The Merkel Cell in the Labial Ridge Epidermis of the Anuran Tadpole. II. Electron Microscope Observations on the Appearance and Differentiation of the Merkel Cell

Tamiko TACHIBANA

Department of Oral Anatomy (Prof. T. NAWA), Iwate Medical University School of Dentistry,
Morioka, Japan

Received August 3, 1978

Summary. The appearance and cytodifferentiation of the Merkel cell were studied electron microscopically using the developing labial ridges of *Rana japonica*. The most primitive Merkel cell was identified with specific cored granules with an average diameter of about 90 nm. Such a primitive Merkel cell was found in the basal layer of the primitive labial ridge epithelium. As the primitive labial ridge continued to mature, the primitive Merkel cell accumulated an increased number of the specific granules, developed digital shaped cytoplasmic processes and came into contact with nerve fibers. When the labial ridge was sufficiently developed, the cell showed typical morphological features of the mature Merkel cell except for a small accumulation of tonofilament-like structures and glycogen particles. It was also observed that the position of the Merkel cell in the epithelium changed from the basal layer to the third basal layer during development. On the other hand, it was difficult to find the Merkel cell in the mesenchymal layer of the developing labial ridges. From the results, it was suggested that the cytodifferentiation of the Merkel cell in the labial ridge of an anuran tadpole occurs within the epithelium.

The Merkel cells in the epidermis have been reported in various species of vertebrates (CAUNA, 1962; MUNGER, 1965; MUSTAKALLIO and KIHSTALA, 1967; SMITH, 1967; KUROSUMI et al., 1969; SMITH, 1970; BREATHNACH, 1971; LYNE and HOLLIS, 1971; HASHIMOTO, 1972a, b; NAFSTAD and BAKER, 1973; FOX, 1974; WHITEAR, 1974; BUDTZ and LARSEN, 1975; HALATA, 1975 and many others). However, there is still a difference of opinion as to the development of the Merkel cell. Some have considered that the Merkel cells differentiated from the undifferentiated basal epidermal cells since they usually possess tonofilaments and desmosomes (MUNGER, 1965; KUROSUMI et al., 1969; SMITH, 1970; LYNE and HOLLIS, 1971; ENGLISH, 1974). On the other hand, some have observed the Merkel cell in the dermis of the human fetus (BREATHNACH and ROBINS, 1970; BREATHNACH, 1971; HASHIMOTO, 1972b), and have proposed the dermal origin hypothesis of the Merkel cell. HASHIMOTO (1972b) suggested that the Merkel cell might originate from the neuro-ectodermal cell.

To resolve this discrepancy, it seems to be necessary to clarify the life cycle of the Merkel cell. The author previously found that the labial ridge epithelium of the anuran tadpole involved numerous Merkel cells in uniform arrangement (TACHIBANA,

1978). The present electron microscopical observation aims to clarify the early development of the Merkel cell in the anuran labial ridge.

MATERIALS AND METHODS

The larvae of *Rana japonica* were raised from eggs in the laboratory. Three developmental stages were used for the study: stage 21 (the opening of the stomodeum), stage 23 (operculum fusion, the appearance of the labial ridges) and stage 25 (the appearance of the hind limb buds and horny teeth columns). The developmental stages of embryos were determined according to the normal table of *Rana japonica* by TAHARA (1959).

The head portions including the primordial mouth were cut from each embryo within a Petri dish containing glutaraldehyde (2.5% in phosphate buffer). The specimens were immediately immersed in 2.5% glutaraldehyde and fixed for 2 hrs at 4°C. After being washed with phosphate buffer, specimens were further fixed with 1% osmium tetroxide for 1 hr. Then they were dehydrated with an ethanol series and embedded in epon 812.

To ascertain if the Merkel cell is present in the primordial labia or not, two or three pieces of ultrathin sections were cut at intervals of 5μ in the sagittal plane of the stage 21 embryos, from a length of about 500μ of the primitive labium. On the other hand, the middle portions of the primitive labium of the stage 23 and 25 embryos were sectioned to about 100μ in length. Ultrathin sections were cut serially, interspersing occasionally with semithin sections. This serial sectioning was done to clarify the structure, distribution and the relation to the nerve fibers of the Merkel cells in these stages.

