the regeneration of phenotypically normal plants will greatly facilitate studies of gene expression and regulation in plants.

ROBERT B. HORSCH
ROBERT T. FRALEY
STEPHEN G. ROGERS
PATRICIA R. SANDERS
ALAN LLOYD
NANCY HOFFMANN

Monsanto Company,
St. Louis, Missouri 63167

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Dependence of Thymus Development on Derivatives of the Neural Crest

Abstract. *Elimination of limited areas of the cephalic neural crest in stage 9 or 10 chick embryos markedly reduced the size of the thymus gland or resulted in its absence. Small thymic lobes contained both thymocytes and epithelial cells but showed delayed development. Parathyroid and thyroid glands sometimes were reduced in size or missing from the normal location on one or both sides. Heart defects were consistently present. Thymus development may depend on direct interaction of mesenchymal derivatives of the neural crest with pharyngeal epithelium. Multiple defects, such as the Di George syndrome, may result from failure of neural crest derivatives to migrate and interact properly.*

Aplasia or dysplasia of the thymus gland may be associated with abnormal development of other pharyngeal pouch derivatives, facial features, and the heart (*1*). The neural crest contributes connective tissue to the cephalic region and thymus (*2*). It was recently demonstrated that the cephalic neural crest contributes to development of the cardiac outflow tract; extirpation of the crest led to abnormalities of the heart and great vessels (*3*). The occurrence of clusters of congenital anomalies of head structures, heart, and thymus, which have in common the incorporation of contributions from the cephalic neural crest, led to the hypothesis that failure of sufficient quantities of cephalic neural crest cells to migrate and interact with these developing organs results in their abnormal development. In support of this hypothesis for the thymus, we report here that defective development of the thymus accompanies cardiac anomalies after extirpation of the cephalic neural crest in chick embryos.

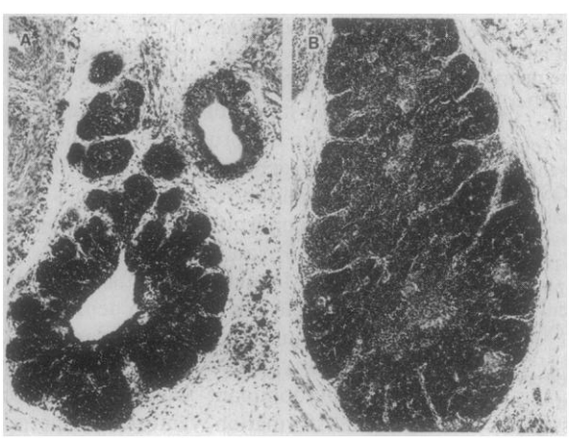

Fig. 1. Photomicrographs of sections of embryonic chick thymus taken (A) after extirpation of the cephalic neural crest and (B) after sham surgery (both ×100). Remnants of primitive pharyngeal pouch endoderm are evident in two areas in (A).

The neural crest is produced from ectoderm in the avian embryo after the neural folds appose each other to form the primitive neural tube. Neural crest cells migrate ventrolaterally from the area of the neural folds and differentiate to form a variety of structures, including cranial, spinal, and autonomic ganglia. The cephalic neural crest, that portion from fifth somite forward, also contributes a large proportion of cells that differentiate into mesenchymal cells. Bones and cartilage of the visceral skeleton, dermis in the face and ventrolateral side of the neck, walls of large arteries derived from branchial arches, and connective tissue of the lower jaw and tongue all originate from the neural crest (*2, 4*). In addition, mesenchymal components of the glandular pharyngeal derivatives have the same origin. Cells derived from the neural crest form the parafollicular cells of the thyroid gland and are found in the interlobular spaces and medulla of thymic lobes and in the connective tissue between cords of parathyroid cells (*2*). The cephalic neural crest contributes to the formation of the periocular tissues and, as mentioned above, the outflow tract of the heart (*2, 3, 5*).

