

Is Developmental Ganglion Cell Loss in Hirschsprung Disorders Inherently Linked to Myofiber Hypercontractility?

M. Lawrence, Agius

Department of Pathology, St Luke's Hospital, Gwardamangia, Medical School,
University of Malta, Msida, Malta, Europe

Abstract: Systems of participation in the development of a state of essential aganglionosis of Hirschsprung's type might primarily relate to a segmental complex of maldevelopmental processes ranging from bowel wall musculature to related vasculature to systems of asynchronized patterns of innervation and of disturbed trophic influence. The distal spastic segment of aganglionic bowel would be associated with the immediately proximal dilated bowel segment in terms particularly of failed continuity of the neural levels of innervating networks that necessarily impair even transformation of muscle wall tone and segmenting contractions to propulsive peristalsis of such bowel segments. It is in terms of such a primary segmentation of the disease process in Hirschsprung's that one might consider different schemes of potential developmental progression resulting in aganglionosis of the bowel. Even with regard, however, to a failed innervation pathway of bowel segments in Hirschsprung's, one would include various subsequent steps in the development of a segmental bowel spasticity that constitutes both dysfunctional and mechanistic bases for intestinal obstruction. Indeed, a central concept of selective vulnerability of intramural ganglion cells of bowel segments would account for a developmental series of multiple participants as a failed recruitment of and progressive loss of ganglion cell subpopulations in the bowel wall beyond simple contractility or spasticity effects. In terms of potential establishment of such segmental aganglionosis of the bowel wall, sources of failed viability of such ganglion cells might ultimately relate more to actual loss of actively interacting ganglion cells with bowel wall musculature and with vasculature smooth myofibers.

Key words: Myofiber hypercontractility, ganglion cell, loss hirschsprung

INTRODUCTION

An essential transformation of enteric musculature dependence on innervation supply in hirschsprung's disease: Hirschsprung's disease is a polygenic disorder with nonMendelian inheritance patterns and generally incomplete penetrance of gene expression indicative probably of modifier loci^[1] including, for example, those coding for vasoactive intestinal peptide receptivity^[2]. Although often attributed to a failed migration of neural crest cells, this has not been proved^[3]. Particularly interesting is the participation in a strictly regional fashion of glial cell line-derived neurotrophic factor (GDNF) in the development of such a complex neural network as the enteric nervous system. In this regard, also, expression of ErbB3 by colonic epithelium is necessary for postnatal maintenance of enteric neurons and glia^[4]. In addition, neuropeptides such as Neu N^[5] probably constitute significant parameters of this disease^[6]. This phenomenon appears to constitute a highly effective inducing process whereby the whole gastrointestinal tract becomes subsequently innervated after development of the tract as an essentially empty tube^[7].

Explant culture experiments have shown basic phenomena of cellular differentiation and morphogenesis towards such development of the enteric mammalian nervous system. In addition, for example, LICAM, a gene that encodes a neural cell adhesion molecule, might also constitute a modifier locus^[8]. In addition, germline mutation of the RET proto-oncogene might participate in the development of Hirschsprung's disease^[9]. The Ret protein receptor appears crucial to development and maturation of the enteric nervous system. High Ret protein expression and low tyrosine kinase activity are in fact typical of the immature enteric nervous system during early development^[10].

It is the glia and the trophic effects such glia exert on development of the enteric nervous system that would characterize functional roles of such glial networks in both the peripheral and central nervous system. In this regard, SOX 10, a transcription factor, when absent, would appear to induce reduction of the enteric progenitor cells and result in bowel aganglionosis^[11]. Vagal neural crest-derived cells capable of acting as stem cells might be distributed throughout the developing nervous system^[12].

It is interesting in this regard that cells of total colonic agangliosis may relate to either apparent maturity or immaturity of colonic function on barium studies after infancy^[13].

The glia do not appear, in fact, simply to provide essential trophic support but to organize systems of cellular differentiation and morphogenesis of the enteric nervous system related to a pre-existing empty intestinal muscular tube. This in itself would imply an intestinal muscular system that developmentally originates outside neural influence.

