Neurotrophin-3 knocks heart off Trk

Heart defects in NT-3 knockout mice implicate this neurotrophin in cardiac development.

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Congenital heart defects (CHD) represent the most common group of human birth defects and occur in nearly 1 percent of live births and in 10 percent of spontaneously aborted fetuses. Although anatomic and physiologic descriptions of cardiac defects in newborns have existed for centuries, the molecular bases underlying most CHD remain poorly understood. However, the last few years have seen an explosion in our knowledge of the genes involved in the various steps of normal cardiac formation. Most recently, a study by Donovan and colleagues[1] in this month's issue of *Nature Genetics* implicates involvement of neurotrophin-3 (NT-3), a member of the nerve growth factor family. The authors show that (as in clinically important congenital cardiac defects) the heart defects in NT-3-deficient mice allow normal growth *in utero*, but result in cardiac insufficiency after birth. As the function of *NT-3* and other genes that regulate cardiac development are more clearly defined, the challenge will be to relate this information to the pathogenesis of abnormal cardiogenesis in humans.

NT-3 belongs to a family of neurotrophic factors, the neurotrophins, which include nerve growth factor, brain-derived neurotrophic factor and neurotrophin-4 and -5. NT-3 acts as a mitogen and survival factor for neural crest cells; its actions are mediated by the tyrosine kinase C (trkC) receptor, which is present on the surface of NT-3-dependent cells. NT-3 and trkC receptors are coexpressed in diverse neural crest-derivatives including the cardiac neural crest, sympathoadrenal cells, peripheral neurons and enteric ganglia[2]. It was previously reported that mice homozygous for mutations in the *NT-3* allele appeared to be normal at birth, but were then found to be dusky, grew poorly and died as neonates. Although deficits were identified in sympathetic and sensory neurons (both neural crest-derived) the reason for the early demise of *NT-3* mutant mice was not clear[3–5].

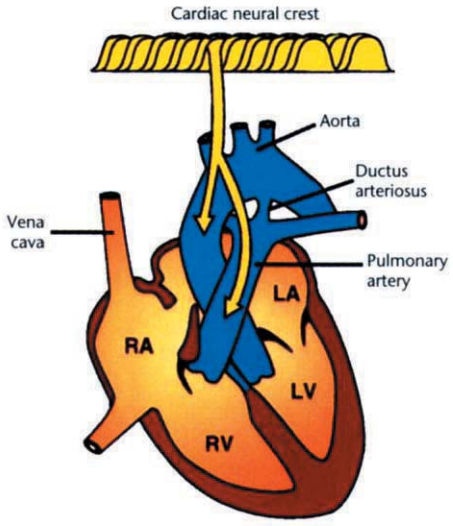

Fig. 1 Contribution of neural crest cells to the developing heart. Cardiac neural crest cells migrate to the heart from the neural folds and populate the aortic arch, proximal pulmonary arteries, ductus arteriosus and the upper portion of the ventricular septum (shown in blue). RA, right atrium; LA, left atrium; RV, right ventricle; LV, left ventricle.

Donovan and associates[1] now report that mice deficient in *NT-3* exhibit a series of cardiac defects that appear to be related to abnormal neural crest development. They found that not only the neural crest-derived peripheral nervous system but also the cardiac neural crest is affected in *NT-3* null mice. A variety of cardiac defects were observed including pulmonary stenosis, tetralogy of Fallot (pulmonary stenosis and ventricular septal defect), persistent truncus arteriosus (failure of separation of pulmonary and aortic arteries) and ventricular septal defects (Fig. 1, 2). These types of abnormalities, which are commonly seen in human pediatric patients, are referred to as cardiac outflow tract defects and are thought to result from defects in neural crest development[6].

