Grafts of the Third Branchial Arch in Chick Embryos

Abstract
The parathyroid glands have been classically considered derivatives of the third and fourth pharyngeal pouches in most species, including humans. The presence of neural crest-derived cells in parathyroid glands connective tissue has apparently been established. However, our previous studies have provided a new hypothesis on the origin of these glands in human and chick embryos. To determine the true origin of the third parathyroid (parathyroid III) gland in the chick embryo, pieces of the third branchial arch from donor chick embryos at Hamburger and Hamilton's stage 19 (embryonic day 3) were grafted to host chick embryos at the same stage of development. Starting from Hamburger and Hamilton's stage 27 (embryonic day 5), a structure identified as the parathyroid III appeared in the ectodermal (epipharyngeal) placode of the third branchial arch graft, from which it subsequently became separated at Hamburger and Hamilton's stage 28 (embryonic day 5.5) and continued to develop and mature. Our findings suggest the conclusion that the parathyroid III gland begins to develop from the epipharyngeal placode, so that this gland, from our point of view, could be considered ectodermal in nature.

Introduction
The study of the origin of the 'branchiogenic' or pharyngeal derivatives has been the main focus of our research in recent years [Sanz-Casado et al., 1984; Garcia-Garcia et al., 1985, 1987; Mérida-Velasco et al., 1989]. Our findings, based on descriptive embryology, have provided a new hypothesis on the origin of the third parathyroid (parathyroid III) gland. Garcia-Garcia et al. [1985] showed that in human embryos, the parathyroid III gland clearly originated from the ectodermal (epipharyngeal) placode at the level of the posterior surface of the third branchial groove. In chick embryos, García-Garcia et al. [1987] established that one area of the ectodermal surface forming the floor of the cervical sinus (where the third branchial groove is barely noticeable), at approximately the level of the concavity of the third aortic arch, showed signs of an active process of placodal invagination, to give rise to the parathyroid III gland.

The parathyroid glands have been classically considered as derivatives of the third and fourth pharyngeal pouches in most species, including humans [Norris, 1937; Schriber and Hamilton, 1952; Van Dyke, 1959; Kayser et al., 1961; Nevalainen, 1969; Ribes and Puchades, 1969; Cordier and Haumont, 1980; Hilfer and Brown, 1984]. However, Pearsen and Takor-Takor [1976] provided morphological evidence of a totally ectodermal origin for the anuran (tadpole)
parathyroid, and they maintained that the placodal ectoderm probably constituted the major component of avian parathyroid glands. Thus, they considered the chief cells of the parathyroid glands to be placode-like cells [Pearse and Takor-Takor, 1976].

Pearse [1977] defined the amine precursor uptake and decarboxylation (APUD) system, and included the parathyroid glands in it. Fujita [1980] classified the parathyroid glands as a part of the paraneuron system. Initial results obtained by Le Douarin's group [Ayer-Le Lièvre and Fontaine-Perus, 1982] demonstrated the neural crest origin of carotid body type I cells and calcitonin-containing cells of the avian ultimobranchial body and mammalian thyroid, and confirmed the neural crest origin of the adrenomedullary cells. The neuroectodermal origin of some APUD (pancreatic islets, endocrine enterogastric and endocrine bronchial) and paraneuron (parathyroid) cell types was still to be studied [Ayer-Le Lièvre and Fontaine-Perus, 1982]. Pearse's hypothesis [1977] could provide a satisfactory explanation for various malignant conditions such as multiple adenoma syndromes and carcinomas secreting ectopic hormones, assuming that normally unexpressed capabilities of the neuroectodermal antecedent may reappear in its progeny cells under particular carcinogenic conditions [Ayer-Le Lièvre and Fontaine-Perus, 1982]. The terms 'apudomas' or 'neurolophomas' and 'neurocrinomatoses' were offered as general terms to designate pathological conditions of APUD cells or neural crest-derived cells. In the meantime, further embryological studies showed that several APUD cell types did not share this neuroectodermal origin (gastroenteric, pancreatic and bronchial endocrine cells). Thus a common neuroectodermal origin could not be put forward to explain the common properties of APUD cells [Ayer-Le Lièvre and Fontaine-Perus, 1982]. The explanation for coincidences in the normal development and carcino genesis of APUD cell types could not be sought in a common embryological ancestor, but rather at another level: in the similar biochemical properties of cells from different origins, which indicated a somewhat convergent development and differentiation. Moreover, the paraneuron of Fujita [1980] is based on a number of common morphological and functional properties. Considering these cells as 'receptosecretory', Fujita stressed their 'neural' but at the same time 'glandular' features. This system gathers together a large number of cell types including APUD cells; however, Ayer-Le Lièvre and Fontaine-Perus [1982] showed that parathyroid, pancreatic islets, enterogastric or bronchial endocrine cells did not have a neuroectodermal origin. The hypothesis of a common neuroectodermal origin of these cell types was subsequently disproved [Fujita and Kobayashi, 1979]. On the other hand, Le Lièvre and Le Douarin [1975], Le Douarin [1982], Noden [1983], and Bockman and Kirby [1984, 1985] established the presence of neural crest-derived cells in the parathyroid gland connective tissue.

