Virchows Arch A (1987) 410:547–552

Virchows Archiv A

Lamellar cells of sensory receptors and perineural cells of nerve endings of pig skin contain cytokeratins

Jean-Paul Ortonne [1], Patrick Verrando [1], Gérard Pautrat [2], and Michel Darmon [2]

[1] Centre Hospitalier, Universitair de Nice, Hôpital Pasteur, Laboratoire de Recherches Dermatologiques, Services de Dermatologie, BP 69, F-06002 Nice, France
[2] Centre International de Recherches Dermatologiques (C.I.R.D.), Département de Biologie Cellulaire, Sophia Antipolis, F-06565 Valbonne, France

Summary. The lamellar cells of the sensory corpuscles of the pig dermis must be considered to be epithelial cells as they contain cytokeratins. The cytokeratins detected are similar to those found in simple epithelia. Moreover, lamellar cells are embedded in an extracellular matrix reminiscent of the basement membrane of epithelium since it contains laminin and collagen IV. The perineural cells surrounding the nerves of pig dermis present the same features.

These results suggest that lamellar cells and perineural cells have the same origin. The nature of the lamellar and perineural cells of the rabbit or human dermis is not as clear since cytokeratins were not detected in those cells. These results, together with recent observations on Merkel cells, may indicate that epithelio-neuronal junctions are a general feature of cutaneous sensory receptors.

Key words: Lamellar cell – Perineural cell – Sensory receptor – Cytokeratin – Desmosomes – Pig skin

Introduction

Peripheral sensations originating at the level of the skin are transducted towards the sensory nervous centers by several types of nerve terminals: (1) free nerve-endings; (2) expanded tip endings such as the Merkel's touch corpuscle; (3) encapsulated endings such as Meissner's or Pacini's corpuscles (Bourlond 1968). These corpuscular receptors consist of clusters of sensory nerve fibers closely associated with specialized lamellar cells (Breathnach 1977). Stimuli received at the level of keratinocytes, Merkel cells, or lamellar cells of the corpuscles must be transmitted to the axons through junctions similar, at least in their function, to synapses. The histological nature and origin of Merkel cells has been controversial for a long time (Breathnach 1980). Merkel cells were recently shown to be of epithelial nature, since they contain desmosomes and cytokeratins (Saurat and Didierjean 1984; Ortonne and Darmon 1985). The lamellar cells of the corpuscles are of unknown origin, although it has been suggested that they derive from Schwann cells (Cauna and Ross 1960; Hashimoto 1973) or perineural cells (Low 1976). In the present paper, we demonstrate that both lamellar cells and perineural cells of the pig dermis express simple epithelial cytokeratins. Cytokeratins were not detected in similar cells of human or rabbit skin. These results suggest indicate that epithelio-neuronal junctions are a general feature of cutaneous sensory receptors.

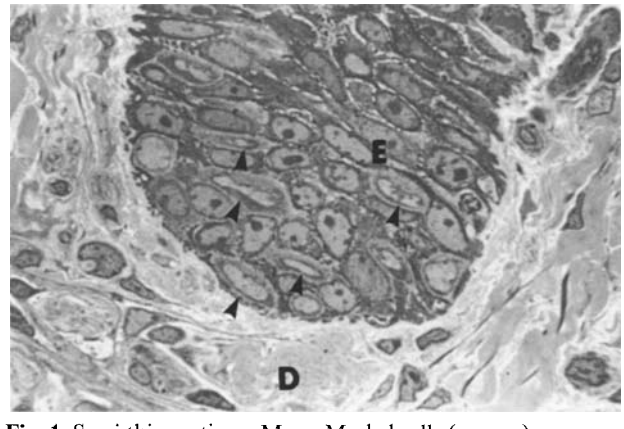

Fig. 1. Semi thin sections. Many Merkel cells (arrows) are present in the epidermis (*E*). *D* = dermis (× 250)

