Neuron-Specific Enolase in the Merkel Cells of Mammalian Skin

The Use of Specific Antibody as a Simple and Reliable Histologic Marker

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Merkel cells are specialized skin receptor cells, characterized by their particular location in the epidermis and close association with nerve terminals. Although they can be distinguished ultrastructurally by their small, electron-dense secretory granules, there is no specific and reliable method for identifying them by light microscopy. Using antibodies to neuron-specific enolase (NSE), the authors have shown sparsely distributed groups of specifically immunostained cells and associated nerve terminals in the nose skin of cats and rats. These cells were easily distinguished from other epithelial cell types, including melanocytes and Langerhans cells and had all the morphologic features of Merkel cells and their so-called neurite complexes, including the characteristic cytoplasmic secretory granules (60 nm in diameter). NSE immunostaining is a simple and reliable method for the specific light-microscopic staining of Merkel cells and provides further evidence for NSE as a marker for the diffuse neuroendocrine system. (Am J Pathol 1981, 104: 63–68)

MERKEL CELLS, first discovered by Dr. Merkel[1] in 1875, are thought to be specialized receptor cells[2] present in the skin. The population of Merkel cells is very small. For example, in the back skin of the rat[3] there are only about 150 Merkel cells/sq cm. In various sensory receptive areas of the mammalian body, such as the nose, fingertips, etc., more Merkel cells are found, but they are still far from abundant. They tend to form groups and are mainly situated within the epithelial basement laminas of touch corpuscles (Haarscheiben) at the junction of the dermis and epidermis.[1–3]

Merkel cells can be distinguished by their orientation in the epithelium. Their longest axis is usually parallel to the surface of the skin and the basement membrane so that the columnar epithelial cells immediately above them lie in a perpendicular plane.[3] In mammals, the Merkel cells are very often directly associated with tylotrich hairs,[4] which are longer than other hairs and have follicles surrounded by aggregations of fibroblasts. They are mostly innervated by long myelinated fibers of Type I sensory neurons, forming the so-called epidermal Merkel cell neurite complexes.[3,5,6]

There is, at present, no specific staining method for Merkel cells, and the most reliable means of identifying them has been the electron microscope.[2,3] It has, however, been suggested that Merkel cells may belong to the diffuse neuroendocrine (APUD) system.[7,8] A newly described common characteristic for the components of this system is the presence of neuron-specific enolase (NSE).[9] NSE is an enzyme that was originally regarded as being present exclusively in neurons[10,11] but was later found in a number of APUD cells.[9]

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This study was carried out to determine whether NSE is present in Merkel cells and if so, whether immunostaining for NSE can identify these cells at the light-microscopic level.

Materials and Methods

Fresh specimens (0.2 × 0.2 sq cm) of skin were taken from the noses of cats (n = 5) and rats (n = 8) and immediately frozen in Arcton (−150 C). They were then freeze-dried overnight at −40 C, fixed in benzoquinone vapor at 60 C for 3 hours, and embedded in paraffin wax. Sections (5μ) were cut, in a plane perpendicular to the surface of the skin, and mounted on uncoated slides. In adition, some sections were cut transversely, parallel to the surface of the skin, and mounted in the same way. In this way, Merkel cells were sectioned both longitudinally and transversely.

Production of Antiserums

Neuron-specific enolase (NSE) was isolated from rat and human brain,[12,13] and antiserums to NSE were raised in New Zealand white rabbits by the intradermal injection of purified NSE emulsified in Freund's complete adjuvant.[12,13] Antiserums were also raised to rat and human nonneuronal enolase (NNE), and these antiserums showed no cross-reactivity with NSE, or vice versa.[13]

Immunocytochemistry

The unlabeled antibody enzyme (peroxidase-antiperoxidase, PAP) method[14] was performed with the use of a procedure described elsewhere.[14] Sections were incubated for 14–18 hours at 4 C in a moist atmosphere with rabbit antirat NSE antiserum, diluted 1:4000. The second layer, unconjugated goat antirabbit globulin, was used at a dilution of 1:200 for 30 minutes at room temperature. The third layer consisted of PAP complex, diluted 1:300, and incubation was again for half an hour at room temperature. The diaminobenzidine (DAB) method of Graham and Karnovsky[15] was used to visualize the final reaction product. Sections were lightly counterstained with hematoxylin.

