

Role of viral infections in the etiopathogenesis of systemic sclerosis

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Received on June 25, 2013; accepted in revised form on June 26, 2013.

Clin Exp Rheumatol 2013; 31 (Suppl. 76): S3-S7.

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Key words: systemic sclerosis, scleroderma virus, parvovirus B19, cytomegalovirus (CMV)

ABSTRACT

Systemic sclerosis or scleroderma (SSc) is a clinically heterogeneous disease of the connective tissue characterised by vascular, immune/inflammatory and fibrotic manifestations. Despite extensive investigations, the key pathogenic links between these disease hallmarks remain obscure, as well as the etiology underlying the beginning of this complex disorder. As for other diseases characterised by prominent autoimmune phenomena, the search for infectious agents responsible for immune tolerance breaks or molecular mimicry events has been a long-pursued issue. In this review, we summarise the current knowledge regarding the association of different viral infections with SSc, focusing mainly on those reports describing a mechanistic interplay between the viral agents and the pathogenesis of SSc. Moreover, we speculate on how viral infections may trigger additional pathogenic mechanisms recently proposed to contributing to SSc phenotype.

Introduction

Autoimmune diseases are characterised by a loss of self-tolerance of the immune system, which can be caused by either genetic or environmental factors or a combination of both. Systemic sclerosis or scleroderma (SSc) is a peculiar member of this group of diseases, because the humoral and cellular immunologic dysregulation, witnessed by the occurrence of autoantibodies to nuclear antigens in 95% of patients (1), must be placed in a complex pathological network involving cellular players as diverse as fibroblasts and endothelial cells, whose functional alterations lead to skin fibrosis and obliteration of the lumen of small arteries, just to mention two of the main features of this multi-

system disorder (2). Viral agents, given their tropism for many, if not all, cellular subtypes, including immune cells, and their ability to transform virtually any cells into antigen-presenting cells, have been traditionally considered as potential etiopathogenic triggers of SSc. Even vaccinations with viral preparations have been claimed as potentially responsible for development or worsening of SSc or other connective tissue diseases, without confirmation from well conducted studies (3, 4). Herein we summarise the most meaningful evidences supporting the role of different viral infections in the etiopathogenesis of SSc lesions and discuss additional pathogenic mechanisms proposed to contributing to SSc phenotype, possibly triggered by viruses.

CMV and vascular alterations in SSc

Vascular injury is an early event in scleroderma. It precedes fibrosis and involves small vessels, particularly the arterioles (5, 6). The vascular damage, which occurs in virtually any organs (7, 8), consists of large gaps between endothelial cells, loss of integrity of the endothelial lining, and vacuolisation of endothelial-cell cytoplasm. In addition, there are several basal lamina-like layers, perivascular infiltrates of mononuclear immune cells in the vessel wall, obliterative microvascular lesions, and rarefaction of capillaries (5, 6, 9, 10) which may become dramatic as the disease progresses, leading to a characteristic paucity of small blood vessels in later SSc stages. A viral agent known for its ability to damage vessel walls is cytomegalovirus (CMV). Supporting evidence includes epidemiological reports indicating that chronic CMV infections in humans may play an important role in the pathogenesis of vascular diseases such as atherosclerosis

Competing interests: none declared.

(11) and systemic sclerosis (12, 13). A possible pathogenic mechanism underlying CMV damage of vessel walls is reported by Hamamdžić *et al.* (14) that employed IFN- γ R deficient mice subjected to whole body irradiation as an animal model of experimental arteritis triggered by murine cytomegalovirus (MCMV) infection. IFN- γ R^{-/-} mice whole body irradiation two months after infection developed severe vasculopathy characterised by extensive adventitial and medial infiltrate and significant neointima formation, a prominent feature of autoimmune vasculopathies in humans. Conversely, no vascular pathology was observed in any of the immunodeficient control groups, suggesting that MCMV infection was the critical factor. Infected immunocompetent animals exhibited only perivascular inflammation, suggesting that infection and immunosuppression were co-requisites of neointima formation. Apoptosis and active proliferation of myofibroblasts and infiltrating cells were detected in the intimal layer of affected aortas of these mice. To further corroborate the analogy of this pathological picture with human SSc, the experimental disease model was characterised by up-regulation of growth factors (TGF- β 1, PDGF-A and B) classically involved in SSc pathogenesis. Induction of TGF- β 1, the canonical pro-fibrotic cytokine (15), by human CMV (HCMV) was reported by other authors (16), implicating that a primary endothelial cell infection by HCMV may induce myofibroblast activation in the vessel wall under the effect of this cytokine. In addition, proliferation of vascular smooth muscle cells highly contributes to increased thickness of the vascular wall in SSc. These cells can be infected and activated by HCMV, with subsequent induction of proinflammatory mediators such as interleukin-1 β (17) and the classical chemoattractant leukotriene LTB₄ (18). Notably, the immediate early gene products of HCMV, among which the chemokine receptor US28 (19) increase vascular smooth muscle cell migration, proliferation, and expression of PDGF beta receptor (20), a receptor overexpressed and hyperactivated in SSc vasculopathy (21).

