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Topography of the enteric nervous system in Peyer's patches of the porcine small intestine

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Abstract. The mechanisms of intercommunication between the immune and nervous systems are not fully understood. In the case of the intestine, the enteric nervous system is involved in the regulation of immune responses. It was therefore decided to employ immunohistochemical techniques to investigate the structural organization of the enteric nervous system in Peyer's patches of the porcine small intestine. Using antibodies against various nervous system-specific markers (protein gene product 9.5, neuron-specific enolase, neurofilament 200, S-100 protein and the glial fibrillary acidic protein), an intimate and specific structural association could be demonstrated between enteric nerves and the compartments of Peyer's patches: follicles, interfollicular regions and domes. Peyer's patches have a close topographical relationship to the two submucosal plexuses. Enteric nerves are located around the follicle in the interfollicular area – the so-called "traffic area" – and in the dome area, which plays an important role in the uptake and presentation of antigens.

Key words: Enteric nervous system – Autonomic nervous system – Peyer's patches – Gut-associated lymphoid tissue (GALT) – Immune system – Pig

Introduction

There is substantial evidence that the nervous system and the immune system interact in a functionally meaningful way (Dantzer and Kelley 1989; Dunn 1989; Jankovic 1989; Irwin et al. 1990). The complex bidirectional communication between the two systems is mediated by hormonal and paracrine signals, and by direct contact between nerve fibers and cells of the immune system (Bateman et al. 1989; Bellinger et al. 1990; Weigent et al. 1990).

The intestine contains one of the largest accumulations of lymphoid tissue in the form of aggregates in Peyer's patches, solitary follicles, and lymphocyte populations scattered throughout the epithelium and the lamina propria: the so-called gut-associated lymphoid tissue (GALT) (Bienenstock and Befus 1985; Doe 1989). Peyer's patches are typically organized lymphoid structures of the gut. They are not only typical of the ileum, but are also present in the duodenum and jejunum in some species. Peyer's patches play a central role in the uptake of antigens, the induction of an immune response, the proliferation of B lymphocytes and the synthesis of immunoglobulins (Pabst 1987).

The innervation of the intestine clearly differs from that of other peripheral organs. Intestinal function is regulated by its own intramural nervous system – the enteric nervous system – which has distinctive structural and functional features. It is relatively independent of the central nervous system. During the past few decades the organisation of this highly complex system has been elucidated by use of a number of different techniques (for a general review, see Gabella 1987; Costa et al. 1987; Wood 1987; Furness et al. 1988; Stach 1989; Timmermans et al. 1990; Krammer and Stach 1991; Scheuermann et al. 1991). The involvement of the enteric nervous system in intestinal immune phenomena is only partly recognized (Castro 1989). For this reason, knowledge of the microanatomical relationship of enteric nerves to cells of the intestinal immune system is of basic importance. It was therefore the aim of this study to provide a detailed description of the innervation of Peyer's patches in the porcine small intestine.

Materials and methods

The small intestine of five-week-old and adult domestic pigs was perfused in the retrograde direction via the abdominal aorta with oxygenated Krebs-Ringer solution at 37° C for 5 min. Segments of the terminal ileum and jejunum, together with their Peyer's patches, were dissected out and immersed in a fixative containing 3% paraformaldehyde and 2% picric acid in phosphate-buffered saline (PBS) at room temperature.

The intestine was further processed to improve the conditions for immunohistochemistry as described by Scheuermann et al.