To determine whether thymus development depends on neural crest derivatives, we ablated regions of the neural folds of chick embryos before cephalic neural crest migration and subsequently examined the thymus. Fertile Arbor Acre chicken eggs (Central Soya of Athens) were incubated for 30 hours in a humidified atmosphere at 38°C. A window was made in the shell and the embryos, at stage 9 or 10 (*6*), were lightly stained with neutral red. The vitelline membrane was torn over the embryos and all or part of the neural folds over somites 1 to 5 were removed bilaterally with a modified Wenger vibrating needle (*7*) or a microcautery needle. Histological monitoring has shown that this technique causes minimal damage, limited to a few cell widths in the dorsal neural tube and adjacent surface ectoderm. After surgery the eggs were sealed, returned to the incubator, and allowed to develop for an additional 13 to 15 days (total incubation age, 14 to 16 days). Sham-operated embryos were processed in parallel with each group of experimental embryos. Fifteen experimental and 16 control embryos were used. On being

killed, all the embryos appeared grossly normal, except that the necks of experimental embryos were somewhat shortened, in two embryos the occipital region of the skull was defective, and in one embryo there was abnormal limb development.

The thymus of control chicks, on dissection under the stereomicroscope, consisted of variably joined lobes distributed along the jugular vein, common carotid artery, and vagus nerve bilaterally in the posterior half to two thirds of the neck. Thyroids were located at the caudal extent of each thymus and parathyroids were adjacent to the thyroids, dorsal to the bifurcation of the brachiocephalic artery.

After extirpation of the neural crest the quantity of thymus tissue was reduced notably. There were fewer lobes and the lobes frequently were smaller. Thymus characteristically was distributed posteriorly in the neck. In three embryos no thymus tissue could be found. In others, tiny lobes were present on one or both sides. In two embryos the distribution of thymus was similar to that in controls, but the quantity was reduced. Total thymus weights for each animal are presented in Table 1. Mean weight after neural crest extirpation (5.4 ± 1.8 mg) was significantly less than that of controls (29.6 ± 2.6 mg). Maximum deficiency was caused by ablating the neural crest over the first three somites. Ablation of additional lengths did not lead to significantly smaller thymus weights.

Table 1. Weights of thymus at various times after ablation of the chick embryo cephalic neural crest and after sham surgery. Wet weights were determined after fixation and separation of thymus tissue from fat, nerves, and blood vessels.

Incubation (days)	Weight of thymus (mg)	
	Experimental embryos	Control embryos
13	2.0	17.3
	1.5	10.0
14	3.1	29.6
	0.0	21.5
	0.0	28.0
	0.0	34.6
		44.9
15	6.8	32.7
	9.5	40.3
	3.7	23.7
	9.2	25.2
		24.2
16	4.0	29.8
	18.3	42.0
	1.5	
	5.4 ± 1.8*	29.6 ± 2.6*

*Means ± standard errors, significantly different at $P < 0.001$ (Student's t-test).

Thymus tissue from five experimental animals was prepared routinely and embedded in epoxy resin for examination by light microscopy (semithin sections stained with toluidine blue) and electron microscopy. Tissue from another experimental animal was fixed in Formalin and examined after routine paraffin preparation. The soft tissue from each side of the neck of two additional experimental animals was fixed en bloc and 10-μm-thick paraffin serial sections were examined by light microscopy. Macroscopic examination did not reveal thymus tissue in the remaining experimental animal. Tissue from controls was processed and examined in parallel. The microscopic lobules from experimental animals frequently appeared smaller and less well developed than those of controls. In three of the eight experimental animals examined histologically, remnants of the primitive pharyngeal cavity, with surrounding epithelium, were observed in the thymus (Fig. 1A). Although thymus in experimental embryos frequently appeared not to have developed as fully as in controls (Fig. 1B)—an observation that might be interpreted as a delay in development—lymphocytes were always present in thymuses that were large enough to be dissected. Histological and electron microscopic observation indicated that lymphocytes were proliferating in an epithelial reticulum and that cortex-medulla differentiation occurred. No thymic corpuscles were present in either group.

Heart defects were present in all experimental animals and were absent in the controls. The anomalies included persistent truncus arteriosus and transposition of the great vessels (*5*). Parathyroids or thyroids (or both) frequently could not be found or were reduced in size on at least one side (*8*).