Control sections were stained with primary antiserums preabsorbed with specific antigen. The absorbed antiserums were applied to sections serial to those that had been immunostained with antiserums to NSE. Further controls included the use of nonimmune rabbit serum as the first layer or the PAP complex alone. Sites of possible nonspecific attachment were visualized with DAB.

Enzyme Histochemistry

The modified oxidase method[16] was used to distinguish the melanocytes from the NSE-immunoreactive cells. Fresh specimens of skin (0.5 × 0.5 sq cm) were taken from the same areas and fixed in 10% formalin in phosphate-buffered saline (0.01M,pH7.1) for 2 hours at room temperature. They were then processed through graded alcohols and xylene and embedded in paraffin wax. Sections 5μ thick, perpendicular to the surface of skin, were cut and mounted on uncoated slides. The sections were then dewaxed and placed in an incubating solution, consisting of 100 mg of DL-3,4 dihydroxyphenylalanine (DL-DOPA) in 100 ml of 0.1 M phosphate buffer (pH 7.4) at 37 C for 1 hour. The sections were then changed to a fresh incubating solution possessing the same constituents for another 4 hours. After a wash in running tap water for 5 minutes, the sections were counterstained with methyl green, dehydrated, and mounted in DPX.

For electron-microscopic examination, fresh tissue was cut into 1-mm cubes and placed immediately into 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.1), then postfixed in 1% osmium tetroxide for 1 hour at 4 C. The tissue was then dehydrated through graded alcohols and embedded in Araldite plastic resin. Thin sections were stained with uranyl acetate and lead citrate, then examined on an AEI 6B model electron microscope.

Results

NSE Immunostaining

A number of cells, located just above the basement membrane, were strongly reactive with NSE antiserums (Figures 1 and 2). The cells were found in very few sections, ie, about 1 out of 20 or 30. The immunostained cells tended to form groups of 3–10, separated by unstained epithelial cells (Figure 2). Most of the immunostained cells lay in a plane parallel to the basement membrane and thus at right angles to the columnar epithelial cells. Immunostained nerve fibers were clearly visible in the subcutaneous layer. They penetrated the basement membrane and were closely associated with the basal part of each immunostained cell (Figures 1 and 2). At times the nerve fibers surrounded the NSE immunoreactive cells, and some projected further toward the surface of the skin (Figure 1). The innervated cells were situated at the bottom of the basal laminas of touch corpuscles and were nearly always associated with tylotrich hairs (Figure 1).

In some cases, when the papillae of the skin were cut transversely, some immunostained cells with a distinctive shape could be seen in the epithelium, at the edge