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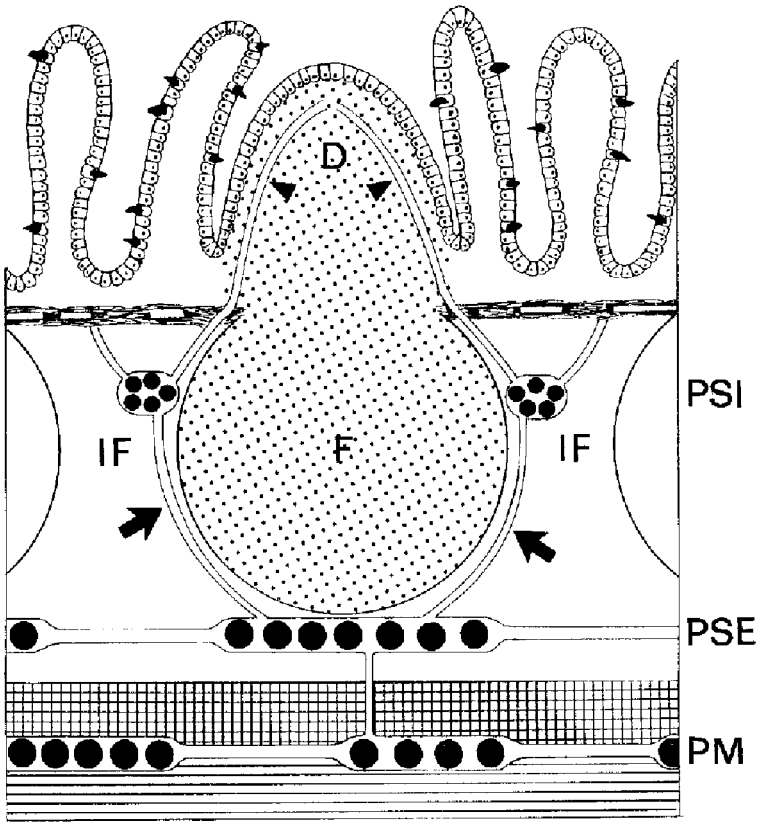

Fig. 1. Schematic representation of the topography of the enteric nervous system in a Peyer's patch. Note plexus myentericus (*PM*) in the tunica muscularis. The plexus submucosus externus (*PSE*) is situated close to the base of the follicle (*F*); nerve fibers (*arrows*) extend from its ganglia to the plexus submucosus internus (*PSI*) in the interfollicular area (*IF*). Nerve fibers (*arrowheads*) arising from these ganglia contact the dome (*D*)

(1987). Whole-mount preparations (tissue-sheet preparations) of the tunica mucosa and tela submucosa including the Peyer's patches were dissected out under stereomicroscopic control and further treated with 10% normal goat serum (Dakopatts; X 907) for 30 min. They were incubated twice in a primary antiserum raised against the following different markers: protein gene product 9.5 (Ultraclone RA 95101, diluted 1:400), neuron-specific enolase (Dakopatts A 589, diluted 1:400), neurofilament 200 (Sigma N 4142, diluted 1:100), S-100 protein (Dakopatts Z 311, diluted 1:400) or glial fibrillary acidic protein (Dakopatts Z 334, diluted 1:400) for 17 h. Goat anti-rabbit IgG (Dakopatts Z 421, diluted 1:100) was used as the secondary antiserum. The specimens were then incubated in a solution containing a peroxidase-anti-peroxidase complex (Dakopatts Z 113, diluted 1:100) for 12 h. 4-Cl-1-naphtol was used as the chromogen for the peroxidase reaction.

For sectioning, the tissues were fixed and processed in a similar fashion to the whole-mounts. Sections (15 µm thick) were collected on chromalum-gelatine-coated slides. Incubation took place in a humid chamber at concentrations and for times similar to those for immunohistochemistry of the whole-mounts. For control of specificity, the reaction was tested by omission of the primary antiserum.

Results

Peyer's patches are found in two distinct segments of the small intestine of the pig: a long continuous aggregate in the terminal ileum, and several discrete patches in the jejunum and upper ileum. Peyer's patches are organized into follicles and interfollicular regions, each follicle being covered by a follicle-associated epithelium and its underlying dome. The location and topography of the enteric nervous system in Peyer's patches can clearly be demonstrated by immunostaining for neuronal and glial markers, both in whole-mount preparations and in sections. Among the various markers used to identify enteric nerves in Peyer's patches, protein gene product 9.5 and glial fibrillary acidic protein provided the best results.

