Immunohistochemical Study of Cyst Structures in Chick Ultimobranchial Glands

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Summary. In order to clarify the functional significance of cyst structures located in ultimobranchial glands, chick ultimobranchial glands of various ages were investigated by an immunoperoxidase method using anti-calcitonin, anti-somatostatin and anti-19S-thyroglobulin antisera. At hatching, the cysts were consistent features of the chick ultimobranchial glands. With aging, they gradually increased in volume and number. In adult chickens, a major portion of ultimobranchial glands was occupied by cysts of various sizes, shapes and luminal contents. Some cyst epithelium consisted of calcitonin-positive cells alone, and a portion of the cystic contents was intensely immunoreactive to the calcitonin antiserum. Neither cyst epithelium nor luminal contents were stained with the 19S-thyroglobulin or the somatostatin antiserum. The present study indicates that some cyst structures of chick ultimobranchial glands are related to the synthesis, secretion and accumulation of calcitonin.

In mammals, C (parafollicular) cells which synthesize and secrete calcitonin are distributed within the thyroid gland, whereas in lower vertebrates—including birds—they are grouped in separate organs, the ultimobranchial glands. Besides the existence of calcitonin-producing cells, a characteristic feature of the ultimobranchial glands of submammalian vertebrates is the presence of cystic cavities containing a colloid-like material (Watzka, 1933; Sehe, 1965). As is well known, chick ultimobranchial glands consist of cysts and cell cords. Although there are detailed studies on the ultrastructure of the cysts in chick ultimobranchial glands (Isler, 1973; Chan, 1978; Hodges, 1979), little information is available with regard to immunohistochemical reactions of the cysts, and their functional significance is still unknown. In dog C cell complexes, which are remnants of embryonic ultimobranchial bodies, cyst structures also occur consistently (Kameda, 1971, 1982a), and the colloid-like substance in cyst lumina exhibits an intensive immunoreaction to thyroglobulin (Kameda, 1982a). In order to clarify the functional significance of the cyst structures located in ultimobranchial glands, the present study examines them in chickens of various ages by an immunoperoxidase method using anti-calcitonin, anti-somatostatin and anti-19S-thyroglobulin antisera.
MATERIALS AND METHODS

Chickens (White Leghorn) of various ages, from newly hatched to 6-month-old specimens were used, as well as adult animals.

The ultimobranchial glands, together with the surrounding tissues, were fixed in Bouin's solution for 24-48 hrs, embedded in paraffin and then cut into 5 μm thick serial sections. Some sections were stained with hematoxylin-eosin and periodic acid-Schiff (PAS) reaction. For immunological staining, an unlabeled antibody-enzyme bridge technique was used as previously described (KAMEDA and IKEDA, 1978). Three specific antisera were employed: anti-porcine calcitonin, anti-somatostatin and anti-chick 19S-thyroglobulin antisera. The preparation and characterization of each antiserum have been described previously (KAMEDA and IKEDA, 1979; KAMEDA et al., 1982; KAMEDA, 1984). All antisera were diluted to various titers (1:20 to 1:1000 dilutions) and used. Control reactions included replacing the primary antiserum with normal (nonimmune) rabbit serum and absorbing the primary antiserum with an excess of the antigen.

RESULTS

As described elsewhere (KAMEDA, 1984), chick ultimobranchial glands during embryonic development are arranged as a compact body consisting of solid cell clusters with a well developed blood supply. At 18 days of incubation, cystic cavities begin to appear in the ultimobranchial glands. At 1 day after hatching, the cyst structures become a consistent feature of the ultimobranchial glands. With maturation, as the ultimobranchial glands are enlarged, the cysts gradually increase in volume and number; there is also an increase in invasion by loose connective tissues.