Offprint requests to: J.-P. Ortonne at the above address

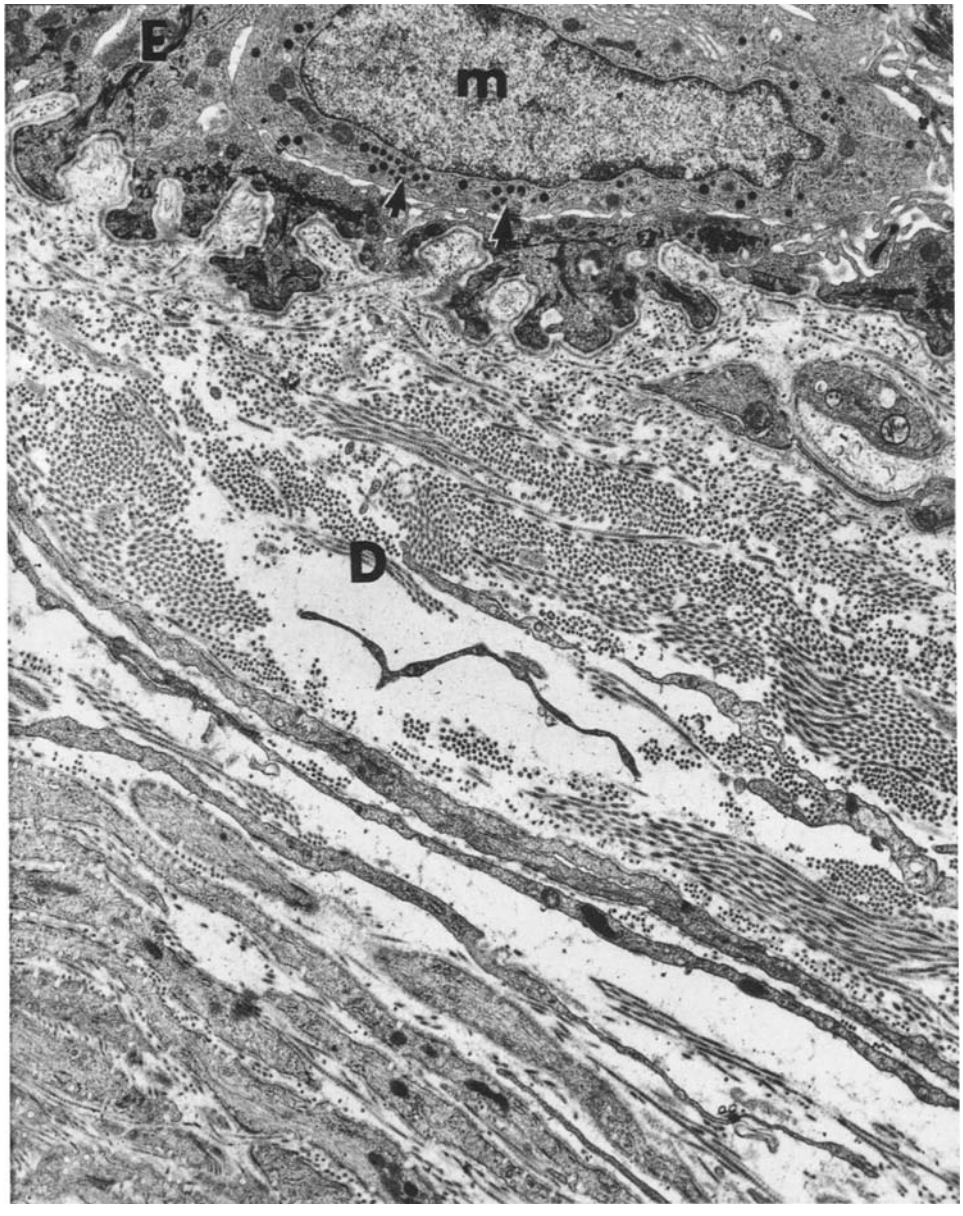

Fig. 2. Electron microscopy (*pig snout*). Merkel cells (*m*) with typical neurosecretory granules (*arrows*). *E* = epidermis; *D* = dermis; *L* = lamellar cells (× 4000)

Material and methods

Tissues. The following tissues were studied: rabbit lip (10 animals), mini pig snout (5 animals), minipeg skin (5 animals), human finger tip skin (3 individuals). All samples were snap frozen and stored at −20° C until use for immunofluorescence studies, or fixed for electron microscopy examination. These tissues are rich in Merkel cells and in sensory receptors.

