Infections, connective tissue diseases and vasculitis

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Received and accepted on January 18, 2008.

Key words: Systemic lupus erythematosus, polymyositis-dermatomyositis, antiphospholipid antibody syndrome, vasculitis, Wegener's granulomatosis, infections, immunosuppressants.

Competing interests: none declared.

ABSTRACT

In genetically predisposed individuals, viruses, bacteria, or parasitic infectious agents are suspected of inducing autoimmunity and/or exacerbating autoimmune rheumatic diseases (ARD) once self-tolerance is broken. Although direct evidence for this association is still lacking, numerous data from animal models as well as from humans support the hypothesis of a direct contribution of pathogens to the induction of several ARD.

This review focuses on the possible role of infectious agents as triggers of autoimmunity in systemic lupus erythematosus, polymyositis-dermatomyositis, antiphospholipid antibody syndrome, and primary vasculitis. Indeed, vasculitis may be a clinical manifestation of an infectious disease (secondary vasculitis).

In addition, immune response abnormalities and immunosuppressive medications may be responsible for the high percentage of infectious complications in ARD patients. Recent therapeutic approaches aimed at lowering doses of cytotoxic agents and shortening duration of treatment with the most toxic drugs, have proved to be as effective as conventional regimens. New drugs and strategies aimed at preventing infections could further improve the outcome of ARD patients.

Introduction

The aetiology of autoimmune diseases is not completely understood, but immunological, hormonal and environmental factors interplay important roles in the induction of disease in genetically predisposed individuals. In those subjects, viral, bacterial or parasitic infectious agents can be responsible for aberrant immune response (1-3).

Accordingly, several studies have been carried out in order to investigate the link between infections and autoimmunity, and a number of hypotheses have been formulated. The microorganism which might be responsible for autoimmunity could interact in the host with cellular proteins inducing changes in the proteins which are no longer recognized as "self" by the host immune system; or it could make clustered cellular antigens accessible to the immune system which has never learned to recognize them as self proteins; otherwise it could induce the production of human antibodies carrying pathogenic idiotypes (anti-idiotypic antibodies); lastly it could have antigenic sites that "mimic" aminoacid sequences in the normal host proteins (molecular mimicry hypothesis) (4).

During infections, numerous autoantibodies are produced as a result of non-specific activation of low-affinity autoreactive B cells, but it is not clear whether this phenomenon can lead to the development of autoimmune disease (5). It has been demonstrated that chronic infections can activate autoreactive B cells as well as create conditions that can drive them to differentiate into memory cells which may initiate autoimmunity in autoimmune-prone individuals (5).

Clinical, epidemiological and experimental evidence supports the hypothesis that autoimmune rheumatic diseases (ARD) are triggered by infections and many autoimmune disorders such as systemic lupus erythematosus (SLE) (6), polymyositis-dermatomyositis (PM-DM), anti-phospholipid syndrome (APS), and vasculitis have been associated with a variety of infectious agents representing the initiating event of autoimmunity. On the other hand, infections frequently occur during the course of ARD as a result of immune response abnormalities as well as immunosuppressant use. All these aspects are reviewed herein.

Systemic lupus erythematosus

A growing body of experimental and clinical evidence supports the pivotal

role of infections in the induction and/or exacerbation of SLE (6).

SLE occurs when an environmental trigger induces a dysfunction of immune system in a genetically predisposed individual leading to a loss of tolerance towards native proteins. Infections, particularly viral infections, seem to play a relevant role as environmental triggers in SLE development (7). Cytomegalovirus (CMV), Epstein-Barr virus (EBV) and Parvovirus B19 are the viruses most frequently involved either in the production of a favourable immune milieu for initiating autoimmune phenomena or in breaking immune tolerance to self molecules.

One mechanism that could be responsible for the generation of lupus autoantibodies is "cross reactivity" or molecular mimicry, in other words, a normal immune response towards a foreign epitope that mimics a common antigenic target of an autoantigen.