Figures 1-3 show an ultimobranchial gland from a 2-month-old chicken. Almost all of the epithelial cells distributed in the ultimobranchial glands exhibited the immunoreaction for calcitonin (Fig. 2). The calcitonin cells were also distributed in thymus tissues located in the ultimobranchial glands (Fig. 2, 4). A majority of epithelial cells bordering the cyst lumina and some of the luminal contents reacted with the calcitonin antiserum (Fig. 2, 4). No immunoreaction for somatostatin was detected in the ultimobranchial glands; neither epithelial cells nor the cyst contents were stained with the somatostatin antiserum (Fig. 3).

Since both connective tissues and cysts increased more and more with age, the number of calcitonin-positive cells per unit area was very small in adult chickens as compared with that in young animals; small groups of calcitonin cells were interspersed among the cysts and connective tissues in adult ultimobranchial glands (Fig. 5, 6). A major part of the adult ultimobranchial glands was occupied by the cyst structures.
Fig. 1-3. Legends on the opposite page.
Fig. 4. Higher magnification of the insert in Figure 2. Single squamous cells of cyst epithelium and some of the luminal contents (arrow) are immunoreactive to the calcitonin antiserum. The calcitonin cells are also distributed in thymus tissue ($T$) located in the ultimobranchial gland. C cysts.  × 290

Fig. 5-7. Legends on the opposite page.
Cysts in Chick Ultimobranchial Glands

Fig. 5-7. An ultimobranchial gland from an adult chicken stained by three different methods. C: cyst. **Fig. 5.** Hematoxylin-eosin staining. There are a large number of cysts varied in size, shape and luminal content. The epithelial cells are interspersed among the cysts and connective tissues and the number of the cells per unit area is very small. ×90. **Fig. 6.** Immunoperoxidase staining using anti-calcitonin antiserum. The cyst epithelium is intensely immunoreactive to the antiserum. ×290. **Fig. 7.** Immunoperoxidase staining using anti-19S-thyroglobulin antiserum. Secretory products of the cyst lumina are non-reactive and there are no cells immunoreactive to the antiserum. ×180
Y. KAMEDA: exhibiting various sizes, shapes and luminal contents (Fig. 5). The cysts were frequently round in shape, occasionally occurring as tubes or ducts which might be straight, curved and branched. They were usually lined with a single layer of cuboidal cells, though sometimes also with single or stratified squamous cells (Fig. 6, 8). Cysts covered with mucous cells were very rarely observed (Fig. 9). The interior of the cysts either appeared empty, or contained variable proportions of an amorphous secretion (Fig. 5). The secretory products in the cyst lumina usually displayed coarse reticular or granular features, and materials resembling the colloid of thyroid follicles were also observed. They were PAS-positive in variable degrees, from weak to intense (Fig. 8). In a large number of cysts, either whole epithelial cells bordering cyst lumina were immunoreactive to the calcitonin antiserum or numerous calcitonin-positive cells were dispersed in the cyst epithelium (Fig. 6). The calcitonin cells were contiguous with the cyst lumen. The immunoreactivity for thyroglobulin was not detected in any of the cysts examined, and there were no cells immunoreactive to the 19S-thyroglobulin antiserum in the ultimobranchial glands (Fig. 7).