Electron microscopy. Specimens for electron microscopy were fixed for 2 h in buffered 2% glutaraldehyde. The samples were rinsed in cacodylate buffer (pH 7.2), post-fixed in 1% osmium tetroxide, deshydrated in a graded ethanol series and embedded in Epon. The tissue blocks were trimmed and sectioned with an ultramicrotome Reichert Om-U3. 2 μm sections were stained with toluidine blue and studied in a light microscope. Thin sections were stained with 3% uranyl acetate and lead citrate and examined with a JEOL electron microscope at voltage of 80 KV.

Antibodies. To identify the intermidiate filaments present in the corpuscles, the following antibodies were used: (1) Anti-neurofilament antibodies, namely anti-70 kD, anti-150 kD and anti-200 kD MAb (monoclonal antibody), (2) anti-vimentin and anti-GFAP MAbs, (3).

Anti-keratin antibodies, namely anti-total epidermal keratin PAb (Viac et al. 1980a), anti-67 kD PAb (Viac et al. 1980b), anti-endoA PAb and anti-endoB PAb (Oshima 1982), TROMA-1 and TROMA-3 (Kemler et al. 1981), LE61 (Lane 1982) and KL-1 MAbs (Viac et al. 1983). Axons were also stained with HNK-1 MAb, which reacts with a peripheral nerve ganglioside (Caillaud et al. 1984; Kruse et al. 1984). Desmo-

somes were identified with the antidesmoplakins (N° 881 147-Boehringer-Mannheim, FRG) MAb. To characterize the type of extracellular matrix of the corpuscle, anti-laminin and anti-collagen IV polyclonal antibodies (Institut Pasteur, Lyon) were used.

Indirect immunofluorescenc (I.I.F.). Cryostat sections (4–5 μm) were mounted on microscopic slides and air-dried at room temperature. The following procedures were performed at 4° C. Each section was incubated with 50 μl of an optimal dilution of antiserum in 0.15 M phosphate buffered saline (PBS), pH 7.2, for 20 min, followed by two 15 min washes in PBS. According to the polyclonal (PAb) or monoclonal (Mab) antibodies used, the sections were incubated with 50 μl of a 1.30 dilution of either fluorescein isothiocyanate (FITC) conjugated swine anti-rabbit immunoglobulins (DAKO PATTS) or FITC conjugated rabbit (DAKO PATTS) antimouse immunoglobulins for 30 min, then washed three times in PBS.

For double-staining IIF, the following conjugates were also used: Rhodamine (TRITC) conjugated rabbit antimouse immunoglobulins (NORDIC) and TRITC conjugated swine antirabbit immunoglobulins (DAKO PATTS). Some sections were counterstained with propidium iodide (Ockleford et al. 1981). They were then mounted in PBS buffered glycerol. All preparations were examined by epi-illumintion with ZEISS Universal Microscope. Photographs were taken with a ZEISS MC 63 camera system and Kodak Ektachrome 400 ASA film. Negative and positive controls for each antibody were included in all experiments.

