Endothelins, pseudo-obstruction and Hirschsprung’s disease

It has been known since the 1950s that the enteric nervous system is formed from cells that arise from the neural crest. The enteric neurones mainly arise from the vagal neural crest of the developing hind brain and colonise the gut in a rostro caudal migration but some seem to arrive in the hind gut from the lumbosacral level via a caudo rostral wave of colonisation. The neural crest cells that migrate and colonise the gut are committed to become neuroblasts or neuronal support cells, glioblasts; however, differentiation into neurones and glial cells seems not to take place until they have reached their final resting places in the gut. Movement through the gut mesenchyme, survival in the gut and differentiation into mature cells is strongly influenced by contacts with the microenvironment which consists of other cells in the mesenchyme, neural crest, and the extracellular matrix. The extracellular matrix components provide directional clues to migrating neural crest cells and together with neighbouring cells provide some of the signals for crest cell differentiation. For example, the appearance of neural crest cells in the gut is preceded by expression of extracellular matrix molecules and other factors such as glial derived neurotropic factor (GDNF) ensure survival of committed neuroblasts. Thus defects of the neural crest cells themselves or alteration of the micro-environment of the migratory pathway may result in defects of development of the enteric nervous system. In humans this disordered development results in the most commonly presenting forms of chronic idiopathic intestinal pseudo-obstruction, congenital enteric neuromuscular disease. In Hirschsprung’s disease’s defects in at least two different cell signalling systems, ret/GDNF and endothelin-3/endothelin B receptor, cause the aganglionosis.

The endothelin system’s important role in the development of the enteric nervous system has become apparent in the past four years or so when mice with targeted disruption of endothelin B receptor (ETR-B) and endothelin-3 (ET-3) were found to have congenital distal intestinal aganglionosis. The endothelins are a family of three peptides, endothelin-1, -2 and -3, coded for by distinct but related genes and act on cells via two G protein coupled receptors ETR-A and ETR-B. The endothelins are synthesised as much larger proproteins which are cleaved by an endothelin converting enzyme (ECE-1) to produce the active 21 amino acid peptide. Of the three endothelins it is ET-3 which is so important in the enteric nervous system and binding of ET-3 to ETR-B on vagal neural crest cells is required for colonisation of the hind gut.

Mutations of either ETR-B or ET-3 have been identified in several naturally occurring animal models of Hirschsprung’s disease, the piebald lethal mouse and the lethal spotted mouse respectively. The ovoar-ovale white foal also has a significant mutation of ETR-B with a single amino acid substitution in the first transmembrane spanning domain of the ETR-B gene. The lethal spotted mouse carries a mutation in its ET-3 gene which prevents proteolytic activation of the peptide. Mutation analysis of children with Hirschsprung’s disease has shown that about 10% carry mutations of either ETR-B, ET-3 or ECE-1. The effects of these genetic defects is to curtail neural crest migration in the distal colon and this is associated with localised overexpression of extracellular matrix molecules. Using transgenic lines of mice which are either ETR-B deficient or ET-3 deficient, Kapur and colleagues have shown that in ETR-B deficient mice enteric nervous system precursors can colonise the murine hind gut when they are surrounded by wild type enteric nervous system precursors. Further wild type enteric nervous system precursors will fail to colonise the hind gut when surrounded by ETR-B deficient ones. This strongly suggests that the enteric nervous system precursors signal ETR-B activation to those nearby and that when this signal is of sufficient intensity an ETR-B deficient crest cell can develop normally. It is thus clear that the interaction between the migrating neural crest cells and the mesenchymal environment of the hind gut is of critical importance in achieving normal innervation of the colon. The mechanism of the terminal aganglionosis that occurs either in the absence of ET-3 or ETR-B however remains unclear.

Despite the increasing understanding of the role of endothelins in the developing enteric nervous system, little work has been done in normal mice or men regarding the timetable of activity or the spatial orientation of these molecules in the developing embryonal gut. On page 246 of this issue Leibl et al describe the temporal and spatial expression of ET-3 and ETR-B in CD1 mouse embryos. They show clearly that ETR-B is confined to migrating neural crest cells and ET-3 to mesenchymal cells initially of the caecum but with a gradient extending rostrally into the small intestine and caudally into the proximal colon. Interestingly by 14 days postcoitum the ETR-B mRNA signal in the colon was stronger than in the more proximal part of the gut at this or earlier stages, perhaps, suggesting that ETR-B is expressed by both vagal and sacral neural crest cells.