A fundamental aspect of innervation of gastrointestinal musculature would appear to concern especially dynamics of relaxation in terms of relief or prevention of a maintained spastic state as seen in Hirschsprung bowel segments. Acquired agangliosis may, rarely, develop following pullthrough procedures for Hirschsprung's disease, due perhaps to vascular compromise of the distal bowel segment^[14].

Effective peristalsis of the enteric tract would perhaps require a coordinated series of contractions and relaxations of the gastrointestinal tract presumably requiring relaxation of the immediately distal segments of the enteric wall. Essential coupling of contraction of the circular and longitudinal enteric musculature involving a relaxation of the immediately distal bowel segment would be primarily targeted in Hirschsprung's disease.

Functionally, there is loss of enteric neuromuscular inhibition. In fact, aganglionic distal colon reveal disrupted ganglia and increased nerve fibers, with selective preservation of nitric oxide synthesizing neurons^[15].

Developmental biology of the enteric nervous system would perhaps serve as an essential ineffectiveness of relaxation of an immediately distal segment of bowel in Hirschsprung's disease that is linked to failure of cellular differentiation and morphogenesis of the enteric nervous system. The role of loss of function mutations^[16] affecting the RET (glial cell line-derived neurotrophic factor receptor)^[17] signal transduction pathway in the developing mammalian enteric nervous system in Hirschsprung's disease would fail primarily as a result of an intrinsic defect in the enteric wall musculature

Glial involvement in such failed trophic stimulation and support of the developing enteric nervous system might be implicated in Hirschsprung's disease. In particular, specific interactions between mutations of RET and EDNRB pathways would constitute an underlying mechanism for the development of the complete Hirschsprung disease^[18].

EDN3/EDNRB signals are not required prior to arrival of crest cells in the gut. EDNRB stimulation elicits distinct cellular responses from RET or RAS activation^[19].

Musculature would serve as a primary source of trophic stimulation evolving in terms of established innervation. Such trophic influence appears not fully dependent on subsequent evolving contractility of the musculature. Morphogenic defects in innervation of bowel segments as seen in Hirschsprung's would perhaps arise primarily as expressed consequences of segmentally failed trophic influence of developing bowel wall musculature.

Essential dyssynchrony in neurotransmitter release patterns underlying vascular spasm and hirschsprung's diseases:

The therapeutic role of injected Botulinum neurotoxin A in conditions ranging from detrusor sphincter dyssynergia to achalasia and focal hyperhidrosis would perhaps incorporate an important modulatory effect.

As such, cases of dystonia and of focal spasticity might serve as main therapeutic effects exerted by Botulinum neurotoxin that is related to a reduction rather than cessation of trans-synaptic neurotransmission.

Any disorder characterized particularly by an asynchrony of axonal impulses would implicate also asynchrony of neurotransmitter release or receptivity in such systems with potentially beneficial toxin effects.

Lowering the firing level of neurons with consequent lowering of synaptic neurotransmitter release might actually serve towards correction of such asynchrony.

Muscle spasticity would perhaps arise as a result of asynchronous neuronal firing in fact, skeletal muscle spasticity, occurring as a component of a wide range of primary and secondary skeletal muscle disorders, would constitute an important manifestation of asynchronous release of neurotransmitters (acetylcholine) at the neuromuscular junction. Also, vascular spasm would reflect asynchronous stimulation of vascular smooth myofibers.

Severe and sustained vascular spasm would perhaps evolve as a function of primarily asynchronous systems of myofiber stimulation.

Vascular smooth myofibers might respond to stimulation by neurotransmitter and chemical agonists in terms specifically of a smooth myofiber contractility that evolves as a possible progression, precipitation and maintenance of abnormally sustained vascular spasm.

Abnormally sustained severe vasospasm would be analogous to esophageal achalasia, infantile pyloric stenosis and urinary bladder outlet dysfunction; abnormal intestinal peristalsis might arise as systems of asynchrony in the established progression of Hirschsprung's disease.

The commonly encountered spastic state affecting distal aganglionic segments of bowel in cases of typical

Hirschsprung's disease might constitute asynchrony rather than complete lack of neurotransmitter release in bowel wall.