Results

Pig snout epidermis is rich in Merkel cells (Fig. 1 and 2) and many mucocutaneous end organs are present in the dermis (Fig. 3). In these sensory corpuscles there are stacks of lamellar cells. These cells contain an abundant network of intermediate filaments (Fig. 4). Desmosome-like structures are observed at intervals along the borders of lamellar cells (Fig. 5), and these cannot be stained with the anti-desmoplakins MAb. The axons, but not the lamellar cells, were stained with anti-neurofilament antibodies of all types (data not shown). However, the lamellar cells were stained with several anti-keratin antibodies, namely anti-EndoA and anti-EndoB PAb, TROMA-3 and LE61 MAbs (Fig. 6), but not with the other anti-keratin antibodies. These results (Table 1) show that lamellar cells contain keratins of simple epithelium similar to the human keratins 8, 18 and 19 (Moll et al. 1982), but not the keratins of stratified epithelia. Anti-EndoA PAb and TROMA-1 are known to react against the same basic keratin polypeptide, human keratin n° 8. Positive reaction with anti-EndoA PAb but not with TROMA-1 can thus be due either to an absence of the particular epitope in the pig or to a masking of this epitope in the lamellar cells. The second hypothesis is probably true since both TROMA-1 MAb and anti-EndoA PAb stain Merkel cells. The touch corpuscle was not stained

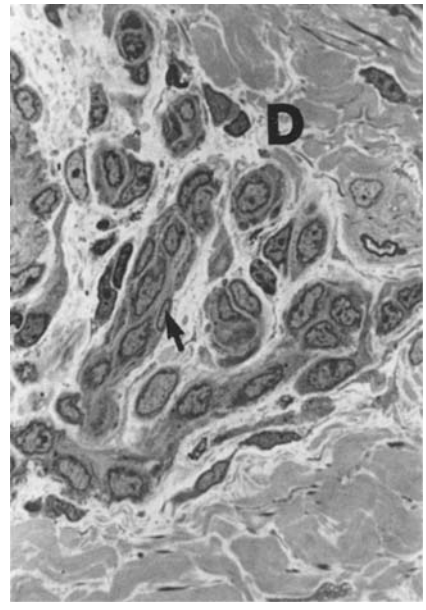

Fig. 3. Semi thin section. Mucocutaneous end organs. Lamellar cells (*arrows*). *D* = dermis (× 250)

by anti-vimentin or anti-GFAP antibodies, showing the absence of fibroblasts or astrocytes in this structure. A basement membrane of approximately 300 A° surrounded individual lamellar cells (Fig. 4) but was sometimes difficult to discern since it merged with a substance of similar electron density which intervened between lamellae. Antilaminin and anticollagen IV antibodies gave a strong staining (Fig. 7), showing that extracellular macromolecules of lamellar cells are similar to those found in basement membrane of epithelia.

Since the lamellar cells of corpuscles derive from perineural cells, it was of interest to study these cells in nerves of pig dermis. Perineural cells were stained by antikeratin antibodies, anti-EndoA, anti-EndoB PAb, TROMA-3 and LE61 MAbs (Fig. 8). They thus have the same characteristics as lamellar cells.

Touch corpuscles of human digital skin or rabbit lip were not stained by our anti-keratin antibodies. The same results were obtained with perineural cells of human or rabbit dermis. It should be noted however that Merkel cells of human and rabbit skin contain the same cytokeratins as Merkel cells, lamellar and perineural cells of pig skin.

Discussion

The results described in this paper show that both lamellar cells and perineural cells of pig dermis must be considered to be authentic epithelial cells since they contain cytokeratins. It is thus possible