Normal development of the thymus requires precisely timed migrations and interactions (Fig. 2). Neural crest cells originally epithelial in nature become mesenchymal and migrate to the epithelial anlagen of pharyngeal pouches III and IV. Induction of thymic epithelial development presumably is analogous to the induction of cartilage and bone development by neural crest mesenchyme (*9*). It is significant that, although somatic mesenchyme can support thymic differentiation, cephalic mesenchyme serves better to induce and sustain thymopoiesis than mesenchyme from other sources (*10*). Lymphoid stem cells are brought to the developing thymic epithelium through the bloodstream (*10, 11*). The thymus is receptive to these stem cells during a specific 24- to 36-hour period (*10*). Without sufficient quantities of the various elements, with a certain history of interaction, and at a given time, a defective thymus results. The thymus, when found in the experimental animals, was composed of thymocytes proliferating in an epithelial reticulum, although its mass was less than in controls, perhaps in part due to delayed development. This resembles the pattern seen in the third and fourth pharyngeal pouch (Di George) syndrome, in which thymus tissue may be absent or greatly reduced in size with lymphoid development (*12, 13*). In contrast, in patients with severe combined immunodeficiency the thymus is characterized as an "epithelial" organ lacking lymphocytes (*14*). It is likely that the epithelial thymus of severe combined immunodeficiency results from an absence of stem cells and that the hypoplastic thymus produced here and observed in the Di George syndrome results from failure of neural crest derivatives to interact with pharyngeal pouch endoderm in sufficient quantity at the right time. At some critical low level of interaction, thymus development becomes impossible. It is also possible that additional indirect effects, such as metabolic changes affected by branchial arch derivatives, may be involved.

Many clinical syndromes are charac-

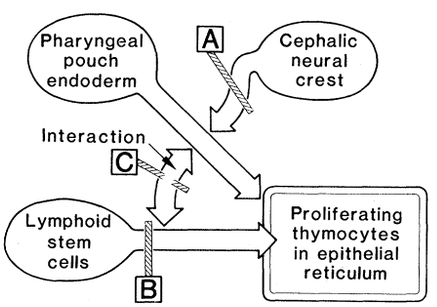

Fig. 2. Conceptual representation of contributions and interactions necessary for the development of a functional thymus. Endoderm from the third and fourth pharyngeal pouches is induced by migrating mesenchymal elements derived from the neural crest. As induced epithelial cells proliferate, they interact with blood-borne lymphoid stem cells, inducing thymocyte differentiation and proliferation in their reticulum. Thymic aplasia or dysplasia, as produced here or in the Di George syndrome, would result from an incomplete contribution by the neural crest (A). The "epithelial" thymus of severe combined immunodeficiency, lacking in lymphocytes, would result from a lack of lymphoid stem cells (B). The epithelial thymus of other lymphoid deficiencies could result from a lack of interaction between epithelium and stem cells (C) or a deficiency in a subpopulation of stem cells.

terized by clusters of developmental defects involving the heart and great vessels along with pharyngeal pouch derivatives and disfigurement of facial features. The observed association of thymus and heart defects and the incidental absence or decreased size of thyroid and parathyroids in some animals suggest that the etiology of these syndromes is related to failure of neural crest cells to migrate and interact in sufficient quantity to support development of the organs in question. The Di George syndrome provides the best clinical correlation for this array of anomalies (*12, 13*). Di George (*13*) originally described this syndrome as "congenital absence of the thymus (and parathyroids)." Micrognathia and hypertelorism have been described in association with heart defects in addition to the characteristic thymus and parathyroid deficiencies (*1*). Other combinations of thymic defects with heart (*1*) and thyroid (*15, 16*) defects have been described in the presence of parathyroids. Numerous examples of heart defects associated with anomalies of head and neck, with or without reported defects in pharyngeal pouch derivatives, have been described (*12, 13*). It is possible that an understanding of the neural crest's role in the development of these organs will lead to increased recognition of clusters of developmental anomalies.

DALE E. BOCKMAN
MARGARET L. KIRBY*
Department of Anatomy, Medical College of Georgia, Augusta 30912

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* To whom request for reprints should be addressed.