Beyond a concept of failure of INNERVATION in the pathogenesis of hirschsprung's disease: Pathogenesis of Hirschsprung's disease would relate to processes responsible for innervation of the developing gastrointestinal tract^[20], particularly in terms of RET receptor tyrosine kinase and endothelin receptor type B EDNRB signaling pathways, amongst other developmental signals in enteric development^[21-23].

How is it that bowel segments participate in the process of their own intrinsic innervation? The bowel would fully participate as processes of involvement implicating neuronal and glial migration, as well as actual development and maintenance of the enteric nervous system itself that concurrently evolve in a context of trophic intestinal musculature influences.

Lack of bowel innervation of bowel segments in Hirschsprung's disease might effectively account for a process more akin to developmental loss of ganglion cells. In addition, there would also develop an abnormal distribution and number of enteric neurons and of interstitial cells of Cajal, in addition to abnormal enteric neurotransmitter expression, as seen also in lethal spotted mice^[24].

Hence, the aganglionosis in Hirschsprung's disease would incorporate not simply an absence of segments of the enteric nervous system, but a defective participation towards the creation of a distinctly pathologic malformation, the megacolon.

A rare cause of Hirschsprung's disease appears to involve a primary disorder of muscle tissue of the large bowel or else denervation atrophy with degeneration of unmyelinated axons^[25].

Even the surgical excision of the aganglionic segment of bowel in Hirschsprung's disease would not fully constitute definitive therapy for a condition that disrupts not only the innervation of the bowel segments but also disturbs innervation patterns that developmentally influence bowel segment formation in the first instance. Innervation of the cholinergic nerves and a loss of nitric oxide mediation of nonadrenergic noncholinergic inhibitory nerves appear to play a significant role in impaired motility of the internal anal sphincter in Hirschsprung's disease^[26]. Hirschsprung's disease would implicate not only a neural set of disturbances but also constitute basic developmental abnormalities of the bowel musculature. In terms of such primary sets of developmental bowel wall abnormalities that arise within the developing bowel mesenchyme and musculature and

subsequently invading neural-crest derived cells^[11], Hirschsprung's disease is primarily segmental. Multiple components in development of the bowel wall would include also vascular myofibers. Mutations or deletions of ZFHXIB (SIPI) would involve a syndromic form of Hirschsprung's disease associated with microcephaly and dystrophic facial features^[27].

Congenital inner ear anomalies associated with colonic aganglionosis would support a role for hereditary defects in neural crest cells in both Waardenburg's syndrome and Hirschsprung's disease^[28].

An essential process of aganglionosis would relate to abnormalities in migration of neural crest-derived cells as a process distinct from bowel segment development. Patients with clinical Hirschsprung's disease would include not only absence of ganglion cells, but also patchy zonal loss, abnormal neurons or neuronal dysplasia. Idiopathic megacolon in childhood may also prove heterogeneous etiopathogenically^[29].

Even a basic operative scheme of growth factors and of neural cell elements invading the developing bowel wall might not fully account for a phenomenon of developmental disorganization affecting both enteric musculature and neural supply with disturbed inter-relationships between these two bowel wall components. Both enteric musculature and enteric innervation would implicate mechanisms of pathogenesis to account for Hirschsprung's disease as primarily a segmental disorder.

Even the proximally dilated bowel that appears normally innervated would constitute perhaps an integral part of the Hirschsprung's disease process arising as a consequence of a bowel pseudo-obstruction that progresses as asynchronous pathways of aberrant developmental morphogenesis.

Hirschsprung's disease reflects loss of viability of enteric ganglion cells independent of abnormal embryonic development: A disturbance in innervation of the smooth muscle of the gastrointestinal tract, particularly of its distal portion, would prove central to pathogenesis of Hirschsprung's disease in terms of a defect in relaxation of the enteric muscle rather than in terms of just a loss of muscle contractility.

In Hirschsprung's disease, essential aganglionosis with hypertrophy of axonal neuronal fibers in the tightly spastic distally involved segment of the bowel would promote pseudo-obstruction involving spastic states inducing mechanical bowel obstruction.