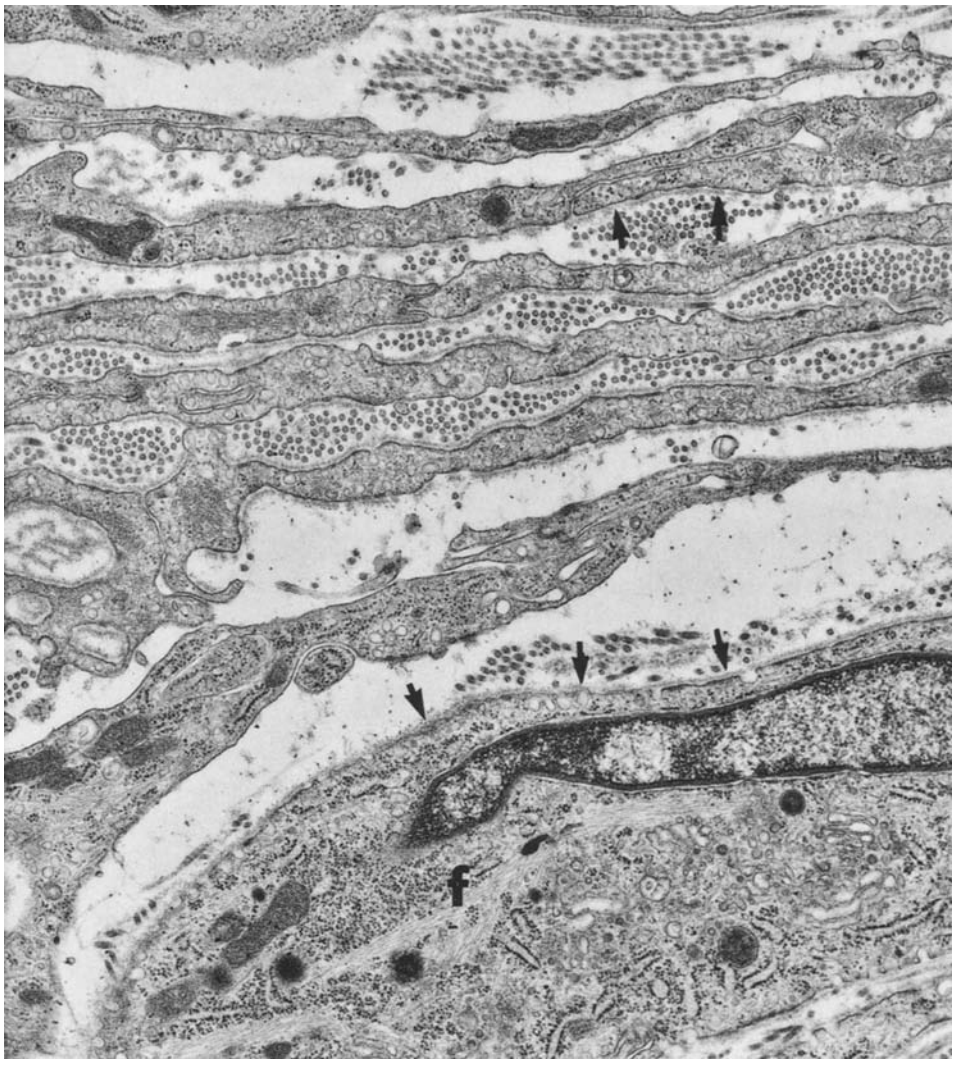

Fig. 4. Electron microscopy. Mucocutaneous end organ. Lamellar cells contain an abundant network of filaments (*f*) and are surrounded by a basement membrane (*arrows*) (× 5000)

