First International Merkel Cell Symposium, Heidelberg, Germany

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SESSION I (SATURDAY, FEBRUARY 19, 1994)

The first session was devoted to general aspects of Merkel cells in vertebrates, including their development, structure, and distribution.

Daisy Kopera (Graz, Austria), co-authored by **K. Holubar (Vienna, Austria)**, submerged the audience into the historical environment of the nineteenth century of Theodor Langhans, Friedrich Sigmund Merkel, and Paul Wilhelm Langerhans, the names of each of whom were taken for the creation of eponyms designating well-known cells, Langhans' giant cells, Merkel cells, and Langerhans' cells as named by Merkel in 1875. Friedrich Sigmund Merkel (1845–1919) was an anatomist whose main place of work was Göttingen. He described "his" cells in the year 1875 as "Tastzellen" (tactile cells) as the simplest form of organ mediating the sense of touch. They are undisputedly referred to by everyone as Merkel cells, whereas there is still some debate on the terminology of the tumors related to these cells.

A major topic of interest was the still not yet definitely resolved question of the embryonic development of Merkel cells. In the case of birds, **Z. Halata (Hamburg, Germany)** reported on transplantation experiments of leg buds from chick to quail embryos and vice versa, two species whose cells can be distinguished easily on the basis of the ultrastructure of the nucleus. These experiments showed that in early grafts Merkel cells exclusively of host origin develop, whereas in later grafts Merkel cells of both host and donor origin can be detected. This is evidence for a migration of Merkel cell precursors, probably arising from the neural crest, to their cutaneous sites, speaking against an epidermal origin. In contrast to mammalian Merkel cells, however, avian Merkel cells are not intraepidermal but form dermal clusters below the epidermis.

W. Hartschuh (Heidelberg, Germany) presented an updated characterization of mammalian Merkel cells. In his material, there was no evidence of transitional cells sharing features of keratinocytes and Merkel cells. In human fetal epidermis of week 11, isolated Merkel cells possessing cytoplasmic processes laden with neurosecretory granules were ultrastructurally detected for the first time in the deep dermis. This finding favors the hypothesis that the Merkel cell is an immigrant cell. Various neuropeptides were localized in mammalian Merkel cells from different species, including not only vasoactive intestinal polypeptide (VIP), pancreastatin, and substance P but also, in the rat, the opioid peptide dynorphin in addition to met-enkephalin. Interestingly, substance P was localized only in Merkel cells from vibrissa hair follicles, but not from epidermal cones, in the pig. These results suggest the existence of different subtypes of Merkel cells in addition to species differences.

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Abbreviations: CK, cytokeratin; SP, substance P; VIP, vasoactive intestinal polypeptide.

Using simple-epithelial cytokeratins as Merkel cell markers, **Ingrid Moll (Mannheim, Germany)** was able to trace the embryogenesis back to week 8 in human skin. In this early stage of a two- to three-layered epidermis, Merkel cells—as identified by the presence of cytokeratin 20—can be found both basally and suprabasally. Thus, these cells appear to arise at approximately the same time as the first stratum spinosum keratinocytes (intermediate layer). These findings suggest that the embryonic basal cell is the precursor of both keratinocytes and Merkel cells; direct proof is, however, still lacking. In the fetus, abundant Merkel cells are present not only in the palmo-plantar epidermis but also in the nail anlage and in the developing hair follicles and are restricted to certain sites in both adnexal structures.

K. Hashimoto (Detroit, USA) characterized the Merkel cells of adult vellus hair follicles. They are localized at the bulge, the attachment site of the arrector pili muscle. This region is difficult to identify in the adult, and is characterized only by knob-like projections and a typical restriction band. He did not detect any association of these hair follicle Merkel cells with nerve endings; abundant nerve fibers were present in the dermis close to the bulge region, but their endings were clearly above the bulge and did not make contacts with Merkel cells. Thus, Merkel cells at this site apparently have no mechanoreceptor function, but rather may exert paracrine functions such as stimulation of hair follicle stem cells, which reside in the bulge, and/or attraction of developing nerve fibers to come together to the arrector pili muscle. In response, Dr. Halata stressed his observations of nerve-associated Merkel cells in the bulge of guard hairs in rodents.

