Distribution of C Cells in Monkey Thyroid Glands as Studied by the Immunoperoxidase Method Using Anti-Calcitonin and Anti-C-Thyroglobulin Antisera

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Summary. The distribution of C cells in the thyroid glands of eight young adult monkeys (4 Macaca fuscata and 4 Macaca irus) was investigated by complete serial sections utilizing the immunoperoxidase method with anti-human calcitonin and anti-dog C-thyroglobulin antisera. The secretory granules of monkey C cells were strongly immunoreactive to both antisera. A markedly uneven distribution of C cells was found. The C cells were distributed in a small area restricted to the dorsomedial portion of the lobe along the longitudinal axis below the parathyroid IV, while the remaining larger portions were devoid of C cells. In the areas where the C cells were concentrated, the cells were scattered, surrounding a large portion or even the whole of the epithelium lining follicles and often forming multiple clusters among the follicles. In these places the C cells exceeded the number of follicular cells.

In the monkey thyroid gland, parafollicular (C) cells are not readily stained by the lead-hematoxylin, HCl-basic dyes and silver impregnation known as selective staining methods for C cells. Accordingly, to date there has been little information on the morphology and distribution of monkey C cells.

The C cells synthesize and secrete calcitonin, a polypeptide hormone which lowers plasma calcium. The cells are specifically stained by immunocytochemical methods using antiserum to calcitonin. In addition, the C cell secretory granules of most of the mammalian species investigated react to the antiserum generated against C-thyroglobulin (C-Tg), a thyroglobulin component having the greatest molecular weight (KAMEDA and IKEDA, 1978a, b, 1979). The present study has established the existence of C cells in monkey thyroid glands by the use of the immunoperoxidase method with the antiserum raised against human calcitonin, and has answered the query of whether or not the C cells in the monkey as well as those in a variety of other mammals reveal immunoreactivity for C-Tg.

There is a considerable variation in the distribution of C cells within the thyroid lobes from species to species: in certain species having no parathyroid IV (e.g., rats, mice) C cells are predominantly observed in the central portions of the thyroid lobes; in others (e.g., dogs) C cells tend to be concentrated around the parathyroid IV, though they are observed in all parts of the thyroid lobe (KAMEDA, 1971a). In some species (e.g., rabbits, cats) they are restricted to the region immediately adjacent to the
parathyroid IV, and the areas far from the parathyroid IV are devoid of C cells (Kameda, 1971b, 1981). In human thyroid glands, the distribution of C cells has been studied by many investigators using specific immunocytochemical methods (Kalina et al., 1970; McMillan et al., 1974; Wolfe et al., 1974) or histological staining methods such as lead-hematoxylin and silver impregnation (Solcia et al., 1970; Roediger, 1973). They, however, have given conflicting data. The monkey thyroid glands seem to be the best material for a comparison with human subjects. Moreover, a systematic study of the whole lobe is easy to perform, as the monkey thyroid is far smaller in size than the human gland. In the present study, the distribution of C cells in monkey thyroid glands is investigated systematically, employing an immunoperoxidase method with anti-calcitonin and anti-C-Tg antisera.

MATERIALS AND METHODS

Eight young adult monkey (4 Macaca fuscata and 4 Macaca irus) of either sex were used. The thyroid lobes were fixed in Bouin's solution and occasionally in GPA solution (25% glutaraldehyde, 1 vol., saturated aqueous solution of picric acid, 3 vol., and acetic acid to give 1%) for 24–48 hrs. The specimens were embedded in paraffin. One lobe from each gland was cut into longitudinal total serial sections 7 μm in thickness. The other lobe was cut in 5 μm nonserial sections. Some sections were stained with hematoxylin-eosin, periodic acid-Schiff (PAS), lead-hematoxylin and Davenport's silver impregnation. The immunocytochemistry was carried out according to the unlabeled antibody-enzyme bridge technique as previously described (Kameda and Ikeda, 1978b). Two specific antisera were employed: anti-human calcitonin and anti-dog C-Tg antisera. The preparation and serological studies for each antiserum have been described previously (Kameda and Ikeda, 1979; Kameda, 1981). Control reactions included replacing the primary antiserum with normal (non-immune) rabbit serum and absorbing the primary antiserum with an excess of the antigens (synthetic human calcitonin and dog C-Tg).

RESULTS

In the two species of monkey investigated (Macaca fuscata and Macaca irus), the thyroid grand consisted of two separated lobes connected with the trace of isthmus. The parathyroid III (external parathyroid gland) lay in the middle between the upper and lower poles of the lobes at the lateral site, or occasionally at a more caudal level. The parathyroid IV (internal parathyroid gland) was embedded within the thyroid parenchyma on the tracheal surface and always located further into the upper portions than the parathyroid III.