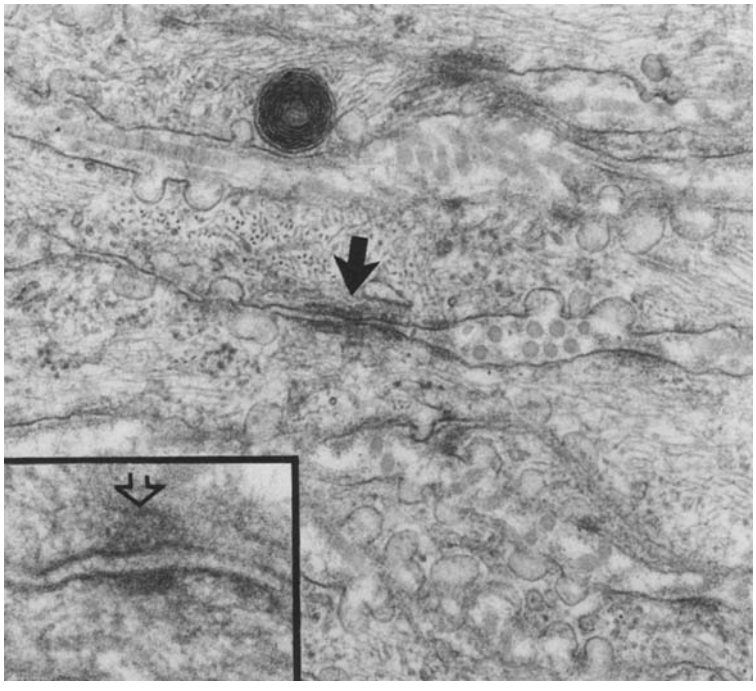

Fig. 5. Electron microscopy. Mucocutaneous end organ. Desmosome-like structures (*arrows*) (× 14000 and inset 45000)

Table 1

Antibodies and corresponding references		Working dilution	Specificities	Staining		
				L.C.	P.C.	M.C.
TK	(PAb)	1:30	Epidermal keratins	−	−	−
67 K	(PAb)	1:30	Component 1	−	−	−
Endo A	(PAb)	1:20	Component 8	+	+	+
Endo B	(PAb)	1:20	Component 18	+	+	+
KG 8.13	(MAb)	1:20	Components 1, 5, 6, 7	−	−	−
KL1	(MAb)	1:50	Components 1, 10	−	−	−
Troma-1	(MAb)	1:10	Component 8	−	−	+
Troma-3	(MAb)	1:10	43 kD, acidic	+	+	+
LE 61	(MAb)	1:50	41–43 kD, acidic	+	+	+
Antidesmoplakins	(MAb)	1:20	Desmoplakins I and II	−	−	N.D.
HNK 1	(MAb)	1:50	Peripheral nerve ganglioside	−		N.D.
Antivimentin[a]	(MAb)	1:50	Vimentin	−	−	−
Anti-GFAP[a]	(MAb)	1:50	Glial fibrillar acidic protein	−	−	−
Antineurofilaments*	(MAb)	1:50	70 kD, 150 kD, 200 kD	−	−	−

(−) Negative; (+) to (+ + +): increasing intensity of labelling; LC: Lamellar Cells; PC: Perineural Cells; MC: Merkel Cells; N.D. = not done

[a] = Dr. D. Paulin, Institut Pasteur, Paris, France

that lamellar cells and dermal perineural cells have the same origin. The keratins found in those cells are present in all simple epithelia, whatever their embryological origin, ectodermal (eg. embryonic skin); mesodermal (eg. kidney), or endodermal (eg. intestine) (Cooper et al. 1985), It is thus difficult to obtain a conclusive view on the embryological origin of lamellar cells or perineural cells of dermis. The same uncertainly applies to Merkel cells although their location in epidermis favours an epidermal origin (Breathnach 1980). It remains to be checked whether the lamellar cells of the receptors of deep organs (eg. muscle) of the pig also contain cytokeratins.

Since developmental patterns are well-conserved among mammals, it is difficult to understand why lamellar cells and perineural cells of human or rabbit dermis do not contain the cytokeratins found in the pig. They might nevertheless contain other cytokeratins not detected by our antisera. The lack of reactivity of desmosome like structures using antidesmoplakins I and II monoclonal antibody demonstrate that, in addition to their ultrastructural appearance, these structures differ also from desmosomes by their biochemical composition.

In general, these results show that epithelioneuronal interactions seems to be the rule in skin. At the level of epidermis, keratinocytes and Merkel cells interact with free nerve endings and expanded tip endings, respectively, while in the dermis, lamellar cells interact with encapsulated nerve endings. How mechanical, thermal, nociceptive informations are transformed into action potentials remains to be determined. Two lines of investigations should be developed in the future: what is the nature and the function of synapse-like junctions between epithelial cells and nerves? and what is the nature of the neurotransmitters responsible for the transduction of signals?

