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Anatomical Note

Merkel cell mitoses in vibrissae: an ultrastructural study

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INTRODUCTION

The Merkel cell is an epidermal cell with neuro-endocrine properties. It is currently considered to be of epithelial origin (Munger, 1965; Smith, 1967, 1970; Lyne & Hollis, 1971; Tachibana & Nawa, 1980; Tachibana & Ishizeki, 1981; Ochiai & Suzuki, 1981; Saurat *et al.* 1984; Moll, Moll & Franke, 1984). This view is supported by ultrastructural features (desmosomes) and immunohistochemical properties (low molecular weight cytokeratin expression) (Saurat *et al.* 1984; Moll, Moll & Franke, 1984), and by the existence in the epidermis of immature Merkel cells, the so-called 'transitional cells' (Tachibana & Nawa, 1980; Tachibana & Ishizeki, 1981; Ochiai & Suzuki, 1981). These transitional cells share features of both mature Merkel cells and keratinocytes. During the postnatal period, the transitional cells decrease in number proportionally as ultrastructurally characteristic mature cells increase (Tachibana & Nawa, 1980). Moreover, Tachibana & Nawa stated that the presence of centrioles and microtubules in the transitional cells could indicate the possibility of Merkel cell production by means of transitional cell mitosis. Such structures have also been observed in mature cells (Nikai & Cattoni, 1971; Ochiai & Suzuki, 1981; Mérot, unpublished data). An important fact is seldom put forward: to our knowledge, even in experimental conditions, no mitosis of either immature or mature Merkel cells has been described. This might be because:

(i) The Merkel cell does not divide in the epidermis or its adnexae, but behaves as a terminal cell with a long life span, similar to that of neural cells.

(ii) Cell division may occur but is so infrequent that studies conducted to date by electron microscopic techniques could have missed ultrastructural signs of division.

(iii) Merkel cell division does not exist *per se*, but it is an epithelial precursor of the Merkel cell that divides. Differentiation towards a mature cell then takes place. In that event, the most likely candidate for being the precursor would be the keratinocyte.

The present study demonstrates that, although unusual, Merkel cell mitoses do exist in mouse embryo skin, but occur very early in embryologic development.

MATERIALS AND METHODS

Skin specimens

Pregnant NMRI mice were killed by chloroform inhalation and head specimens obtain from 12, 13 and 14 days old embryos. They were fixed *in toto* in 3% phosphate-buffered glutaraldehyde, postfixed in 1·33% collidine-buffered osmium tetroxide, dehydrated in acetone and embedded in Araldite. Semithin sections 0·5–1·0 μm were stained with methylene blue. Ultrathin sections of the skin were cut

with a Reichert OMU3 ultramicrotome, contrasted with uranyl acetate and lead citrate, and observed with a Philips EM300 at 80 kV.

RESULTS

As part of a continuing study of the development and distribution of Merkel cells in the skin of the newborn mouse and mouse embryos, we systematically performed an ultrastructural study of epidermis, hair follicles and vibrissae. In a developing vibrissa of a 12 days old mouse embryo we observed a suprabasal mitotic cell (prometaphase) showing three dense cored membrane-bound granules and rudimentary desmosome-like structures (Fig. 1). Its cytoplasm contained a few organelles, i.e. mitochondria and rough endoplasmic reticulum, as well as glycogen particles, but no tonofilament aggregates were present. Two adjacent cells also contained dense cored membrane-bound granules typical of Merkel cells.

Furthermore, in two developing vibrissal hair follicles of 13 days old mouse embryos, and in another from a 14 days old mouse embryo, we observed three additional mitotic cells with similar granules. All these cells were lacking tonofilament aggregates but possessed rudimentary desmosome-like structures. They were either located in a suprabasal position or were only separated from the basement membrane by a thin keratinocyte expansion.

DISCUSSION

The observation of cutaneous mitotic cells showing dense cored membrane-bound granules, i.e. neurosecretory type granules, is, to our knowledge, the first demonstration of dividing Merkel cells. Indeed, the presence of such granules is a prerequisite condition for the recognition of an epidermal or an adnexal cell as a Merkel cell; no other kinds of epidermal or adnexal cells contain these organelles.

This finding clearly establishes that mitosis can occur in Merkel cells, even if it does not rule out the possibility that mitoses can also occur in a precursor cell without signs of neuro-endocrine differentiation. Mitotic cells were only observed during the early phases of skin development (gestation days 12 to 14) in the mouse muzzle. However, it does not rule out the possibility that Merkel cell mitoses could take place later elsewhere in the integument. Indeed, it is well known that the cells do not appear simultaneously in all the body integument: there is a cranial–caudal progression in their appearance (English, Burgess & Kavka-Van Norman, 1980; Mérot, unpublished data).