The following description is a composite picture gained by observation of the localization of these markers for demonstration of the enteric nervous system. The enteric nervous system in the region of Peyer's patches consists of three ganglionated plexuses: the plexus myentericus, plexus submucosus externus, and plexus submucosus internus. In the other layers of the intestinal wall, the tunica muscularis, the lamina muscularis mucosae and the tunica mucosa, nerve fibers form aganglionated plexuses (Fig. 1). Due to the location of lymphoid follicles in the tela submucosa and tunica mucosa Peyer's patches are in close topographical relationship with the two submucosal plexuses. The plexus submucosus externus is situated close to the base of the follicles (Fig. 2). Nerve fibers run from its ganglia to the plexus submucosus internus between the follicles, in the interfollicular area (Fig. 3). The internal submucosal plexus is located within the innermost part of the tela submucosa. Ganglia of this plexus lie in the interfollicular region and the emerging nerves surround the lymphoid follicles (Fig. 3). Nerve fibers run from these ganglia to the tunica mucosa. An aganglionic plexus, which also surrounds the follicle, is formed in the lamina muscularis mucosae (Fig. 4a). Although we were unable to find nerve fibers in the follicle proper, many nerve fibers run together in the dome area of the tunica mucosa and form a dense network under the follicle-associated epithelium (Fig. 4b).