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Nak

Figure 2
Innervate

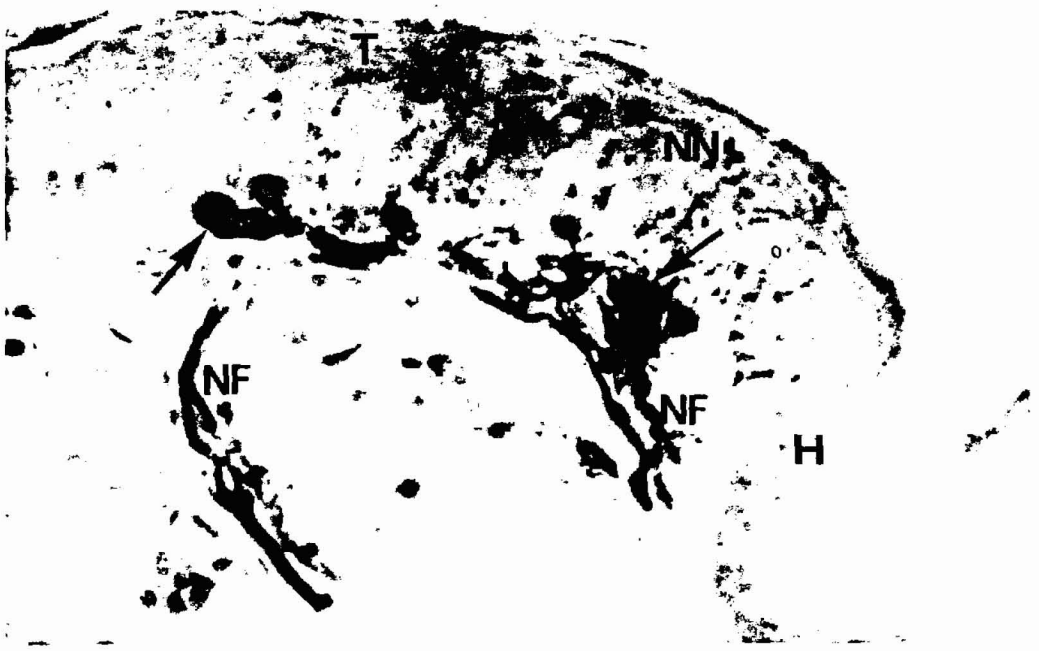

Figure 1—NSE-immunoreactive cells in the nose skin of cat. The immunostained cells (*arrow*) show all the characteristics of Merkel cells which include their number, location, orientation, and association with nerve fibers (*NF*), touch corpuscles (*T*), and a tylotrich hair follicle (*H*). Naked nerve endings (*NN*) can be seen in the epithelium. (×200)

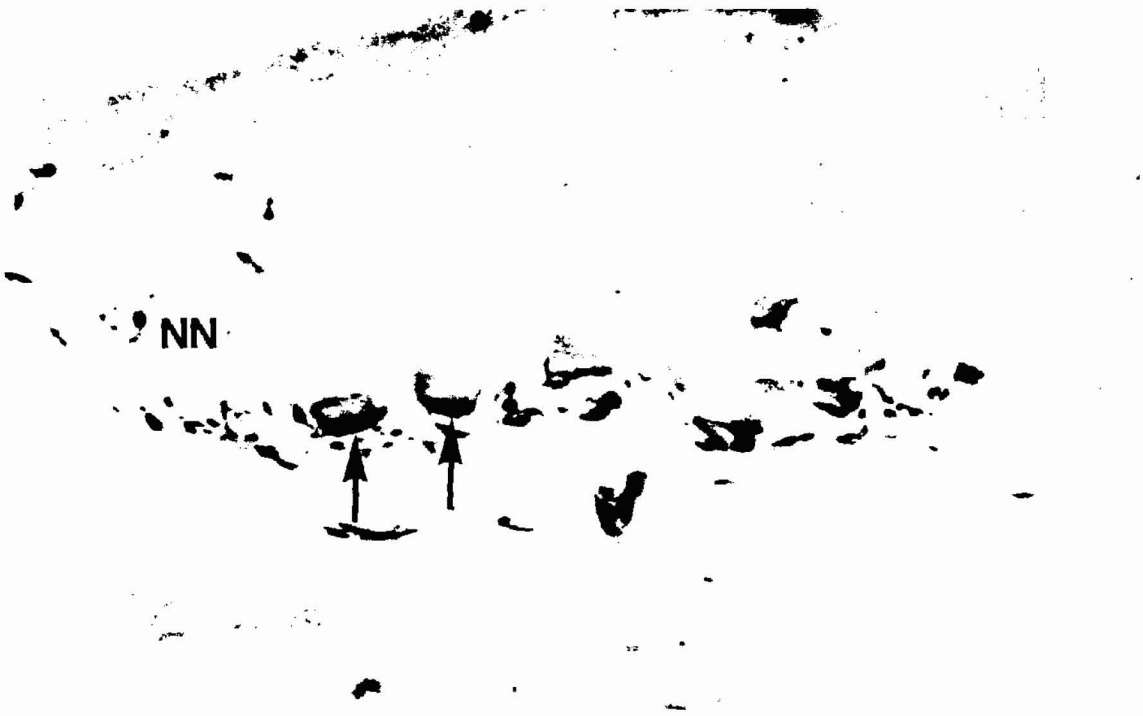

Figure 2—A group of Merkel cells situated within the epithelial basement lamina of a touch corpuscle of feline nose skin. The cells are clearly innervated (*arrow*), and some nerve fibers go further toward the surface of the skin, forming naked nerve endings (*NN*). (×400)

