

# Infectious disease as aetiological factor in the pathogenesis of systemic sclerosis

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## ABSTRACT

Systemic sclerosis is an autoimmune disease characterised by vascular obliteration, excessive extracellular matrix deposition and fibrosis of the connective tissues of the skin, lungs, gastrointestinal tract, heart, and kidneys. The pathogenesis of systemic sclerosis is extremely complex; at present, no single unifying hypothesis explains all aspects. Over the last 20 years increasing evidence has accumulated to implicate infectious agents in the aetiology of systemic sclerosis. Increased antibody titres, a preponderance of specific strains in patients with systemic sclerosis, and evidence of molecular mimicry inducing autoimmune responses suggest mechanisms by which infectious agents may contribute to the development and progression of systemic sclerosis. Here we review the current state of knowledge of infectious risk factors in systemic sclerosis and the possible mechanisms by which infectious exposures might induce pathologic processes.

## KEYWORDS

Systemic sclerosis, pathogenesis, infections

## INTRODUCTION

The cause of systemic sclerosis (SSc) has remained elusive despite intense investigations. Although the disease is not inherited in a classical Mendelian pattern, there is strong evidence that genetic factors contribute to its development and clinical manifestations, as discussed in more detail below. However, it has become apparent that environmental agents play a crucial and more important role than genetic influences. One study reported a remarkably low concordance in the development of SSc among homozygous twins, indicating that the heritability component of the disease was very low and that the most

important factor was of an environmental or acquired origin.<sup>1</sup> Many agents have been postulated as being involved in the cause of the disease. The hypothesis that infectious agents may cause systemic sclerosis has been studied extensively. Some researchers have suggested that the production of specific autoantibodies in SSc is the result of an antigen-driven response caused by molecular mimicry. Molecular mimicry was originally defined as the theoretical possibility that sequence similarities between foreign and self-peptides are sufficient to result in the cross activation of autoreactive T cells by virus-derived peptides.<sup>2</sup> Escaping the process of clonal deletion, certain populations of autoreactive T cells are known to persist in normal individuals and require only an appropriate stimulus to initiate self-directed immune responses and potentially autoimmune disease.<sup>3</sup> The concept of molecular mimicry proposes that antibodies against self-antigens are produced because these antigens contain epitopes that share structural similarities with viral or bacterial proteins. In the immunopathogenesis of SSc, herpesviruses, retroviruses, and human cytomegalovirus (CMV) infections, among others, have been suggested as possible causative agents. Evidence supporting the role of retroviruses includes the demonstration of sequence homologies between certain retroviral proteins and the topoisomerase I antigen, which is the target of anti-Scl 70 antibodies in patients with systemic sclerosis.<sup>4</sup> In addition, it has been shown that the induced expression of retroviral proteins in normal human dermal fibroblasts results in the acquisition of a SSc-like phenotype in the production of extracellular matrix proteins.<sup>4</sup> Furthermore, antibodies to retroviral proteins have been detected in serum specimens from patients with SSc.<sup>5</sup> Another hypothesis has suggested that human cytomegalovirus may be involved in the initial events of SSc. This hypothesis is supported by the observations of a higher prevalence of IgA antihuman cytomegalovirus antibodies in patients with SSc, which

are capable of inducing apoptosis in human endothelial cells; the increased prevalence of anticytomegalovirus IgA antibodies in patients positive for Scl-70 autoantibodies; and the severe fibroproliferative vascular changes and the increased occurrence of antinuclear antibodies with an immunofluorescence pattern similar to that present in serum specimens from patients with SSc in human cytomegalovirus infections.<sup>6,7</sup> Despite intensive study, however, there is no definitive evidence to conclude that SSc has a viral origin.

