Immunohistochemical Demonstration of the Nerves in Human Dental Pulp with Antisera against Neurofilament Protein and Glia-specific S-100 Protein

Takeyasu Maeda,1 Toshihiko Iwanaga,2 Tsuneo Fujita2 and Shigeo Kobayashi1

Department of Oral Anatomy (Prof. S. Kobayashi),1 Niigata University School of Dentistry and Department of Anatomy (Prof. T. Fujita),2 Niigata University School of Medicine, Niigata, Japan

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Summary. Human dental pulp was investigated by an immunofluorescence method using antisera against neurofilament protein (NFP) and glia-specific S-100 protein. Nerve fibers coursing through the pulp were selectively stained with the anti-NFP serum, and their Schwann sheath with the S-100 antiserum. In addition to a definitive demonstration of the subodontoblastic nerve plexus, we found another hitherto unknown nerve plexus composed of delicate fibers at the base of the odontoblastic layer. This study shows that immunohistochemical techniques using antisera against nervous system-specific proteins are superior to previous silver impregnation methods, due to their specificity and constancy in reaction.

Nerves in the dental pulp are an important topic for dentistry as they relay pain sensation from the teeth and because their terminal distribution remains to be clarified. Yet the pulpal nerves are not easy to stain. Many investigators have studied them by means of urea silver impregnation methods according to Ungewitter (1951) and Powers (1952). Although these methods may be useful for demonstrating the distribution of nerve fibers (Fernhead, 1970; Itoh, 1976; Gunji, 1982), they are, as all silver techniques are, capricious and unspecific in nature. For that reason, misinterpretations were often ascribed to the microscopic structure and distribution of the pulpal nerves. Moreover, the silver impregnation methods give us little information concerning the Schwann sheath of the nerves.

Through advances in biochemistry and immunohistochemistry, numerous proteins have been discovered to be specific for nervous tissues (Bock, 1978). The nervous system-specific proteins are classified into two main groups: neuron-specific and glia-specific proteins. Neurofilament protein (NFP), a representative of the former group, constitutes the neuronal cytoskeleton (Schlaepfer and Lynch, 1977). On the other hand, S-100 protein is contained in astrocytes, oligodendrocytes, and ependymal cells of the central nervous system, as well as in Schwann cells and satellite cells of the peripheral nervous system (Ludwin et al., 1976; Cocchia and Michetti, 1981; Stefansson et al., 1982). Immunohistochemistry using antisera against the neuron and glia-specific proteins has been shown to be a useful tool for identifying neuronal and glial elements.
and their derivatives (Ludwin et al., 1976; Schlaepfer and Lynch, 1977; Cocchia, 1981; Cocchia and Michetti, 1981; Stefansson et al., 1982; Fujita et al., 1983).

The present study reports the applicability of immunohistochemical techniques using NFP and S-100 antisera to the study of human dental pulp. This paper also deals with the distribution of pulpal nerves, especially a nerve plexus at the base of the odontoblastic layer which has not yet been clearly identified in previous impregnation studies.

MATERIALS AND METHODS

Human third molar teeth were used in this study. The extracted teeth were immersed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.3, for 6 hrs. After fixation, the teeth were fractured bucco-lingually with a chisel and hammer, and their pulp carefully extirpated from the teeth. They were rinsed in 30% sucrose solution overnight and rapidly frozen in dry ice-acetone. Sections were cut at 40–50 μm thickness in a cryostat. The floating sections were processed for an indirect immunofluorescence method according to Coons et al. (1955). After treatment with 0.3% Triton-X in 0.01 M phosphate buffered saline, the sections were incubated in an anti-NFP serum or an anti-S-100 serum which were both diluted in 1:150. The anti-NFP serum was raised in a rabbit by injections of a NFP subunit of 145 K molecular weight purified from rat brains according to a modification of Yen and Fielder (1981). The anti-S-100 serum was obtained from a rabbit by injecting S-100 purified from bovine cerebra (Masuda et al., 1983). The specificity of the immunoreactions was tested by use of antisera pre-incubated overnight at 4°C with the corresponding antigens (10 μg/ml diluted antiserum).

For the purpose of comparison, we observed specimens of the dental pulp fixed in Bouin’s fluid and impregnated with the Powers’ method (1952).

RESULTS

Numerous nerve fibers showing NFP-immunoreactivity were observed in the human dental pulp (Fig. 1). The immunopositive nerve fibers in the root pulp were bundled, and ascended with blood vesseles. Most of them were distributed throughout the whole coronal pulp splitting into numerous nerve fasciculi apart from the blood vessels and, ultimately, into single fiber branches. Individual fibers positive for NFP formed a plexus in the vicinity of the cell-rich zone; this corresponds to the subodontoblastic nerve plexus of Raschkow (1835) (Fig. 2a). Some nerve fibers extending from the plexus terminated in the marginal pulp, and others formed a fine network structure at the base of the odontoblastic layer, which we can consider as a nerve plexus (Fig. 1, 2b). From here, fibers passed perpendicularly through the odontoblastic layer toward the pulpodentinal junction (Fig. 1). The subodontoblastic plexus of Raschkow also directly projected some branches into the odontoblastic layer. The fibers immunostained for NFP were clearly more numerous and denser in distribution than those impregnated with Powers’ method. The newly described, odontoblastic plexus could be identified only by this immunohistochemical method.

