Olfactory Receptor Cells: Immunocytochemistry for Nervous System-Specific Proteins and Re-evaluation of Their Precursor Cells

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Summary. The immunohistochemical localization of nervous system-specific proteins and cytokeratin in the olfactory mucosa is described mainly in humans and guinea pigs. Immunoreactivity for neuron-specific enolase (NSE) was demonstrated in olfactory receptor cells including their dendrite and axon. Immunoreactivity for neurofilament protein (NFP) was contained in the dendrite and perikaryon of the olfactory cells, but not in the axon. Immunoreactivity for spot-35 protein, a kind of neuron-specific proteins was selectively localized in several flask-shaped cells of the olfactory epithelium only in the guinea pig. These spot-35 protein-positive cells were suspected of being microvillar cells, considered the second type of receptor cells dispersed in the olfactory epithelium. Immunoreactivity for glia-specific S-100 protein was demonstrated in the Bowman’s glands as well as Schwann cells associated with the olfactory nerves in the lamina propria. Supporting cells were not immunolabelled with any antisera against nervous system-specific proteins. Cytokeratin, a useful marker for epithelial cells, was expressed exclusively in basal cells.

Axotomy of olfactory nerves induced the disappearance of the olfactory receptor cells with NSE immunoreactivity 3 days after the operation. Regenerating cells showing NSE immunoreactivity were first recognized in the lower portion of the epithelium within 7 days. The epithelium was completely repaired 3-4 weeks later and came to contain many NSE-reactive mature receptor cells. Bromodeoxyuridine (BrdU), administered during regeneration of the olfactory epithelium, was immunohistochemically detected in the cells of the layer above the basal cell layer, but not in the latter itself. This result does not support the general contention that the receptor cells originate from the basal cells.

Olfactory receptor cells have been regarded as a type of neuron, though they are clearly discriminated from ordinary neurons in certain respects. With their apical ends called the olfactory vesicle, the cells protrude into the nasal cavity to perceive odors. The receptor cells are widely accepted to regenerate after chemical destruction or after degeneration caused by olfactory axotomy; furthermore they undergo turnover under normal circumstances. The receptor cells are generally believed to originate from precursor cells present in the basal portion of the epithelium (GRAZIADEI and METCALF, 1971; GRAZIADEI and OKANO, 1979).

In consideration of these properties, the olfactory receptor may be called a neuron with paraneuron overtones or may even deserve to be counted as a paraneuron (FUJITA, 1977; see also FUJITA et al., 1988). The unique features of the “regenerating neuron” have led us to a number of experiments whose results may serve our understanding of the neuron-paraneuron system as a whole. This study deals with immunocytochemical characteristics of various cells constituting the olfactory mucosa, and with the regeneration process and the precursor of the receptor cells.

HISTOLOGY AND IMMUNOHISTOCHEMICAL FEATURES OF THE OLFACTORY EPITHELIUM

The human olfactory mucosa occupies a small superior region of the nasal cavity, roughly estimated to be 0.5 – 1 cm² in area on each side in adults. It is much wider in other mammalian species; in the rodents or carnivores, one third of the nasal cavity is covered by the olfactory mucosa. Three main cell types have been identified in the olfactory epithelium among
mammalian species: olfactory receptor cells, supporting (sustentacular) cells, and basal cells.

The olfactory receptor cell displays a bipolar neuron. The perikaryon extends a short dendrite to the epithelial surface forming the olfactory vesicle, and a long axon towards the olfactory bulb.

The olfactory receptor cell which is regarded as a primary sensory neuron shares some neuron-specific substances with other authentic neurons. Neuron-specific enolase (NSE) is a brain-specific isozyme of the glycolytic enzyme enolase. This substance is characterized by its exclusive localization in neurons (MARANGOS and SCHMECHEL, 1980). We revealed the existence of NSE immunoreactivity in the olfactory receptor cells of human fetuses (TAKAHASHI et al., 1984) and confirmed this finding in human adults and guinea pigs (Figs. 1, 6). Neurofilament protein (NFP) is an important protein forming the cytoskeleton of neurons. Immunohistochemistry using anti-NFP sera has been utilized to specifically stain neurofilaments in neurons (YEN and FIELDER, 1981). We also found NFP immunoreactivity to be contained in the olfactory receptor cells in human fetuses and in some animals including rats, guinea pigs and dogs (Fig. 2), although we could not demonstrate the immunoreactivity in human adult cells (TAKAHASHI et al., 1984 and our unpublished data). It was common among mammalian species for the NFP immunoreactivity to be restricted to the perikaryon and dendrite of the receptor cells, and absent in the axon (TAKAHASHI et al., 1984).

Our study (YAMAGISHI et al., 1986) also revealed that an antiserum against keratin (molecular weight: 56 and 64 kilodaltons) isolated from the human skin selectively labelled the basal cells in humans and guinea pigs (Fig. 7). Other cellular elements including receptor cells and supporting cells were negative in reaction. Immunostaining for keratin appears to be useful for specifically detecting basal cells.