All ultrathin sections were collected serially, counterstained with uranyl acetate and lead citrate, and observed under the electron microscope. The semithin sections were stained with toluidin blue and observed with the light microscope.

Fig. 1. Sagittal section of the mouth of *Rana japonica* embryo at stage 21 stained with toluidin blue. The buccopharyngeal membrane (*bm*) is opening. The primordium of labia (*l*) and jaws (*j*) can be seen. $\times 300$

RESULTS

Stage 21

At stage 21, the buccopharyngeal membrane was ruptured, two ectodermal protuberances appeared on both the dorsal and ventral sides of the anterior border of the primary mouth (Fig. 1). The outer protuberances indicated the primordia of the labia, the inner ones indicated the primordia of the jaws. Each protuberance was composed of stratified epithelium and underlying mesenchyme (Fig. 1). The surface layer of the epithelium of every protuberance was covered by secretory cells. Two types of secretory cells, ciliated and non-ciliated cells were present in a random arrangement. On the other hand, the basal layer of each epithelium was composed of immature ectodermal cells which were characterized by numerous free ribosomes, microtubules, small Golgi complexes, yolk platelets and pigment granules. The

Fig. 2. Sagittal section of the mouth of *Rana japonica* at stage 23 stained with toluidin blue. Three protuberances of the primary ridges can be seen on the ventral labium (*vl*). Note that the surface and basal layers are distinguishable in each epithelium of the ridges. *dl* Dorsal labium, *j* jaw. $\times 370$

Fig. 3. Sagittal section of the ventral labium of *Rana japonica* at stage 25 stained with toluidin blue. Three triangular and one round ridge are seen. In the triangular ridges, the columnar arrangement of the horny tooth cells is formed incompletely in the middle zone (arrows). $\times 600$

horny tooth cell and horny beak cell did not yet appear in the epithelium: therefore it was difficult to distinguish the primary jaws from the primary labia by the epithelial structure at this stage.

The accumulation of the mesenchymal cells was observed under the epithelium of each protuberance (Fig. 1). In the cytoplasm of the mesenchymal cells, yolk platelets and pigment granules were present, but other cytoplasmic organelles were insufficiently organized.

In spite of close observation, it was difficult to discover the nerve fiber, the Merkel cell or the presumptive progenitor cell of the Merkel cell in the mesenchymal layer as well as in the epithelial layer.

Stage 23

By stage 23, the jaws and labia became more distinctive, the epithelium of the jaw showed characteristic arrangement of the epithelial cells which indicated the differentiation of the horny beak cells (Fig. 2).

Simultaneously, the ventral labium formed three low consecutive protuberances of the primary labial ridges, but the dorsal labium was still only a single protuberance (Fig. 2). The three ridges of the ventral labium slightly differed from each other in developmental degree. The outermost ridge was covered by two (or three) layered round epithelium, but the two other ridges were covered by multi-layered triangular epithelium (Fig. 2). The surface layer of every ridge's epithelium was

Fig. 4. Surface layer of the primordial labial ridge epithelium in a stage 23 embryo of *Rana japonica*. Two secretory cells without cilia are shown. Thin nerve fibers are seen in the intercellular spaces of the secretory cells (arrows). ld Lipid droplet. $\times 8,800$

composed of non-ciliated secretory cells and a small number of ciliated cells, whose ultrastructure was not so different from those of stage 21. On the other hand, the rest of these epithelium were chiefly composed of the undifferentiated ectodermal cells which included yolk platelets, pigment granules, microtubules and lipid droplets in their cytoplasm. The mitochondria, granular endoplasmic reticulum and free ribosomes were more numerous than the basal cells in stage 21. The mitosis of the epithelial cell was frequently observed in the basal layer of each primary labial ridge. In both the basal and the surface layer of the epithelium, a number of nerve fibers were observed in the intercellular spaces (Fig. 4, 5).