To date, the most compelling evidence for molecular mimicry is by the EBV (8, 9). It has been demonstrated that the PPPGRRP sequence of EBV Nuclear Antigen-1 (EBNA-1) is partially cross reactive with PPPGMRPP which seems to be the first epitope of SmB antibody response (10). Immunization of experimental animals with either PPPGMRPP or PPPGRRP was able to induce anti-Sm antibodies (11). Moreover, a cross reactivity between EBNA-1 (GGSGSGPRHDGVRR) and the sequence TKYKQRHGWSHKD which seems to be the first epitope of 60 kD Ro to be bound by anti-Ro antibodies has been reported (10). The immunization of New Zealand white rabbits (a strain resistant to EBV infection) with either EBV or Ro cross reactive peptides induced antibodies to both 60 kD Ro and EBNA-1 followed by epitope spreading with progressive accumulation of multiple reactivity and, eventually, by lupus manifestations (10).

In addition, EBV involves a number of other mechanisms altering the normal immune response, including aberrant T cell responses characterized by an increase of interferon (INF)-producing CD4 positive EBV-specific cells and dysfunctional CD8 responses (6).

In line with these observations, an association of SLE with viral EBV DNA from peripheral blood in children as well as with previous EBV exposure in adults has been reported (11). By contrast, in these studies no consistent relationships between SLE and other common Herpes viruses, including CMV, *Herpes simplex* 1 or 2 were found (11). Since a large percentage of healthy subjects is infected with EBV, an association of SLE with EBV could explain only a small, albeit crucial part of the risk of developing lupus (11). Noteworthy, recent data show that the fine specificities of EBNA-1 antibodies formed in SLE patients are different from those formed in healthy individuals (11).

One of the first steps in the pathogenesis of SLE and other autoimmune conditions is the over-expression of type 1 INF genes, called "interferon signature", which have been identified in most patients (12). INF-α can contribute to SLE through various mechanisms, including direct and indirect effects on antigen presenting cells (APCs), T cells and B cells. INF-α is largely produced by immature dendritic cells (DCs), now known as plasmocytoid DCs (pDCs).

Viruses induce pDCs to produce INF-α which up-regulates expression of Toll-like receptor (TLR) 7 by B cells, promotes cell death increasing the release of certain RNA and DNA autoantigens and primes pDCs to respond more effectively to immunecomplexes (13). Autoreactive B cells – for example, induced by EBV through molecular mimicry – bind the autoantigens released from apoptotic cells using B-cell receptor (BCR) which delivers RNA autoantigen to TLR7. The engagement of TLR7 leads to proliferation and differentiation of these autoreactive B cells with the consequent generation of autoantibodies. Autoantibodies bind their cognate autoantigens to form immunocomplexes (IC) which, in turn, bind the receptors for the Fc portion of IgG (FcγR) at the surface of pDCs. Once internalized, IC engage TLR7 leading to the production of more INF-α by pDCs and, therefore, contributing to a self-perpetuating feedback loop for autoantibody formation (13).

Recently, the possible important role of hypomethylated plasma DNA in the pathogenesis of SLE has been highlighted. Bacterial and viral DNA, which are hypomethylated, can induce various immune changes that are similar to those observed in SLE, including activation of TLR 9 signalling leading to the over expression of type 1 INF genes, polyclonal B cell activation and production of autoantibodies such as anti-DNA antibodies, production of interleukin (IL)-6 and resistance to apoptosis that potentially allows the survival of autoreactive cells (14). Indeed, induction and/or aggravation of SLE is well known to occur after bacterial or viral infection.

Infections are very common in SLE patients where they are responsible for 30-50% of morbidity and mortality (15). All types of infections, including bacterial, viral and opportunistic infections, have been reported (16, 17). It is noteworthy that opportunistic infections are emerging as an important cause of death in SLE patients in developed as well as developing countries (15).

There are a number of factors, which genetically predispose SLE patients to infections (6, 15).

SLE patients with early complement deficiencies have a higher risk for infections due to *Streptococcus pneumoniae*, whereas those with late complement deficiencies are at increased risk for infections caused by *Neisserie meningitides* and *Neisserie gonorrhoeae*. Moreover, SLE patients with mannose-binding lectin (MBL) deficiency, associated with homozygous MBL variant alleles, have an increased risk for developing infections such as pneumonia by *Staphylococcus pneumoniae*. A similar susceptibility to infections might be expected in patients with C reactive protein (CRP) deficiency, which could also be genetically determined. However, no data on the risk of infections in patients with lower levels of CRP have been reported to date.