Why do *NT-3* mutant embryos develop normally *in utero* but have cardiac compromise soon after birth? During the transition from fetal to neonatal circulation in mice and humans, previously asymptomatic cardiac defects are often manifested. Primary defects of the cardiac muscle generally result in heart failure and early embryonic demise and are therefore rarely seen at birth. In contrast, cardiac defects that do not primarily involve the muscle but rather involve abnormal atrio-ventricular or ventriculo-arterial connections may not result in physiologic dysfunction during fetal life. The dramatic physiologic changes that occur at the time of birth include the transition from a circulation that flows in series to two separate circulations flowing in parallel. Because the lungs are not aerated *in utero*, little blood is pumped to the lung (pulmonary circulation) and most instead is redirected to the body (systemic circulation) via a small vessel, the ductus arteriosus, which connects the pulmonary artery to the aorta; additional venous blood is directed toward the systemic circulation via an opening in the atrial septum (Fig. 1). Thus, both ventricles are pumping blood to the body with little regard for separation of blood between the pulmonary and systemic circulations, a situation that would function well *in utero* with only one ventricle or one outflow tract. Soon after birth the lungs are inflated, the atrial opening closes as does the ductus arteriosus, and deoxygenated blood flows to the lungs where it is oxygenated before returning to the left side of the heart to be pumped to the body. The result is two separate circulatory beds (pulmonary and systemic) that require two separate pumps. The majority of clinical congenital cardiac defects are compatible with fetal life but, because of poor separation of pulmonary and systemic circulations after birth, result in cardiac problems in the neonatal period.

A subset of neural crest cells, known as the cardiac neural crest, participates in division of the systemic and pulmonary circulations[7]. Neural crest cells are a unique population of migratory cells that arise from the neural folds during em-

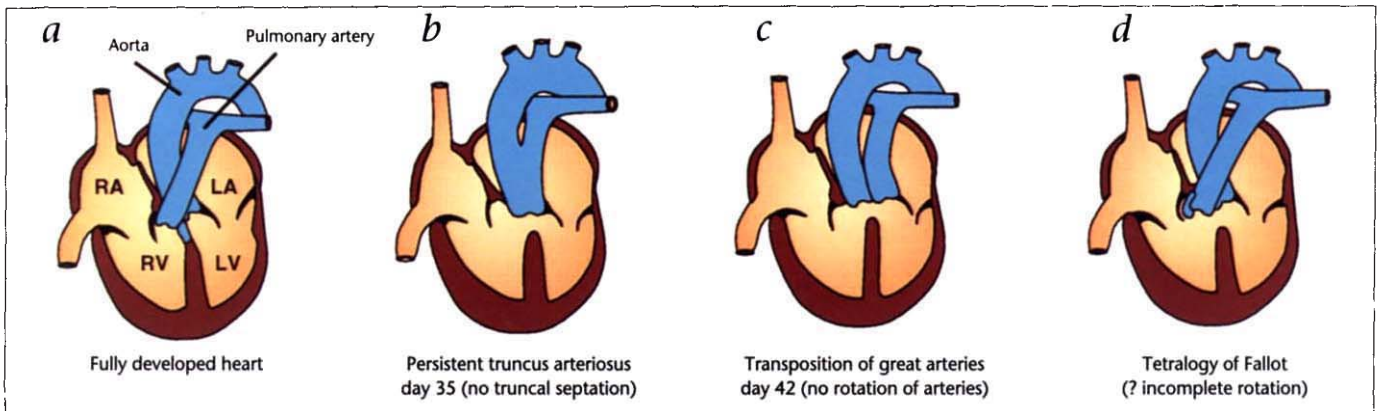

Fig. 2 Cardiac defects of neural crest-derived structures. Many congenital heart diseases resemble a normal developmental stage of the cardiac outflow tract and great vessels during embryogenesis. *a*, Normal mature heart with the aorta arising from the left ventricle (LV) and the pulmonary artery arising from the right ventricle (RV). *b*, Persistent truncus arteriosus represents an arrest around day 35 of human gestation due to a lack of truncal septation into a pulmonary artery and aorta. *c*, Transposition of the great arteries may result from lack of rotation of the great vessels after septation, as seen around day 42 in human embryos. *d*, Tetralogy of Fallot (as seen in some *NT-3*-deficient mice) may represent incomplete rotation of the great arteries, which occurs around human embryonic day 45, resulting in malalignment of the vessels with rightward deviation and a narrowed right ventricular outflow tract. Regions populated by neural crest cells are shown in blue. RA, right atrium; LA, left atrium.