Specific cells containing osmiophilic cored granules with a diameter of 60–120 nm were distinguished among the epithelial cells in each of the primary labial ridges. In the outermost ridge these cells were present in the base of the epithelium and showed a spherical shape with a large irregular nucleus. There were Golgi-like structures composed of an agranular endoplasmic reticulum near the nucleus. A few free ribosomes, mitochondria and microtubules were scattered throughout the cytoplasm. There were also some yolk platelets, lipid droplets and pigment granules in the cytoplasm (Fig. 6). The serial sections revealed that the specific cells in the

Fig. 5. Basal layer of the primordial labial ridge epithelium in a stage 23 embryo of *Rana japonica*. Numerous nerve fibers (*n*) are seen in the subepithelial mesenchymal layer and in the intercellular space of the basal epithelium. A nerve fiber is invading the epithelium in **B**. *ld* Lipid droplet, *ml* mesenchymal layer, *y* yolk platelet.

A: $\times 83,00$; B: $\times 15,400$

Fig. 6. Primitive Merkel cell in the basal layer of the primary labial ridge epithelium (corresponding to A in Fig. 2) at stage 23 of *Rana japonica* embryo. The Merkel cell granules (arrows) can be seen. Note the reticular Golgi apparatus (G) and desmosome (double arrow). *pg* Pigment granule, *ml* mesenchymal layer, *y* yolk platelet. $\times 18,000$

Fig. 7. Basal layer of the epithelium of the inner primary labial ridge (corresponding to *B* in Fig. 2) at stage 23 of *Rana japonica* embryo. The Merkel cell (*M*) is present in the second cell layer from the base. An increased number of Merkel cell granules is noted. *ld* Lipid droplet, *ml* mesenchymal layer, *pg* pigment granule, *y* yolk platelet. $\times 8,300$

outermost ridge did not attach to the basal lamina of the epithelium or to the nerve ending, but they formed desmosomes with the neighboring epithelial cells (Fig. 6, double arrow).

On the other hand, the specific cells in the two inner primary ridges were chiefly located in the second layer from the base of the epithelium (Fig. 7, 8). The cells were spherical or elliptical in shape and included a slightly higher number of the osmiophilic cored granules (Fig. 7). The Golgi apparatus was composed of well developed smooth vesicles, coated vesicles and cored granules (Fig. 8). A few finger-shaped cytoplasmic projections were sometimes observed in these cells (Fig. 8,

Fig. 8. Peripheral region of the Merkel cell shown in Figure 7. Well developed Golgi apparatuses (*G*) include various types of vesicles. A typical cytoplasmic process of the Merkel cell (arrow) and nerve fibers (*N*) can be seen. $\times 16,700$

arrow). Furthermore, nerve fibers could be found in contact with the cell (Fig. 8).

In this developmental stage, each primitive labial ridge was shallowly indented by the underlying mesenchymal tissue (Fig. 2). There were many unmyelinated nerve fibers without Schwann's sheath in the mesenchymal layer (Fig. 5). Nerve fibers invading the epithelium were frequently observed (Fig. 5). However, no other immigrating cell was observed in the epithelial-mesenchymal junction. The presumptive immature Merkel cell was not found in the mesenchymal layer either.

Stage 25

By stage 25, a fourth protuberance of the labial ridge was added to the three ridges of the ventral labium already formed (Fig. 3), and all primordia of the ventral labial appendages had been formed. On the other hand, the dorsal labium formed two ridges. Ultrastructural observation was limited to the ventral labium.

Fig. 9. Merkel cell (*M*) in the third cell layer from the base of the epithelium of the inner labial ridge (marked by thick arrow in Fig. 3) at stage 25 of *Rana japonica* embryo. Merkel cell granules are increased, and other cytoplasmic organelles are further developed. Note that the Merkel cell-neurite complexes are formed and the Merkel cell granules are accumulated near the complexes (enlarged in **B**). Arrows indicate cytoplasmic processes. A: $\times 12,500$; B: $\times 38,000$

In the cross section, the epithelium of the inner three ridges of the ventral labium showed a triangular arrangement of the epithelial cells, while the outermost ridge was round in shape (Fig. 3). The round ridge indicated the future labial fringe without the horny teeth. In the triangular epithelium of the three ridges the horny tooth cells initiated cytodifferentiation in the middle zone, but their characteristic columnar arrangement was still incomplete. The immature keratinocytes which included a few tonofilaments covered the rest of the epithelium. The secretory cells had disappeared from the apical region of the triangular epithelium, except for the surface epithelium of the groove between the triangular ridges and the round ridge.