Risk factors for infections in SLE are leucopenia, the presence of severe disease manifestations, such as glomerulonephritis or central nervous system (CNS) involvement, and corticosteroid and cyclophosphamide (CYC) use (where the risk of developing infection is dose-dependent).

Recent epidemiological evidence has clearly shown that short and medium-term survival rate of patients affected with SLE has greatly improved in the last decades, but unfortunately long-term prognosis still remains very poor (18). This means that the current treatment for SLE is more effective than that used in the past, but in the long-term, complications of the disease itself and/or its treatment occur. Notably, among long-term complications a high percentage is represented by infections (17). Therefore, in order to improve long-term prognosis in SLE we should adopt, early in the disease course, some preventive strategies to avoid drug side effects, including infections (19, 20).

Patients with a lymphocyte count lower than 200/mm^3 are more susceptible to develop *Pneumocystis jiroveci (ex carinii)* pneumonia. A prophylactic treatment with co-trimoxazole (160 mg trimethropin/800 mg sulpamethosazole twice a week) concomitantly with the use of corticosteroids and immunosuppressants is recommended (19).

Moreover, patients should be systematically screened for *Mycobacterium tuberculosis* and for some viral infections, including hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) before treatment.

In patients treated with high dosage corticosteroids and immunosuppressants, an adequate prophylaxis against some infections should be carried out, when indicated (19).

No prophylactic treatments for HCV and HIV in immunocompromised patients are currently available. In HCV-RNA carriers we should use immunosuppressants with low effect on viral replication, such as cyclosporine A (21) and mycophenolate mofetil (22, 23). In HIV carriers we should try to balance SLE and HIV treatment according to the number of CD4$^+$ cells and the levels of viremia.

Vaccination is still an open field (16, 24). Inactivated and component vaccines, particularly pneumococcal and influenza vaccinations, seem to be safe and effective in SLE, whereas live attenuated vaccines should be avoided, particularly in patients taking immunosuppressants or prednisone at a dosage higher than 20 milligrams/day (25). Immunosuppressants should be withdrawn 3 months before vaccination and reintroduced 2-4 weeks after vaccination.

Polymyositis-dermatomyositis and infections

Infections are strongly associated with PM and DM, whether as causative agents directly involved in the pathogenesis of the disease or as a complication of the disease because of the impairment of the immune system and the immunosuppressive drugs used to treat these patients (26).

The role of infections in the initiation of the disease is supported by several evidences. Many infectious agents have been proposed as initiating factors, including Coxsackie viruses, Parvoviruses, Enteroviruses, Retroviruses, in particular Human T-lymphotropic virus (HTLV) and HIV (26).

It has been observed that subsets of PM and DM patients tend to develop as well as to relapse the disease at different times of the year (27), indicating that a common environmental factor such as bacterial or viral infections could trigger disease onset.

Moreover, a clear seasonal pattern could be found in groups of patients defined by circulating autoantibodies which are specifically detected in these patients, assuming that these antibodies mark the presence of an inciting infection.

In order to better understand the triggering role of viruses in myositis, several experiments using animal models have been conducted.

In the Tucson strain of Coxsackie virus B1 model, infected mice develop an acute viral myositis which progresses to chronic inflammation mimicking the human PM (28). Encephalomyocarditis virus can also induce an inflammation in mice (29) but different mouse strains have different degrees of susceptibility to inflammation and to the consequent disease, thus suggesting that the host factors are also required for the full induction of the disease (29).

In addition, this experimental evidence suggests that acute viral infections can induce inflammation within the muscle (30) which, if persistent, may trigger the chronic process of an idiopathic inflammatory myopathy also in humans.

It is thought that humoral response plays an important role in the development of PM and DM because of the high serum levels of autoantibodies in these patients such as the aminoacyl-tRNA synthetases (31), the nuclear protein Mi-2 (32) and protein components of the Signal Recognition Particle complex (SRP).

These autoantibodies could be considered "footprints" of previous infections: the microbial agent can initiate an immune reaction in the muscle which continues after the microorganism is eliminated and no longer detected within the muscle.

It has also been suggested that anti-tRNA synthetases antibodies result from the interaction of a virus with the tRNA synthetase during the replication of the virus itself. The native protein is presented to the immune system in association with the foreign viral protein, thus breaking the immune tolerance.

