Achalasia

Novel mechanism for impaired nitrergic relaxation in achalasia

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New insights into the pathogenesis of achalasia indicate that incubation with serum from patients with achalasia leads to altered neurochemical coding of the myenteric plexus and impairs the nitrergic response to nerve stimulation

Incomplete relaxation of the lower oesophageal sphincter (LOS) following deglutition and absence of oesophageal peristalsis, in most cases accompanied by an increased resting tone of the LOS, are the manometric hallmarks of idiopathic achalasia.[1–3] The net result of these motor abnormalities is stasis of saliva and food in the oesophagus leading to the typical symptoms of achalasia: dysphagia for both solids and liquids, regurgitation of undigested food, respiratory complications (nocturnal cough and aspiration), chest pain, and weight loss.[4] The treatment of this relatively rare disorder (incidence of approximately 1/100 000 per year) mainly aims at reducing the resistance to flow at the oesophagogastric junction. This can be achieved by reduction of LOS pressure by pharmacological drugs (nitrates, Ca^{2+} channel blockers, botulinum toxin), by forceful dilation using endoscopic techniques, or by surgical myotomy.[5] With the introduction of minimally invasive techniques, the surgical approach in particular has recently gained a significant increase in interest and has become the method of choice in several centres.[6] Large prospective randomised studies comparing the two most widely used therapies—that is, endoscopic pneumatic dilation and laparoscopic Heller myotomy combined with an antireflux procedure—are required to determine the most optimal treatment for patients with achalasia.

Although achalasia is the best characterised oesophageal motor disorder, its pathogenesis is still incompletely understood.[7 8] Histological examination reveals a significant decrease in the number of myenteric neurones, especially inhibitory nitric oxide releasing neurones, in the distal oesophagus and at the level of the LOS,[9] but the underlying mechanism leading to neuronal loss remains unknown. Familial, neurodegenerative, genetic, infectious, and autoimmune mechanisms have all been forwarded as possible explanations.[8] To date, some of these mechanisms have been gathered into a unifying hypothesis. It is suggested that an initial insult, possibly a viral infection, initiates a cascade of events leading to inflammatory changes and damage to the myenteric plexus. Subjects with a certain genetic background[10 11] may develop an autoimmune response leading to the development of autoantibodies and subsequent chronic inflammation with further destruction of inhibitory neurones. More detailed examination of resection specimens indeed shows infiltration of myenteric ganglia with CD3/CD8 positive lymphocytes expressing activation markers.[12 13] In addition, IgM antibodies and evidence of complement activation were shown within myenteric ganglia.[14] Finally, antibodies against myenteric neurones have been repeatedly shown in the serum of achalasia patients,[15 16] especially in patients with a specific HLA genotype, namely those carrying the DQA1*0103 and DQB1*0603 alleles.[11]

These findings all indicate an autoimmune origin of the myenteric ganglionitis observed in achalasia. However, the exact stimulus initiating this autoimmune response or the antigen targeted remain to be identified. Some studies suggest a previous viral infection with varicella zoster or measles as a possible trigger but others have failed to confirm this.[14 17–19] Other investigators have also demonstrated antineuronal antibodies in serum from patients with gastro-oesophageal reflux disease (GORD), suggesting that the antineuronal antibodies are generated in response to tissue damage and thus represent an epiphenomenon.[16]