DISCUSSION

Cystic structures storing a colloid-like substance are characteristic features of the ultimobranchial gland in lower vertebrates, including birds (WATZKA, 1933; SEHE, 1965).
In adult chickens, the largest proportion of the ultimobranchial glands was occupied by numerous cysts of various sizes, shapes and luminal contents. During embryonic development, however, the ultimobranchial glands consist of solid clusters of small polygonal cells, and cysts are very rarely present in them (KAMEDA, 1984). After hatching, the cyst structures rapidly increase in size and number and become a constant feature of the ultimobranchial glands. The ultrastructure of ultimobranchial cysts in chickens has been reported by several authors (STOECKEL and PORTE, 1969; ISLER, 1973; CHAN, 1978; HODGES, 1979). They reported that the cyst epithelium consists of two (STOECKEL and PORTE, 1969; ISLER, 1973) or four (CHAN, 1978) cell types; in any case, the secretory cells corresponding to the mammalian thyroid C cells are never in contact with the cyst lumen. In contrast to these observations, the present immunocytochemical study clearly indicates that in some cysts the whole epithelium can consist exclusively of the calcitonin-positive cells and some of the secretory products in cyst lumina exhibit a very intensive immunoreaction for calcitonin. Even when a few calcitonin cells were dispersed in the cyst epithelium, they remained in contact with the cyst lumen. However, there were also numerous cysts showing no immunoreaction for calcitonin. It can be considered, therefore, that in chick ultimobranchial glands, some cysts are involved in the synthesis, secretion and accumulation of calcitonin. This is not surprising, since in the fish and amphibian ultimobranchial glands present a follicular gland, as they typically consist of a single follicle or cyst with a central lumen and the cells containing secretory granules send microvillous processes out into the lumen (COLEMAN, 1975; HOOKER et al., 1979). In trout ultimobranchial glands, a large proportion of the cyst epithelium demonstrate an immunoreaction for calcitonin (McMILLAN et al., 1976). In dog thyroid glands there are C cell follicles which are lined solely by C cells and which accumulate a colloid-like substance in the luminal cavities (KAMEDA, 1982b). The colloid-like substance of C cell follicles is PAS-positive and immunoreactive to the calcitonin antiserum in variable degrees (KAMEDA, 1982c). Thus, the cyst structures of chick ultimobranchial glands, whose whole epithelium is composed of calcitonin-positive cells alone, seem to be comparable to the C cell follicles of dog thyroid glands.

The embryonic ultimobranchial bodies of mammalian species disperse into the thyroid gland to form C cells. In dogs, however, they remain partially separate to form C cell complexes (KAMEDA et al., 1980). In C cell complexes there are follicular cells in various stages of differentiation, i.e., the cell clusters not yet organized into follicles, primordial follicles with small lumina and comparatively enlarged follicles storing plentiful amounts of colloid (KAMEDA, 1971; KAMEDA and IKEDA, 1980). The follicular cells in these C cell complexes reveal an immunoreaction for 19S-thyroglobulin; the follicles also have an ability to incorporate radioiodine (KAMEDA et al., 1981). Therefore, in mammals, functional thyroid follicles can arise from the ultimobranchial bodies. In addition, the C cell complexes usually contain cyst structures lined mainly by a single layer of cuboidal cells and storing variable amounts of secretory products in their lumina; they are intensely immunoreactive to the 19S-thyroglobulin antiserum but hardly accumulate any silver grains after injection of Na\(^{125}\)I (KAMEDA, 1982a). Calcitonin-containing C cells are distributed in the cyst epithelium of C cell complexes, but the luminal contents of the cysts are not immunoreactive to calcitonin. The cysts in C cell complexes seem to synthesize and store a thyroglobulin-like glycoprotein, though they are not directly involved in thyroid hormone synthesis. In contrast to the cysts of dog C cell complexes, cyst structures of chick ultimobranchial glands were not stained with the 19S-thyroglobulin antiserum. Furthermore, there were no follicu-
lar cells immunoreactive to the 19S-thyroglobulin antiserum. In short, chick ultimobranchial glands show no specific thyroidal properties. It has been reported that the cysts of ultimobranchial glands in some reptilia and birds—including the chicken—do not incorporate radioiodine (Sehe, 1965; Almqvist et al., 1971). Thus, the properties of ultimobranchial glands in lower vertebrates are somewhat different from those of mammalian ultimobranchial remnants.

Mammalian thyroid C cells do reveal an immunoreaction for somatostatin, though the somatostatin contents in the thyroid glands vary from species to species (Kameda et al., 1982). In chick ultimobranchial glands, no immunoreactivity for somatostatin was observed; neither the calcitonin cells nor cyst structures were stained with the somatostatin antiserum.

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