It is well known that human pathogens often express proteins which show high homologies with human proteins. This phenomenon named molecular mimicry increases (33) the potential antigeneicity of the foreign viral proteins (34).

A sequence of homology between group A streptococcal type 5 M protein and myosine of human skeletal muscle has been found, and it has been shown that these shared sequences are the target of immune responses in patients with juvenile DM (34). Molecular mimicry between a major antigen of the bacterium and the target of the disease is the basis for the development of juvenile DM by aberrant immune reactions to streptococcus.

Several studies have been carried out to search for the viral genome in the affected tissue from PM and DM patients, but the data are controversial (35) since in not all of these studies the direct evidence of the presence of viral RNA in muscles from these patients has been found. Besides this point, it should be considered that in individuals who cannot mount inflammatory response because of genetic defects, viral proteins could persist in muscle even if the viral genome is no longer present,

and viral infection could initiate the inflammatory process and becoming self-sustaining.

Infectious complications have been described in up to 30% (15, 36) in case series of PM and DM patients, which result from immune abnormalities and organ system manifestation associated with these diseases and their treatment with immunosuppressive medications.

Several types of infections can occur in these patients. Aspiration pneumonia produced by Gram-positive and anaerobic bacteria is the most common infection in about 20% of PM and DM patients (36) and in some cases it represents also the main cause of death due to related esophageal motor involvement and ventilatory insufficiency.

About 11% of PM and DM patients develop opportunistic infections (37). Fungi, mainly *Pneumocistis Jiroveci (ex carinii)* and *Candida albicans*, are responsible for opportunistic infections in more than 50% of cases after the onset of PM and DM, and during the active phase of disease (37). *Herpes zoster* is common in PM and DM patients usually occurring during the inactive phase of disease (38). A relationship between Enteroviruses (such as HCV, HIV), Coxsackie viruses (*i.e.*, B1) and Parvoviruses (*i.e.,* B19) infections and the onset of PM and DM has been described, but the direct implications of these viruses in the pathogenesis of the disease is still not confirmed (28).

Finally, calcinosis represents a predisposing factor for the development of bacterial infections. This is a known risk for the development of staphylococcal soft tissue and dermal infections due to *Staphilococcus aureus* in the area of calcinotic lesion (36).

Antiphospholipid antibody syndrome

APS was described in the 1980s as the association of vascular thrombosis and/or pregnancy loss with persistently positive antiphospholipid antibodies (39). Antiphospholipid antibodies (aPL), are now detected by lupus anticoagulant (LA), anticardiolipin antibodies (aCL) and anti-beta 2 glycoprotein I antibodies (anti-β2GPI) (40) but they were first observed in patients with infective diseases. In fact the critical antigen of nontreponemal serological tests syphilis (STS), described in 1906, was identified in 1942 as an anionic phospholipid derived from bovine heart and named *cardiolipin*. In patients with autoimmune disease, aCL antibodies bind a complex of negatively charged phospholipids and β2GPI, which is now considered the main target of autoimmune aPL. In contrast, the antibodies present in the majority of patients with infectious diseases recognize cardiolipin *per se*, independently from β2GPI; they are usually described as transient and not associated with thrombosis. Recently, however, several epidemiological and experimental studies have underlined a relationship between APS occurrence and infective disease, suggesting that viral or bacterial infection might also induce autoimmune aPL (41-44).

Several viral infections (Parvovirus B19 (45), Cytomegalovirus, Varicella-zoster virus, EBV, HCV and HIV) have been associated with the occurrence of vascular thrombosis related to aPL. Importantly viral infection can trigger aPL and thrombosis episodes in children that generally do not have other common risk factors for thrombosis (*e.g.*, atherosclerosis, cigarette smoking, contraceptive medications) (46). In pediatric cases of varicella-zoster, venous thrombosis and cerebrovascular disease were reported as being associated to the transient positivity of LA and anti β2GPI. In addition, an association between LA and protein S deficiency due to anti protein S autoantibodies was reported in children with post-varicella purpura fulminans or thrombosis. In these conditions, positive antibody titre are generally temporary, but they were shown stable for more than 12 weeks in some patients with thrombosis. Most aPL detected during viral infection are not directed to β2GPI (β2GPI independent) however those found associated to CMV, HIV and Parvovirus B19 infection recognize β2GPI as well as the antibodies typically characterizing autoimmune diseases (47). In particular, Parvovirus B19 infection with β2GPI dependent aCL and with a clinical profile including thrombocytopenia, haemolytic anemia, arthritis and miscarriages has been indicated as a trigger for the development of autoimmune APS (48).