Hirschsprung's disease would involve mechanical bowel obstruction due to abnormalities of developmental systems influencing musculature, vascular and neural bowel components. Indeed, abnormal prenatal

development of neural crest-derived stem cells appears central to abnormal enteric innervation in Hirschsprung's disease^[12].

On the other hand, however, Hirschsprung's disease might evolve in some cases at least as an essential failure of survival of ganglion cells rather than as failure of migration of such ganglion cells.

The distal bowel generally would be normally populated by ganglion cells derived from the sacral neural crest rather than from the vagally derived neural crest migratory precursors.

Moreover, loss of smooth muscle relaxation might lead directly to the creation of truly mechanical types of intestinal obstruction at the site of such induced aganglionosis.

Such interface between spastically contracted smooth myofibers of the distal enteric wall and of the developing ganglion cell network would perhaps become disrupted as a result of permanent loss of whole ganglion cell subsets. Ganglion cell loss would constitute a smooth muscle dysfunction based on a disturbed coupling of spasticity and relaxation cycles of activity of the distal enteric tract that subsequently impairs developmental viability of the ganglion cells.

Loss of viability of ganglion cells in the distal enteric tract would be associated with mutations of glially derived neurotrophic factor, its preferred receptor GFR α 1 or its signaling component, RET, in certain cases of Hirschsprung's disease. This would be relevant in terms particularly of a linking up of sacral and vagally derived migratory ganglionic systems of innervation of the developing bowel musculature and vasculature.

The innervation process of enteric smooth muscle would ensure viability of ganglion cells that are participating in such innervation and which are central to the pathogenesis of the aganglionosis and also to a lack of relaxation of the distal enteric wall in Hirschsprung's disease.

Failed innervation of smooth muscle would involve failed relaxation as a dysfunction of developmental systems affecting vascular and bowel wall musculature and also neural migratory dynamics. These appear inter-related as essentially viable or nonviable participants of an enteric developmental progression. There is evidence of a functional defect of the vascular endothelin B receptor in patients with Hirschsprung's disease^[30].

Intrinsic and extrinsic neural supply and intraluminal contents of bowel segments in segmenting versus peristaltic wave contraction: The intrinsic contractility of the bowel muscle wall and particularly the transformation of such contractility to peristaltic bowel contractions would be central to the establishment of gastrointestinal

motility. This would determine, in turn, subsequent morphogenesis of various bowel wall components particularly on a segmental basis.

A pacemaker functionality involving the alimentary tract would account for slow contractile activity of the bowel wall when its lumen is empty. Indeed, the bowel wall musculature possesses both intrinsic muscle tone and also intrinsic contractility.

Muscle wall tone in some way would progress to a state of contractility that implicates a primarily segmental progression of peristaltic waves of contraction.

Motility of the gastrointestinal tract would appear based not only on pacemaker rhythmic discharge from the interstitial cells of Cajal but also on contractile functionality of musculature implicating a coordination of enteric smooth muscle layers. Cytoskeletal proteins would tend to be either absent or markedly reduced in small myofibers of the spastic aganglionic bowel segment in Hirschsprung's disease^[31].

Some forms of transformation would affect intraluminal bowel contents that convert segmenting contractions to peristaltic activity. Indeed, enteric muscle tone musculature would increase in terms particularly of an initially increasing contractility affecting the inner circular muscle layer.

Contractility of the bowel wall would involve a squeezing action on luminal contents that is coordinated with more forceful contractions that transform segmental to propulsive peristaltic contracting activity.

A threshold value might separate physiologically slow segmental contractions of the bowel musculature from a propulsive peristaltic wave. Generation of peristalsis would be analogous to action potential generation in neuronal firing.

Bowel obstruction in Hirschsprung's disease would involve complex pathologic interactions between myogenic and neurogenic influences that go beyond simple pathogenetic considerations of aperistaltic obstruction in this disorder^[32].

Segmenting contractions as a phenomenon restricted to only a specific bowel segment would primarily depend on an integrally continuous tube of smooth circular and longitudinal muscle from stomach to colon.