To determine the true origin of the parathyroid glands in the chick embryo, we grafted the third branchial arch (including the epiphyaryngeal placode), with or without the third pharyngeal pouch, to establish the participation of ectodermal and endodermal tissues in the origin of the parathyroid glands.

Materials and Methods

Fertilized White Leghorn chick embryos were incubated at 37.8 °C. The donor embryos were windowed at stage 19 of Hamburger and Hamilton (HH) [1951], i.e. on embryonic (E) day 3. The ventral half, dorsal half or entire third branchial arch was isolated, with a microscalpel made from a needle. The isolated piece of the third branchial arch was grafted onto the anterior limb bud (50%) or the celomic cavity (50%) of an HH stage 19 host embryo (E3) as described previously [Mérida-Velasco, 1991]. Figure 1A describes the different steps of the operation: (1) surgical removal of the entire third donor branchial arch, (2) placement of the isolated piece of the graft in Tyrode's solution [Moscona and Moscona, 1952] and division in ventral and dorsal halves, and (3) implantation of the piece of the donor embryo into the host: ventral half (41 cases), dorsal half (15 cases), or entire third branchial arch (11 cases). A total of 67 chick embryos were grafted. Figure 1B, C shows, in a frontal section of the ventral (fig. 1B) and dorsal (fig. 1C) half of the third donor branchial arch, the tissues included in the graft, i.e. the epiphyaryngeal placode, the third arterial arch, and the ectomesenchyme (fig. 1B), or the third pharyngeal pouch, the third arterial arch, and the ectomesenchyme (fig. 1C). The eggs were returned to the incubator to allow further development of the embryos. Grafted embryos were harvested at HH stages 22–36 (E3.5–E10); 39 of them survived (the ventral half: 29 cases, dorsal half: 6 cases and entire third branchial arch graft: 4 cases). Immediately after removal from the shell, the embryos were fixed in 15% neutral formaldehyde for 6–10 days, embedded in paraffin and cut transversely into 10-μm sections. After dewaxing, the sections were stained with Harris' hematoxylin-eosin [McManus and Mowry, 1968].

Results

The morphogenetic time table of the chick embryo parathyroid III gland was described by García-García et al. [1987]. To determine the origin of this gland, we grafted the third branchial arch. In 29 cases, the grafted piece, which was from the ventral half of the branchial arch, was composed of the epiphyaryngeal placode and the ectomesenchyme (fig. 1B). In 6 cases (when the grafted piece corresponded to the dorsal half of the branchial arch), the structures that subsequently formed were the third pharyngeal pouch and the ectomesenchyme (fig. 1C). The graft
Fig. 1. A. Scheme showing the different steps of the operation: (1) surgical removal of the entire third donor branchial arch; (2) placement of the isolated piece of the graft in Tyrode's solution, and division in ventral (a) and dorsal (b) halves, or placement of the whole third branchial arch (c); and (3) implantation of the piece of the donor embryo into the host. B. HH stage 19, E3. Frontal section of the ventral half of the third donor branchial arch. Observed tissues included the epipharyngeal placode (E), the third arterial arch (T), and the ectomesenchyme (M). There was no evidence of the third pharyngeal pouch. C. HH stage 19, E3. Frontal section of the dorsal half of the third donor branchial arch. Observed tissues included the third pharyngeal pouch (6), the third arterial arch (T), and the ectomesenchyme (M). There was no evidence of the epipharyngeal placode.
Fig. 2. Grafts of the ventral half of the third branchial arch. HH stage 27, E5 (A) and enlargement of A (B): The epipharyngeal placode of the grafted third branchial arch began to invaginate forming a cylindrical structure (1) with a lumen that remained anchored to the ectodermal layer. There was no evidence of the third pharyngeal pouch. 2 = Anterior limb bud; 4 = peritoneal cavity. HH stage 28, E5.5 (C) and HH stage 30, E6.5 (D): In these cases we observed a cord-like structure which we interpreted as the parathyroid III gland (3). There was no evidence of the third pharyngeal pouch. 2 = Anterior limb bud; 3 = right bronchus.

fused with the host tissues and continued to develop, allowing us to analyze the participation of both ectoderm and endoderm in parathyroid III gland organogenesis.