After syphilis, which was the first bacterial infection associated to aPL, bacteria were described in several other of these conditions including streptococcal and staphylococcal infection, *Mycoplasma pneumoniae* (49), *Coxiella burnetii*, *Escherichia coli*, other Gram-negative bacteria, tuberculosis, and leprosy. Interestingly, in leprosy aCL, LA and anti-β2GPI, with a prevalence of IgM isotype, were reported without the occurrence of any APS related clinical manifestation (50). In general, patients affected by bacterial infections, despite being positive for aPL, do not suffer thrombotic events with the same frequency observed in patients with viral infections.

Not much has been reported on parasitic infections as a trigger for aPL production. In patients with malaria, β2GPI independent aCL antibodies were reported, and in patients with leishmaniasis anti- β2GPI antibodies were observed in the absence of aCL, suggesting that in this case antibodies might be directed to different epitopes on the β2GPI molecule.

Occasionally, patients undergoing vaccinations may produce aPL. This has been proven in a group of normal subjects immunized with recombinant hepatitis B vaccine (51). In some SLE patients undergoing repeated influenza vaccinations a progressive increase of anti-β2GPI has been shown suggesting that these subjects need a careful clinical and laboratory follow-up.

The most convincing evidence of an existing link between infections and APS is the well-known possibility that about a quarter of the so-called catastrophic APS are preceded by infections (52). Catastrophic APS is a rare and life-threatening variant characterized by microvascular thrombosis causing organ failure especially in the kidneys, heart, lungs and central nervous system. This finding is in agreement with the epidemiological observation that the most frequent cause of death in 1000 APS patients followed for 5 years is the occurrence of bacterial infections (53).

All the above quoted epidemiological observations support a possible infectious pathogenesis of APS. Experimental data suggest that a mechanism of molecular mimicry between microbial antigens and β2GPI molecule can cause autoantibody production and eventually APS (43). The immunization with a CMV derived synthetic peptide, sharing structural similarities with the phospholipid-binding site of β2GPI, induced pathogenic aPL in mice. In addition a peptide (TLRVYK), with high homology for various viral and bacterial proteins, was shown to be the target of pathogenic anti-β2GPI monoclonal antibodies and anti TLRVYK antibodies were detected in mice immunized by *Haemophilus influenzae*, *Neisseria gonorrhoeae* and *Tetanus toxoid*. These antibodies can cause APS related symptoms in animal models. Molecular mimicry was also suggested to explain the aPL production during persistent Parvovirus B19 infections in patients with rheumatic diseases. In fact, the minor capsid protein VP1, with phospholipase A2-like activity, may contribute to the inflammatory tissue reaction by generation of abnormal cleavage products from cellular phospholipids compounds, which in turn induce aPL in combination with a particular genetic predisposition (54).

Therefore, infections may induce synthesis of aPL, and occasionally, these antibodies may be accompanied by thrombotic events. This picture seems to be related to viral more than bacterial infection. Data on the real duration of positive postinfectious aPL are rare, so it is not clear if some of them acquire the features of persistent autoimmune aPL. Several reports underline that the target of post infectious aPL is not only the phospholipid molecule, in fact anti-β2GPI antibodies were reported in different infective diseases. If these are always directed to the same epitopes of anti- β2GPI present in patients with autoimmune disease or if they may bind different parts of β2GPI molecule has still to be clarified. In the meanwhile, it might be important to screen patients with APS for possible infectious agents, taking into account that in this case the disease might be only temporary, and therefore it could be considered possible the withdrawal of the anticoagulant treatment with evident benefit for the patients.