In the present issue of *Gut*, Bruley des Varannes and colleagues[20] show that incubation with serum from patients with achalasia leads to altered neurochemical coding of the myenteric plexus and impairs the in vitro nitrergic response to nerve stimulation of the human gastric fundus ***(see page 319)***. Tissue was obtained from patients who underwent surgery for adenocarcinoma of the oesophagus and was incubated overnight for 16–18 hours with serum from healthy subjects, from patients with achalasia, or from GORD patients. Only serum from achalasics altered the chemical coding of myenteric neurones, with a significant decrease in nitric oxide synthase (NOS) positive neurones and an increase in cholinergic neurones. Interestingly, these immunohistochemical changes corresponded with impaired inhibitory motor response to nerve stimulation of isolated muscle strips. Although the identity of the factor responsible for these changes is unclear, it seems rather unlikely that antineuronal antibodies are involved. Only 12% of the sera studied were found to contain antineuronal antibodies. Instead, the authors suggest that circulating cytokines (for example, interleukin 8) may be involved, especially as they observed an increase in interleukin 8 levels in the sera of achalasia patients compared with controls (unpublished results). Unfortunately, data on GORD serum are rather limited ($n = 5$) and no experiments were performed evaluating the effect on motility. Nevertheless, the study is unique as, in contrast with previous studies, the effect of serum from achalasia patients was studied on human tissue and morphological data were correlated with functional data. Ideally though, LOS or oesophageal tissue should have been used, although from a practical point of view it should be emphasised that this would have further complicated the present study due to the scarce availability of this tissue. Therefore, the authors need to be congratulated for completing this rather difficult study providing new insight into the pathogenesis of achalasia.

Several crucial issues however need to be addressed. Firstly, although the study shows changes comparable with those observed in achalasia (that is, impaired nitrergic neurones with increased/maintained cholinergic innervation), no decrease in the number of myenteric neurones was observed, one of the typical findings in achalasia.[13] The described reduction in NOS positive neurones thus most likely results from impaired expression of the enzyme due to transcriptional downregulation of neuronal NOS, as previously reported in the rat stomach in response to endotoxin.[21] One may argue that the incubation period of 16–18 hours is too short to induce neuronal death and the typical loss of neurones. Therefore, it would be interesting to search for early signs of neuronal cell death using apoptotic markers such as DNA

fragmentation or caspase-3 like activity, providing further evidence that the serum of achalasics indeed contains a ''neurotoxic'' substance. Alternatively, one may speculate that in the very early stage of achalasia, neuronal loss may possibly be preceded by the changes observed in the present study. Clearly, given the rather long delay of several years between the onset of symptoms and diagnosis, it may be impossible to gain insight into these very early changes.

Secondly, accepting that a circulating factor contributes to changes in chemical coding and eventually neuronal loss, why then are neurones mainly in the oesophagus and LOS affected? This typical presentation most certainly implies region specific mechanisms, making it less likely that circulating cytokines or neurotrophic factors are involved. Previous studies have suggested a role for specific autoantibodies to myenteric neurones[11 15 16] although the targeted antigens have not been identified and a causal relationship or data on the effect of these antibodies on neuronal survival/neurochemical coding and motor responses, as reported by Bruley des Varannes and colleagues,[20] are lacking. Furthermore, antineuronal antibodies have also been reported in the serum of GORD patients,[16] suggesting that these antibodies may simply result from tissue damage secondary to inflammation. Other investigators propose a role for neurotropic viruses, especially for viruses with a predilection for squamous epithelium.[8] This would provide a better explanation for the selective loss of neurones in the oesophagus. As the family of herpes viruses possess these properties, some investigators have focused on a possible role of such viruses in the pathogenesis of achalasia. Data from studies focusing on the presence of viral antibodies in the serum of patients or viral DNA in oesophageal tissue showed rather conflicting results.[14 17–19] A recent study used an original approach in evaluating the possible role of herpes simplex virus 1 (HSV-1).[18] Mononuclear cells were purified from oesophageal tissue obtained during Heller myotomy and were brought into contact with either inactivated HSV-1 or poliovirus. Circulating HSV-1 antibodies in patients with achalasia did not differ from controls. In contrast, mononuclear cells isolated from the LOS from achalasia patients showed a higher proliferative index in response to HSV-1 compared with controls. In line with this finding, challenge with HSV-1 antigen resulted in higher release of interferon γ, illustrating that HSV-1-reactive immune cells are present in LOS muscle, possibly contributing to neuronal damage. Additional experiments to date have further confirmed these data supporting a causal role for a subpopulation of cytotoxic lymphocytes activated by HSV-1 antigens or antigens on neurones similar to HSV-1 (Zaninotto and Castagliuolo, unpublished results, personal communication).

In conclusion, although achalasia was described as early as the 17th century, its pathogenesis still remains unclear and continues to be a challenge for scientists. More studies like those reported by Bruley des Varannes and colleagues[20] should eventually unravel this interesting motor disorder.

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