Finally, CMV-associated chronic endothelial cell inflammation and damage result also from chemokine-mediated immunopathogenic effects such as the recruitment of natural killer (NK) cells (22), a population involved in collagen vascular disease (23).

CMV and autoimmunity in SSc

Since anti-endothelial cell and anti-fibroblast pathogenic autoantibodies in SSc have been described (24-26), HCMV may be involved in the pathogenesis of SSc not only through direct infection of endothelial cells or fibroblasts, but also through autoimmune events triggered by molecular mimicry, that is one of the mechanisms linking infections and autoimmunity (27-29). A remarkable molecular mimicry paradigm involved in the pathogenesis of SSc was reported by Lunardi *et al.* (30), who identified in the serum of SSc patients IgG that specifically recognised the HCMV late protein UL94 and the endothelial cell surface integrin-NAG-2 protein complex, thereby inducing endothelial cell apoptosis. This is a formal demonstration that a host antiviral response, primarily directed against a HCMV protein expressed in infected cells, may become self-reactive toward autoantigens, endothelial ones in this case, triggering SSc. Later on, the same group showed that anti-HCMV antibodies may be linked to the pathogenesis of SSc not only by inducing endothelial cell activation and apoptosis, but also by causing activation of fibroblasts. In fact, they showed that NAG-2 is expressed as surface molecule also on dermal fibroblasts and that anti-UL94 antibodies bind to fibroblasts. Following anti-UL94 antibody stimulation, dermal fibroblasts acquired a "scleroderma-like" phenotype with up-regulation of several genes involved in extracellular matrix deposition (31). Moreover, humoral autoimmunity can also be elicited by non-specific B cell activation. In fact, CMV is a polyclonal B-cell activator *in vitro*, and the B cell hyperresponse does not require viral replication (32). Thus, additional pathogenic autoantibodies in SSc (33) might arise as a consequence of exces-

sive activation of autoreactive B cell clones (34) triggered by CMV. In addition, CMV interacts with toll-like receptor (TLR) 7 and/or 9 in human plasmacytoid dendritic cells (DCs), leading to secretion of IFN- α and B cell proliferation (35). These DC-mediated events might facilitate polyclonal B cell activation and autoantibody production in SSc during CMV infection (36, 37). Also, a potential role for type I IFN-activated monocyte/macrophages in the pathogenesis of SSc has been hypothesised (38) and these cell types can be commonly infected and activated by CMV *in vivo* (39, 40).

CMV and SSc-like disease

B cell hyperactivation has clinical implications for infected patients, as demonstrated in transplant recipients, wherein autoantibodies contribute to the development of graft-versus-host disease (GVHD) in CMV-infected allogeneic stem cell transplant (allo SCT) patients and to graft rejection in solid organ recipients (41-44). HCMV infection and its reactivation are associated with an increased risk for the development and the worsening of extensive chronic cGVHD (45, 46), characterised by SSc-like lesions in the skin and internal organs associated with the presence in the serum of SSc-specific autoantibodies such as anti-topoisomerase I (47). In a recent work by Lunardi's group (48), plasma from 18 SCT patients was tested for anti-UL94 and/or anti-NAG-2 antibodies by ELISA. Both donors and recipients were anti-HCMV IgG positive, without autoimmune diseases. 11/18 patients developed cGVHD and all of them showed skin involvement, ranging from diffuse SSc-like lesions to limited erythema. 8/11 cGVHD patients were positive for anti-UL94 and/or anti-NAG-2 antibodies. Remarkably, 4/5 patients who developed diffuse or limited SSc-like lesions had antibodies directed against both UL94 and NAG-2; their anti-NAG-2 IgG bound HU-VECs and fibroblasts inducing both endothelial cell apoptosis and fibroblasts proliferation, similar to that induced by purified anti-UL94 and anti-NAG-2 antibodies obtained from SSc patients. These data suggest a pathogenetic link

between HCMV infection and SSc-like skin cGVHD in SCT patients through a mechanism of molecular mimicry between UL94 viral protein and NAG-2 molecule, as observed in SSc patients.