Recently, MORAN et al. (1982a, b) demonstrated a fourth cell type in the human olfactory epithelium by electron microscopy, calling it the “microvillar cell”. The presence of cells related to “microvillar cells” had been already reported in other mammals: OKANO (1973) observed the same cell type in the dog bearing microvilli under the electron microscope. ANDRES (1969) briefly reported on a “microvillar cell” as an independent cell element in the cat and considered it a type of receptor cell. This cell is characterized by its position near the free surface and flask-like shape. Its apical end is covered with short and thick microprojections which are apparently different from the microvilli of the supporting cell. Despite these detailed morphological examinations, the function of the microvillar cells remains to be elucidated. We found that the flask-shaped microvillar cells strongly immunoreacted with antisera against spot-35 protein in guinea pigs; some cells extended an axon-like cytoplasmic process towards the basement membrane (Fig. 3). Spot-35 protein is a soluble protein which has been shown to occur specifically in cerebellar Purkinje cells (YAMAKUNI et al., 1984). IWANAGA et al. (1985) demonstrated the existence of an immunoreactivity for this protein in some sensory paraneurons. In their study, only a small number of immunoreactive cells were found in the olfactory epithelium of the guinea pig and were judged as olfactory receptor cells. However, further immunohistochemical examinations by us showed that the immunoreactive cells corresponded to the microvillar cells rather than the olfactory receptor cells. The existence of spot-35 protein in the microvillar cells seems to support the view that the cells may be a second receptor cell in the olfactory epithelium.

The lamina propria of the olfactory mucosa contains Bowman’s gland and two types of nervous elements, olfactory and trigeminal nerves. Our immunohistochemical staining using anti-NSE antiserum demonstrated various sizes of nerve bundles throughout the lamina propria in human fetuses (TAKAHASHI et al., 1984). On the other hand, the anti-NFP antiserum specifically stained nerve bundles that were thick and located in the lower (deeper) region of the lamina propria. These NFP-positive bundles appear to correspond to the trigeminal nerves, because the axons of the olfactory receptor cells are immunonegative for NFP (TAKAHASHI et al., 1984). We confirmed the same immunohistochemical finding for nerves in guinea pigs. These immunohistochemical results indicate that NFP could be a good marker for the trigeminal nerves in the lamina propria, NFP-negative and NSE-positive nerves being assigned as olfactory nerves. Both types of nerve bundles were intensely immunolabelled with antisera against the glia-specific protein, S-100 protein due to the reaction of their Schwann sheaths. We further demonstrated that Bowman’s glands reacted with the S-100 antiserum (Fig. 4). Immunoactivity for S-100 protein has been detected in glandular and ductal cells of some exocrine glands including the salivary gland (MOLIN et al., 1984; HAIMOTO et al., 1987) and nasal gland in the respiratory mucosa (SUZUKI et al., 1988). These results suggest that S-100 protein may somehow be involved in the secretory function in some exocrine glands.
REGENERATION OF OLFACTORY RECEPTOR CELLS

Although the olfactory receptor cells show neuronal characteristics, they undergo regeneration or turnover which is usually not seen in authentic neurons. NAGAHARA (1940) first examined morphological changes in the olfactory mucosa after cutting the olfactory nerve in amphibians and found extensive degeneration of the receptor cells. In his experiment most of the receptor cells disappeared in 3 days, but the olfactory epithelium including intact receptor cells had completely regenerated within 90 days after the operation. Later several investigators confirmed these degeneration and regeneration processes in the frog (SMITH, 1951), monkey (SCHULTZ, 1960), rabbit (MULVANEY and HEIST, 1971) and pigeon (GRAZIADEI and OKANO, 1979).

Fig. 1. Neuron-specific enolase (NSE) immunoreactivity in the olfactory epithelium of a human adult. NSE immunoreactivity is found in olfactory vesicle, dendrite and perikaryon of the olfactory receptor cells. ×600

Fig. 2. Neurofilament protein (NFP) immunoreactivity in the olfactory epithelium of a human fetus. NFP immunoreactivity is restricted to the perikaryon and dendrite of the receptor cells, and absent in the axon. ×750

Fig. 3. Spot-35 protein immunoreactivity in the olfactory epithelium of a guinea pig. Immunoreactivity is restricted to a flask-shaped cell with an axon-like cytoplasmic extension. ×850

Fig. 4. S-100 protein immunoreactivity in the olfactory mucosa of a human adult. Nerve bundles (arrow) and Bowman's glands in the lamina propria are immunoreactive for S-100 protein. ×460
Fig. 5. NSE immunoreactivity in the olfactory epithelium of a guinea pig 7 days after olfactory axotomy. NSE-reactive cells (arrows) are present in the atrophic and thin epithelium; some of them reach the surface of the epithelium. ×850