## PARVOVIRUS B19

Parvovirus B19 has been proposed as a causative agent in rheumatoid disease and other vascular injury syndromes, including Wegener's granulomatosis,<sup>8</sup> microscopic polyarteritis nodosa,<sup>9</sup> Henoch Schonlein purpura,<sup>10</sup> and dermatomyositis<sup>11</sup> in SSc.

Ferri *et al.* first suggested the possible involvement of parvovirus B19 in SSc. They found that antiparvovirus B19 NS-1, which could be a marker of persistent parvovirus B19 infection, was frequently detected in the serum of patients with SSc.<sup>12</sup> However, circulating parvovirus B19 DNA was detected in only 4% of SSc patients. They subsequently showed parvovirus B19 infection of bone marrow in SSc.<sup>13</sup> The presence of parvovirus B19 DNA was demonstrated in a significant percentage of bone marrow biopsies from SSc patients and was never detected in the control group. SSc patients with bone marrow parvovirus B19 infection showed a shorter mean disease duration than parvovirus B19-negative patients. These patients showed the most severe active endothelial injury and perivascular inflammation. The historical record was reported to be consistent with the finding of parvovirus B19 infection of bone marrow in SSc patients.<sup>14</sup> Ray *et al.* showed that incubation with parvovirus B19-containing serum induced an invasive phenotype in normal human synovial fibroblasts.<sup>15</sup> A direct correlation between the extent of degenerative endothelial cell alterations and the degree of B19 RNA expression suggested a causal role of B19 in the propagation of the endothelial cell dysfunction.<sup>16</sup> Endothelial injury in patients infected with B19 likely reflects a combination of direct viral cytotoxicity and humoral immunity. It has been shown that B19 exerts a cytotoxic effect on infected cells through a non-structural protein designated NS-1.<sup>17</sup> The ability of parvovirus B19 to persistently infect SSc fibroblasts might be responsible for important cell alterations, as suggested by phenotypic changes observed in normal human synovial infected *in vitro* by parvovirus B19.<sup>18,19</sup> Zakrzewska *et al.* showed some differences in the rate of persistence of B19V DNA, in the simultaneous persistence of two genotypes and in the pattern of viral expression among SSc patients and controls.<sup>20</sup>

## CYTOMEGALOVIRUS

CMV infection may play a part in SSc pathogenesis due to its ability to infect both endothelial and monocyte/macrophage cells and through the upregulation of fibrogenic cytokines and induction of immune dysregulation.<sup>21,22</sup> It has been proposed as accelerating factor in autoimmune vasculopathy, allograft rejection and coronary restenosis.<sup>22</sup> CMV infects vascular endothelium, and this infection is characterised by latency, reactivation, and shedding of the virus to distal tissues. In both rat and mouse models, it was shown that infection with CMV leads to the development of intimal lesions.<sup>23-25</sup> In addition, indirect evidence of a role for CMV in SSc includes an association between increased serum levels of CMV-specific antibodies and the prevalence of SSc-related autoantibodies in patients with SSc.<sup>26,27</sup>

The appearance of SSc shortly after an acute episode of viral infection suggested CMV as a possible trigger for SSc.<sup>28</sup> CMV infection is characterised by latency, reactivation, and downstream shedding of the virus.<sup>24</sup> Infection of endothelial cells selectively alters the expression of integrins, downregulating  $\alpha 5\beta 1$  and  $\alpha 2\beta 1$  and upregulating  $\alpha 6\beta 1$  and  $\alpha 3\beta 1$  integrins.<sup>29</sup> It also induces the expression of fibrogenic cytokines and contributes to immune dysregulation and possibly to autoimmunity against selective epitopes such as centromere, Scl-70, RNP, and anti-RNA polymerases.<sup>30</sup> The most direct evidence of a link between CMV and SSc is the presence of high-titre IgG antibodies to the polyglycine motifs of CMV.<sup>31</sup>