The present results add to the growing body of work emphasising the importance of the gut mesenchyme in determining regional identity along the gut primordium and also in the regulation of region specific innervation of the gastrointestinal tract. The mechanisms that regulate expression of ET-3 and ETR-B genes are currently unknown. It is clear however that the rostro-caudal specification of the gastrointestinal tract is likely to involve a spatial, temporal and combinatorial patterns of expression of homeobox genes, the so called enteric hox code. In chick embryos there is clearly overlapping expression of the genes Hox A-9, -10 and -11 and we have recently produced some preliminary data demonstrating specific spatial, temporal and combinatorial expression patterns of hox genes A4, B4, D4, A5 and C5 in developing murine gut. The relation between caecum specific hox gene expression and ET3 and ETR-B is currently unknown but they are certainly candidate downstream molecules for these developmental control genes. A number of transgenic animal models provide evidence of the importance of homeobox genes in the control of morphogenesis of the gut and these include the “knock out” of ENX, causing increased innervation of the hind gut and over expression of hox A4, resulting in megacolon. Thus this family of genes and their
downstream targets are of importance within the genetic hierarchy of gut morphogenesis. Delineation of the genes comprising the enteric hox code, their downstream targets and their spatiotemporal patterns of expression is an essential and integral part of understanding the molecular events underlying the devastating diseases which cause pseudo-obstruction and Hirschsprung’s disease in humans. Such knowledge may enable antenatal diagnosis in some families and will be essential for the development of neuronal transplant strategies for the treatment of enteric neuropathic diseases.

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Back to the whale bone?

Most doctors with any practical experience of achalasia would be willing to admit that the disorder often provides considerable professional satisfaction. Firstly, it can be very satisfying to make the diagnosis. Far too often, patients will have suffered from gradually worsening dysphagia for many years and the diagnosis will have been missed at earlier consultations. The second moment of satisfaction can be enjoyed when the symptoms are relieved immediately after a result of successful dilatation using a whale bone.

Malfunction of the lower oesophageal sphincter (LOS) plays a key role in the genesis of dysphagia in achalasia, in which it usually maintains an abnormally high resting tone. This malfunction is the main reason for the relaxation defect of the lower oesophageal sphincter (LOS) pressure at rest and during swallowing. Such knowledge may enable antenatal diagnosis in some families and will be essential for the development of neuronal transplant strategies for the treatment of enteric neuropathic diseases.

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Malfunction of the lower oesophageal sphincter (LOS) plays a key role in the genesis of dysphagia in achalasia, in which it usually maintains an abnormally high resting tone. More importantly, however, the LOS does not relax sufficiently on swallowing, causing a persistent barrier to food boluses. In addition, the oesophageal body lacks normal propagation of contractions. Presently there are four therapeutic options in achalasia, all of which are directed at lowering the tone of the LOS.

In what is considered to be the first report on treatment of achalasia, Sir Thomas Willis in 1672 described the successful dilatation of the sphincter using a whale bone. Since then several types of dilating instruments have been used, but today endoscopically guided pneumatic dilatation with a low-compliant polyethylene balloon is preferred by most gastroenterologists. The occurrence of gastro-oesophageal reflux disease (GORD) after pneumatic dilatation is rare. Pneumatic dilatation with modern balloons is mainly reserved for short periods—for example, while the patient is on a waiting list for a more definitive procedure.

Since 1994, a fourth option has emerged, namely intrasphincteric injection of botulinum toxin type A, a toxin produced by Clostridium botulinum that inhibits acetylcholine release from nerve endings. This approach was shown to lead to short term symptom relief in up to 90% of patients. By six months, 20 (65%) of the 31 patients treated were still in remission. The endoscopic injection procedure is simple and no major complications have been reported as yet. A question that remained unanswered in the early studies with botulinum toxin concerns the duration of its effect in comparison with surgery and pneumatic dilatation.

In this issue (see page 231) Vaizei et al describe a randomised study in which they compared the immediate and long term efficacy of botulinum toxin with pneumatic dilatation. Their study is the first that formally compares these two treatment modalities in a prospective way. The most important result of the study, from a clinical point of view, is that botulinum toxin injection resulted in a significantly lower remission rate at 12 months than did pneumatic dilatation (32% compared with 70% of patients in complete remission). As an explanation for the apparent difference between the outcome of their study and those of some previous reports, the authors rightly highlight the fact that in previous studies of botulinum toxin in achalasia, repeat injections were given to patients who relapsed shortly after the initial injection. In an earlier study that compared botulinum toxin injection with pneumatic dilatation, only patients who did not respond to botulinum toxin treatment were pneumodilated, rendering the comparison unfair.

