Review article: pathogenesis and clinical manifestations of gastrointestinal involvement in systemic sclerosis.

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Recommended Citation
Kumar, Sumit; Singh, Jagmohan; Rattan, Satish; DiMarino, Anthony J.; Cohen, Sidney; and Jimenez, Sergio A., "Review article: pathogenesis and clinical manifestations of gastrointestinal involvement in systemic sclerosis." (2017). Department of Dermatology and Cutaneous Biology Faculty Papers. Paper 74.

https://jdc.jefferson.edu/dcbfp/74
Role of Functional Autoantibodies and microRNAs in the Pathogenesis of Gastrointestinal Involvement In Systemic Sclerosis

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Keywords:
Oesophagus < Organ-based, Basic science < Topics, Stomach and duodenum < Organ-based, Motility < Topics
Alimentary Pharmacology & Therapeutic
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Running title: Pathogenesis of gastrointestinal involvement in systemic sclerosis

Keywords: systemic sclerosis, gastrointestinal, auto antibody, dysmotility
The gastrointestinal tract is the most common internal organ involved in systemic sclerosis. Any part of the gastrointestinal tract from the mouth to the anus can be affected. Despite extensive investigation the pathogenesis of gastrointestinal involvement in systemic sclerosis remains elusive. Dysmotility of the gastrointestinal tract causes the majority of symptoms. Recent investigations have identified a novel mechanism in the pathogenesis of gastrointestinal tract dysmotility mediated by functional anti-muscarinic receptor autoantibodies. Furthermore, the role of microRNA in the pathogenesis of SSc gastrointestinal involvement is being intensively studied. Although treatment currently remains symptomatic, an improved understanding of novel pathogenic mechanisms may allow the development of potentially highly effective approaches including intravenous immunoglobulin and microRNA based therapeutic interventions.
INTRODUCTION

Systemic sclerosis (SSc) is a chronic systemic autoimmune disorder characterized by a severe and often progressive fibrotic process affecting the skin and numerous internal organs. Tissue fibrosis in SSc is accompanied by fibro-proliferative alterations in the microvasculature and various humoral and cellular immunological alterations leading to production of autoantibodies, tissue infiltration with chronic inflammatory cells and abnormalities in innate immunity.1 Gastrointestinal Tract (GIT) involvement occurs in greater than 90% of SSc patients.2 Any part of the GIT from the mouth to the anus can be involved with dysmotility being the cardinal pathological abnormality contributing to the majority of symptoms.3 GIT involvement occurs in both the diffuse and limited cutaneous subsets of SSc. Patients may present with symptoms of SSc GIT involvement in the absence of cutaneous disease.

Pathogenesis of SSc

The exact mechanisms involved in SSc pathogenesis are not well understood. However, it is apparent that the clinical and pathologic manifestations of the disease are the result of three distinct processes: 1) Fibroproliferative vascular lesions of small arteries and arterioles, 2) fibrosis of skin and various internal organs induced by the increased production of various profibrotic growth factors such as transforming growth factor-β (TGF-β), connective tissue growth factor, and insulin-like growth factor, and 3) multiple alterations of innate, humoral and cellular immunity resulting in the accumulation of lymphocytes and macrophages in affected tissues and the production of numerous autoantibodies.8-12 Although the putative etiologic agent and the exact mechanisms involved have not been determined, recent studies have provided novel information about the early events in SSc pathogenesis. As a result of those studies it has been postulated that there is a sequence of alterations initiated by unknown etiologic factors in a genetically receptive host. The earliest events induce structural and functional endothelial cell abnormalities affecting selectively the microvasculature.13,14 These alterations cause increased
production and release of numerous cytokines, chemokines and polypeptide growth factors that result in the recruitment of bone marrow-derived and circulating fibrocytes and in the phenotypic conversion of quiescent tissue fibroblasts, and of epithelial and endothelial cells into activated myofibroblasts, the cells ultimately responsible for the fibroproliferative vasculopathy and progressive tissue fibrosis. These alterations also result in chemokine and cytokine-mediated attraction of specific inflammatory cellular elements from the bloodstream and bone marrow to the affected tissues and in the activation of resident tissue macrophages resulting in the establishment of a chronic inflammatory process. This sequence of events is diagrammatically illustrated in Figure 1.