Indeed, intrinsic neural networks within the bowel wall and also an operative extra-intestinal neural connecting network, would contribute towards defining actual threshold values at which bowel segmenting contractions would transform to propulsive peristaltic waves. Any retrograde movement of luminal contents would perhaps be inhibited by intrinsic muscle tone and also by an initially stronger wave of contractions extending distally.

A persistent state of peristaltic contraction at a more distal adjacent site of the bowel wall would adapt to dynamics of a spasticity process that is prone to further sustained persistence in Hirschsprung bowel segments.

In the endothelin B(-/-) receptor null rat, increased contractility of smooth muscle as well as increased thickness of the intestinal muscular wall would contribute to the bowel obstruction in a manner similar to that associated with aganglionosis of Hirschsprung's disease^[33].

Hence, an initial site of stronger peristaltic contraction would tend to limit the degree of retrograde movement of luminal contents in conjunction with a weaker peristaltic contractility affecting the immediately distal bowel wall segment.

Predominantly inhibitory control exerted by tonically contracted myofibers in motility of gastrointestinal contents: Control, modulation and effective induction of motility of the gastrointestinal tract would implicate physiologic relationships between the smooth muscle cell and the interstitial cell of Cajal within one overall scheme.

Interstitial cells of Cajal would give rise to either myenteric or muscular interstitial cells of Cajal under the influence of the kit ligand that is provided both by neuronal and non-neuronal cells. Dysmotility patterns of bowel might themselves alter distribution of such interstitial cells of Cajal in Hirschsprung's disease^[34].

A considerable variety of receptor types (particularly G-protein coupled receptors) and the different types of G protein-mediated second messenger pathways occur on smooth muscle fibers of the gastrointestinal tract. The receptors that transmit messages via different second signaling pathways would implicate largely independent pathways of control of myofiber contractility. The presence of the interstitial cell of Cajal, particularly in the deeper layers of the tunica muscularis of the gastrointestinal wall, might constitute a mechanism that coordinates, modulates and in general determines effective patterns of control of contractility response on the part of the enteric smooth myofiber.

The interstitial cell of Cajal would determine voltage phenomena in terms of regulators of Ca^{+2} , K^{+} , Na^{+} ion channels that are in turn responsive for both activated Ca^{+2} channels and for Ca^{+2} sparks that are induced from intracellular Ca^{+2} stores.

Ca^{+2} levels within the cell, both as direct effects on the contractile apparatus of the smooth muscle fiber and also indirectly in activating, through changes of transmembrane voltage, the opening of Na^{+} and K^{+} ion channels, would target the interstitial cell of Cajal.

A positive feedback mechanism would predominantly determine the functional control of Ca^{+2} input and release from the smooth myofiber that would depend on an integration of ionic current membrane phenomena centered on such Ca^{+2} controlled voltage changes.

Inhibitory effects exerted by the interstitial cell of Cajal on the smooth myofiber, together with the general inhibitory roles of cyclic AMP, ATP, VIP (vasoactive intestinal peptide) and PACAP (pituitary adenylate cyclase-activating peptide) would all primarily tend to inhibit stimulation by acetylcholine and neurokinin. This aspect regarding motility of the gastrointestinal tract would relate to a fundamental change in physiologic control affecting enteric smooth myofiber contractility.

Perhaps much of the effector mechanisms controlling enteric muscle contractility would induce attenuation of a persistent state of acetylcholine stimulation of the enteric myofiber.

In addition, it would appear that SK3-immunoreactive interstitial fibroblast-like cells, that are distinct from the Kit-immunoreactive interstitial cells of Cajal, would control excitability of enteric musculature^[35].

Effective motility of the gastrointestinal tract might largely be dependent on a controlled modulation of a basal state of stimulated contractility of gastrointestinal myofibers. All the inhibitory mechanisms including the interstitial cell of Cajal as a coordinator for such inhibition, would constitute a mechanism of control of both contractility and of motility of the gastrointestinal tract.

The wide diversity of G-protein coupled receptors, their multiple inhibitory mediators and phosphorylation of the intracellular domains of such receptors would tend to mediate inhibitory effects; all would exert a predominant inhibitory influence as modulated control of gastrointestinal motility.

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