A. Beiras (Santiago, Spain) reported on immunostaining of Merkel cells for the neural cell adhesion molecule; he further showed examples of a stronger albeit heterogeneous immunoreactivity in Merkel cell carcinomas. He suggested that neural cell adhesion molecule may serve as a marker for both normal neuroendocrine cells and neuroendocrine tumors.

K. Toyoshima (Kitakyushu, Japan) reported on how gustatory nerves entering the tongue find their way during morphogenesis of taste buds in the frog and rat and in particular on the origin and possible function of cells containing both serotonin and neuron-specific enolase. These cells are modified Merkel cells and may act as target sites for the gustatory nerves.

In lower vertebrates such as frog and salamander, Merkel cell–like can be observed in the fetal taste buds of the tongue epithelium and are known to produce serotonin and neuron-specific enolase. They are innervated, and they show microvillous projections, sometimes even crossing the basal lamina. They also make desmosomes with neighboring epithelial cells. In the fetal taste buds, these Merkel cell–like cells coexist together with gustatory sensory cells. This special type of Merkel cell thus may play a role in the initiation of taste bud morphogenesis in some vertebrates. A droll-appearing animal, the Japanese shrew-mole, possesses a protruded snout and was impressively presented by

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S. Shibanai and H. Iseki (Tokyo, Japan). In shrew-mole snout epidermis, Merkel cells can be found in the Eimers' organ as epidermal swellings where these cells reside in the basal cell layer and are innervated. Below this structure, there are dermal laminated corpuscles. Interestingly, in the embryonic mole snout, immature Merkel cells appear to make holes through the basement membrane through which pseudopod-like processes protrude, and it appeared that the processes led nerves into the epidermis and onto the Merkel cell itself. Similar phenomena were observed in the Merkel cells of the house shrew embryo snout. This may be important for the formation of the nerve terminal on the Merkel cell.

This session thus made evident that when different body sites and particularly different species are considered, cells referred to as Merkel cells may show, besides common ones, quite different and special characteristics. Association of these cells with nerve endings appears to be the rule but is obviously not without exception. Thus, additional autocrine or paracrine functions are items of ongoing debate, but definite proof of such functions is still awaited.

SESSION II (SATURDAY, FEBRUARY 19, 1994)

According to **E. Weihe (Mainz, Germany)** neuropeptides and neuropeptide-processing enzymes are localized in neural and non-neural elements of mammalian skin including man. Prominent neuropeptides in cutaneous nerves include substance P (SP), calcitonin gene-related peptide, neuropeptide Y, and VIP. Neuropeptide Y appears to be specific for sympathetic innervation, SP is characteristic for small diameter sensory fibers, and calcitonin gene-related peptide marks small and large diameter sensory fibers as well as cholinergic postganglionic sympathetic sudomotor nerves. Opioid-, galanin-, somatostatin-, and cholecystokinin-containing nerves are relatively sparse and are expressed weakly. The pan-peptidergic nature of sensory and autonomic cutaneous innervation is reflected by pan-neural expression of chromogranin A, which is involved in neuropeptide packaging and storage.

M. Schäfer (Mainz, Germany) in addition demonstrated region-specific neuropeptide expression in neural and non-neural elements matched by region-specific patterns in the expression of neuropeptide processing enzymes such as PC1 and PC2. Expression of these enzymes and of CGA in specific layers of the epidermis suggests that keratinocytes represent previously unrecognized sources of neuropeptide synthesis *in vivo*. There is evidence for inflammation-induced plasticity of neuropeptide-receptor expression in various non-neural cutaneous cells including subsets of immune cells. Taken together, the data suggest that neuropeptides, neuropeptide receptors, and neuropeptide processing enzymes may play a major role in neuroimmune mechanisms of cutaneous disease.

The potential paracrine activity of neuropeptides derived from Merkel cells and cutaneous nerves and the implications for skin diseases were reviewed by **U. Wollina (Jena, Germany).** In cell culture experiments of a permanent human keratinocyte cell line Dr. Wollina was able to confirm results of other investigators that VIP may act as a potent growth factor for human keratinocytes. In addition he was able to demonstrate that VIP-induced proliferation of keratinocytes occurred only in serum-free culture medium. In contrast, addition of serum to the culture medium reduced proliferation, suggesting pleiotropic actions of VIP. This mechanism might be important in the regulation of homeostasis.