One difficulty in making a clear association between CMV and SSc is the fact that 60 to 90% of adults show serological evidence of past CMV infection, yet SSc affects at most three in 10,000 people. Recent identification of genes controlling CMV susceptibility in mice through genetic analysis may be able to shed light on this issue by facilitating studies of genetic susceptibility to CMV in humans. In the immune-compromised mouse model of CMV-induced neointimal formation, genetically resistant mice (the C57BL/6 strain) do not develop any vascular pathology in response to the viral infection, whereas CMV-susceptible mice (129 interferon- $\gamma$ R-/-) form reproducible neointimal lesions in a dose-dependent response to CMV infection.<sup>23</sup> Another possible mechanism that may explain sporadic development of SSc in response to CMV infection and the 8:1 ratio of SSc-affected women to SSc-affected men is the microchimerism hypothesis. Microchimerism refers to prolonged survival of allotypic lymphocytes (foetal T cells acquired during pregnancy or cells received by blood transfusion or organ transplant) usually in circulating blood. Microchimeric T cells of foetal origin were verified 27 years after giving birth in one woman and have been shown to be more common

and more numerous in women with SSc than in healthy, age-matched controls.<sup>32</sup> The pathogenic effects of these cells are not known, but microchimeric cells were also found in the cellular infiltrate of sclerodermatous lesions.<sup>33</sup> The engraftment and survival of these cells are dependent on the complex relationships between the tissue antigens of the mother and offspring (or host and donor) and are highly variable as a result.<sup>34</sup> Support for the idea that CMV may induce the proliferation of microchimeric cells comes from *in vitro* studies that show that T cells exposed to allotypic endothelial cells become more highly activated and proliferate to a greater extent if the endothelial cells are infected with CMV.<sup>35</sup> Therefore, in people with circulating microchimeric T cells, the vascular endothelium represents an allotypic stimulus to those cells. However, if the endothelium is infected with CMV, proliferation and cytokine expression may be amplified, possibly triggering a cascade of endothelial activation, vascular inflammation, and neointimal formation in the same fashion as transplanted T cells do in graft-versus-host disease.

#### HELICOBACTER PYLORI

The most recent research on the involvement of bacterial infections in the pathogenesis of SSc focuses on *Helicobacter pylori* (*H. pylori*) which has been implicated in other vascular diseases.<sup>36</sup> Studies have investigated *H. pylori* infections for an association with Raynaud's phenomenon, Sjögren syndrome, and SSc. In a study of patients with primary Raynaud's phenomenon, eradication of *H. pylori* infection was associated with complete disappearance of the episodes of Raynaud's phenomenon in 17% of treated patients and a reduction in symptoms in an additional 72%.<sup>37</sup> Although this study was not double blinded, it is intriguing that symptoms of Raynaud's phenomenon did not improve in those patients in whom eradication of *H. pylori* failed. A more recent trial of comparable design reported very similar results.<sup>38</sup> One study identified higher incidence rates of serological evidence of *H. pylori* infection in patients with rheumatological diseases, including SSc.<sup>39</sup> In contrast, three larger studies found no difference in *H. pylori* infection rates between patients with SSc with Raynaud's phenomenon compared with healthy controls.<sup>40-42</sup> However, even if it were true that *H. pylori* infection rates do not correlate with SSc, this does not necessarily rule out its involvement in SSc. A recent study<sup>43</sup> indicated that, despite the absence of a difference in *H. pylori* infection rates between SSc patients and control subjects, 90% of patients with SSc were infected with the virulent CagA strain compared with only 37% of the infected control subjects. Therefore, confounding factors such as coinfections, differences in *H. pylori* strains, and immunological and genetic host factors will have to be