On the other hand, S-100 immunoreactive elements were also distributed densely throughout the pulp (Fig. 3). The anti-S-100 serum was found to stain Schwann cells
with slender cytoplasmic processes (Fig. 4). Observation of the cross-sectioned nerve bundles revealed that an S-100-positive thin layer surrounded the S-100-negative axons, suggesting the existence of the Schwann sheath being selectively stained. The S-100-positive elements formed a plexus located adjacent to the cell-rich zone, corresponding to the subodontoblastic plexus of NFP-positiv enerve fibers. Passing through the plexus, some of the S-100-positive fibers ramified to terminate in the marginal pulp, while others reached the odontoblastic layer. Corresponding to the finding of NFP immunoreactive fibers, the newly described odontoblastic plexus was identified as a network of very delicate, S-100-positive fibers.

It was noticed that S-100-positive fibers showed, here and there, spindle-shaped swellings. These, without doubt, corresponded to the nuclear portion of Schwann cells.

**DISCUSSION**

The present study revealed the dense distribution of nervous elements showing NFP
Fig. 2.  

a. A subodontoblastic nerve plexus in a tangential section. This plexus represents a coarse network of NFP-positive fibers. ×200.  
b. Photomicrograph, taken by changing focus, of the same region as Figure 2a. Newly described plexus at the base of the odontoblastic layer is demonstrated, composed of much more delicate fibers than the Raschkow’s plexus. ×200
and S-100 immunoreactivities in the human dental pulp. As NFP-immunoreactive nerve fibers were observed more abundantly than the fibers stained in silver-impregnated sections with the same thickness, it is supposed that the immunohistochemistry for NFP is more sensitive for detecting nerve fibers. A majority of the

Fig. 3. A subodontoblastic nerve plexus of Raschkow immunostained with an anti-S-100 serum. The S-100-positive elements are distributed densely under the odontoblastic layer (OL). Fine fibers reach the odontoblastic layer and one may identify the delicate odontoblastic plexus (arrow). ×205

Fig. 4. S-100 immunopositive nerves in the dental pulp. Stained Schwann cells with slender cytoplasmic processes and spindle-shaped thickenings are visible. Arrows indicate the localization of the nuclei. ×150
NFP-positive nerve fibers, except the fibers supplying the vessels, are considered to be sensory nerves, since several experimental studies to resect the inferior alveolar nerve have suggested a sensory origin for the pulp nerves (Corpron et al., 1972; Arwill et al., 1973). Sensory nerves showing intense NFP-immunoreactivity were also found in the skin (Iwanaga et al., 1982; Dalsgaard et al., 1984) and iris (Seiger et al., 1984).

We were able to demonstrate that fine NFP-positive fibers, after passing through the subodontoblastic nerve plexus of Raschkow, formed a construction deserving the name of nerve plexus at the base of the odontoblastic layer. As far as we are aware, this plexus has not been reported yet. We assume that this delicate network of fibers has been difficult to find in silver-impregnated preparations due to insufficient stainability. Moreover, such a network structure has been difficult to identify in silver-impregnated sections which have been usually 10–15 μm in thickness; our immunohistochemical sections can attain more than 40 μm.

Some fine NFP-positive fibers extending from this plexus and directly from the nerve plexus of Raschkow run through the odontoblastic layer toward the pulpodentinal junction, suggesting the penetration of the fibers into the predentin. Many researchers have reported by means of electron microscopes that nerve fibers can enter the predentin and dentin beyond the pulpodentinal junction (Arwill, 1967; Frank and Frank, 1972; Dahl and Mjör, 1973; Gunji, 1982). Furthermore, these dentinal nerve fibers have been proposed to form a mechanoreceptive complex with the odontoblastic processes (Gunji, 1982). In the specimens used in this study, these problems could not be studied as the dentin had been separated beforehand. We are searching new techniques to make immunostainable specimens of the pulp combined with dentin.

In this study, the authors confirmed that S-100 protein in the dental pulp is contained specifically in Schwann cells. Since immunostaining for S-100 demonstrated fibers in the same staining pattern as the NFP-positive fibers, it is suggested that all NFP-positive fibers possess a Schwann sheath. Even the newly identified odontoblastic nerve plexus as well as the fine fibers ascending in the odontoblastic layer can be visualized with the S-100 antiserum.

In conclusion, the present study showed that immunohistochemistry using anti-NFP serum and anti-S-100 serum is a useful technique to detect the neuronal and glial elements in the human dental pulp. Especially is immunostaining for NFP expected to take the place of previous silver impregnation methods.

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