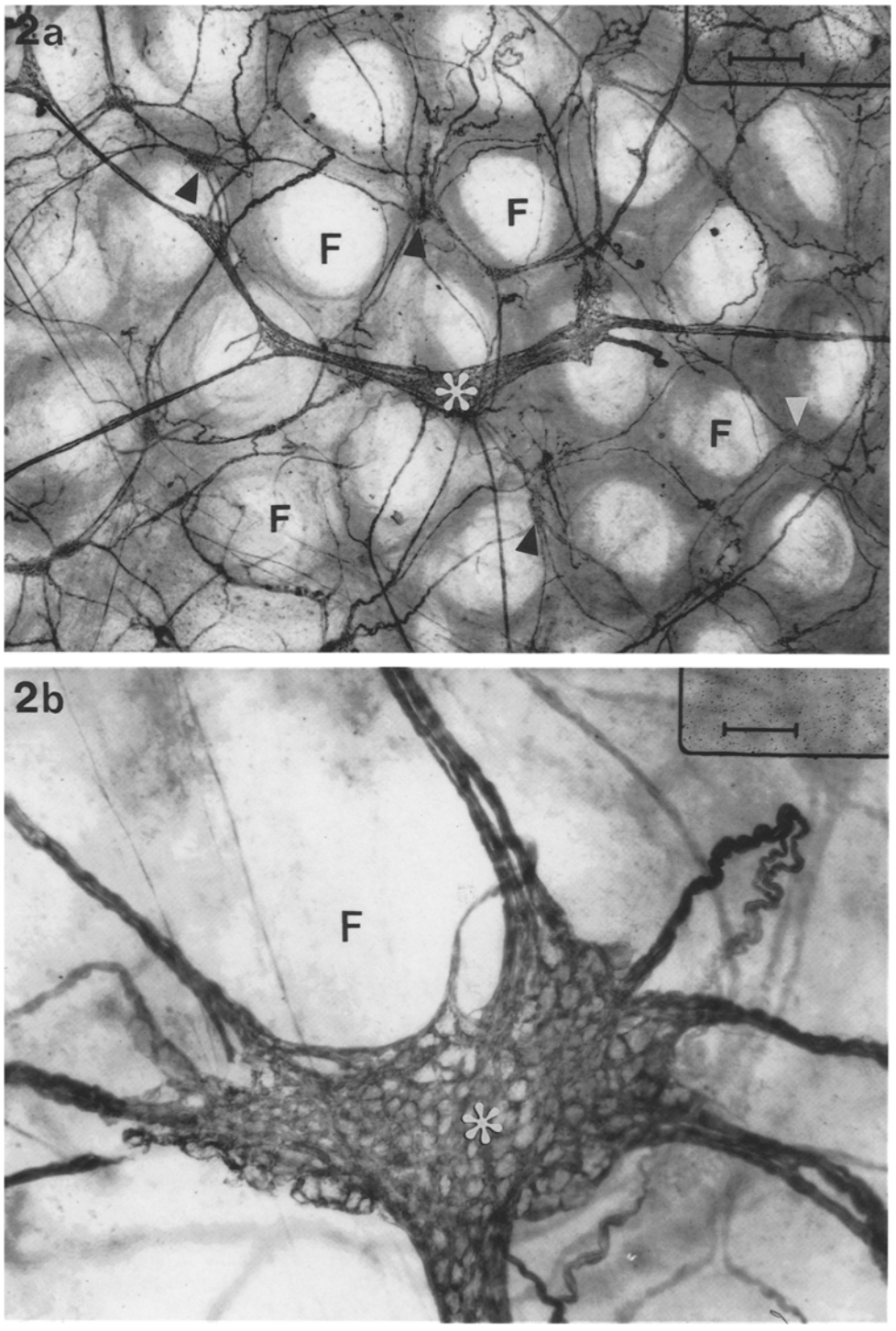

Fig. 2a, b. Immunohistochemistry for GFAP in a whole-mount preparation of the tela submucosa in Peyer's patches. (**a**) The plexus submucosus externus (*asterisk*) and the plexus submucosus internus (*arrowhead*) have a close topographical relationship with the Peyer's patches. (**b**) A ganglion of the plexus submucosus externus (*asterisk*) is situated adjacent to the base of the follicle (*F*). **a** × 25, *bar*: 400 μm; **b** × 100, *bar*: 100 μm

Discussion

The most convincing evidence for the immuno-modulatory effects of the nervous system comes from studies on the cholinergic and postganglionic sympathetic innervation of the primary and secondary lymphoid organs (Felten et al. 1985; Kendall et al. 1988; Ackerman et al. 1986; Madden et al. 1989; Van Tits et al. 1990).

Evidence for the peptidergic innervation of lymphoid tissue has not been so thoroughly established as for classical transmitters (Fink and Weihe 1988; Lundberg et al. 1985; Bellinger et al. 1990). In particular, the origin, distributional pattern and target relations of the nerves in lymphoid tissue are still incompletely elucidated (Kurkowski et al. 1990; Weihe et al. 1991).

Strong support for the influence of neuropeptides on the intestinal immune system comes from the investigations of neuropeptide receptors on cells of the immune system, e.g., receptors for substance P, somatostatin and vasoactive intestinal polypeptide. Furthermore, these substances have been shown to modulate processes in the intestinal immune system such as proliferation and