bryogenesis. They give rise to diverse cells including peripheral neuronal cells, melanocytes, and adrenal chromaffin cells, as well as connective and chondrogenic cells that contribute to craniofacial structures. Lineage tracings indicate that neural crest cells populate the outflow tract of the heart (conotruncus), the aortic arch, proximal pulmonary arteries and the ductus arteriosus (Fig. 1). Cardiac neural crest cells undergo an ectomesenchymal transformation and form the walls of the aortic arch arteries and participate in septation of the outflow tract. Ablation of the cardiac neural crest in chick embryos results in cardiac defects similar to those seen in *NT-3* mutants, with a predominance of cardiac outflow tract and aortic arch defects. Groups of patients with these types of abnormalities share in common microdeletions of chromosome 22 and the acronym CATCH-22 has been used to describe this syndrome[8]. The critical region of chromosome 22 has been narrowed to 1.5 Mb, and it is clear that identification of the genes located in this chromosomal region and others involved in their pathways will lead to an understanding of the mechanisms of cardiac neural crest defects.

The cardiac phenotype of the *NT-3* mutant mice suggests that this growth factor plays a role in such a pathway. However, other genes of marked diversity have been knocked out in mice resulting in very similar phenotypes. Such genes include *endothelin 1*, which encodes a signaling peptide; *endothelin 1*-deficient mice have cardiac outflow defects and craniofacial abnormalities[9]. Mice lacking the retinoic acid receptor[10] or the homeodomain protein Pax3 (ref. 11) also show cardiac and pharyngeal neural crest defects, reminiscent of those seen in the human syndrome, CATCH-22. It remains unclear how such seemingly unrelated factors regulate similar processes during development, although a simplistic model might implicate each of these gene products in overlapping pathways leading to a common end point.

The cardiac phenotype described in the *NT-3* mutant mice is the first demonstration of an essential nonneuronal role for NT-3. This finding suggests a commonality in the development of phenotypically divergent and functionally distinct populations of neural crest-derived cells. Are there common factors involved in the basic process of neural crest cell migration or are there conserved steps involved in proliferation or differentiation of neural crest cells after they arrive at their final destination? When migratory defects exist, are there abnormalities in extracellular matrix or signaling molecules rather than cell autonomous defects? Accurate cell fate mapping of the neural crest using chimeric mouse models of wild-type and mutant cells should answer these questions in the future.

In vitro and *in vivo* data suggest that NT-3's role may lie in promoting the survival and proliferation of neural crest cells; introduction of NT-3 into cultures of neural crest cells results in cell proliferation[12]. Since NT-3 serves as a ligand for the cell-surface trkC receptor, it is believed that NT-3's effects are mediated through this interaction. Indeed, in the *NT-3* knockout the survival of certain trkC-expressing cells is diminished, suggesting that maintenance of these cells is dependent on interaction with NT-3. Not surprisingly, mice deficient in the trkC receptor have a phenotype similar to that of NT-3-deficient mice with regard to their survival and neuronal loss[13]. Absence of growth factors may result in loss of certain neural crest cells leading to the observed phenotype. Whether the decreased number of cells is due to decreased proliferation or increased apoptosis has not been clearly established.