The C cells of monkey thyroid glands were specifically stained by immunoperoxidase method using anti-human calcitonin antiserum; the cytoplasm was filled with numerous secretory granules immunoreactive to the antiserum (Fig. 1). They were clearly identifiable and distinguishable from nonreactive follicular cells, colloid and connective tissues. The secretory granules of C cells were also stained densely with the anti-dog C-Tg antiserum (Fig. 2). The reaction pattern of monkey C cells to the anti-C-Tg antiserum was completely identical with that to the anti-calcitonin antiserum (Fig. 1, 2). Individual C cells were of an oval to polyhedral shape. They were distributed as a small group of cells or as single cells located close to the basal portion of the follicular epithelium or in the interfollicular position.

The C cells were distributed in a small zone restricted to the dorsomedial parts of
the thyroid lobes along a longitudinal axis below the parathyroid IV, and extensive portions of the thyroid parenchyma were devoid of C cells (Fig. 3, 4). They were most markedly concentrated in portions directly beneath the parathyroid IV, and from that level the frequency of occurrence of C cells diminishd gradually towards the lower pole. The distribution of C cells throughout the lobes was similar in all the eight thyroid glands examined. The distribution of C cells is diagrammatically shown in Figure 5.

Fig. 1 and 2. Immunoperoxidase stainings of adjacent serial sections of a monkey thyroid gland. Fig. 1 was stained with human calcitonin antiserum and Fig. 2, with dog C-Tg antiserum. The secretory granules of C cells are strongly immunoreactive to both antisera. Although anti-C-Tg antiserum is absorbed with 19S-thyroglobulin, a faint immunoreaction of follicular cells and luminal colloid is still observed. F follicle. × 720
Fig. 3. Distribution pattern of C cells in a monkey (*Macaca irus*) thyroid gland, stained by the immunoperoxidase method using anti-calcitonin antiserum. Coronal section. C cells are concentrated below the parathyroid IV (PT IV) at the medial site. PT III parathyroid III, F follicle. ×25

Fig. 4. Distribution pattern of C cells in a monkey (*Macaca fuscata*) thyroid gland, stained by the immunoperoxidase method using anti-calcitonin antiserum. A parasagittal section through the midregion of the parathyroid IV. The C cells are scattered, forming a band-like stream along the longitudinal axis of the lobe. The larger portions of the thyroid lobe are devoid of C cells. F follicle. ×25

Fig. 5. Diagrammatic representation of C cell distribution in total serial sections of the right thyroid lobe as seen from a lateral view. Each dot represents an area of several C cells. Note the limited distribution of C cells in the dorsomedial portion of the lobe along the longitudinal axis below parathyroid IV (PT IV). PT III parathyroid III.
In the thyroid regions where the C cells were numerous, there were many follicles of which a large portion or even the whole of their epithelium was surrounded with the C cells, and among the follicles multiple clusters of C cells were present (Fig. 6). In these places C cells predominated over the number of follicular cells.

**Fig. 6.** Immunoperoxidase staining with anti-calcitonin antiserum of the thyroid area containing numerous C cells. C cells surround almost all portions of the follicular epithelium. F follicle. × 290

**Fig. 7.** Accessory parathyroid gland (PT) situated below the parathyroid IV, stained by the immunoperoxidase method using anti-calcitonin antiserum. C cells filled with immunoreactive secretory granules are also dispersed among the parathyroid cells. F follicle. × 290
No C cells were found in the parathyroid III and parathyroid IV. However, in the accessory parathyroid glands located below the parathyroid IV, C cells were again encountered (Fig. 7).

DISCUSSION

Immunoreactivity for C-Tg has been observed in C cells of the thyroid gland in a wide variety of mammalian species, i.e., dogs, rats, rabbits, hamsters, mice, cats, goats, cows and humans (KAMEDA and IKEDA, 1978a). As in these species, the secretory granules of C cells in the monkey were strongly immunoreactive to the anti-C-Tg antiserum. Thus, the relationship of C-Tg to C cells was also present in monkey thyroids. Thyroglobulin, which is a glycoprotein essential for the formation of thyroid hormones, is heterogeneous. In addition to the main protein component, 19S-thyroglobulin, there are several components with slower and faster sedimentation coefficients. Among these thyroglobulin components, only the antiserum generated against C-Tg specifically reacts to the secretory granules of C cells in addition to luminal colloid and follicular cells (KAMEDA and IKEDA, 1978b, 1979). It is believed that C cells, in addition to secreting calcitonin, synthesize a glycoprotein resembling thyroglobulin, although the C cells have no ability to incorporate radioiodine and are not functionally related to the thyroid hormone synthesis (KAMEDA et al., 1981). In fact, the C cell follicles, i.e., small follicles composed solely of C cells, are found in dog thyroid glands; the lumina of C cell follicles store a colloid-like substance which is PAS-positive and immunoreactive to the anti-C-Tg antiserum (KAMEDA, 1982).

