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Hirschsprung's ENS transplantation

Transplanting the enteric nervous system: a step closer to treatment for aganglionosis

Michael D Gershon

Autologous transplantation can be used to treat Hirschsprung's disease by implantation and proliferation of the crest-derived stem cells in vitro

One might think that Hirschsprung's disease (congenital megacolon) should be passé as a medical problem. After all, many genes, including *RET*, *GDNF*, *NRTN*, *EDNRB*, *EDN3*, *ECE1*, *PHOX2b*, *SOX10*, *PAX3* and *SMADIP1* (SIP1, ZBFX1B),[1–7] have successfully been linked to its pathogenesis. Knowledge of the actions and interactions of these genes and their products has enabled the processes by which the bowel is colonised to be, if not completely understood, at least comprehended in general terms.[3 8–10] Effective treatment for Hirschsprung's disease, moreover, exists in the surgical removal of the aganglionic segment of bowel.[11–13] Unfortunately, the medical problems posed by Hirschsprung's disease continue despite the lengthy list of genes implicated in its generation and the progress that has recently been made in understanding enteric nervous system (ENS) development. Unresolved medical problems continue because advances made in comprehending genes and pathogenesis have not been translated into new and improved methods of treatment; moreover, although surgical techniques are evolving and associated morbidity is decreasing,[12 13] the surgical treatment of Hirschsprung's disease essentially converts an otherwise lethal defect into a chronic condition with which many, if not most, patients must learn to cope.[14 15]

Hirschsprung's disease occurs when a variable length of terminal bowel is congenitally aganglionic. Because the reflexes and behaviours mediated by the ganglionated plexuses of the ENS are essential for propulsive motility and normal secretion,[16] aganglionosis results in a pseudoobstruction that, if left untreated, is incompatible with life. Reliable modern statistics on untreated Hirschsprung's disease are not available because failure to treat it is immoral; however, aganglionosis is lethal to animals with genetic defects that model the condition.[17–23] Because the aganglionic region of the bowel lacks the inhibitory neurotransmitter nitric oxide some authors have speculated that the aganglionic zone goes into spasm and narrows to become obstructive[24]; however, it is more likely that motor patterns simply fail to propel luminal contents through the aganglionic zone so that the ganglionated bowel proximal to the aganglionic segment dilates. Removal of the aganglionic portion of the gut is thus obviously necessary in the treatment of Hirschsprung's disease, but in many patients it is not sufficient.

The greatest problems faced by patients after the definitive surgical correction of the aganglionosis of Hirschsprung's disease include faecal soiling,[15] constipation and postoperative enterocolitis.[25] Studies vary in the reported incidence of these complications, and the type of surgery used to carry out the repair undoubtedly matters; however, soiling has been reported in as many as 76% of patients.[15] A transanal one-stage pull-through operation may be advantageous for rectosigmoid aganglionosis[13] even though it carries a high risk of postoperative enterocolitis because a single surgical procedure is preferable to two[12 25]; however, a modified Duhamel procedure has been advocated as superior to any other for total colonic aganglionosis.[26] Whatever procedure is used, faecal soiling is a serious risk, which over the long term causes surprisingly less psychiatric morbidity than would be expected, given the social stigma attached to that particular defect; nevertheless, faecal soiling gives rise to a great deal of concern in families and is highly distressing to patients.[15] The outcomes of treatment are worse for patients with total colonic aganglionosis than for those with short-segment disease, and patients with total colonic aganglionosis tend to perceive themselves as less well adjusted than their matched pairs with a shorter aganglionic region of gut.[27] Additional surgeries, including posterior myotomy/myectomy, can be undertaken to lessen the effect of persistent soiling, but these procedures are not invariably successful.[28] Clearly, no treatment that carries a high risk of faecal soiling can be considered to be perfect, and one has to examine what we know about the pathogenesis of Hirschsprung's disease and the development of the ENS to determine whether anything can be done that is better than what is now being done to treat Hirschsprung's disease.

The gut is colonised by precursors that migrate to it from the neural crest. The

premigratory crest is a heterogeneous structure, in that it seems to contain both pluripotent and fate-restricted precursor cells.[29–34] The population of postmigratory cells that colonises the gut is multipotent when it arrives in the bowel,[35–37] although individual cells within this population may already be committed to a single fate. The observation that a subset of the serotonergic neurones of the mouse bowel are born as early as embryonic day 8.5, which precedes the migration of any crest-derived cells into the gut, strongly suggests that some cells of the colonising population of crest-derived cells are committed and even postmitotic when they enter the bowel.[38] Despite the fact that the crest-derived cell population that colonises the gut contains members that are postmitotic, the group also contains cells that are self-renewing, multipotent stem cells. In fact, it is of particular interest that stem cells are still present in the postnatal bowel.[39–41] Both hope and logic suggest that these neural crest-derived stem cells may ultimately provide a solution to the unsatisfactory current status of treatment for Hirschsprung's disease.