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The Edge Cell, a Possible Intraspinal Mechanoreceptor

Abstract. *In the lateral edge of the "white matter" in the lamprey spinal cord, there is a group of nerve cells referred to as edge cells. The results of a combined physiological, light microscopical, and electron microscopical study suggest that these cells serve as intraspinal mechanoreceptors. Edge cells are depolarized on stretch of the lateral margin of the spinal cord, and they have nestlike ramifications in this region oriented in a rostrocaudal plane. These cells exhibit a close structural similarity with the crayfish stretch receptor.*

Retzius has described a group of nerve cells in the spinal cord of the cyclostome located far out in the "white matter" along the lateral edge (*1*). These cells in the lamprey were further described by Kolmer (*2*), Tretjakoff (*3*), and Rovainen (*4*) and are now referred to as edge cells. They have "dendrites" extending toward the lateral margin in a rostrocaudal plane and an axon ascending on the ipsi- or contralateral side for some segments, and they receive synaptic input from a few sources. It has recently been demonstrated that mechanosensitive elements

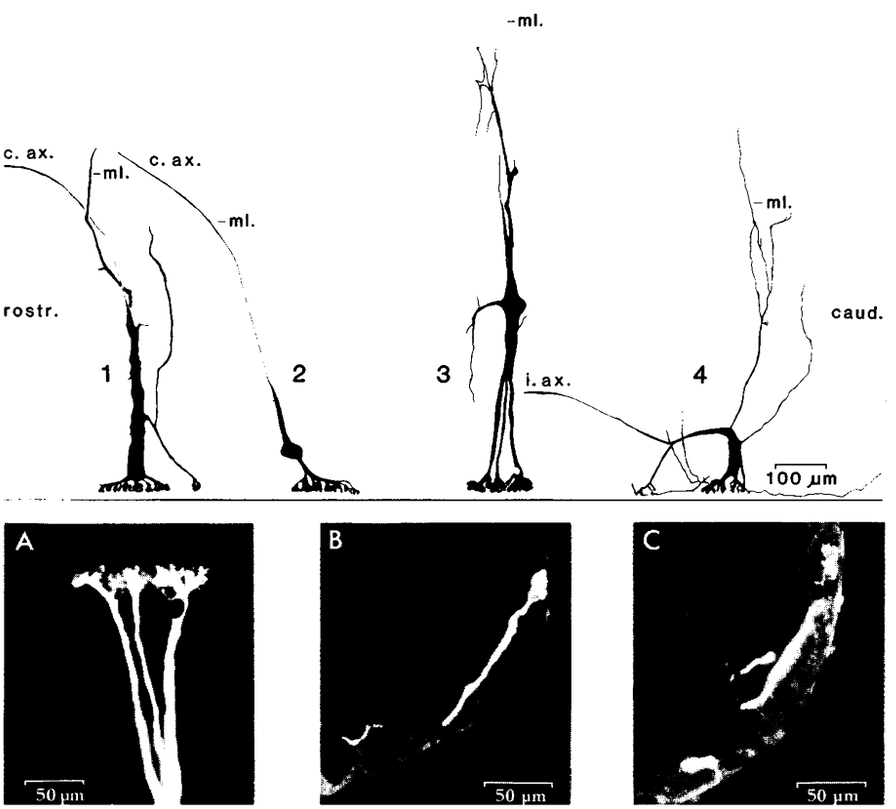

Fig. 1. Reconstruction of edge cells. The neurons were reconstructed (dorsal view) from four different Lucifer yellow preparations (1 through 4) and are oriented along the lateral margin (downward). The midline (*ml.*) is at different distances from the lateral margin because of differences in the sizes of the spinal cords. Rostral (*rostr.*) is to the left. Axons projecting to the ipsi- or contralateral side of the spinal cord are indicated with *i.ax.* or *c.ax.*, respectively. (A, B, C) Photographs of the lateral ramifications of cell 3. (A) Photograph showing the nests of fine terminals (Lucifer yellow, dorsal view). (B and C) Photographs of the same lateral processes in transverse section. Note the close proximity to the lateral edge and how the ramifications course among the individual axons.