Fig. 6. NSE immunoreactivity in the olfactory epithelium of a guinea pig 28 days after axotomy. The epithelium becomes thick, numerous olfactory receptor cells being immunoreactive for NSE. The immunopositive nerve bundles with various size are seen in the lamina propria. ×280

Fig. 7. Basal cells in the olfactory mucosa of a normal guinea pig react strongly to an anti-keratin antiserum. ×380

Fig. 8. Bromodeoxyuridine (BrdU) immunoreactivity in the olfactory mucosa of a guinea pig 7 days after axotomy. Cells uptaking BrdU (arrows) are dispersed in the lower portion of the epithelium. Note that they are located in the second cell-layer apart from the basement membrane. ×480
Fig. 9. Olfactory epithelium of a human adult with olfactory disturbance after viral infection. Cells having irregular-shaped nucleus (arrows) are arranged between the basal cell-layer and a layer containing nuclei of receptor cells and supporting cells. O olfactory receptor cell, S supporting cell, B basal cell. ×10,000
We analyzed the regeneration of the olfactory receptor cells in the guinea pig by use of an immunohistochemical method. The olfactory mucosa was obtained from the superior-posterior portion of the nasal septum 3, 7, 14, 21 and 28 days after olfactory axotomy at the level of the lamina cribrosa. Within 3 days, NSE-immunoreactive cells had completely disappeared, while supporting cells and keratin-immunoreactive basal cells remained. New receptor cells reacting with the anti-NSE antiserum were found within one week not in the very basal cells but in the cells directly above them (Fig. 5), and 3-4 weeks later the epithelium recovered to its normal state, containing many NSE-immunoreactive receptor cells (Fig. 6).

PRECURSOR OF OLFACTORY RECEPTOR CELL

In his pioneering study, Nagahara (1940) noted that the precursor cells were situated in the basal area of the olfactory epithelium and called them “resting cells”. Nagahara’s finding was supported by an electron microscopic study by Andres (1969) who called the cells “blastema cells”. Graziaedi and Metcalf (1971) believed, on the basis of their autoradiographic studies with 3H-thymidine, that the basal cells should be regarded as the precursors of both olfactory receptor and supporting cells; After administration of 3H-thymidine, the labelling was concentrated in two types of cells in the epithelium. One type of cells lay on the basal lamina, while the other was located between the basal cells and the middle cell-layer where nuclei of the receptor cells gathered. Graziaedi and Metcalf (1971) thus regarded the former cells as undifferentiated basal cells, and the latter as differentiated cells, and considered that “resting cells” or “blastema cells” might be elements under differentiation from the basal cells to mature receptor cells.

This view has been accepted by many investigators, while our previous study has demonstrated that all basal cells strongly reacted to an anti-keratin antiserum (Yamagishi et al., 1986). This result suggested that this cell type could be characterized as a mature epithelial component. We therefore cast doubt on the view that the receptor cells originate in the basal cell.

To determine the precursor of the olfactory receptor cells, we performed an experiment using a new technique to label dividing cells by bromodeoxyuridine (BrdU). BrdU, a thymidine analogue is incorporated into DNA in dividing S-phase cells, and then the existence of BrdU in the tissue can be detected by using a monoclonal antibody against it (Gratzner, 1982). BrdU was administered intraperitoneally to guinea pigs 1 h before decapitation. Specimens were obtained 7, 14, and 21 days after axotomy in guinea pigs. At the same time, an anti-keratin antiserum was used to identify the basal cells. When BrdU was administered to guinea pigs after axotomy, immunostaining for BrdU labelled some cells which were different from the basal cells and located just by one layer more apically than the latter (Fig. 8). The BrdU-immunoreactive cells were most numerous at 7 days; fewer immunoreactive cells were found at 14 days, and still fewer at 21 days. The basal cells were never immunolabelled with the BrdU antibodies during the regeneration process. We conclude therefore that the precursors of the receptor cells are located between the receptor cells and the basal cells.

Electron microscopic observation revealed unidentified cells with an irregular-shaped nucleus occurring between the perikaryon of receptor cells and basal cells in human olfactory epithelium (Fig. 9). We could frequently observed the similar cells under some pathologic conditions such as viral infection and head trauma. This type of cell was originally reported by Yamamoto (1976) by electron microscopy in bats and regarded as an intermediate type developing from the basal cell to the receptor cell. It is reasonable to consider that these cells in humans and bats correspond to our BrdU-uptake cells recognized in guinea pigs.

In conclusion, we propose that the basal cells are keratin-rich mature epithelial cells and do not represent the precursor cells of the olfactory receptor cells. Our NSE immunocytochemistry and BrdU uptake study indicate that the cells immediately above the basal cell layer should be the precursors.

REFERENCES

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