Immune–endothelial–nerve interaction: an explanation for the failure of the gastrointestinal system in systemic sclerosis?

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In this issue, Kawaguchi *et al*¹ (see page 710) report the occurrence of antimuscarinic 3 receptor (M3R) antibodies correlating with gastrointestinal involvement and severe dysmotility in systemic sclerosis (SSc). Contractility of the gastrointestinal system is controlled, in the myenteric plexus, by the autonomic nervous systems—sympathetic and parasympathetic—that are both essentially sustained by cholinergic transmission. The data reported by Kawaguchi *et al*¹ might suggest that these antibodies impair enteric cholinergic neurotransmission, thus fostering enteric dysmotility in SSc. Clearly, this report focuses interest on the role that the immune system, with the production of autoantibodies, may play in generating the dysmotility of the gastrointestinal system in SSc. It is also important to focus attention on the technical details. It is well known that the technique for antibody detection is critical and may lead to controversial results, as already shown in Sjögren syndrome.² This aspect is even more important because these antibodies are found in high titres in Sjögren syndrome,^{3,4} remarking the absence of a connective tissue disease control group in the study by Kawaguchi *et al*.¹

Howe *et al*⁵ have previously shown the presence of circulating antibodies to myenteric neurons in SSc, with a positive correlation with the presence of Raynaud's phenomenon but not with gastrointestinal involvement. Moreover, Eaker *et al*⁶ have injected antimyenteric neuronal antibodies separated from SSc serum into an immunosuppressed rat model (with chronic indwelling intestinal

electrodes to measure intestinal myoelectric activity) to observe the effects on intestinal motility. A prolongation in activity front duration and interval and a disruption were seen after SSc antibody injections. The authors hypothesised that these antibodies could account for the gastrointestinal neuropathic motility disturbances seen in SSc.⁶

It is well known that in SSc the involvement of the gastrointestinal tract is one of the earliest events appearing almost as early as Raynaud's phenomenon.⁷ It is characterised by neural damage, smooth muscle fibrosis, atrophy and collagen deposition of the submucosal space all along the gastrointestinal tract.⁷ The very early endothelial damage⁸ is followed by the deposition of collagen and intracellular matrix produced by fibrogenic clones of tissue fibroblasts. Most of the gastrointestinal complications originate from the consequences of decreased gastrointestinal motility (oesophageal dysmotility and reflux oesophagitis, gastroparesis, intestinal pseudo-obstruction, diarrhoea/constipation and fecal incontinence).

The morphological changes of the gastrointestinal system in SSc are due to a complex interaction among the vascular, neural and immune system, and profibrotic cytokines seem to play a major role in the damage of the gastric wall of SSc patients.⁹ Modifications of the immune and microvascular systems may account for tissue damage in the gastric wall. T-cell infiltration is a prominent finding with the CD4/CD8 T-cell ratio significantly increased.¹⁰ T cells are found in both lymphocyte aggregates and diffuse infiltrates and strongly express the activation markers very large antigen-4, leucocyte function antigen-1 and intracellular cell adhesion molecule-1, whereas endothelial cells show strong expression of vascular cell adhesion molecule-1 and intracellular cell adhesion molecule-1.¹⁰ Mature B cells may also be frequently observed arranged in aggregates.¹⁰ These

data demonstrate that the endothelial/lymphocyte activation and CD4 T-cell infiltration may play a key role within the gastric wall of patients with SSc,⁸ indicating that the disease may at least partly be triggered by the immune system. The effect of antineuronal antibodies inhibiting muscarinic neurotransmission and the cytokines released upon the stimulation of the immune system may also alter different gastrointestinal functions. These complex early events are already present in the preclinical phase of SSc, before the onset of the clinical symptoms. The target organ in the early phase is the oesophagus in approximately 70% of patients.⁷ It has been suggested that scleroderma-associated gastrointestinal dysmotility may begin primarily as a neuropathic process and later becomes a myopathic process.⁷ A neurogenic disturbance has been suggested after the demonstration that the lower oesophageal sphincter smooth muscular response was intact after stimulation with methacholine (directly acting at the cholinergic receptor), edrophonium (enhancing acetylcholine effects) and gastrin I (proposed to increase the release of acetylcholine).¹¹ Therefore, the early involvement of the oesophagus is characterised by lower oesophageal sphincter dysfunction that is purely due to a dysfunction of the autonomic nervous system. In this phase, the patient may be symptomless and only later may the typical symptoms—dysphagia, heartburn and regurgitation—appear.⁷