During the HH stage 27 (E5), organization of the parathyroid III gland from the epipharyngeal placode began, as we had previously established (García-García et al., 1987). In the HH stage 27 (E5) host embryos, we observed two significant features. The epipharyngeal placode of the grafted third branchial arch began to invaginate, forming a cylindrical structure with a lumen that remained anchored to the ectodermal layer (fig. 2A, B). There was no evidence of the third pharyngeal pouch (fig. 2B), because the graft was from the ventral half of the third branchial arch. Hence, the structure that appeared was necessarily ectodermal, not endodermal, in nature, as the latter tissue was not present in the graft.

From the HH stage 28 (E5.5) on, the parathyroid III gland continued to develop into a solid cord-like structure. This pattern of development was clearly observed in the graft in HH stage 28 (E5.5, fig. 2C) and 30 (fig. 2D) host embryos. In these cases, because the graft was from the ventral half of the third branchial arch, there was no evidence of the third pharyngeal pouch. Hence, the cord-like structure which we interpreted as the parathyroid III gland could not be endodermal in nature, but must have been ectodermal. However, when the graft was from the dorsal half of the third branchial arch, we observed only the organization of the third pharyngeal pouch (fig. 3).
In eleven embryos (four of them survived) we grafted the whole third branchial arch to study the development of the epipharyngeal placode together with the third pharyngeal pouch. In these four cases we observed the presence of the third pharyngeal pouch, in the vicinity of the third branchial arch cartilage (fig. 4A). Caudally to the pharyngeal pouch, we observed a cord-like structure which we interpreted as the parathyroid III gland (fig. 4B).

Discussion

Our findings suggest that the epipharyngeal (ectodermal) placode of the grafted third branchial arch (and not the third pharyngeal pouch) gives rise to the parathyroid III gland. During the HH stage 27 (E5) the gland was formed from the ectodermal layer (epipharyngeal placode) of the grafted ventral half of the third branchial arch. Further development and structuring took place through a process of invagination, during which the gland remained joined to the epipharyngeal placode from which it developed. This structure could not have developed from endodermal material, because no part of the third pharyngeal pouch was present in the graft.

Fig. 4. Grafts of the entire third branchial arch. HH stage 34 (E8). A Donor third pharyngeal pouch (6). B Caudally to the latter we observed the parathyroid III gland (3).
When we grafted the dorsal half of the third branchial arch, we observed the organization of a pouch-like structure, i.e., the third pharyngeal pouch; however, the parathyroid III gland did not develop. On the other hand, when we grafted the entire third branchial arch, we observed the organization of a pouch-shaped structure in the middle of the graft, and a cord-like (glandular) structure (parathyroid III) in an eccentric part of the graft. There was no connection between the two of them, hence the latter is unlikely to have developed from the former.

Our findings disagree with those of a number of studies [Weller, 1933; Politzer and Hahn, 1935; Norris, 1937; Schrier and Hamilton, 1952; Van Dyke, 1959; Kayser et al., 1961; Nevalainen, 1969; Ribes and Puchades, 1969; Cordier and Haumont, 1980; Hilfer and Brown, 1984] which claimed an endodermal origin for the parathyroid III gland. Moreover, with regard to studies in human embryos, we established [García-García et al., 1985] that the absence of the third pharyngeobranchial membrane may suggest a common origin for the thymic and parathyroid III anlagen. The parathyroid III, in this case, appears to be nothing more than a diverticular expansion of the dorsal wall of the thymic anlage, to the degree that their respective lumina can be clearly interconnected, and may open to the exterior of the embryo [see fig. 7 in García-García et al., 1985]. We believe that this is what has induced previous authors to support the theory that the parathyroid III developed from the dorsal surface, whereas the thymus arose from the ventral surface of the third pharyngeal pouch. This misinterpretation of ontogenetic events was most likely based upon other investigators' reluctance to suggest an objective explanation for the continuity between the two lumina, which in our opinion is the result of the absence of the third intermediate pharyngeobranchial membrane, and of the apposition and fusion of the opposing surfaces of the third branchial cleft. These two phenomena together give the impression that the two anlagen form a single structure. Schrier and Hamilton [1952] marked the third and fourth visceral clefts and pouches of the chick with particles of carbon on embryonic day 4. Subsequently, the carbon was found either inside the parathyroids or the thymus on the marked side, or in the mesenchyme immediately surrounding these glands. The authors concluded that the parathyroids are derived from the third and fourth visceral pouches. We believe these authors misinterpreted their results, because it is hard to understand how the appearance of carbon marks inside the parathyroids after marking of the ectodermal surface of the embryo can be taken as evidence that the parathyroids III developed from the third pharyngeal pouch. Cordier and Haumont [1980], in their study on Naval Medical Research Institute (NMRI) mouse embryo- and nude mice at 10.5 days of development, claimed that the ectoderm of the dorsal part of the fourth arch, bounded by the third and fourth clefts, was thickened and protruded into the underlying mesenchyme. They considered this invagination to be the cervical vesicle, which does not derive from the closing of the cervical sinus. According to these authors, the ventral part of the cranial edge of the cervical vesicle was an extension of the third cleft, which was fused with the midportion of the third pouch. These authors reported that on the day 11, the ectoderm of the third branchial cleft proliferated, mainly in NMRI mice, to cover the distal edge and a small part of the cranial and caudal walls of the third pouch. The cervical vesicle then rotated and became attached to the endoderm. As a result, the cervical vesicle was situated at the cranial pole of the complex, of which it occupied the distal part. Between days 11 and 11.5, the parathyroid primordium appeared at the same time in nude and NMRI mice as a cluster of modified epithelial cells within the cranial wall of the third endodermal pouch. We think that the ectodermic cervical vesicle described by Cordier and Haumont [1980], which became attached to the endoderm was actually the parathyroid gland primordium. Hilfer and Brown [1984], in their study of pharyngeal endocrine organs in mouse and chick embryos, proposed that 'when the embryo is cleaved in cross-section the pouch can be oriented to allow inspection of the inner surface, revealing two evaginations, one dorsal and the other ventral. These are the thymic and parathyroid primordia respectively. Cleaving the embryo or sectioning through the level of the evaginations results in distortion to the extent that the evagination becomes recognizable in the chick.'

