Role of Neural Crest in Congenital Heart Disease

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Historical Background

In the preface for his monograph on neural crest in 1950, Horstadius\(^1\) wrote, "Most text-books of embryology or anatomy make only passing mention of the neural crest as the source of origin of one or two structures in the vertebrate body. Few books devote even as much as a page to this interesting structure." Although the passage of 40 years has seen a revolution in our understanding of the neural crest in basic and clinical sciences, Horstadius' statement is still applicable to the paucity of information about neural crest in textbooks. Unfortunately, among scientists and clinicians not directly involved in neural crest research, this has led to almost profound ignorance about the neural crest and its role in development.

The neural crest is an unusual structure that appears early in development and has only a transient existence because the cells of the neural crest quickly migrate throughout the body and differentiate into other cell types. Its transience and widespread migration have made neural crest derivatives a technical challenge to study. Because of these difficulties, knowledge of neural crest derivatives progressed somewhat haltingly through the last half of the 19th century and the first half of the 20th century, dependent on development of novel techniques with which to study this labile population of cells. In the past 40 years, with the availability of tissue culture, autoradiography, interspecies transplantation, immunohistochemistry, and in situ hybridization, we have begun to understand much more about neural crest derivatives.\(^2\)

The neural crest has been recognized primarily for its contribution to the peripheral nervous system, although the largest portion of Horstadius'\(^5\) 1950 monograph\(^1\) is devoted to non-neural derivatives of the neural crest in the vertebrate head and branchial region. In 1888, Kastschenko first claimed that some mesenchyme of the head originated from the neural crest.\(^1\) Platt (1893–1897) showed that cartilage of the visceral arches and dentine of the teeth were derived from neural crest ectoderm.\(^1\) She proposed the term "mesectoderm" for mesenchyme that originates from the ectodermal rather than the mesodermal germ layer. This term has been revised to "ectomesenchyme" to emphasize the origin of the mesenchyme from ectoderm rather than its usual source.\(^1\) Under normal circumstances, we know of no source of ectomesenchyme other than the neural crest; however, as will be discussed below, when the neural crest is removed, other areas of the ectoderm might have the capability of producing ectomesenchyme.

The importance of neural crest in the cardiovascular system was first documented by Drs. Christine Le Lièvre and Nicole Le Douarin in 1975.\(^3,4\) These investigators transplanted quail neural crest into chick embryos and discovered that the entire musculoconnective tissue wall of the large arteries arising from the heart in the chick was made of ectomesenchymal cells of quail (neural crest) origin. They also noted that "the transition zone between the bulbus arteriosus and the aortic trunks...contains a mixture of quail and host (neural crest and non-neural crest) cells." Rychters\(^5\) and Thompson and Fitzharris\(^6\) also presaged the discovery that neural crest is involved in development of the cardiac outflow septation. Rychters\(^5\) noted that pigment placed between the third and fourth pharyngeal arches migrated into the base of the aorta and pulmonary trunk in the aorticopulmonary septum. Thompson and Fitzharris\(^6\) showed the dual origin of mesenchymal cells in the developing outflow tract. One population of cells was derived from the endocardium, and a second population migrated into the outflow tract from the pharyngeal region.

The relation between neural crest and outflow tract septation became clear when various parts of the cranial premigratory neural crest were ablated in chick embryos.\(^7\) This proved to be the critical step in understanding the significance of the presence of these cells in the outflow tract, in that removal of the neural crest resulted in outflow tract malformations.\(^7\) This relation has been explored more thoroughly in the last 7 years, and the findings will be reviewed below. Practically all of the work on neural crest has been performed on the chick embryo for three reasons: The embryo is accessible for surgery early in development; it has a short developmental period of 21 days; and interspecies transplantation can be

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FIGURE 1. Diagrammatic representation of the neural crest highlighting the region of the cardiac neural crest. Division between trunk and cranial neural crest is at somite 5. Cranial neural crest has ectomesenchymal cells, whereas trunk neural crest has few of these cells. The cardiac area is located between the midotic placode and somite 3. This area of neural crest migrates into pharyngeal arches 3, 4, and 6 as designated.

performed from the Japanese quail embryo, which has a distinctive nuclear marker that makes it easy to trace after transplantation.