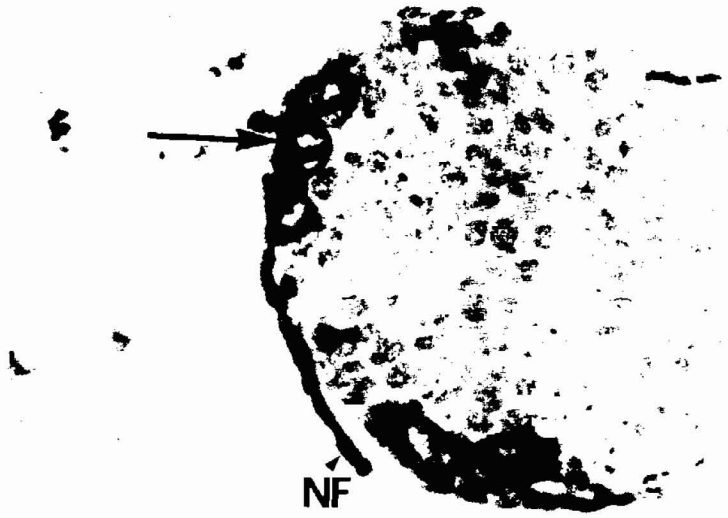

Figure 3—A group of Merkel cells (*arrow*) immunostained by NSE antiserum, at the edge of a transversely cut papilla of feline nose skin. The cells have a ringlike appearance. A nerve fiber (*NF*) can be seen nearby in the subcutaneous layer. (×300)

Figure 4—Single uninnervated Merkel cell immunostained by NSE antiserum in feline nose skin. The cell is located at the junction of the dermis and epidermis, and its longest axis lies parallel to the surface of the skin. The cell is associated with a touch corpuscle (*T*) (Haarscheiben) and a tylotrich hair follicle (*H*). (×300)

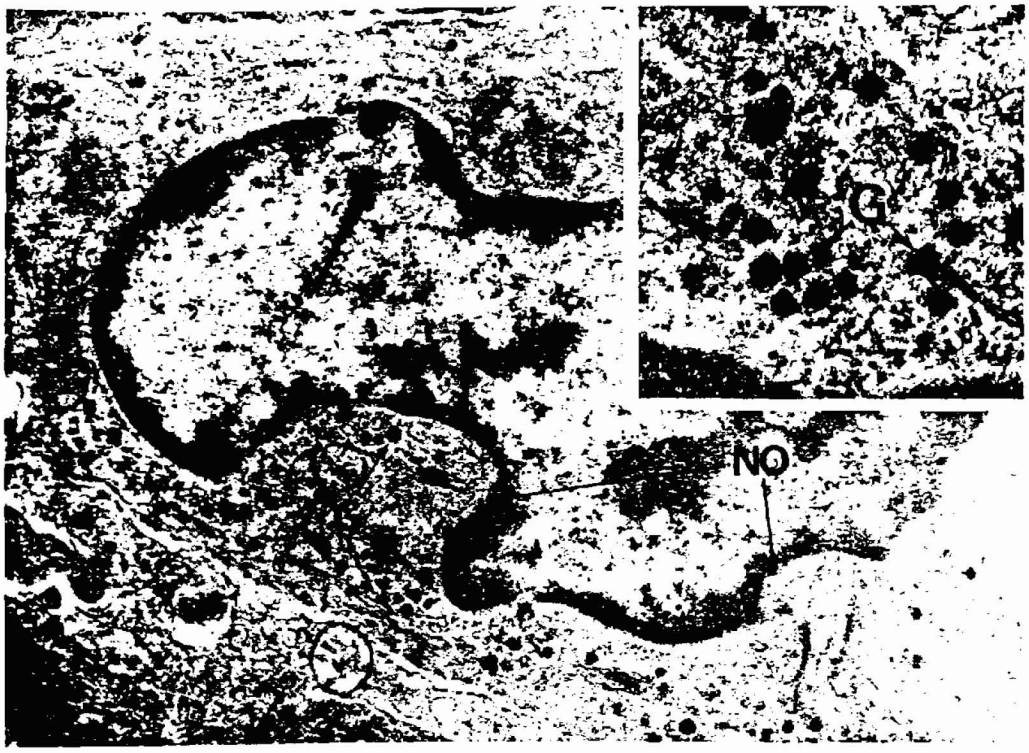

Figure 5—Ultrastructurally identified Merkel cell with electron-dense granules (*G*) and irregular nuclear outline (*NO*) were found in the identical areas. (×35,000) **Inset**—The secretory granules. (×85,000)