In each labial ridge including the round ridge, Merkel cells were found in the basal second or third layer of the epithelium. They were usually elliptical in shape, their long axis was oriented parallel to the basal lamina of the epithelium. Synaptic contacts with nerve endings were usually observed (Fig. 9). The osmiophilic cored granules (Merkel cell granules) were accumulated in the cytoplasm facing the axon terminals (Fig. 9). The Merkel cell granules measured 60–120 nm in diameter (average 90 nm). Several cytoplasmic processes of digital shape occurred on the cell, most of them were directed to the apex of the ridge (Fig. 9). The Golgi apparatus was well organized and the granular endoplasmic reticulum was slightly developed. Glycogen particles and tonofilament-like filaments, which are other cytological features of the mature Merkel cell of the tadpole, were scarcely observed in this stage. It was seen that a few large lipid droplets persisted in these Merkel cells.

DISCUSSION

The developmental origin is one of the problems to be settled concerning the Merkel cell. Two different hypotheses have been proposed. MUNGER (1965), KUROSUMI et al. (1969), SMITH (1970) and others postulated that the Merkel cells differentiated from undifferentiated epidermal cells because tonofilaments and desmosomes were usually included. On the other hand, BREATHNACH and ROBINS (1970) and HASHIMOTO (1972b) observed that the Merkel cells were present not only in the epidermis but also in the underlying mesenchyme of human fetal skin, and postulated that they were immigrated dermal cells. These hypotheses were deduced from morphological observations of mature or immature materials. However, few studies have been made on the early development of the Merkel cell until now. This was attempted in the present study, using the developing labium of the anuran tadpole.

Definitive Merkel cells were first identified in the epithelium of the labial ridge at stage 25 when the labial ridges were almost developed. At stage 23, specific cells containing a number of osmiophilic granules of about 90 nm diameter were recognized in the epithelium of the primary labial ridge. These specific cells slightly varied in morphology with the developmental degree of the primordial labial ridge. In the most primitive ridge, the cells could be distinguished from other immature epithelial cells only by the occurrence of the granules. On the other hand, in the more developed ridge, the cells sometimes showed other structural features, i.e., the digital processes and synaptic contact with nerve fibers. In such cells the cored granules were also increased and more closely resembled the granules of the mature Merkel cell. The appearance of the digital process and the increasing number of

cored granules suggest the gradual cytodifferentiation of the Merkel cell.

TACHIBANA (1978) showed that the Merkel cell in the anuran tadpole accumulates tonofilament-like cytofilaments and glycogen particles in the cytoplasm. These changes, however, could not be found in the Merkel cell at stage 25. This may mean that the accumulation of the cytofilaments and glycogen belongs to a later stage of the Merkel cell differentiation.

In the present study it was difficult to distinguish any presumptive progenitor cell of the Merkel cell in the mesenchymal layer of the primordial labial ridge. It is thus concluded that differentiation of the Merkel cell of the anuran tadpole occurs not in the mesenchymal layer but within the epithelium. However, it does not necessarily indicate that the Merkel cell is of epithelial origin. The possibility may not be excluded that a neural crest cell which immigrates into the epithelium at a very early developmental stage may be the progenitor cell of the Merkel cell. The exact origin of the Merkel cell must be clarified by experimental studies.

無尾両生類幼生の齒列ひだ上皮におけるメルケル細胞について

II. 透過電子顕微鏡によるメルケル細胞の出現と分化の研究

立 花 民 子

ニホンアカガエルの後期胚を用い、発生中の齒列ひだにおけるメルケル細胞の出現と分化を電子顕微鏡で観察した。

もっとも未分化なメルケル細胞は 直径 約 90 nm の有芯顆粒の存在によって 他の未分化な細胞から識別され、このような細胞は 未分化な齒列ひだの上皮中に見いだされた。齒列ひだ原基の形態分化にともない、未分化メルケル細胞は、有芯顆粒の増加、指状細胞質突起の形成、神経線維との接触など、細胞分化の進行を思わせる変化を示した。齒列ひだの形態分化が ほぼ終了したステージ25では、メルケル細胞の分化はかなり進み、トノフィラメント様細胞内線維とグリコゲン粒子の蓄積がまだみられないほかは、成熟メルケル細胞に酷似する形態を示した。また メルケル細胞は細胞分化中に齒列ひだ上皮の基底層から第3層目に移動する。一方 いずれの発生段階でも、齒列ひだ原基の下層の間葉組織中には、メルケル細胞や未分化メルケル細胞と思われるものは認められなかったので、無尾両生類の齒列上皮のメルケル細胞は 上皮内分化を遂げるものと考えた。