The development of a well-formed parathyroid III gland in host embryos suggests that interactions between mesenchyme and epithelia were responsible for its organization, as hypothesized by Bockman and Kirby [1985]. These authors had previously shown [Bockman and Kirby, 1984] that ablation of the neural crest was frequently followed by the nonformation or unilateral reduction of the parathyroid glands. Therefore, normal development of noncardiovascular structures in the pharyngeal apparatus was also dependent upon cardiac neural crest-derived cells, i.e., a portion of the neural crest between the midotic placode and the caudal boundary of somite 3, in pharyngeal arches 3, 4 and 6 [Bockman and Kirby, 1984; Van Mierop and Kutsche, 1986; Kirby and Waldo, 1990; Kuratani and Kirby, 1991]. These structures include the thymus, parathyroid and thyroid glands.

On the other hand, Adelman [1925] and Da Costa [1931] noted that the neural crest in the rat and the cerebral nerve
ganglia beneath the epithelium in guinea pig caused local thickenings in the ectoderm through processes of induction. These observations imply that neural crest cells must participate in parathyroid gland development [Le Lübvre and Le Douarin, 1975; Le Douarin, 1982; Noden, 1983; Bockman and Kirby, 1984, 1985; Weber et al., 1991]. In agreement with these authors, our findings suggest that the parathyroid III gland begins to develop from the epitharyngeal placode of the third branchial arch, presumably in concert with inductive forces of cardiac neural crest-derived cells.

Thereafter, the developing parathyroid III gland separates from the epitharyngeal placode (HH stage 28, E5.5), and presumably together with neural crest-derived cells that give rise to its mesenchymal components [Le Lübvre and Le Douarin, 1975; Le Douarin, 1982; Noden, 1983; Bockman and Kirby, 1984, 1985], subsequently forming the parathyroid III gland as seen in our grafts from the HH stage 28 (E5.5).

Our hypothesis regarding the ectodermal origin of the parathyroid III gland is further supported by the presence in the parathyroid glands of chromogranin-A [Morrissey et al., 1980; Cohn et al., 1981; Cohn and Elting, 1983], which is co-stored and co-secreted by the parathyroid glands together with parathormone [Cohn et al., 1982; Arps et al., 1987; Bajpai and Hamilton, 1990]. Moreover, as established by Le Douarin [1982], peptide-secreting cells should be considered part of the nervous tissue, just as the adrenal medulla and the neurohypophysis. Because of the multiple cellular source of several recently discovered peptides, the APUD concept has now been developed and the neural crest is no longer considered as the only possible source of polypeptide-hormone-secreting cells [Le Douarin, 1982].

These latter are in fact derived, as are all neurons, from the neuroectoderm, which includes the neural tube, the neural crest and also the specialized ectoderm of the placodes [Pearse, 1976; Pearse and Takor-Takor, 1976]. According to our conception of the origin of the parathyroid III gland, this gland can be included in the APUD [Pearse, 1977] or the paraneuronal [Fujita, 1980] system.

This new concept of the origin of the parathyroid III gland is currently being investigated in our laboratory with immunocytochemical techniques, to elucidate how neural crest-derived cells participate in the organization of the parathyroid glands, and to determine whether participation of the epitharyngeal placode in the development of the parathyroid glands is concurrent with the organization of the nodose ganglion.

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References


