediction and prevention of autoimmune diseases is a rapidly emerging subject of debate. The preventive treatment modalities include the administration of aspirin in APS, ursodeoxycholic acid in primary biliary cirrhosis (PBC), and vitamin D in SLE and autoimmune thyroid disease (AITD) (23). Another possibility is that \textit{H. pylori} may protect against rather than induce the development of autoimmune diseases, as mentioned above in relation to IBD. There are also conflicting data concerning the relationship between multiple sclerosis (MS) and \textit{H. pylori}, which has been described as both an inducer and possible protective factor against conventional MS (24). Moreover, there are findings suggesting that \textit{H. pylori} infection may play a protective role against the development of systemic lupus erythematosus (SLE), and that immunoregulatory events leading to \textit{H. pylori} seropositivity correlate inversely with the risk of developing it, which was suggested when an association was established between the disease and \textit{H. pylori} seronegativity (15). Finally, although there is a possible mechanism by which \textit{H. pylori} induces autoimmune gastritis (AIG), there are also findings indicating that it has an inhibitory effect on the development of AIG in mice, thus suggesting that it may also play a role in protecting humans (26).

In any case, it is now almost universally accepted that microbial agents play a major role in the pathogenesis of many autoimmune diseases and it is clear that, in genetically susceptible subjects, a certain environmental factor (especially an infective agent) may induce or exacerbate them. As described above, \textit{H. pylori} is frequently associated with various autoimmune diseases, although its precise role in many of them is still controversial. Depending on factors such as genetic susceptibility, time of infection and disease type, it may act in different ways: it can be involved in pathogenesis and trigger the development of overt disease, or it may be protective. However, further studies are required in order to clarify its effects on autoimmunity.

\textbf{Infectious agents and antiphospholipid syndrome}

Anti-phospholipid antibodies (aPLs) are associated with recurrent thrombosis and fetal losses, and are formal diagnostic markers of both antiphospholipid syndrome (APS) and pathogenic antibodies. It is, in fact, widely accepted that APS is an autoantibody-mediated autoimmune disorder (27). There is strong evidence that innate immunity plays a role in the pathogenesis of APS: for example, complement (C') activation is apparently required in both aPL-mediated fetal loss and experimental models of thrombosis. It has been reported that pro-inflammatory cytokines (e.g., TNF-\(\alpha\)) and chemokines are important in the aPL-mediated model of fetal loss (28-32) and, accordingly, it is well known that fetal demise and premature labour are associated with an active inflammatory response (33). A comparable role for inflammatory mediators has also been suggested in experimental models of aPL-induced thrombosis (31, 32). The arterial infusion of anti-\(\beta_{2}\)GPI IgG fractions in naïve rats does not induce any vascular effects in the mesenteric microcirculation when given alone, but becomes thrombogenic if a small amount of lipopolysaccharides (LPS) is also injected intra-peritoneally. As LPS per se does not induce any significant effect, it has been suggested that aPLs may be a first hit (necessary but not sufficient to trigger clotting) and that a second hit – represented by the local injection of LPS – is required in order to reveal the thrombogenic activity of the antibodies. The role of this second inflammatory hit is further underlined by the fact that, although the antibodies can bind to vessel walls and co-localise in tissues with C' component deposition, they are not thrombogenic without the addition of LPS.

The two-hit theory well fits the clinical observation that aPLs are persistently present in the circulation but thrombotic events only take place occasionally. In this regard, it is interesting to speculate on the reported association between acute thrombotic events and a recent or concomitant infectious process in APS patients, or traumatic inflammatory stimuli in experimental models (34). In line with the role of inflammation in APS, there is evidence that innate immunity receptors capable of triggering inflammatory responses are involved in its pathogenesis as it has been found that Toll-like receptors (TLRs) – particularly TLR-4 – trigger the endothelial cell activation mediated by anti-\(\beta_{2}\)GPI antibodies in vitro (35). It is widely accepted that this is
one of the main mechanisms responsible for the thrombophilic state, and this finding has recently been supported by the demonstration that mice showing a spontaneous single point mutation of the tlr4 gene associated with defective cell signalling are protected against the thrombogenic effect of aPLs. Humans also carry polymorphisms of the tlr4 gene, which are associated with an impaired response to inflammatory stimuli. We have reported preliminary evidence showing that the prevalence of such “protective” tlr4 polymorphisms is significantly reduced in APS patients who have experienced previous thrombotic events (36), which suggests that a strong inflammatory response may increase susceptibility to thrombosis in the presence of aPLs.