REFERENCES

- Breathnach, A. S.: Embryology of human skin: a review of ultrastructural studies. J. invest. Dermatol. 57: 133-143 (1971).
Breathnach, A. S. and J. Robins: Ultrastructural observations on Merkel cells in human foetal skin (Abstract). J. Anat. 106: 411 (1970).
Budtz, P. E. and L. O. Larsen: Structure of the toad epidermis during the moulting cycle. II. Electron microscopic observations on *Bufo bufo*. Cell Tiss. Res. 159: 459-483 (1975).

- Cauna, N.:** Functional significance of the submicroscopical, histochemical and microscopical organization of the cutaneous receptor organs. *Anat. Anz.* 111 (Erganz.-ht.): 181-197 (1962).
- English, K. B.:** Cell types in cutaneous type I mechanoreceptors (Haarscheiben) and their alterations with injury. *Amer. J. Anat.* 141: 105-126 (1974).
- Fox, H.:** The epidermis and its degeneration in the larval tail and body of *Rana temporaria* and *Xenopus laevis* (Amphibia, Annura). *J. Zool. Lond.* 174: 217-235 (1974).
- Halata, Z.:** The mechanoreceptors of the mammalian skin. *Adv. Anat. Embryol. Cell Biol.* 50: 7-75 (1975).
- Hashimoto, K.:** Fine structure of Merkel cell in human oral mucosa. *J. invest. Dermatol.* 58: 381-387 (1972a).
- : The ultrastructure of the skin of human embryos. X. Merkel tactile cells in the finger and nail. *J. Anat.* 111: 99-120 (1972b).
- Kurosumi, K., U. Kurosumi and H. Suzuki:** Fine structures of Merkel cells and associated nerve fibers in the epidermis of certain mammalian species. *Arch. histol. jap.* 30: 295-313 (1969).
- Lyne, A. G. and D. E. Hollis:** Merkel cells in sheep epidermis during fetal development. *J. Ultrastr. Res.* 34: 464-472 (1971).
- Munger, B. L.:** The intraepidermal innervation of the snout skin of the opossum. *J. Cell Biol.* 26: 79-97 (1965).
- : Neural-epithelial interactions in sensory receptors. *J. invest. Dermatol.* 69: 27-40 (1977).
- Mustakallio, K. K. and U. Kiistala:** Electron microscopy of Merkel's "Tastzelle," a potential monoamine storing cell of human epidermis. *Acta dermatovenerol.* 47: 323-326 (1967).
- Nafstad, P. H. J. and R. E. Baker:** Comparative ultrastructural study of normal and grafted skin in the frog *Rana pipiens*, with special reference to neuroepithelial connections. *Z. Zellforsch.* 139: 451-462 (1973).
- Smith, K. R.:** Fine structure and function of the Haarscheibe. *J. comp. Neurol.* 131: 459-474 (1967).
- : The ultrastructure of the human Haarscheibe and Merkel cell. *J. invest. Dermatol.* 54: 150-159 (1970).
- Tachibana, T.:** The Merkel cell in the labial ridge epidermis of anuran tadpole. I. Fine structure, distribution and cytochemical studies. *Anat. Rec.* 191: 487-502 (1978).
- Tahara, Y.:** Table of the normal developmental stages of the frog, *Rana japonica*. *Jap. J. exp. Morphol.* 13: 49-60 (1959).
- Whitear, M.:** The nerves in frog skin. *J. Zool. (Lond.)* 172: 503-529 (1974).

立花 民子

〒020 盛岡市中央通1丁目3の27
岩手医科大学歯学部
第二口腔解剖学教室

Tamiko TACHIBANA

Second Department of Oral Anatomy
Iwate Medical University School of Dentistry
Morioka, 020 Japan