Vasculitis

Vasculitis can be defined as an inflammatory process of blood vessels. It can be secondary to other conditions or constitute a primary, in most cases, idiopathic disorder. Underlying conditions in the secondary vasculitides are infectious diseases (55), connective tissue diseases, and hypersensitivity disorders (Table I). IC, supposedly composed of microbial antigens in case of underlying infectious diseases, autoantigens in the connective tissue diseases, and non-microbial exogenous antigens in the hypersensitivity disorders (Table I), are involved, in many cases, in the pathophysiology of the secondary vasculitides. Here, we will consider the role of infections in the primary vasculitides.

The primary vasculitides, classified according to the size of the vessels involved, the histopathology of the lesions, and certain clinical symptoms (Table II), are, as mentioned, idiopathic disorders. Nevertheless, microbial agents have been suggested to be involved in their pathogenesis, although the precise mechanisms have not been fully elucidated (56).

Table I. Secondary vasculitides: antigens presumably involved.

Exogenous antigens:
- Microbial antigens
  - *Bacterial*
    - Streptococci
    - Staphylococci
    - Mycobacterium leprae
    - Treponema pallidum
    - Others
  - *Viral*
    - Hepatitis B/C virus
    - Human immunodeficiency virus
    - Cytomegalovirus
    - Epstein-Barr virus
    - Others
  - *Protozoal*
    - Plasmodia
- Non-microbial antigens
  - Heterologous proteins
  - Allergens
  - Drugs
  - Tumor antigens (?)

Autologous antigens
- Nuclear antigens (antinuclear antibodies)
- Immunoglobulin G (rheumatoid factor, cryoglobulins)
- Others

With respect to large vessel vasculitides (57), Parvovirus B19 and Herpesviruses have been detected in affected temporal arteries of patients with giant cell arteritis but these findings are highly controversial. Based on seasonal and geographical variation in presentation, infectious agents have been suggested but proof for their involvement is lacking. Possibly, different infections may trigger the disease.

With respect to medium-sized vessel vasculitides, polyarteritis nodosa (PAN) associated with HBV infection has been noted for a long time. Depending on the geographical area, in particular the prevalence of HBV infection, different percentages are given, but around 30% of patients with PAN are carriers of HBV (58). Testing for HBV in patients with PAN is important as HBV-infection determines clinical presentation, treatment and outcome. In patients with HBV-PAN, glomerulonephritis, anti-neutrophil cytoplasm antibody (ANCA)-positivity, and relapsing disease are almost never found (58). Treatment now consists of corticosteroids together with antiviral agents and plasma exchange. Treatment should be aimed at attaining seroconversion and stopping viral replication which results in complete remission of the disease without occurrence of relapses and the prevention of long-term hepatic complications (58).

Kawasaki disease is an intriguing inflammatory vasculitis of early childhood in which the coronary arteries are frequently affected (59). It has been suggested that the disease results from an abnormal immunological response to various, still undetermined, microbial agents in genetically susceptible infants. Viruses have been implicated such as the New Haven Coronavirus, HIV, Adenoviruses, etc. (60). Also superantigens derived from *S. aureus* have been implicated (61). Superantigens are proteins that bind to class II MHC molecules on antigen presenting cells and interact simultaneously with

Table II. Primary vasculitides.

I. Large vessel vasculitis	
1. Giant cell (temporal) arteritis	
2. Takayasu arteritis	
II. Medium-sized vessel vasculitis	
1. Polyarteritis nodosa	(HBV)
2. Kawasaki disease	(*S. aureus superantigens,* viruses?)
III. Small vessel vasculitis	
1. Wegener's granulomatosis*	(*S. aureus?)*
2. Churg-Strauss syndrome*	
3. Microscopic polyangiitis*	
4. Henoch Schönlein purpura	(respiratory infections?)
5. Essential cryoglobulinemic vasculitis	(HCV)
6. Cutaneous leukocytoclastic angiitis	

*ANCA-associated

specific Vβ segments of the T-cell receptor (TCR). As such, they are able to stimulate, in an antigen-independent way, all T-cells that utilize a particular group of TCR Vβ segments. In Kawasaki disease, *S. aureus* strains have been isolated expressing various superantigens, in particular the toxic-shock-syndrome toxin-1 (TSST-1) superantigen, and analysis of the Vβ repertoire on the TCR of circulating T-cells showed T-cell expansion compatible with superantigen driven T-cell proliferation (61). However, the exact role of *S. aureus* in Kawasaki disease is far from clarified.