Major problems must be overcome before enteric neural crest-derived stem cells can be successfully used to treat Hirschsprung's disease, and at least some of these problems have successfully been dealt by Almond *et al*[42] (***see page 489***). First, the crest-derived stem cells have to be isolated and then expanded to obtain enough cells for autologous transplantation, which would be the goal. Once instilled into the bowel wall, moreover, these cells would have to migrate to the correct destinations and form appropriate connections with one another so that they can reconstitute the reflexes and integrative neural activity of the normal ENS. It is obviously not enough just to get neurones to form, or even to migrate to correct destinations in the bowel wall; neurones must be functional and in sufficiently good control of effectors so that reflexes are rescued and the pseudo-obstruction of Hirschsprung's disease can be corrected. Almond *et al*[42] have adapted the technique of producing neurospheres from single-cell suspensions to obtain enriched populations of crest-derived stem cells[43–45] and they have been able to expand the size of the original population by maintaining the proliferation of stem cells in vitro. The investigators, furthermore, succeeded in implanting crest-derived stem cells into an aganglionic mouse gut and, after doing so, they observed that the cells fortunately migrate along pathways that are appropriate for cells derived from the neural crest. Strikingly, the implanted crest-derived stem cells differentiate within the aganglionic zone to give rise to end-stage cells that express phenotypic markers identifying them as enteric glia and neurones. The neurones, furthermore, express some of the molecules that characterise the chemical code used for identifying enteric neurones,[46] such as vasoactive intestinal peptide and nitric oxide synthase.

This report is undoubtedly a major step forward, which, for the first time, implies that autologous transplantation of neural crest-derived enteric stem cells is realistic as a prospective treatment for the aganglionosis of Hirschsprung's disease. Of course, one must be cognizant, as are Almond *et al*,[42] of the enormity of the remaining problems that must still be overcome before autologous transplantation replaces pull-through operations as routine treatment for Hirschsprung's disease. Although Almond *et al* demonstrate that some of the correct markers are expressed by the neurones that develop from grafts of neural crest-derived stem cells, they have not shown that the full chemical code[46] is acquired. The minimum number of neurotransmitters and neuromodulators necessary for function is unknown, because it is unclear as to whether or not a serviceable ENS might be formed if some of the elements of the normal chemical code failed to develop. More importantly, Almond *et al* were not yet able to determine whether synaptic connections developed between enteric neurones, and between these neurones and their effectors. In the absence of that information, one can only speculate about whether the newly formed neurones fashion themselves into the complex microcircuits responsible for regulating propulsive and secretory activity. The bottom line restoration of function and clearing of the pseudo-obstruction, furthermore, are still to be demonstrated. There are, however, always many hurdles in the path that leads from scientific discoveries to successful treatment. Although Almond *et al* have not cleared all of them in showing that autologous transplantation can be used to treat Hirschsprung's disease, they have leaped across some daunting hurdles and have thus started the race to the cure.

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Correspondence to: Professor M D Gershon, Department of Pathology and Cell Biology, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA; mdg4@Columbia.edu

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Apoptosis in Crohn's disease

In vivo single-photon emission computed tomography imaging of apoptosis in Crohn's disease and anti-tumour necrosis factor therapy

Alastair J M Watson

It has long been known that apoptosis of T cells is an important mechanism for terminating inflammatory reactions.[1] It was proposed over 10 years ago that defective apoptosis could play a role in the pathogenesis of inflammatory bowel disease.[2] There is now substantial experimental and clinical evidence supporting this hypothesis.[3]

In Crohn's disease there is an expansion in CD4 T-cell populations mediated by tumour necrosis factor (TNF) and γ-interferon (IFN-γ), which activate macrophages to release interleukin-6, interleukin-12 and TNF.[4 5] These cytokines act to perpetuate the inflammatory reaction by reducing the susceptibility of T cells to die by apoptosis.[3] In the uninflammed state, lamina propria T cells have a higher susceptibility to apoptosis than unstimulated T cells from the peripheral blood because of high expression of the apoptosis-inducing receptor Fas.[6] In contrast to this, lamina propria T cells from patients with Crohn's disease are resistant to apoptotic stimuli.[7] These observations suggest that apoptosis limits the number of CD4 T cells in healthy individuals whereas in Crohn's disease expansion of T-cell populations can occur without the restriction of apoptosis.

This resistance to induction of apoptosis is mediated by interleukin-12, the interleukin-6 receptor and TNF. Interleukin-12 is one of the most important cytokines in Crohn's disease promoting Th_1 T-cell differentiation. It also renders T cells resistant to Fas-induced apoptosis, possibly through inhibition of caspase 3 and 9, thereby prolonging T-cell survival.[8] Early clinical studies have shown that antibodies that block the action of interleukin-12 reduce the severity of Crohn's disease.[9] Such antibodies also increase apoptosis in lamina propria T cells and reduce the severity of trinitrobenzene sulphonic acid experimental colitis.[5]

Interleukin-6 secreted by lamina propria macrophages and T cells also promotes the survival of T cells by inhibiting apoptosis. Complexes of interleukin-6/interleukin-6 receptor activate lamina propria T cells expressing the cytokine receptor gp130 on their surface. This activates a signal transduction pathway involving the phosphorylation, by JAK kinases, of the transcription factor STAT3. STAT3 increases the expression of the anti-apoptotic protein Bcl-x_L thereby increasing the resistance of T cells to apoptosis.[4]

Perhaps the most compelling evidence for the importance of apoptosis in Crohn's disease has come from analysis of the mechanism of action of anti-TNF therapy. Though complex, a full understanding of the biology of TNF is essential for an appreciation of its role in the treatment of Crohn's disease. TNF is a cytokine that has many proinflammatory effects. A precursor form called transmembrane TNF-α (mTNF) is expressed on the surface of activated lymphocytes and macrophages. The extracellular 157 amino acids can be cleaved off mTNF and secreted. Both the secreted and transmembrane forms can induce apoptosis. Secreted TNF can bind either of the two TNF receptors, TNF-R1 (p55) or TNF-RII (p75), and activate the extrinsic apoptosis pathway through caspase 8.[10] The transmembrane form can also activate the extrinsic apoptosis pathway by binding to TNF-RII.[11]

However, a third mechanism of inducing apoptosis may be the most relevant for the treatment of Crohn's disease with