It is well known that thyroid C cells are derived from the ultimobranchial bodies (PEARSE and CARVALHEIRA, 1967; JORDAN et al., 1973; KAMEDA et al., 1980). During the early fetal period, the ultimobranchial bodies of many mammals develop from the pharyngeal pouch IV together with the parathyroid IV and the thymus IV. In progressive stages, the ultimobranchial bodies join the thyroid anlage and then move into the thyroid parenchyma to be dispersed as typical thyroid C cells. Thus, the distribution of C cells is dependent upon the fate of the ultimobranchial bodies during development.

By immunoperoxidase staining using anti-calcitonin and anti-C-Tg antisera, monkey C cells were clearly identified and distinguished from the follicular cells, colloid and connective tissues. The present systematic study gave evidence that, in monkey thyroid glands, C cells were distributed in a small area restricted to the dorsomedial parts of the lobe below the parathyroid IV, and larger portions of the lobe were devoid of C cells. The uneven distribution of C cells is the most conspicuous in the monkey thyroids as compared with that of the other animal species reported to date (KAMEDA, 1971a, b).

In the thyroid glands of many mammalian species, C cells tend to be concentrated surrounding the region of the parathyroid IV. Especially in rabbits and cats is their distribution restricted to the region immediately adjacent to the parathyroid IV (KAMEDA, 1971b, 1981). In monkey thyroid glands, C cells were distributed in portions of the lobe lower than the parathyroid IV, and they were not encountered in the upper pole. It is thought that in the monkey the ultimobranchial body develops from a somewhat lower part of pharyngeal pouches than that of other experimental animals, and after joining the thyroid anlage, invades the lobe caudally from its point of origin.

In several mammalian species, e.g., rabbits, cats, goats, dogs, C cells are scattered within the parathyroid IV, though the cells are never found in the parathyroid III (KAMEDA, 1971a, b, 1981). Furthermore, in cats and dogs they are also distributed within
the thymus IV situated adjacent to the parathyroid IV (Kamed a, 1971a, b, 1981). From their developmental relation with ultimobranchial bodies, it is not surprising that the branchiogenic organs such as the parathyroid IV and thymus IV contain C cells. In monkeys, C cells were observed in the accessory parathyroid IV located in portions of thyroid lobe lower than parathyroid IV, though there were no C cells in the parathyroid IV itself.

There has been only one report to date on the distribution of C cells in monkey thyroid glands using histological staining methods, i.e., silver impregnation and lead-hematoxylin (Das and Das, 1978). They described that C cells are located in the central region of thyroid along the median axis. Such a distribution, however, was not observed in the present eight thyroid glands investigated by immunoperoxidase staining. By histological staining methods, the demonstration of monkey C cells is very difficult compared with that of other experimental animals such as dogs, rabbits, cats, mice and rats. The differences may reflect in part these technical factors.

In their systematic immunocytochemical studies Wolfe et al. (1974) and McMillan et al. (1974) described that in human thyroid glands, C cells are concentrated along the central axis of the lateral lobes in their middle thirds. They did not, however, report the topographic relation with the parathyroid IV concerning the distribution of C cells. According to both the present results in monkey thyroid glands and the distribution patterns of C cells in a variety of other mammals reported, it is considered that human C cells are concentrated in the posterior region of the lateral lobes, particularly near the superior parathyroid as described by Solcia et al. (1970) and Roediger (1973).

The distribution of C cells in monkey thyroid glands was restricted to the small areas of the lobe. In these regions the C cells were distributed surrounding a large portion or even the whole of the epithelium lining a follicle and often formed multiple clusters among the follicles. The C cells exceeded the number of follicular cells in these places, though their concentration per the whole lobe was far smaller compared with that of other mammals such as dogs, guinea pigs and rabbits. Unless normal morphologic features are thoroughly grasped by systematic examination of entire thyroid glands, a wrong diagnosis as "C cell hyperplasia" may be made for these distribution patterns of monkey C cells. It has been generally accepted that nodular multifocal C cell hyperplasia precedes medullary thyroid carcinoma, which is the neoplasm derived from C cells, and increased serum or thyroid content of calcitonin usually accompanies such hyperplasia (Wolfe et al., 1973; DeLellis et al., 1977). On the other hand, several investigators (Janzer et al., 1979; Gibson et al., 1981) have described human subjects having no hypercalcemia or parathyroid diseases in whom large C cell nodules are often observed between thyroid follicles; they represent physiologic rather than neoplastic phenomena. In primates, including man, embryonic cellular migration of ultimobranchial bodies seems to be limited and C cells are only concentrated in certain regions of the thyroid lobes. Therefore, for these species having a markedly uneven distribution of C cells, the serial sectioning of thyroid tissues is important for obtaining correct information regarding the distribution and cell biology of C cells.

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