Pathogenesis of GIT involvement in SSc

The pathogenesis of GIT SSc is complex and poorly understood. There is a paucity of studies examining the etiology and pathogenesis of gastrointestinal manifestations in SSc. Fibroproliferative vasculopathy, immune dysfunction and fibrosis have been postulated as pathogenic mechanisms of GIT dysfunction and structural alterations in SSc (Figure 1). In the following sections we highlight the important role of each of these pathogenic processes in relation to the GIT involvement in SSc. Furthermore, we discuss the results of recent studies that have identified and explored unique and novel mechanistic pathways and have allowed to postulate a novel pathogenic model for GIT involvement in SSc.

1. Vasculopathy

Endothelial cell injury has been recently suggested to play a crucial role in SSc pathogenesis and results in increased production of reactive oxygen species and release of chemokines and growth factors, which lead to recruitment of inflammatory T and B cells and pro-fibrotic macrophages in the interstitium of affected tissues. Activated inflammatory cells and tissue hypoxia resulting from structural vascular damage cause further release of reactive oxygen species, cytokines and pro-fibrogenic mediators that propel the disease process.
Studies in GIT SSc have shown evidence of diminished mucosal blood flow in the duodenum and gastric antrum.22 Histological studies of the esophagus and stomach have demonstrated endothelial cell apoptosis, perivascular infiltrates and basement membrane thickening.23,24 Vascular lesions such as gastric antral vascular ectasia (GAVE) and telangiectasia of the digestive tract are also representative of the diffuse vasculopathy affecting the GIT as illustrated in Figure 2.25,26

2. Humoral immunity

Although the presence of numerous autoantibodies is one of the critical manifestations of SSc, the occurrence of functional autoantibodies capable of causing significant dysfunction of mouse colonic smooth muscle contraction by M3-R inhibition was not described until quite recently when Goldblatt and colleagues demonstrated the presence of such autoantibodies in the serum of SSc patients.27 Subsequent studies revealed that immunoglobulins isolated from the serum of SSc patients (SSclgGs) interfere with neurally (cholinergic) mediated contraction of the GIT. This was evidenced by the demonstration that SSclgGs caused specific attenuation of the simultaneous rat colonic contractile response and release of acetylcholine induced by electric field stimulation. SSclgGs were also found to abrogate the M3-R agonist-induced contractile response of rat colonic smooth muscle in a dose dependent manner. 28,29 Most recently, it was shown that SSclgGs lead to GI cholinergic dysfunction by binding at the M3-R on the GI smooth muscle and the myenteric cholinergic neurons of the rat colon. (Figure 3) Further analysis revealed that IgG’s isolated from SSc patients early in their disease course bind with greater intensity to the myenteric neurons. As the disease duration increases, there is progressive increase in binding at both the smooth muscle and myenteric neurons. Of substantial therapeutic significance were the observations that the neural and myogenic effects of the SSclgGs were consistently and reproducibly abrogated by the administration of pooled human intravenous immunoglobulin (IVIG) and its antigen binding fragment F(ab')2 .30
3. Cell-mediated immunity (CMI)

Numerous studies have demonstrated strong evidence for the role of CMI in SSc.31-33 Recent studies have shown that alterations in CMI are important participants in GIT involvement in SSc. Gastric biopsy specimens have demonstrated accumulation of immune cell infiltrates mainly consisting of CD4+ T lymphocytes with a pronounced increase in the CD4+/CD8+ T cell ratio.34 SSc in general has a predominant Type 2 helper (Th2) polarization of CD4+ T cells.1 IL-4 causes naïve CD4+ cells to differentiate into Th2 cells by downstream activation of STAT6 and GATA3, which lead to further production of IL-4 and IL-13, the classic Th2 cytokines.35 IL-4 in a positive feedback amplifies its own response and also up-regulates humoral immunity by inducing immunoglobulin production and isotype switching (Figure 1). IL-13 along with IL-6 released from various cells are pro-fibrotic cytokines that cause fibrosis by direct pro-fibrotic effects as well as by activating TGF-β. 33 The presence of increased CD4+ cells in gastric biopsy specimens could be responsible for excessive production of pathogenic autoantibodies and the extensive GIT fibrosis, as described above.