Cardiac Neural Crest

Normal Distribution of Neural Crest Cells in the Heart

The neural crest is divided into two major regions known as cranial and trunk neural crest (Figure 1). The cranial neural crest extends from the mid-diencephalon to the caudal limit of somite 5, whereas the trunk neural crest proceeds from somite 5 to the caudal extent of the embryo (Figure 1). The region of the cranial neural fold between the midotic placode and the caudal limit of somite 3 generates the neural crest cells that migrate into the cardiac outflow tract. This region of neural crest has been called "cardiac neural crest" for convenience. The largest population of neural crest cells in the outflow tract is derived from the region of the neural fold that will populate pharyngeal arch 4. The cardiac neural crest migrates from the neural fold into pharyngeal arches 3, 4, and 6. In the pharyngeal arches, the cells derived from the neural crest provide support for the endothelium of the aortic arch arteries. Some cells migrate from the pharyngeal arches into the outflow tract where they form the aorticopulmonary septum and populate the truncal folds (Figure 2). The neural crest-derived ectomesenchymal cells are arranged in ventral and dorsal columns in the truncal folds that are elongations from the aorticopulmonary septum. Late in development, the ectomesenchymal cells are located between the proximal aorta and pulmonary trunk. A few ectomesenchymal cells are scattered in the cusps of the arterial valves (Kirby, unpublished observation). The caudal extent of migration of neural crest-derived ectomesenchymal cells is unclear. Downstream from the semilunar valves, the ectomesenchyme forms smooth muscle of the tunica media of the large arteries derived from the aortic arch arteries (Figure 2). At the same time, ectomesenchymal cells interact with the pharyngeal endoderm in formation of the other derivatives of the pharyngeal apparatus such as the thymus and the parathyroid and thyroid glands (Figure 2).
That the cardiac neural crest is a unique population of cells was shown recently. When the cardiac neural crest is removed and replaced by mesencephalic (cranial) or trunk (caudal) neural crest (Figure 3), persistent truncus arteriosus (nonseptation of the outflow tract) results. This is a surprising result in that other areas of the neural crest have been presumed to be plastic. This indicates that the cardiac neural crest is predetermined early in development. Noden also showed early predetermination of the cranial neural crest. When he replaced presumptive arch 3 neural crest with arch 2 neural crest, he found that arch 2 derivatives were repeated in the arch 3 region. This finding prompted Noden to suggest that the ectomesenchymal derivatives of the cranial neural crest may direct patterning in the head.

The addition of mesencephalic neural crest to the cardiac neural crest also results in persistent truncus arteriosus (Figure 3), whereas heart development proceeds normally after addition of trunk to cardiac neural crest. This indicates that the mesencephalic neural crest may actually interfere with the normal development of the cardiac neural crest during outflow septation.

In another series of experiments, ectomesenchyme was shown to be generated from the nodose placode after removal of the cardiac neural crest (Figure 4). The placodes are a series of ectodermal thickenings in the head region of the embryo that generate portions of the sensory ganglia of the head and viscera. One of the most prominent of these is the otic placode that generates the vestibulocochlear ganglion and portions of the inner ear. The nodose placode is immediately caudal to the otic placode and generates the neurons that carry sensory information from the thoracic and abdominal viscera to the brain.
stem. Under ordinary circumstances, the nodose placode has no non-neuronal derivatives. In the absence of the cardiac neural crest, the ectomesenchyme produced by the nodose placode migrates to the aortic sac and the truncal cushions, but it is not capable of causing outflow septation. The cells derived from the nodose placode mimic the cells derived from the neural crest in that they ultimately assume smooth muscle phenotypes in the walls of the great arteries.

The fact that these alternate populations of cells are not capable of supporting outflow tract septation strongly supports the uniqueness of the ectomesenchymal cell population in the cardiac neural crest.

**Neural Crest-Related Malformations**

To appreciate the effects of neural crest ablation completely, it is important to understand that the early studies involved removal of the neural crest at 24–30 hours of incubation followed by reincubation until days 8–11 of incubation. At that point, the hearts are large enough to analyze macroscopically. The embryos that survived until the time of analysis represent a self-selected population because only a fraction of the operated embryos actually lives to days 8–11. This same situation is true clinically where the infants cared for by pediatric cardiologists constitute a selected population; that is, the embryos or fetuses that are able to compensate for their defects live to the time of analysis or birth.