further identified and controlled in order to understand the role of *H. pylori* in Raynaud's phenomenon, SSc, and other vascular phenomena. The association between *H. pylori* infection and Raynaud's syndrome<sup>41,43</sup> has been attributed to increased levels of cytokines and acute phase reactants, such as C-reactive protein and fibrinogen, resulting in vasospasm and platelet aggregation. Kalabay *et al.*,<sup>44</sup> who found a high prevalence of *H. pylori* infection in patients with systemic sclerosis (78%) (n=55), attempted to explain the preferential occurrence of *H. pylori* infection in SSc in two ways. First, an increased prevalence of *H. pylori* infection might be favoured by the disturbed gastrointestinal motility, a clinical phenomenon well known in patients with SSc. The second explanation may be that *H. pylori* infection and the immunological mechanisms operative in the course of SSc may be related to each other. We recently performed a study aiming to evaluate the possible association between *H. pylori* infection with disease activity, biochemical and serological data.<sup>45</sup> Our preliminary results suggest that *H. pylori* infection is implicated in activity of SSc, especially in skin involvement of this disease. This study may indicate *H. pylori* infection as a possible cofactor in the development of SSc. Clinical trials are still necessary to define the pathogenesis and confirm the increase in association between *H. pylori* and SSc.

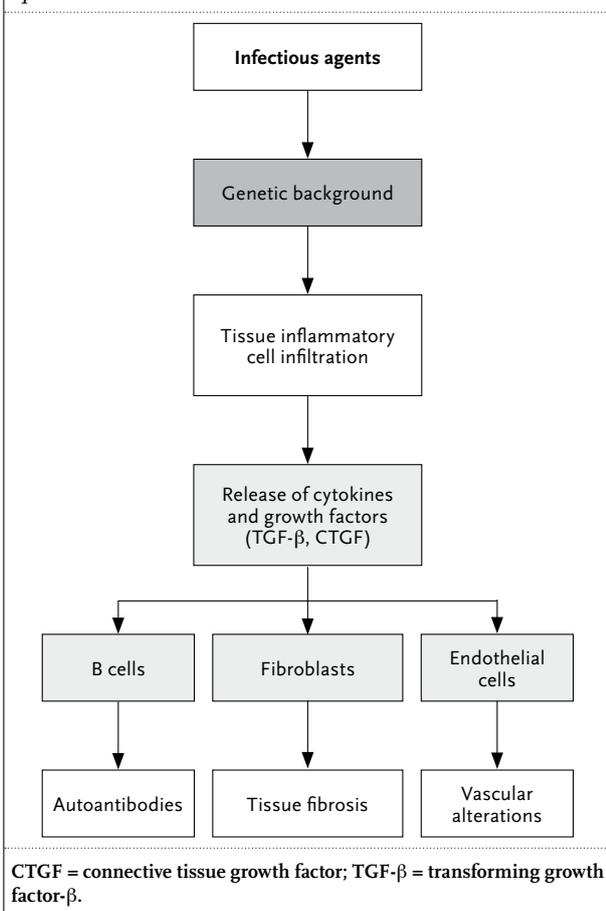
#### DISCUSSION

An increasing body of evidence suggests that there are many potential environmental triggers for SSc and that host factors determine the susceptibility of the host to disease in response to these triggers.<sup>46</sup> Infectious agents, both bacterial and viral, have long been suspected as a contributing factor in the development and progression of SSc. The rationale for this infection hypothesis is that many SSc-like symptoms are transiently elicited by infectious agents in otherwise healthy individuals. There are two general lines of evidence implicating bacterial infections in the pathogenesis of SSc. One is anecdotal evidence that treatment with antibiotics relieves SSc symptoms in some patients. The other is that graft-versus-host disease, which is recognised as having many similarities to SSc, cannot be induced in germ-free animals and is significantly reduced in children pre-treated with antibiotics to eradicate their normal bacterial flora.<sup>47</sup> Arson *et al.* recently assessed serological reactivity against various infectious agents in patients with SSc and compared them with healthy controls.<sup>48</sup> Serological samples obtained from 80 patients with SSc were compared with 296 compatible healthy controls; all samples were tested for the presence of antibodies directed against hepatitis B virus, hepatitis C virus, toxoplasmosis, rubella, CMV, Epstein-Barr virus (EBV), and *Treponema*