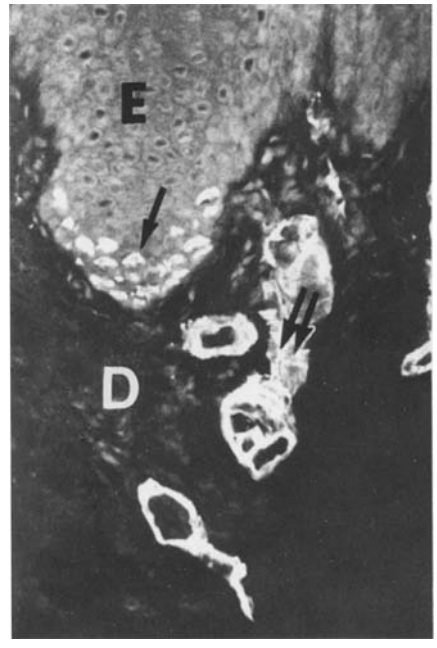

Fig. 6. Indirect immunofluorescence Monoclonal Antibody LE61. Intraepidermal Merkel cells (*arrows*) as well as lamellar cells of mucocutaneous end organs (*Double arrows*) are strongly stained (*E* = epidermis, *D* = dermis) (× 180)

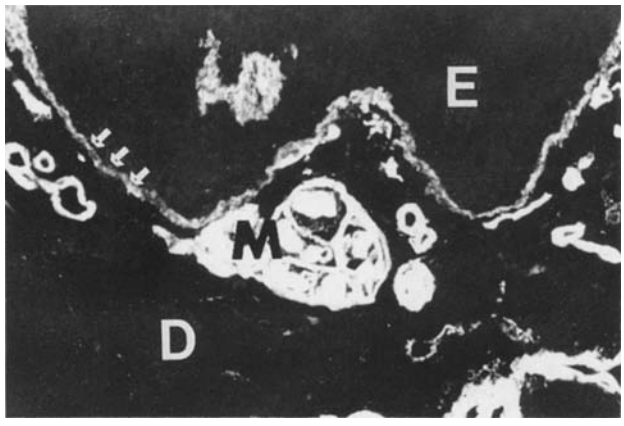

Fig. 7. Indirect immunofluorescence Polyclonal Anti-collagen IV antibody. The dermoepidermal junction (*arrows*) as well as the extracellular matrix of mucocutaneous end organ (*M*) are stained. *E* = epidermis, *D* = dermis (× 250)

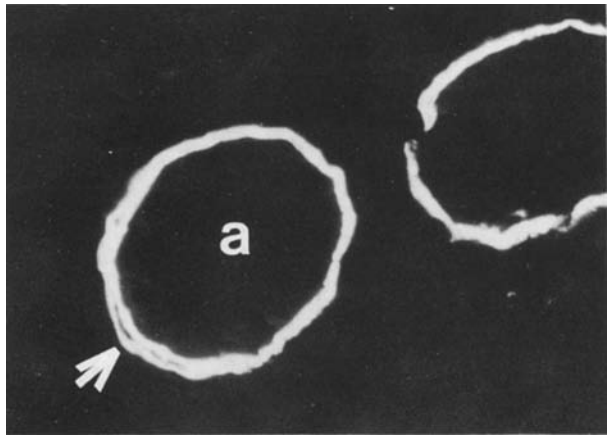

Fig. 8. Indirect immunofluorescence Monoclonal antibody LE61. Strong staining of perineural cells (*arrows*). Axon: *A* (× 300)

Acknowledgements. We are grateful to Drs B. Geiger, B. Habibi, R. Kemler, B. Lane, R.G. Oshima, D. Paulin, M.J. Staquet and J. Viac for kindly providing us with antibodies. We wish to thank Anne Pisani and Françoise Bernerd for excellent technical assistance. This work was supported by a grant from F.N.C.L.C.C. (Federation Nationale des Centres de Lutte contre le Cancer).