**Outflow Tract.** Ablation of the premigratory cardiac neural crest results in a variety of malformations of the heart and great vessels (Figure 5), depending on the extent of the ablation. For example, persistent truncus arteriosus results very predictably if the entire cardiac neural crest is removed. In this paradigm, the persistent truncus arteriosus can take any of a number of morphological manifestations. However, an obligatory component for the positive identification of persistent truncus arteriosus has been the presence of a single outflow valve. This single valve typically has four to six cusps. The presence or absence of the aorticopulmonary septum appears to depend on the completeness of the cardiac neural crest ablation. On the other hand, a ventricular septal defect is always a component of this defect. The position of the single outflow vessel with respect to the ventricles is variable; that is, it can arise entirely from the right or left ventricle or can straddle the ventricular septum. In the most common configuration, the vessel arises entirely from the right ventricle. This does not appear to correlate with the presence of any other morphological abnormalities, such as nonpersistence of the right or left aortic arch arteries.

Ablation of a smaller area within the cardiac neural crest, or of cranial neural crest outside the cardiac area, results in a spectrum of defects that have been classified under the generalized title of dextroposed aorta. These malformations include double outlet right ventricle, tetralogy of Fallot and Eisenmenger's complex. Transposition of the great arteries occurs infrequently after neural crest ablation.

**Aortic arch arteries.** Associated with each of these cardiac defects is a variable occurrence of interrupted aortic arch, or anomalies of the other great arteries, including persistence of some vessels that should otherwise disappear (Figure 5). In the chicken, the defin-
Aortic aorta (fourth arch artery) is on the right rather than on the left as it is in humans. After neural crest ablation, the aorta on the left side occasionally persists so that there are bilateral aortic arches or persisting left-sided aorta with disappearance of the right-sided aorta. The carotid vessels (third arch arteries) are also frequently interrupted. In very severe cases, there is a single persisting vessel that connects the heart with the dorsal aorta. These embryos die early in development and have not been seen in the detailed morphological studies on days 8–11 of incubation.

**Inflow anomalies.** Neural crest ablation also causes changes in atrioventricular alignment (Figure 5). The inflow tract anomalies that have been seen include tricuspid atresia, tricuspid stenosis, straddling of the tricuspid valve, and double inlet left ventricle. Atrioventricular septal defects occur infrequently after neural crest ablation. In the neural crest model of heart malformations, there is an obligatory relation between inflow and outflow anomalies. Chick embryos with inflow anomalies caused by ablation of the neural crest always have coexisting persistent truncus arteriosus or one of the dextroposed aorta anomalies. This is quite different from the occurrence of these defects in humans where outflow tract anomalies very rarely appear with inflow anomalies. On the other hand, outflow anomalies in the chick occur most commonly with no signs of inflow anomalies. Atrial anomalies are very difficult to analyze in chick embryos, and a systematic study of the atria after neural crest ablation has not been performed to date. However, our impression is that the atrial septation and other details of atrial morphology are normal.

**Veins.** Even though there are severe defects in the heart and arteries derived from the aortic arch arteries, neither systemic nor pulmonary venous anomalies occur after cardiac neural crest ablation (Figure 5).

**Noncardiovascular defects.** Removal of the cardiac neural crest affects development of all the structures in the pharyngeal arches for which it is destined (Figure 5). Hence, the thymus, parathyroid, and thyroid glands, all of which obtain their stroma from neural crest in the pharyngeal arches are affected. The neural crest migrating into arches 1 and 2, that will develop into the lower face and upper neck, is cranial to the cardiac region of neural crest; thus, facial structures are not usually affected by cardiac neural crest removal.

**Parasympathetic innervation.** Although not mentioned previously, the cardiac parasympathetic innervation is also derived from the cardiac neural crest. One may expect a deficiency in parasympathetic innervation of the heart after ablation of the cardiac neural crest. This, however, is not the case because cells from the nodose placodes migrate into the heart and reconstitute the cholinergic cardiac plexus in the absence of the cardiac neural crest.

**Early Changes in Cardiovascular Development Induced by Neural Crest Ablation**

**Morphological Changes**

Changes in heart and aortic arch artery morphology occur while the heart is still in the looped tube stage, long before septation of the outflow tract normally occurs. Distinct changes are evident by the third day of incubation, and these changes persist in a manner that precludes formation of a normal cardiovascular system.
Cardiac tube. At this stage in development, conotruncal shape is altered even though the heart is a looped tube and septation has not yet begun. Leatherbury et al further noted dilation of the primitive ventricle and incomplete looping of the cardiac tube.