4. Fibrosis

Fibrosis disrupts the normal tissue architecture and leads to a non-functioning GIT. TGF-β produced by activated fibroblasts and immune cells leads to extensive fibrosis by Smad-dependent and Smad-independent pathways36, and in conjunction with endothelin-1 (derived from endothelial cells) participates in the conversion of fibroblasts into myofibroblasts.11,16 Myofibroblasts produce excessive fibrillar type I and type III collagen along with other extracellular matrix components, initiate expression of α-smooth muscle actin and are the cells ultimately responsible for the fibrotic process.9,37-39 Gastric wall biopsies of SSc patients show generalized patchy fibrosis, increase deposition of type I and type III collagen in the muscularis mucosae, submucosa and muscularis propria, and strong expression of fibrogenic cytokines including TGF-β, connective tissue growth factor and the myofibroblast marker α-smooth
muscle actin. (Figure 4) As collagen has a higher elastic modulus than smooth muscle, its deposition in the intestinal wall leads to increased stiffness, subsequently causing impaired muscle contractility. Furthermore, increased tissue stiffness has been recently shown to represent a potent stimulus for further fibrotic deposition. Besides fibroblasts, telocytes (peculiar type of stromal cells with long cytoplasmic processes) essential for extracellular matrix scaffolding are damaged and severely reduced in fibrotic areas of gastric muscle in SSc. Recent studies have also highlighted the role microRNA (miRNA) in SSc pathogenesis. Differentially expressed miRNAs that target both inflammation and fibrosis have been identified in SSc. These miRNAs may be either anti- or profibrotic. Of these miRNAs, the ones that modulate TGF-β signaling and the expression of related genes encoding collagens, metallopeptidases, and integrins are the most significant. The miR-29 family has been extensively studied in relation to SSc. Indeed, miR-29, which targets collagen gene expression and regulates fibrosis, has been shown to be severely reduced in SSc skin biopsies and reduced levels of miR-29 have been suggested to lead to increased tissue deposition of collagen.