CMV and epithelial-mesenchymal transition in SSc.

Fibrosis in SSc is not only due to activation of tissue-resident fibroblasts and their transdifferentiation into myofibroblasts, but also to differentiation of bone marrow-derived fibrocytes, and transition of endothelial and epithelial cells, pericytes and adipocytes into activated mesenchymal cells. Epithelial cells from different sources can transition into fibroblasts and myofibroblasts in response to transforming growth factor beta and other growth factors/cytokines. This is called epithelial-mesenchymal transition (EMT) (49, 50).

Along with endothelial cells, fibroblasts and smooth muscle cells, epithelial cells are the predominant targets for virus replication (51), which might induce EMT. Also pericytes, that can differentiate into vascular smooth-muscle cells, fibroblasts, and myofibroblasts (52) are permissive to HCMV infection with subsequent upregulation of pro-inflammatory cytokines (53) possibly mediating EMT.

Parvovirus B19 and SSc.

In patients with SSc, Ferri et al. have demonstrated the presence of Parvovirus B19 in bone marrow and/or skin biopsy specimens from a significant number of unselected subjects (54, 55). Interestingly, the same authors found high levels of Parvovirus B19 DNA and TNF-alpha expression in endothelium and fibroblasts of SSc patients using an in situ RT-PCR technique (56). Furthermore, the degree of viral transcript expression correlated with active endothelial cell injury and perivascular inflammation, relevant features in the initial phases of the disease, suggesting that the SSc tissue injury may be a consequence of a direct viral cytotoxicity (57, 58). On the other hand, Parvovirus B19 infection has been associated with production of antibodies directed against a vast array of autoantigens including nuclear antigens, rheumatoid

factor, neutrophils cytoplasmic antigens, mitochondrial antigens, smooth muscle, gastric parietal antigens and phospholipids (59-62). Thus, an alternative explanation for Parvovirus B19 role in the pathogenesis of SSc may be the induction of autoantibodies endowed with a pathogenic action as demonstrated for HCMV. However, such hypothesis is still awaiting confirmation.

Viruses and defective vasculogenesis in SSc.

Notwithstanding the progressive loss of blood vessels and high plasma levels of vascular endothelial growth factor (63, 64) caused by the adaptive response to hypoxia, SSc is characterised by a defect in vasculogenesis. (65, 66). The molecular mechanisms underlying this paradox is unknown: both angiogenic (63, 64) and angiostatic (67, 68) factors have been detected in early SSc. Parvovirus B19 and HCMV infections in bone marrow (54, 69) might account for the defective vasculogenesis observed in SSc, due to their ability of causing myelosuppression (70, 71). In fact, one of the main pathogenic hypothesis is that the production or recruitment of hematopoietic endothelial progenitor cells from bone marrow might be impaired in SSc patients thus contributing to endothelial dysfunction and poor vasculogenesis in this disease (72-74).

Concluding remarks and future perspectives.

We have summarised the most compelling evidences in favor of a viral etiopathogenesis of SSc, that are limited to CMV and Parvovirus B19. Given the high number of viral agents capable of infecting human tissues (75), it may be provokingly argued that researchers in the SSc field should increase their efforts in order to gather new insight on the possible mechanistic associations between the many viral species commonly infecting humans and SSc. Being the etiology of SSc still largely obscure as well as largely unexplained are the early pathogenic steps of the different tissue lesions characterising this multisystem disorder, we believe this topic deserves attention. Research for

additional viral triggers of SSc should benefit from upcoming tools enabling the study of the human virome (76) and from novel transgenic animal models suitable for *in vivo* testing of identified candidate viral culprits, following the example of other rheumatic diseases with a suggested etiopathogenic relationship with viral infections (77). Remarkable, from this perspective, is the seminal work of Stappenbeck and Virgin groups (78) on the multifactorial etiopathogenesis of Crohn's disease. This study represents a paradigm of the complex interplay between the different components underlying an immune-mediated chronic inflammatory disease, where specific and non-specific agents concur to disease onset and progression, and clarifies how environmental factors including infections may select in a pool of individuals with common genetic backgrounds (79) those who will develop disease from those who will remain unaffected. Finally, novel concepts on the role of viral infections in altering the normal immunoregulatory mechanisms, such as the interaction between autoantigen-presenting cells and autoreactive effector cells (80), should be instrumental to the identification of further links between viral agents and SSc development.

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