In small vessel vasculitides, infectious agents have been suggested to be involved in disease pathogenesis in various disorders.

HCV infection, having a global prevalence of around 2%, is associated with mixed cryoglobulins in around 50% of infected patients, and with cryoglobulinemic vasculitis in 5-10% of patients. The clinical spectrum of this form of vasculitis is variable ranging from purpura to severe proliferative glomerulonephritis (62). How HCV induces B-cell activation and proliferation, resulting in the production of monoclonal rheumatoid factor, an essential component of mixed cryoglobulins, is presently not clear (63). However, the detection of HCV in patients with essential cryoglobulinemic vasculitis is of utmost importance as it has therapeutic consequences. A combination of antiviral treatment (INF-α with ribavirin) and immunosuppressive treatment (rituximab alone or with steroids, cyclophosphamide and even plasma exchange in very severe cases) has now been suggested (62).

Henoch Schönlein purpura (HSP) is a frequently occurring form of systemic vasculitis in childhood, characterized by the deposition of IgA within the vessel wall (64). Its etiology is unknown but its development or relapse has been described in conjunction with infections related to a multitude of microorganisms.

Wegener's granulomatosis

Wegener's granulomatosis (WG) is an ANCA-associated necrotizing granulomatous vasculitis that predominantly affects small-sized vessels. Two facets of the potential role of infection in WG etiology must be considered: an infectious disease as the cause of WG, and the consequences of WG and its treatment on infection occurrence.

We know very little about the etiology(ies) of WG but infectious hypotheses have been proposed since the earliest descriptions of the disease (65). After WG diagnosis and treatment initiation, many infectious side effects were reported, mainly resulting from immunosuppressant use. Before the 1970s, the 1-year mortality rate for systemic necrotizing vasculitides exceeded 80%. Although the combination of steroids and cytotoxic agents, usually CYC, dramatically improved the outcome, it favored the development of major infections, *e.g.*, septicemia, *P. jiroveci* pneumonia (PJP) or herpes zoster infections (66). Despite systematic PJP prophylaxis with co-trimoxazole and less intensive treatment, infections remain a major concern. Their numbers and severity have progressively declined since pulse CYC replaced oral intake and when CYC induction duration lasts only 3–6 months (67). Maintenance therapy with low-dose steroids, azathioprine, methotrexate or another immunosuppressant is less toxic than oral CYC and fewer infections are observed than in the past.

Infection as a cause of Wegener's Granulomatosis

T-cell activation can persist in active and remitted WG, and are suggestive of persistent antigenic stimulation. Several exogenous factors (*i.e.*, airway dust, silica or heavy metals; antileukotriene agents or microbes) have been suspected of playing a role in the development of ANCA-positive vasculitides (68). The potential role of microorganisms was investigated and *S. aureus* was retained as a possible pathogenic agent of WG. In addition to the high number of ear, nose and throat (ENT) infections in WG, chronic nasal carriage of *S. aureus* was associated with higher relapse rates (69). *S. aureus* carriage was also associated with the ANCA-positivity of patients in remission under immunosuppressive treatment. Those findings were corroborated by the observation that co-trimoxazole provided effective maintenance therapy, achieving lower WG relapse rates (70), perhaps through its anti-infectious rather than its antimetabolite properties. In addition, Mayet *et al.* (71) demonstrated that WG patients' T cells were reactive to proteinase-3 (PR3) and *S. aureus*, but not to myeloperoxidase, *S. epidermidis* or *S. pyogenes*.

S. aureus-secreted toxins act as superantigens (SAg) and have been thought to play a role in WG pathogenesis. Several studies focused on the potential role(s) of these superantigenic toxins, strong T-cell activators, which could explain this persistent lymphocyte activation. Popa *et al.* found high carriage rates of *S. aureus* strains harboring genes encoding the SAg toxic shock syndrome toxin-1 (TSST-1), staphylococcal enterotoxin and exfoliative toxin A in WG patients (72): among 62