of the papilla. The periphery of these cells was immunostained more intensely than the center, giving the cells a ringlike appearance (Figure 3). It was not possible, using sections cut in this plane, to show that the cells were intimately innervated, but very often one or two nerve fibers were found lying nearby in the subcutaneous layer.

Occasionally, single or grouped immunostained cells (Figure 4) were seen, lying on the basement membrane, with or without association with a touch corpuscle. These were generally not shown to be innervated. Sometimes only immunostained nerve fibers were seen, penetrating the basement membrane and ending on or near the surface of the epithelium, forming naked nerve endings.

The sections incubated with preabsorbed antiserum were completely negative, as were the other control specimens.

DOPA Oxidase Method

The melanocytes were strongly stained brownish-black by the DOPA oxidase method. These cells were mainly located in the basal layer of the epithelium. They had a central cell body, containing the nucleus and numerous long, thin, branching cytoplasmic processes. In addition to having a different shape, the melanocytes were also far more abundant than the NSE-immunoreactive cells.

Electron Microscopy

Parallel ultrastructural studies showed the presence of scattered cells with a distribution identical to that of the NSE-positive cells and morphologic features similar to those described for Merkel cells. Thus, they contained characteristic intracytoplasmic, round, secretory granules, surrounded by a limiting membrane and with an average diameter of 60 nm. Other characteristics of Merkel cells were also seen in these cells, including irregular nuclear outline and spindlelike cytoplasmic processes (Figure 5).

Discussion

We have demonstrated here the presence of NSE in Merkel cells. These cells were identified by the use of both light- and electron-microscopic methods.

There are three types of nonkeratinocytes in the skin, namely melanocytes, Langerhans cells, and Merkel cells. The melanocytes, stained in this study by the DOPA oxidase technique and described before by other investigators,[16] have morphologic characteristics entirely different from those of the NSE immuno-

reactive cells. The Langerhans cells are located in the suprabasal layers of the epithelium, which lie in the area above the NSE-immunoreactive cells, and have a star shape with long, irregular processes.[17] Both of these cells can be easily distinguished from the NSE-immunoreactive cells.

At the light-microscopic level, it would seem that, since Merkél cells are the only cells in the epithelium that are characteristically innervated,[18] the NSE-immunoreactive innervated cells must be Merkel cells. In addition, these innervated NSE-positive cells display all the morphologic characteristics of Merkel cells, which include association with touch corpuscles and tylotrich hairs and the tendency to form groups, as well as the location, number, and orientation of the cells. Moreover, parallel electron-microscopic studies showed the presence of electron-dense secretory granules, a characteristic of Merkel cells. Thus, it may be concluded that these innervated NSE-immunoreactive cells are Merkel cells and not melanocytes, Langerhans cells, or any other cell type.

The immunostained cells with a ringlike appearance are only seen in transversely sectioned papillae. It is likely that they are Merkel cells, peripherally located immunostaining giving the ringlike pattern. The associated nerve fibers could not be demonstrated in the same sections. This problem may be due to the plane in which the tissue was cut and may also explain the fact that there were always some nerve fibers lying nearby in the subcutaneous layer. It has been shown that not all Merkel cells are innervated; neither are they all associated with touch corpuscles.[2,3,5,6] Thus, although the single or grouped noninnervated NSE-immunoreactive cells may represent a cell type not previously described, it seems more likely that they, too, are Merkel cells.

The finding of this newly discovered biochemical marker in Merkel cells supports the earlier suggestion that those cells are members of the diffuse neuroendocrine system.[7,8] The presence of this neuronal antigen in these cells further supports the functional relationship of neuroendocrine cells with neurons.

We therefore conclude that NSE immunostaining is a reliable, simple, and highly specific method for demonstrating not only Merkel cells but also their neurite complexes.

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