References

Bourlond A (1968) L'innervation cutanée. Masson, Paris

Breathnach AS (1977) Electron microscopy of cutaneous nerves and receptors. J Invest Dermatol 69:8–26

Breathnach AS (1980) The mammalian and avian Merkel cell. In: Spearman RIC, Riley PA (eds) The skin of vertebrates, Linnean Society, Symposium Series, pp 282–291

Caillaud JM, Benjelloun S, Bosq J, Braham K, Lipinski M (1984) HNK-1 defined antigen detected in paraffin embedded neurectoderm tumors and those derived from cells of the amine precursor uptake and decarboxylation system. Cancer Res 44:4432–4439

Cauna N, Ross LL (1960) The fine structure of Meissner's touch corpuscles of human fingers. J Biophys Biochem Cytol 8:467–482

Cooper D, Schermer A, Sun TT (1985) Classification of human epithelia and their neoplasm using monoclonal antibodies to keratins: Strategies, applications and limitations. Lab Invest 52:243–256

Hashimoto K (1973) Fine structure of the Meissner's corpuscle of human palmar skin. J Invest Dermatol 60:20–28

Gigi O, Geiger B, Eshhar Z, Moll R, Schmid E, Winter S, Schiller D, Franke W (1982) Detection of a cytokeratin determinant common to diverse epithelial cells by a broadly cross-reacting monoclonal antibody. EMBO J 1:1429–1437

Kemmler R, Brulet P, Schneleben MT, Gaillard J, Jacob F (1981) Reactivity of monoclonal antibodies against intermediate filament proteins during embryonic development. J Embryol Exp Morphol 64:45–60

Kruse J, Mailhammer R, Wernecke H, Faissner A, Sommer I, Goridis C, Schachner M (1984) Neural-cell adhesion molecules and myelin-associated glycoprotein share a common carbohydrate moiety recognyzed by monoclonal antibodies L_2 and HNK-1. Nature 311:153–155

Lane EB (1982) Monoclonal antibodies provide specific intramolecular markers for the study of epithelial tonofilament organisation. J Cell Biol 92:180–189

Low FN (1976) The perineural and connective tissue of peripheral nerve. Landon DN (ed) London, Chapman and Hall

MacDonald DM, Schmitt D (1979) Ultrastructure of the human mucocutaneous end organ. J Invest Dermatol 72:181–186

Moll R, Franke W, Schiller D, Geiger B, Krepler R (1982) The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 31:11–24

Montagna W, Yun JS (1964) The skin of the domestic pig. J Invest Dermatol 43:11–21

Mowafy M, Cassens RG (1975) Microscopic structure of pig skin. J Anim Sci 4:1281–1290

Ockleford CD, Hsi BL, Wamely J, Bradley RA, Whyte A, Page Faulk W (1981) Propidium Iodide as a nuclear marker in immunofluorescence. Use with tissue and cytoskeleton studies. J Immunol Meth 43:261–267

Ortonne JP, Darmon M (1985) Merkel cells express desmosomal proteins and cytokeratins. Acta Derm Venereol 65:161–164

Oshima RG (1982) Developmental expression of murine extraembryonic endodermal cytoskeletal proteins. J Biol Chem 257:3414–3421

Saurat JH, Didierjean L (1984) The epidermal Merkel cell in an epithelial cell. Dermatological 169:117–120

Viac C, Staquet MJ, Goujon C, Thivolet J (1980a) Experimental production of antibodies against stratum corneum keratin polypeptides. Arch Dermatol Res 267:179–188

Viac J, Schmitt D, Staquet MJ, Thivolet J, Ortonne JP, Bustamante R (1980b) Binding specificity of guinea-pig anti-a-keratin polypeptide sera on human keratinocytes: comparison of their receptors with those of human epidermal cytoplasmic antibodies. Acta Derm Venereol (Stockh) 60:189–196

Viac J, Reano A, Brochier J, Staquet MJ, Thivolet J (1983) Reactivity pattern of a monoclonal antikeratin antibody (KL-1). J Invest Dermatol 81:351–354

Accepted September 26, 1986