The study of duodenal myoelectric activity showed that impaired intrinsic cholinergic pathways were involved.¹² The capacity of octreotide to provoke a response of enhanced propagating small bowel strongly indicates that the disease process is a neuropathic event, because organised small bowel motor activity is mediated via the myenteric plexus.¹³

The severity and clinical presentation of gastrointestinal dysmotility in SSc is variable and may remain clinically occult. One problem is that patients often have major abnormalities in gastrointestinal function that are subclinical and become apparent after invasive recording techniques are applied. SSc patients, with minimal or absent skin disease, may already have severe gastrointestinal dysmotility, whereas other patients with severe skin disease, and despite the duration of the disease, may have no symptoms linked to gastrointestinal dysmotility. Howe *et al*⁵ observed that six of their patients, although strongly positive for antimyenteric neuronal antibodies, denied

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gastrointestinal symptoms and did not undergo extensive motility testing. Indeed, 90% of SSc patients tested with oesophageal manometry during some course of their illness showed oesophageal aperistalsis in the lower two-thirds of the oesophagus (the smooth muscle portion) or decreased lower oesophageal sphincter pressure¹⁴ with or without subjective dysphagia.

The sequence of events currently leading to the damage of autonomic pathways, involved in the main reflexes necessary to make the autonomic nervous system work properly in the gastrointestinal tract of SSc patients, remains unknown.¹⁵ The work of Kawaguchi *et al*¹ is now taking us closer to the possibility that M3R antibodies may play a role in gastrointestinal involvement, but the mechanisms leading to the loss of function of the autonomic nervous system remain uncertain. It is not clear whether there is a direct link between the presence of these antibodies and the development of neuronal damage in the gastrointestinal tract. In the clinic we frequently miss the symptoms, derived from the gastrointestinal tract, reflecting gastrointestinal dysmotility. This demonstrates clearly our inability to link the presence of these myenteric plexus neuronal antibodies with gastrointestinal symptoms and the downstream clinical implications, in particular in different phases of the disease, are far from being elucidated.

Moreover, it is not clear whether the role of the immune system in the gastrointestinal tract is mainly due to the production of antibodies only or also to direct the infiltration of immune cells into the enteric wall. The complex endothelial-immune-nerve interaction remains a fascinating but still poorly understood network that plays a role of paramount importance in the genesis as well as the evolution of SSc. Besides the cross-sectional studies, follow-up investigation of samples derived from different geographical areas also seems to be required to clarify the importance of antimuscarinic antibodies in the development of gastrointestinal manifestations. The fine epitope specificity of these particular antibodies, the functional consequences of receptor binding, and the pathway(s)

utilised by antimuscarinic antibodies at the cellular level should also be determined. In general, this is also true for other autoantibodies described in SSc, including anti-endothelial cell, antifibroblast and antiplatelet-derived growth factor antibodies.^{16–18} Future work may help to solve the current controversies and clarify the exact role of autoantibodies targeting different structures in the pathogenesis of SSc.¹⁹

The present work does not address the problem of whether antimyenteric antibodies may play a role in the early phase of the disease, when puffy fingers, Raynaud's phenomenon, lower oesophageal sphincter dysmotility and capillaroscopic abnormalities are present and antinuclear and anticentromere or anti-topoisomerase I antibodies are positive. If this can be demonstrated in the future a specific strategy reducing the source (anti-B-cell agents)²⁰ or blocking the effect downstream of these antibodies (intravenous immunoglobulins)²¹ could be developed barring the way to progression to dysmotility and then to bacterial overgrowth, fibrosis and muscle atrophy with eventual intestinal paresis.

All these issues are waiting for urgent answers in order to fight the silent evolution of gastrointestinal involvement adequately and open a "window of opportunity" to prevent the evolution of disease advancement in SSc patients.

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