WG patients, 51 were *S. aureus* carriers and 37 (72.5%) of them harbored at least one SAg-positive *S. aureus* strain. SAg presence and type influenced the risk of relapse, which was markedly higher when TSST-1 was present (relative risk: 13.3, 95% confidence interval: 4.2–42.6). Eradication of TSST-1-positive *S. aureus* would provide definitive proof if it prevented WG relapses (73). Those findings seem to support a possible link between WG clinical activity, and SAg, activation and expansion of T cells-expressing SAg-binding T-cell receptor (TCR) Vβ chains. However, that connection has not yet been clearly established. A few years ago, Popa *et al*. found that T-cell expansions were not associated with *S. aureus* or its SAg and they concluded that the deleterious effects of *S. aureus* are probably governed by mechanisms other than superantigenic T-cell activation. While T-cell expansions may be seen in WG, no T cell-repertoire abnormalities suggestive of superantigenic activation were observed (73). Grunewald *et al*. showed that $BV8^+CD4^+$ T cells with a common complementarity-determining region-3 (CDR3) motif recognized a specific antigen expressed by DR4 molecules, thereby suggesting the possibility of a shared antigen (74). Their observation argues against superantigenic stimulation. However, the role of *S. aureus*-produced toxins remains controversial and no significant differences have been found between WG patients and healthy subjects (73). If *S. aureus* were really implicated in WG pathogenesis, the bacterium might require additional mechanisms to induce its deleterious effects.

Other biological findings suggest that *S. aureus* could be involved in WG pathogenesis. First, staphylococcal acid phosphatase could bind to endothelial cells through charge interactions, as observed in glomeruli of WG patients. In addition, antibodies to staphylococcal acid phosphatase might be induced, leading to endothelial cell activation, neutrophil attraction, oxidative burst, PR3 release and, finally, breakdown of PR3 tolerance. Second, Fcγ-receptor polymorphisms could be implicated by decreasing macrophage phagocytosis of *S. aureus*, thereby favoring chronic carriage, and, hence, associated with higher relapse rates.

Recently, it has been shown that peptides from *S. aureus* show strong homology with complimentary proteinase 3 (PR3), a peptide translated from the antisense DNA strand encoding for proteinase 3 (75). In addition, the authors showed that immunization of mice with cPR3 induced also autoantibodies to PR3. So, peptides from *S. aureus* could, by molecular mimicry, induce autoantibodies to cPR3 which, in turn, could induce antibodies to proteinase 3, the characteristic autoantibodies in WG (75).

Taken together, all these findings corroborate the potential role of an infectious agent, particularly *S. aureus*, in WG pathogenesis through ENT infections. That possibility could be highly relevant because nasal lesions could initiate systemic WG manifestations. Indeed, Voswinkel *et al*. recently showed that selection of autoreactive B cells and their affinity maturation might start in nasal tissue granulomatous lesions in WG patients. These autoreactive B cells might be able to produce anti-PR3 autoantibodies, the most specific ANCA for WG. However, a link between such granulomatous lymphoid tissue and *S. aureus* remains to be proven.

Although numerous data have been reported, the role of *S. aureus* in WG pathogenesis remains debatable. Because no experimental animal model of WG exists, it is difficult to determine the precise pathogenic effects of infectious agents, especially *S. aureus*, in WG development.

Infections during Wegener's Granulomatosis

Infections are a major cause of WG-patient's deaths. The majority of our patients received CYC pulses, and fewer infectious side effects were observed than in patients treated with oral CYC for more than 1 year: 53 infections occurred in 35 of our 113 patients and only 2 patients died from infections (personal data). PJP, which had been fatal in the past, is no longer a risk because co-trimoxazole prophylaxis is systematically prescribed. None of our patients died of PJP.

However, the number of infections remains too high and clinicians must still strive to prevent infections and reduce their severity. Standard treatment of WG and other ANCA-associated vasculitides has been largely modified to maintain, at least the same efficacy, while limiting treatment side effects. Lower immunosuppressant cumulative doses given for shorter durations and new treatments are now being evaluated. Although biotherapies might limit the number of infections, they also carry their own risks. Pertinently, progressive multifocal leukoencephalopathy has been reported in patients with autoimmune diseases treated given biologics and, recently, when rituximab was prescribed for vasculitis (manufacturer's warning). Those events demonstrate that clinicians must continue to search for new therapies and emphasize that no treatment is completely harmless. Therefore, at present, our objective to lower the number of infections and their impact remains elusive, and their disappearance, unfortunately, seems improbable.

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