The etiopathogenesis of APS is apparently multifactorial: it involves both adaptive and innate immunity responses that are supported by a specific genetic background, and triggered by environmental factors – characteristics that are shared by most autoimmune disorders. Environmental factors are particularly important: e.g., β2GPI – a PL-binding plasma protein that is one of the main antigenic targets of aPLs – has extensive amino acid sequence homology with various bacterial and viral components. There is evidence that aPLd (particularly anti-β2GPI antibodies) cross-react with different peptides derived from infectious agents, that these peptides can induce the production of cross-reactive antibodies in experimental animal models, and, more importantly, that the induced antibodies mediate some of APS manifestations (34). In other words, it has been suggested that antibodies cross-reacting with viral/bacterial components and β2GPI may be the consequence of molecular mimicry, and so genetically predisposed subjects may start procuring these autoantibodies persistently and thus develop the disease. Infectious agents therefore seem to play a dual role in the etiopathogenesis of APS: i) they initially trigger the production of anti-β2GPI antibodies (the first hit); and ii) induce an inflammatory response that is theoretically capable of acting as the second hit responsible for the vascular manifestations.

**SAPHO syndrome and infections**

SAPHO (synovitis, acne, pustulosis, hyperostosis and osteitis) is a syndrome whose most typical form is characterised by particular osteo-articular manifestations and various chronic dermatological conditions, particularly palmo-plantar pustulosis (PPP) and severe acne (SA), although Sweet’s syndrome, pyoderma gangrenosum and other neutrophilic dermatoses have been described less frequently. The inclusion of *psoriasis vulgaris* as the only skin lesion is still being discussed. Osteitis and hyperostosis are the striking features that can be observed in any involved skeletal segment. The main localisation is the anterior chest wall (ACW), although the spine, pelvic girdle, peripheral bones and that mandible may be other affected sites. A variety of articular manifestations may co-exist, arising from the extension of adjacent osteitis or in the form of peripheral mono or oligo-arthritis (37).

SAPHO is still considered rare, but its real prevalence may be underestimated because of its confusing symptoms and the possible absence of skin manifestations (purely osteitic-hyperostotic subsets). Curiously, most of the reported cases come from North Europe and Japan, and a few have been described in the English language medical literature (38). Its nosological framework is still a matter of debate, but its relationship to psoriasis is particular interesting and a link with psoriatic arthritis has been suggested (9). Although repeatedly related to the family of spondyloarthropathies (SpA), emerging evidence indicates that SAPHO could be a primary inflammatory osteitis (39, 40). Various stimuli have been implicated as triggers, particularly a low-virulence pathogen, *Propionibacterium acnes* (alive or as dead antigens), but an autoimmune or auto-inflammatory mechanism cannot be ruled out (4). Although the bone lesions are usually sterile, the role of *P. acnes* (a bacterium involved in the pathogenesis of comedones and acne) is still being investigated. Positive clues are the discovery of *P. acnes* in bone lesions from some patients with osteitis associated with acne, and its isolation from synovial tissue and fluid in patients with SAPHO syndrome (41).

*P. acnes* is a gram-positive, motionless, non-spore-forming bacillus with maximum growth in anaerobiosis. The microorganisms involved in human disease have five biotypes, of which biotypes I and III are the most frequently involved in the etiopathogenesis of acne. They form part of the normal flora of the oral cavity, large intestine, conjunctiva, external ear conduit and the skin, particularly the sebaceous follicles (42). There have been occasional reports of the identification of *P. acnes* in bone biopsies taken from subjects with SAPHO syndrome, which have led to the hypothesis that it may play a part in the pathogenesis of the disease, although its exact role is still unknown. If we include the diseases related to SAPHO syndrome, it has been reported in only 24 of the 69 studied cases (5), possibly because of its ability to persist in bone lesions in a form that is incompatible with culturing. It has also recently been identified in the inter-vertebral disc material of patients with severe sciatica, thus suggesting that the chronic inflammation leading to the symptoms may be triggered by a low virulence infection (43). This hypothesis is based on the possibility that low virulence microorganisms may have access to bone, and can thus start or stimulate a chronic inflammatory response with accompanying symptoms. A number of studies have shown that *P. acnes* may trigger the non-specific activation of cell-mediated immunity, an immunological response that may be an attempt to eliminate the germ perpetuating the inflammation (44). The complete genome sequence has been detected and clearly reveals numerous gene products involved in degrading host molecules. This would explain its ability to colonise and survive in human skin and a wide spectrum of other environments, including bone and synovial fluid (45, 46). On the other hand, possible contamination cannot be excluded in the largest reported series because *P. acnes* is an ordinary skin saprophyte (42).

As *P. acnes* is often involved in the pathogenesis of comedones and acne, its presence in osteitic lesions could...
indicate a subset of SAPHO patients characterised by a considerable response to antibiotic therapy. Given the occasional isolation of *P. acnes* and other microorganisms in specimens taken from bone lesions and synovial tissue, antibiotics (especially macrolides, tetracycline and co-trimoxazole) have been used to treat SAPHO but the conflicting results have not led to a turning point in therapy. The different responses to anti-biotic therapy may also reflect different pathogenetic mechanisms underlying the symptoms of SAPHO syndrome insofar as *P. acnes* could simply trigger osteitis and hyperostosis, and the damage may continue after its elimination. In conclusion, at least in some cases, *P. acnes* may represent the prime mover of SAPHO syndrome, with the ost-eitic lesions being due to a reaction to a low-virulence infection.

References
36. COLINA M, LO MUNACO A, KHODJEI R, TROTTA F: Propionibacterium acne and S-31