Novel developments in the Pathogenesis of SSc GIT involvement

We recently proposed that GIT dysfunction in SSc is a staged process beginning with neuropathy and progressing to myopathy, with eventual fibrosis. The initial neuropathic damage leads to disrupted contraction with normal amplitude in manometric assessment, which is followed by a substantial reduction in amplitude of contractions signifying the development of myopathy. The earliest evidence for neuropathy was obtained in a study that showed contractile response of the esophageal smooth muscle of SSc patients to the direct acting muscarinic agonist methacholine but not to edrophonium, which acts indirectly. These and numerous subsequent studies suggested that in the early stages of SSc GIT involvement circulating M3-R autoantibodies block cholinergic neuro-transmission by inhibition of
Acetylcholine release at the myenteric cholinergic nerves (neuropathic damage), and that progression of the disease leads to myopathy via inhibition of acetylcholine action at the gastrointestinal smooth muscle cell. Some of these studies further suggested a temporal increase in binding of SScIgGs to the neural and myogenic M3-R with disease duration, observations that may account for the progressive nature of GIT involvement in SSc. The demonstration of autoantibody-mediated inhibition of neurotransmission provides a cogent rationale to the well-demonstrated observations that SSc patients may develop GIT manometric abnormalities long before the occurrence of histological changes in the GIT. As acetylcholine is the principal excitatory neurotransmitter in the GIT, inhibition of its release results in the absence of response of the smooth muscle in the GIT and its inability to contract in response to physiological stimuli. However, intestinal dysfunction at this stage is still reversible if the M3-R autoantibodies can be removed from the plasma or neutralized. Subsequently, loss of GIT contractile function may be the result of disuse muscle atrophy and progressive SSc related tissue fibrosis. (Figure 4) Once fibrosis ensues the smooth muscle is unable to respond to any type of external stimuli and treatment of dysmotility at this stage is futile. The fibroser and dysfunctional GIT leads to reflux of gastric acid contents into the esophagus, a dilated and non compliant stomach, overgrowth of bacteria in the small intestine, colonic dilatation and a non-functional internal anal sphincter, alterations that manifest clinically as Barrett’s esophagus, gastroparesis, severe malabsorption, and fecal incontinence, respectively. Increased accumulation of fibrillary collagens and reduced expression of matrix metalloproteinase-1 along with GIT dysmotility lead to development to wide mouth diverticula, which have been reported in the esophagus, small intestine and the colon. The pathogenesis of GAVE and vascular ectasia in the small intestine has been suggested to be analogous to the vasculopathy responsible for the cutaneous and other vascular manifestations of SSc. (Figure 3) IVIG could potentially have multiple beneficial
effects in SSc not only by anti-idiotypic mediated neutralization of muscarinic autoantibodies but also by causing reduction of pro-fibrotic cytokines or by counteracting the deleterious effects of anti-fibroblast or anti-endothelial cell circulating antibodies. IVIG is relatively safe compared to immunosuppression and has been shown to be beneficial in halting progression of GI symptoms in observational studies and to reverse cholinergic dysfunction induced by M3-R autoantibodies in vivo.29,30,103-105 Targeting miR-29 by anti-miRs, which are chemically modified oligonucleotides, is another promising therapeutic option in the future.46,106 However, rigorous studies are needed before patients can be benefited from these novel interventions.

CONCLUSION

GIT involvement is the most common non-cutaneous organ system involved in SSc and is associated with substantial morbidity and mortality. Our current understanding of the pathogenesis and treatment of this entity is still far from complete, owing to the limited number of studies that have focused on histo-pathological, cellular and molecular changes in the GIT. Autoantibody mediated dysmotility offers a new avenue for further research into the pathogenesis and treatment of gastrointestinal SSc. Moreover, there is lack of non-invasive tests or biomarkers, which can identify patients who are at high risk for development of GIT manifestations. Early identification and symptomatic treatment of patients is crucial until more targeted and effective disease-modifying therapeutic approaches become available. Improved understanding of the pathogenesis of GIT manifestations of SSc employing focused and intensive cellular and molecular studies, research endeavors aimed at identification of novel disease biomarkers, and extensive efforts to develop personalized medical therapies should allow achieving these goals in the near future.
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Figure Legends

Figure 1: Pathogenesis of SSc: Unknown environmental factors in a genetically susceptible host trigger initial endothelial cell injury. This results in release of reactive oxygen species, chemokines, cytokines, and growth factors. The local cytokine milieu leads to recruitment and activation of chronic inflammatory cells, including T- and B-lymphocytes and macrophages. B cells evolve into plasma cells that produce M3-R antibodies, which occupy the extracellular loop-2 of the M3-R on the intestinal smooth muscle and prevent acetylcholine released from the synaptic terminal to act on the receptor. The CD4+ T cells under influence of IL-4 differentiate into Th2 subtype. Th2 cells secrete pro-fibrogenic cytokines IL-4 and IL-13, which along with IL-6 stimulate TGF-β production. Fibroblasts are activated into myofibroblasts by action of TGF-β. Myofibroblasts produce excess collagen, which causes structural damage and fibrosis, distorting the normal architecture of the tissue, leading to dysmotility.

Figure 2: SSc IgG binding to colon: Transverse section of rat colon demonstrating specific binding of SSc IgG to the smooth muscle and myenteric plexus as evidenced by increase immunofluorescence (green) intensity.
STATEMENT OF INTEREST

The work was supported by Grant Numbers R01-AR19616 (to SAJ) and R01-DK-035385 (to SR) from the National Institutes of Health and an institutional grant from Thomas Jefferson University.
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