Vaizei and colleagues also examined the response of a number of objective parameters to treatment with botulinum toxin and pneumatic dilatation. Changes in diameter and length of the barium column at radiographical examination of the oesophagus paralleled changes in symptom scores. Somewhat surprisingly, however, botulinum toxin, in contrast to pneumatic dilatation, did not have a statistically significant effect on LOS pressure. Even at one month after botulinum injection no reduction in LOS pressure was found. This finding is in contrast with observations made in earlier studies. The possibility that
measurement of mid-expiratory rather than of end-
expiratory LOS pressure might have obscured an effect of
botulinum toxin is discarded in the discussion section of
the paper, although the authors do not provide us with the
measurements.

The results of the study by Vaezi and colleagues raise
more doubts on the clinical value of botulinum toxin treat-
ment in achalasia than hitherto expressed. It is important
to those actively involved in the treatment of patients with
achalasia to consider the results of Vaezi et al’s study care-
fully. As the aim of treatment in achalasia, a life-long
disease, is to reduce symptoms with a minimum number of
interventions during the patient’s lifetime, a new treatment
that has to be repeated frequently is likely to be less satis-
factory, both to the patient and to the doctor, than the
existing range of potential treatments.

As has been the case with many other new therapeutic
options for various other diseases, the initial enthusiasm for
botulinum toxin treatment in achalasia may have been too
great. It seems that the pendulum is swinging back again. It

Genes means pancreatitis

Identifying the molecular mechanisms responsible for
acute and chronic pancreatitis in humans is one of the most
difficult problems in modern science. Major obstacles
include the inaccessibility of the human pancreas to obser-
vation, the unpredictability of disease onset, the non-
specific nature of abdominal pain early in the course of
acute pancreatitis, an inability to biopsy the pancreas
safely, difficulty in distinguishing initiating events from the
concomitant inflammatory response, and the obvious
problems of investigating a tissue that self-destructs during
the disease process. Even fundamental questions as to
whether pancreatitis begins in the acinar cell or through
pathology related to the pancreatic ducts continue to be
debated. Animal models also fail to provide critical
insights, partly because of the artificial methods used to
induce pancreatitis. The discovery of the mutations in the cationic trypsino-
gen gene responsible for hereditary forms of pancreatitis in
American and European kindreds provided tremendous
insights into the mechanism of acute and chronic pancrea-
titis in these families. It was hypothesised that the cationic
tripsinogen R117H mutation eliminates a key hydrolysis
site on the chain connecting the two globular domains of
trypsin that is part of a fail-safe trypsin inactivation mech-
anism. Rather than being autolysed, prematurely activated
mutant trypsin remains active within the pancreas, activates
all other digestive enzymes, leads to acinar cell
autodigestion and, therefore, acute pancreatitis. The
second major insight was that the chronic pancreatitis
commonly seen in patients was associated with mutations in
tripsinogen. This observation suggests that recurrent
acute pancreatitis may lead to chronic pancreatitis.

Families with the cationic trypsinogen R117H and N21I
mutations have now been identified in Caucasians
throughout the United States and Europe.

In this issue, Nishimori et al (see page 259) report the
presence of the same two cationic trypsinogen gene muta-
tions in Japanese kindreds with hereditary pancreatitis as
seen in Caucasians. Additional polymorphisms in the cat-
tonic trypsinogen gene were also reported, but they either
fail to result in an amino acid substitution or segregate with

is highly unlikely, though, that it will swing back to the
whole bone approach!

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autoactivate. Humans may differ from experimental animals in that acute pancreatitis in animals may require lysosomal hydrolases, such as cathepsin B, to activate trypsinogen. Thus, in experimental animals, conditions must be met that allow trypsinogen and cathepsin B to co-localise, whereas in humans trypsinogen activation may occur in a variety of locations under relatively milder conditions. However, the conditions that initiate excessive trypsinogen activation and pancreatitis in hereditary and non-hereditary pancreatitis require further investigation.

A final important finding in Nishimori et al’s report was that four of the six families with hereditary pancreatitis did not have mutations in the cationic trypsinogen genes. This observation suggests that at least one additional gene mutation is associated with hereditary pancreatitis. Discovery of this new gene may provide further insights into the mechanisms of acute and chronic pancreatitis.

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withdrawal of cereal proteins from the diet is difficult to reconcile with this concept.

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