What other factors may lie upstream or downstream of the neurotrophins and trkC receptors during development? Several potential candidates exist that are present in the developing neural crest. These include homeodomain-containing proteins such as MHox (ref. 14) and Pax3 (ref. 11); basic helix-loop-helix transcription factors such as MASH-1 (ref. 15) and the HAND (ref. 16) proteins; retinoids and their receptors[10]; and signaling molecules such as the endothelins[9]. Establishment of the hierarchy of genetic control during the cascade of events resulting in migration and differentiation of cardiac and other neural crest-derived cells will be necessary for understanding the pathogenesis of defects related to these cell types. It will be interesting to determine which, if any, of these factors is in-

volved in the same pathway as the yet undetermined gene products of chromosome 22 that are deleted in patients with cardiac neural crest defects.

Although establishing models of human congenital heart disease is exciting, several questions remain regarding the pathogenesis of these defects. Comparison of embryologic and clinical studies of CHD suggest that many defects related to the cardiac neural crest represent an arrest of development rather than abnormal development. The anatomy seen in persistent truncus arteriosus, transposition of the great arteries, ventricular septal defects, and maybe even tetralogy of Fallot can be seen in a normal fetus at some stage of development (Fig. 2). Factors such as NT-3 and other members of a pathway that regulates neural crest development may be responsible for an arrest of neural crest contribution or patterning at varying stages of heart formation, resulting in the spectrum of congenital heart disease observed clinically.

In spite of the interesting discoveries in recent years regarding CHD, will it ever be possible to intervene in the pathologic process? Because the heart forms very early during pregnancy, current technology would limit the application of molecular discoveries to improved genetic testing and counseling for parents. However, more aggressive therapies hold promise for the distant future. Improvements in targeted gene delivery systems may allow specific replacement therapy in developing neural crest cells of the fetus. Alternatively, surgical intervention for cardiac defects (which could cause secondary abnormalities later during development) may be possible and could be augmented with direct gene therapy at the time of surgery. Early results from fetal surgery in humans have been promising[17], and fetal cardiac surgery may represent the next frontier. Great strides will have to be made before any of these types of interventions become a reality, but deciphering the genetic code for heart formation must be the first step. This is no trivial matter for, as in the words of Pascal, "the heart has its reasons which reason does not know."

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Can a killer be arrested?

A promising drug study in a mouse model of Chagas' disease raises hopes that a major health and socioeconomic problem can be eliminated.

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Trypanosoma cruzi, the unicellular parasite that causes Chagas' disease, is transmitted to mammals by hematophagous insects (known as kissing bugs; see figure) but can also be acquired by transfusion of contaminated blood. Prevalent in South and Central America, with occasional cases reported in the United States, this disease comprises a variety of pathological manifestations ranging from the severe, such as cardiopathy (often complicated with aneurysm of the apex of the left ventricle), massive enlargement of the colon or esophagus and damaged nervous tissue, to the insignificant[1]. According to World Health Organization estimates, approximately 25 percent of the Latin American population is at risk of Chagas' disease and 16 to 18 million people are infected[2]. Of these, 30 percent are likely to develop severe clinical symptoms. While underscoring why Chagas' disease is such a significant health problem, these statistics do not reveal the additional, serious socioeconomic impact of this disease upon many chagasic patients who are unable to work and support their families.

Although some attempts to vaccinate laboratory animals against *T. cruzi* infection have been partly successful[3,4], efforts to protect at-risk humans in this way have encountered a stumbling block because of the hotly debated notion that Chagas' disease might have an autoimmune component triggered by similarities between parasite and host tissue antigens[5,6]. Until this controversy is resolved, realistic hopes of combating *T. cruzi* infection lie in the development of effective chemotherapy. Several drugs are currently used to treat chagasic patients, but all of them have severe side effects and are frequently ineffective[7]. Therefore, despite occasional successes with drug treatment, Chagas' disease is widely regarded as incurable. For this reason, the recent report in *Science* by Urbina and coworkers[8] has attracted much attention. They show that treatment with the antifungal agent, D0870 (a bis-triazol derivative that inhibits sterol biosynthesis), eliminates the parasite from infected laboratory mice without causing visible toxicity to the host[8]. These findings are an