Kinetic studies conducted in rabbits (Mérot *et al.* 1986) confirmed this by showing that 0·21 to 2·59% of cells, which could be reasonably considered as Merkel cells by various techniques (Tachibana & Nawa, 1980; Tachibana & Ishizeki, 1981; Saurat, Chavaz, Carraux & Didierjean, 1983), incorporated [^{3}H]thymidine. The cell proliferation rate remained, however, very low and these figures refer only to rabbit lip epithelium.

Our results indicate that proliferation of Merkel cells can take place in the skin, that mitosis can occur in a cell already showing signs of neuro-endocrine differentiation and that mitoses seem to occur preferentially in early embryonic life.

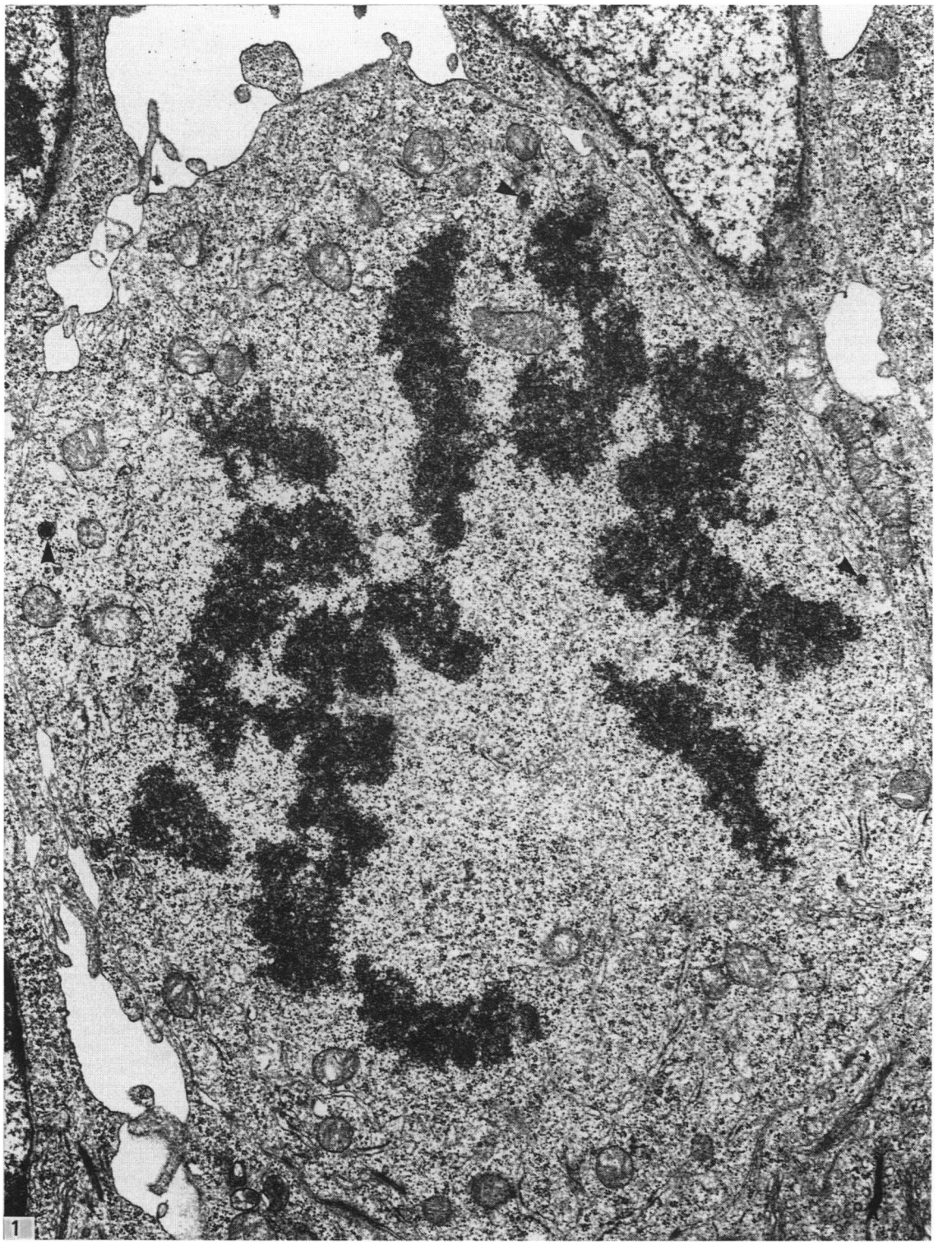

Fig. 1. Vibrissal hair of the lip of a 12 days old mouse embryo. The dividing cell shows 3 dense-cored membrane-bound granules (arrowheads) characteristic of Merkel cell granules. × 28000.

SUMMARY

This is the first ultrastructural description of Merkel cell mitoses. We observed four mitotic cells showing dense cored membrane-bound granules within their cytoplasm, i.e. the unique ultrastructural characteristics of Merkel cells which are not shared by other cutaneous epithelial cell types. These cells were located in vibrissal hair follicles of 12, 13 and 14 days old mouse embryos. This finding indicates that proliferation of Merkel cells can take place in the skin.

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