C. Pincelli (Modena, Italy) gave an update of the role of neuropeptides in the pathogenesis of inflammatory dermatoses, focussing on SP and VIP. By radioimmunoassay, he demonstrated a decrease in SP and an increase in VIP in lesional skin from patients with atopic eczema, psoriasis, or contact dermatitis compared to normal skin. Nerve growth factor, which was shown to be synthesized in the skin and to be secreted during proliferation by human keratinocytes in culture, is assumed to regulate neuropeptide synthesis and thus to underlie this imbalance of neuropeptides.

H. O. Handwerker (Erlangen, Germany) stated that a generally acknowledged theory of itch is still lacking. From neurophysiologic experiments it is known, however, that itch sensations are mediated mainly by unmyelinated peptidergic nerve fibers ending in the superficial layers of the skin when itch is induced, e.g., by intracutaneous histamine injection. The enigma remains that unmyelinated peptidergic nerve endings also mediate pain. Evidence from microneurography experiments in human volunteers was presented showing that itch- and pain-mediating afferents represent overlapping but not identical populations of nociceptors. Hypothetically the different sensory qualities of itch and pain could be explained by different central nervous connections of the peripheral nerve fiber populations mediating itch and pain.

Kathleen B. English (Salt Lake City, USA) showed by immunocytochemical studies that nerve growth factor in adult mammalian skin (rat) is intensely expressed in keratinocytes of specialized touch dome mechanoreceptors. Merkel cells in the basal epidermis of touch domes and their afferent type I neurons stain for the low-affinity nerve growth factor receptor, $P75^{NGFR}$. Analysis of quantitative Northern blots of epithelial cells confirms that nerve growth factor mRNA is present in adult skin. Interestingly, its levels are three times greater in larger cells from the stratum granulosum and spinosum than in smaller basal cells from the stratum germinatieum where Merkel cells are located.

H. Kurzen and Ingrid Moll (Mannheim, Germany) studied the expression of various cytokeratin (CK) polypeptides within the human haarscheiben by immunohistochemistry and two-dimensional gel electrophoresis. The basal clusters of Merkel cells were specifically detected by CKs 8, 18, 19, and 20. Interestingly, in contrast to normal keratinocytes, keratinocytes within haarscheiben expressed CK 17 in the lower and middle epidermal layers. Moreover, the basal epidermal compartment, as characterized by the expression of CK 5, was enlarged, and CK 15 was reduced in basal keratinocytes. Also, various cell adhesion molecules were of basal cell type in the enlarged basal compartment. These results show that haarscheibe keratinocytes express a unique program of differentiation.

Tamiko Tachibana and T. Nawa (Morioka, Japan) investigated ultracytochemical localizations of Ca^{++} in Merkel cell–axon complexes under various experimental conditions. Mechanical stimulation caused a remarkable increase in the C^{++} level of axon terminals, but not of Merkel cells. Ca^{++} levels of Merkel cells varied from low to high, not only in static states but also in stimulative states. However, nifedipine, a blocker of L-type Ca^{++} channels, depleted Ca^{++} from Merkel cells. These results suggest that Ca^{++} channels of Merkel cells are gated by some non-mechanical factor, whereas those of axon terminals are directly gated by mechanical stimulation.

K. Baumann, Eliza Chan, and W. H. Yung (Hong Kong) investigated the effect of adenosine triphosphate on the intracellular calcium concentration in Merkel cells isolated from rat vibrissae and on responses of type I (Merkel endings) receptors to determine whether or not Merkel cells are responsible for the mechano-electrical transduction process. Their results suggest a modulatory effect of adenosine triphosphate on the function of Merkel cell mechanoreceptors in rat vibrissae mediated through the intracellular calcium concentration in Merkel cells. Thus, these findings are consistent with the not generally accepted hypothesis that the Merkel cell acts as a mechano-electric transducer.

SESSION III (SUNDAY, FEBRUARY 20, 1994)

The third session was devoted to the pathology of Merkel cells, in particular cutaneous neuroendocrine (Merkel cell) carcinomas and Merkel cells in diseases.

J.Ph. Lacour, co-authored by J. P. Ortonne (Nice, France) presented an overview of clinicopathologic features of neuroendocrine carcinomas of the skin. He stressed that they are now recognized as very aggressive tumors; in Lacour's series, there were frequent local recurrences, regional and systemic metastases, and a 3-year survival of only 55%. Although the therapeutic strategies still are not clearly defined, he recommended surgical treatment of the

primary tumor by wide local excision (3 cm margin) and postoperative radiation therapy.