*pallidum*. The results of this study demonstrate that antibodies against CMV, HBV, and toxoplasmosis were detected more often in patients with SSc. This association confirms that infectious agents might have a role in disease pathogenesis and expression. In *figure 1*, it is hypothesised that the infectious agents, both bacterial and viral, are the incising factor that acts on a genetically predisposed host and results in the subsequent recruitment and homing of macrophages and T cells to the affected tissues. The most prominent clinical manifestations of systemic sclerosis are caused by the exaggerated accumulation of collagen and other connective tissue components in the affected organs. The inflammatory cells would undergo selective proliferation and expansion, perhaps because of an antigen-driven response, and then release cytokines and growth factors that initiate the process of tissue and vascular fibrosis. Infectious agents cause a profound phenotypic change in various target cells of different lineages (immune cells, fibroblasts, and endothelial and vascular smooth-muscle cells). This phenotypic change could be caused by integration of genetic material (for example, of retroviral origin) within the genetic sequence of the target cells that through unknown mechanisms

would induce the expression of specific regulatory genes, altering the function and behaviour of the target cells. These alterations are manifested by increased collagen and extracellular matrix production in fibroblasts, generation of autoantibodies and cellular immune abnormalities in lymphocytes, and severe fibroproliferative and prothrombotic alterations in endothelial cells. The target cell effects cytokines and growth factors, particularly transforming growth factor- $\beta$  and connective tissue growth factor. Molecular mimicry is a mechanism that may explain the pathogenicity of antibodies against viral proteins in SSc. Infection with HCMV may generate a host-antiviral response that is self-reactive toward autoantigens and endothelial cells. Self-reactive antibodies against a virus may induce endothelial cell apoptosis through interaction with the integrin  $\alpha_3\beta_1$  and  $\alpha_6\beta_1$  NAG-proteins complex.<sup>49</sup> Endothelial injury represents one of the first steps in the pathogenesis of SSc. Endothelial cells may be infected by bacteria or viruses that may be instrumental in inducing vasculitis. After a few days of viral infection, endothelial abnormalities are followed by necrosis.<sup>48</sup> Antibodies against endothelial cells (AECA) induce both upregulation of adhesion molecules with consequent mononuclear cell adhesion and endothelial cell apoptosis.<sup>50</sup> Microbial superantigens could initiate an immediate T cell while it has been shown that B cell response may bind to microbial superantigens to surface class II major histocompatibility complex molecules and become a target of T-helper lymphocytes. Superantigens are proteins that are expressed endogenously in the organism or that are derived exogenously by bacteria.<sup>51</sup> Numerous infectious agents have been proposed as possible triggering factors in SSc but very few infections are as rare as SSc. Therefore, development of SSc is unlikely to depend exclusively on an infectious agent. Instead, it likely occurs as a result of interactions between the infectious agent and a cascade of host-specific factors and events. This is not surprising because immune response to infection is highly individual. It is controlled by multiple genes, age, and the route of infection. It may even be different in the same individual from one day to the next owing to a number of factors, including co-infections, stress, and pregnancy. In addition, polymorphisms in genes unrelated to immunity may cause an infectious agent to induce disease through molecular mimicry in one person and not another. Therefore, in a disease as varied, complex, and rare as SSc, infection prevalence alone should not be expected to provide sufficient evidence for or against a pathological role in the disease. Despite intensive studies there is no definitive evidence to conclude that SSc has a viral or bacterial origin. In SSc, some viral or bacterial products could synergise with other factors in the microenvironment predisposing to SSc development. We definitely need some new studies or experimental

**Figure 1.** Infection hypothesis of the pathogenesis of systemic sclerosis



(animal) models to define precise mechanisms by which an infectious agent contributes to the disease process because a direct association is still missing.

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