Aortic arch arteries. The quantity, as well as the distribution, of mesenchyme is altered in the pharyngeal arches surrounding the aortic arch arteries. In normal embryos, the endothelial tubes of the aortic arch arteries in the pharyngeal arches are centrally located and completely ensheathed in mesenchyme. In experimental animals, a large portion of the endothelium of these vessels is directly apposed to pharyngeal epithelium, without intervening mesenchyme. At this time, the mechanism by which reduced ectomesenchyme in the pharyngeal arches leads to abnormal development of the arch vessels is not known.

Functional Changes

Functional, as well as morphological, changes appear early in development subsequent to neural crest removal. Microcinemaphotography has been used to study ventricular function in chick embryos at stage 18 with the result that a number of functional deficiencies have been noted at this early stage. These include decreased emptying of the bulbus cordis, incompetent truncal cushions, and lack of blood flow in the fourth right aortic arch artery with increased blood flow in the third right aortic arch artery. At the same time, all of the indexes of contractility, including shortening fraction, wall velocity, and ejection fraction are markedly depressed. However, despite these functional changes, cardiac output is normal because of the ventricular dilation. This suggests that an embryo in the process of developing a heart defect is able to compensate for the insult by dilation of the ventricle.

In a more recent study with a slightly altered design, Tomita et al demonstrated some important factors in the self-selection of experimental embryos between days 3 and 11 of incubation. Heart rate, ejection fraction, stroke volume, and cardiac output were measured in all embryos on day 3 of incubation.

**FIGURE 5.** Diagram illustrating the cardiovascular and noncardiovascular malformations induced by removal of the cardiac neural crest.
Embryos that lived until day 11 had significantly higher ejection fraction, stroke volume, and cardiac output on day 3 of incubation than embryos that did not live to day 11.

These findings demonstrate that functional and morphological changes occur before the time when the predicted structural defects of septation would be apparent in the heart. Cardiac function may be maintained during development at the expense of morphology. There may be physiological compensations in response to early morphological alterations that maintain cardiac function within the normal range.25 Leatherbury et al23 further suggest that the mechanism of compensation for depressed contractility is ventricular dilation. This in turn interferes with complete looping of the cardiac tube, which hinders normal alignment of the developing inflow and outflow tracts. The spectra of dextroposed aorta and atrophicventricular malalignment, discussed above, are a result of these alterations.

Blood pressure has also been measured in the ventricle and dorsal aorta.25 With the servonull technique to measure intraventricular pressure in stage 18 chick embryos, experimental animals displayed no significant change in heart rate, peak ventricular pressure, or end-diastolic pressure compared with controls.

Changes in Calcium Current

Calcium currents have been assessed at 11 days of incubation in hearts with persistent truncus arteriosus.26 Compared with normal hearts, hearts with persistent truncus arteriosus were enlarged (26% greater ventricle to whole embryo weight) and had a twofold reduction in the peak magnitude of the L-type or dihydropyridine-sensitive calcium current. At the same time, there was not a concomitant reduction in the number of L channels detected in radioreceptor assays. This indicates that protein synthesis is not affected in production of the L channel but that there is a difference in post-translational modification of the channel protein in experimental and control embryos.26 The change could perhaps be due to an difference in the phosphorylation status of the channel in myocardium developing under the hemodynamic stresses outlined above. Further exploration of the changes in the substrates of electrical activity in the myocardium should help cardiologists and surgeons in dealing with patients who have congenital heart defects. Although the studies have not yet been performed, ventricular characteristics at 3 days of development23 would lead to the prediction that the same depression of calcium current is present much earlier in development.

Extracellular Matrix

Rosenquist and colleagues27-30 attempted to determine what factors are unique to the cardiac neural crest. Elastogenesis is first found in foci at the interface between the myocardial cuff and the ectomesenchyme of neural crest origin on day 4 of incubation (Figure 6). The foci coalesce as development proceeds. Elastogenesis is then propagated downstream along the developing great arteries in an orderly proximodistal sequence by the ectomesenchyme and then the propagation of elastogenesis downstream along the developing great arteries.