Daisy Kopera and J. Smolle (Graz, Austria) were able to follow up 20 cases of Merkel cell carcinomas over a period of 36 months. Six of 20 patients developed metastases, and three patients died of the disease. Among various clinical and pathohistologic factors studied, the age (>78 years) and high mitotic frequency were particularly relevant for prognosis.

According to **W. Hartschuh (Heidelberg, Germany)**, important ultrastructural features of neuroendocrine skin carcinomas include neurosecretory granules, intermediate filaments forming globular aggregates referred to as fibrous bodies, primitive cell junctions, and cytoplasmic processes: the latter were prominent, however, in only two of nine cases. The characteristic fibrous bodies were absent in only one case of this series. Immunohistochemically, chromogranin A is a very good marker, whereas neuropeptides are rarely detected.

R. Moll (Mainz, Germany) showed the recently identified cytokeratin polypeptide 20 to be, within the skin, a specific marker for Merkel cells. This cytokeratin is consistently expressed in Merkel cell carcinomas but is absent in small cell carcinomas of the lung; thus it can be useful for the important differential diagnosis Merkel cell carcinoma versus lung cancer metastasis. Moreover, the peculiar distribution of cytokeratin 20 together with its expression in both Merkel cells and Merkel cell tumors argues strongly for the derivation of these carcinomas from Merkel cells. Merkel cell tumors, in addition, express neurofilaments (NF-L, NF-M, NF-H). This highly unique intermediate filament phenotype of Merkel cell carcinomas was fully maintained in three cell lines derived from such tumors. In addition, these cell lines produce and secrete substance P, vasoactive intestinal polypeptide, and chromogranin A.

Data on seven cases were reported by **W. Back (Mannheim, Germany)**. One patient presenting with lymph node metastasis of a small cell undifferentiated carcinoma in the mediastinum was initially diagnosed clinically with small cell lung cancer. Later, a nodular skin tumor with the same histologic features was recognized on his arm and was shown after autopsy to represent a primary Merkel cell carcinoma with extensive systemic metastasis. The tumor cells expressed cytokeratin 20, like all other cases except one. One tumor was histologically conspicuous by the presence of focal squamous differentiation.

B. Wolf (Mannheim, Germany) focused on the frequency and distribution of Merkel cells in normal and diseased skin. In normal skin (different body sites) 30 to 60 Merkel cells/mm^2 (mean 22.4) were counted, in accordance with reports from other groups. In acute inflammation (e.g., acute ultraviolet erythema) a proportion of Merkel cells migrates upwards to suprabasal sites and subsequently may become destroyed. This would be in line with the findings in various chronic skin diseases (psoriasis, lichen ruber, lymphoma, chronic eczema) where Merkel cell density was markedly decreased, to 0–3 Merkel cells/mm^2 (mean 0.2). Whether regeneration is possible after healing remains to be clarified.

The occurrence of Merkel cells around and within epidermal and adnexal skin tumors was reported by **W. Hartschuh and W. Schulz (Heidelberg, Germany)**. In adnexal tumors they demonstrated intratumoral Merkel cells in nevus sebaceous, trichofolliculoma, trichoepithelioma, and desmoplastic trichoepithelioma. The latter in particular typically contains a high number of Merkel cells that are not innervated. In contrast, basal cell carcinomas contained only rare clustered Merkel cells; most were concentrated around the tumor, and some were in the dermis. In contrast, there was no increase of Merkel cells in condylomata acuminata, molluscum contagiosum, or squamous cell carcinoma.

The last speaker, **V. E. Gould (Chicago, USA)**, broadened the spectrum of neuroendocrine tumors to include other organs. Most important are neuroendocrine carcinomas of the lung, which comprise a spectrum from higher to lower neuroendocrine differentiation. Another organ which typically gives rise to such tumors is the intestine: here somatostatin may be produced and secreted. One should be aware of the existence of primarily non-neuroendocrine carcinomas, including prostate adenocarcinomas and bronchioloalveolar carcinomas of the lung, which exhibit neuroendocrine features that may even lead, through ACTH production, to Cushing's syndrome.