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**Figure 6.** Flow chart illustrating the site of initiation of elastogenesis in the conus cordis at stages 23-24 by the ectomesenchyme and then the propagation of elastogenesis downstream along the developing great arteries.
Application in Humans

All of the heart defects described in chick embryos after neural crest ablation have also been seen in human infants. In the chick embryo, removal of the cardiac neural crest also affects other structures to which that area of neural crest contributes. Isolated heart defects occur rarely, if ever, without changes in thymus, parathyroid, and thyroid glands. It is too early to say whether this is the case in humans. The most obvious correlation in humans is the DiGeorge syndrome. Typically, the DiGeorge syndrome includes hypocalcemia, defective thymic-dependent cellular immunity, anomalies of the face, ears, and palate, and congenital cardiovascular malformations. Van Mierop and Kutsche\(^{31}\) suggested that the DiGeorge syndrome is indeed due to abnormal development involving the neural crest. The interesting observation by Wells et al\(^{22}\) that the thyroid cartilage, a derivative of the fourth pharyngeal arch, is hypoplastic in patients with DiGeorge syndrome or tetralogy of Fallot indicates that other outflow anomalies may be associated with abnormalities of the neural crest. Sulik et al\(^{33}\) suggested that fetal alcohol syndrome should also be included among syndromes in which the cranial neural crest is damaged. Furthermore, she proposes that some cases of DiGeorge syndrome can be directly attributed to ethanol or its metabolites.

Other syndromes have been suggested as linking neural crest abnormalities with heart defects. These include the CHARGE association\(^{34}\) and congenital neuroblastoma with congenital heart disease.\(^{35}\) Retinoic acid embryopathy has also been attributed to damage to the neural crest in humans and animals.\(^{36-38}\)

The evidence has become very strong that neural crest is involved in a number of syndromes and is affected by certain teratogens. Our current concern is to determine what role, if any, neural crest plays in generation of congenital heart defects that are not part of a syndrome. A recent study by Todd and Todd\(^{39}\) showed an increase in the rate of otitis media associated with outflow tract anomalies compared with other heart defects. The outflow defects included transposition of the great arteries, tetralogy of Fallot, and aortic stenosis. They suggested that patients who are prone to otitis media have architectural differences in their eustachian tubes. The eustachian tube develops from pharyngeal arches 1 and 2 and is dependent on ectomesenchyme in those arches: hence, the suggested association between outflow tract defects and middle ear infections. An alternate explanation is that resistance to infection in these children is not adequate because of compromised thymic-mediated immunity.\(^{40,41}\) It is also becoming apparent that children with heart defects have mild facial dysmophogenes.\(^{41-44}\)

It is important to document changes in neural crest–associated tissues in any case of heart defect to begin to understand which of these defects is due to malfunction of the neural crest during development.

Role of Neural Crest in Heart Development

Our current understanding of the cardiac neural crest indicates that it plays a complex role in heart development with at least two major components. The simpler of these seems to be its participation in outflow septation. Although the cellular and molecular events that the neural crest directs have not been elucidated, it is clear that the presence of a certain number of cells in the outflow tract is essential for septation to occur. Below this critical number, septation does not occur. Because the major defects in DiGeorge syndrome are persistent truncus arteriosus and interrupted aortic arch type B, this syndrome can probably be attributed to a lack of the critical number of cardiac neural crest cells in pharyngeal apparatus and the outflow tract. This could be caused by a failure of proper migration into the area or by excessive cell death.

There are still a number of questions with regard to the exact distribution of the neural crest in the heart. These include the possibility and extent of cardiac neural crest involvement with the sinoatrial and atroventricular nodes and conduction system, the coronary arterial system, and the formation of the semilunar valves.

The role of the cardiac neural crest that is more difficult to understand is the one it plays in the pharyngeal arch region. The interplay among all the elements of the pharyngeal arches including endoderm of the aortic arch arteries, endoderm of the pharyngeal pouch, ectoderm of the pharyngeal grooves and arches, and, finally, mesenchyme of non–neural crest origin, must be quite complex. A combination of interactions must determine which arch arteries persist and which disappear. Perhaps the same or a completely different set of interactions determines the hemodynamic characteristics of individual arch arteries that then influence the final normal or abnormal phenotype of the developing heart. The extracellular matrix probably plays a major role in this developmental regulation; however, many other factors may be involved and are yet to be elucidated.

References

5. Rychter Z: Analysis of relations between aortic arches and aortopulmonary septation, in Rosenquist GC, Bergsma D
34. Siebert JR, Graham JM, MacDonald C: Pathologic features of the CHARGE association: Support for involvement of the neural crest. Teratology 1985;31:331–336

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