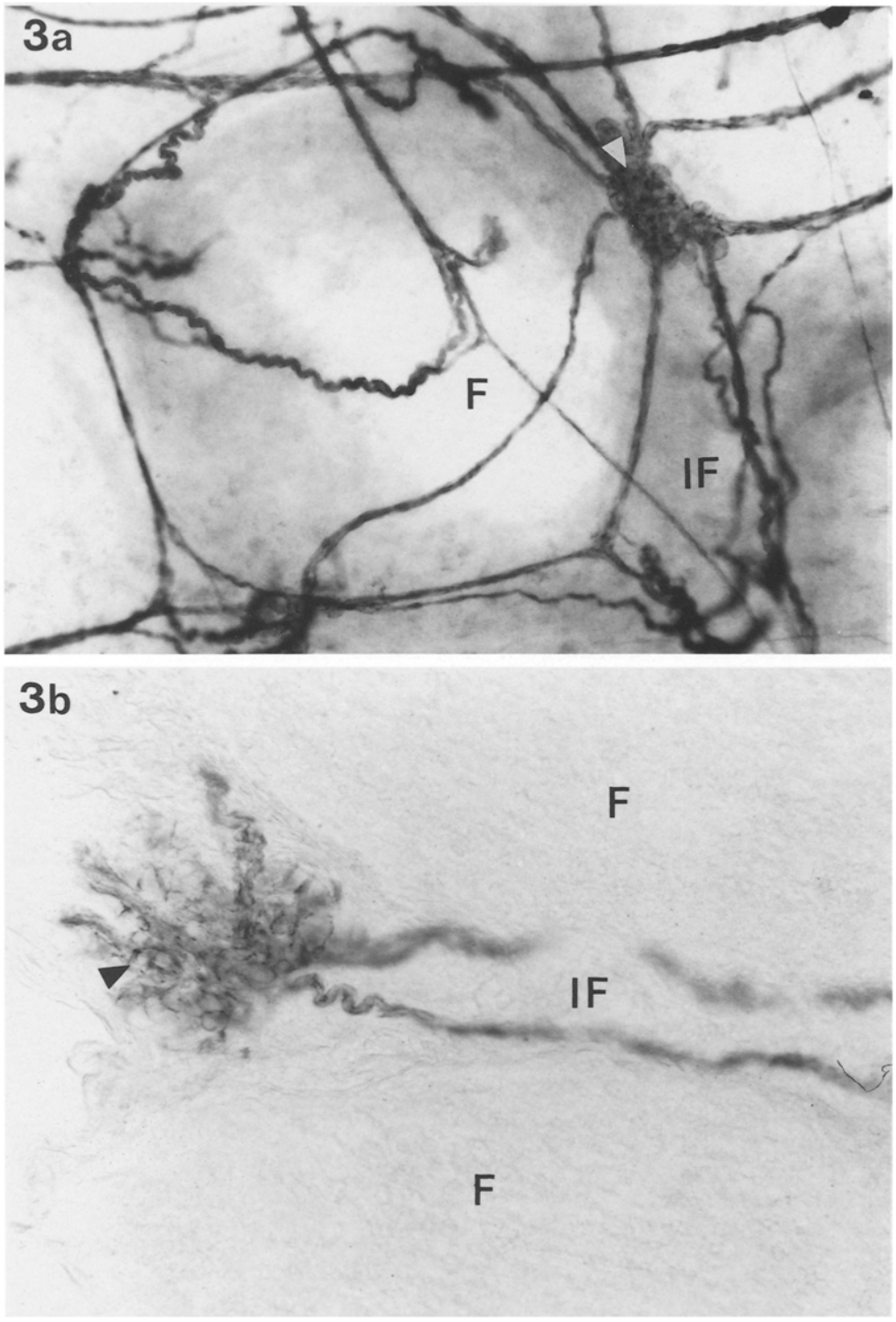

Fig. 3. Immunohistochemistry for GFAP in a whole-mount preparation (**a**) and in a section (**b**) of Peyer's patches. The ganglia of the plexus submucosus internus (*arrowhead*) are located in the interfollicular region (*IF*). Nerve fibers surround the follicle (*F*). **a** × 100; **b** × 250

migration of lymphocytes, and synthesis of immunoglobulins (for review, see Stanisz et al. 1986; Stead et al. 1987; Bellinger et al. 1990). To exert an effect on cells of the intestinal immune system in vivo, neuropeptides must be locally present in concentrations high enough to stimulate a response in the different compartments of Peyer's patches. The four compartments (follicle, corona, interfollicular region, dome) are defined by the typical localization of the lymphocyte subsets and their functions (Spencer et al. 1986; Pabst 1987). It therefore appears crucial to investigate which compartment of the Peyer's patch receives a neural input.

The present study has revealed that many nerve cells and varicose and nonvaricose axons are localized in the interfollicular region. This area is characterized by the presence of specific postcapillary venules, which are lined with high endothelial cells (Yamaguchi and Schoell 1983). Via these venules lymphocytes migrate from the blood throughout the entire follicle (Bjerknes et al. 1986). The anatomical relationship between these blood

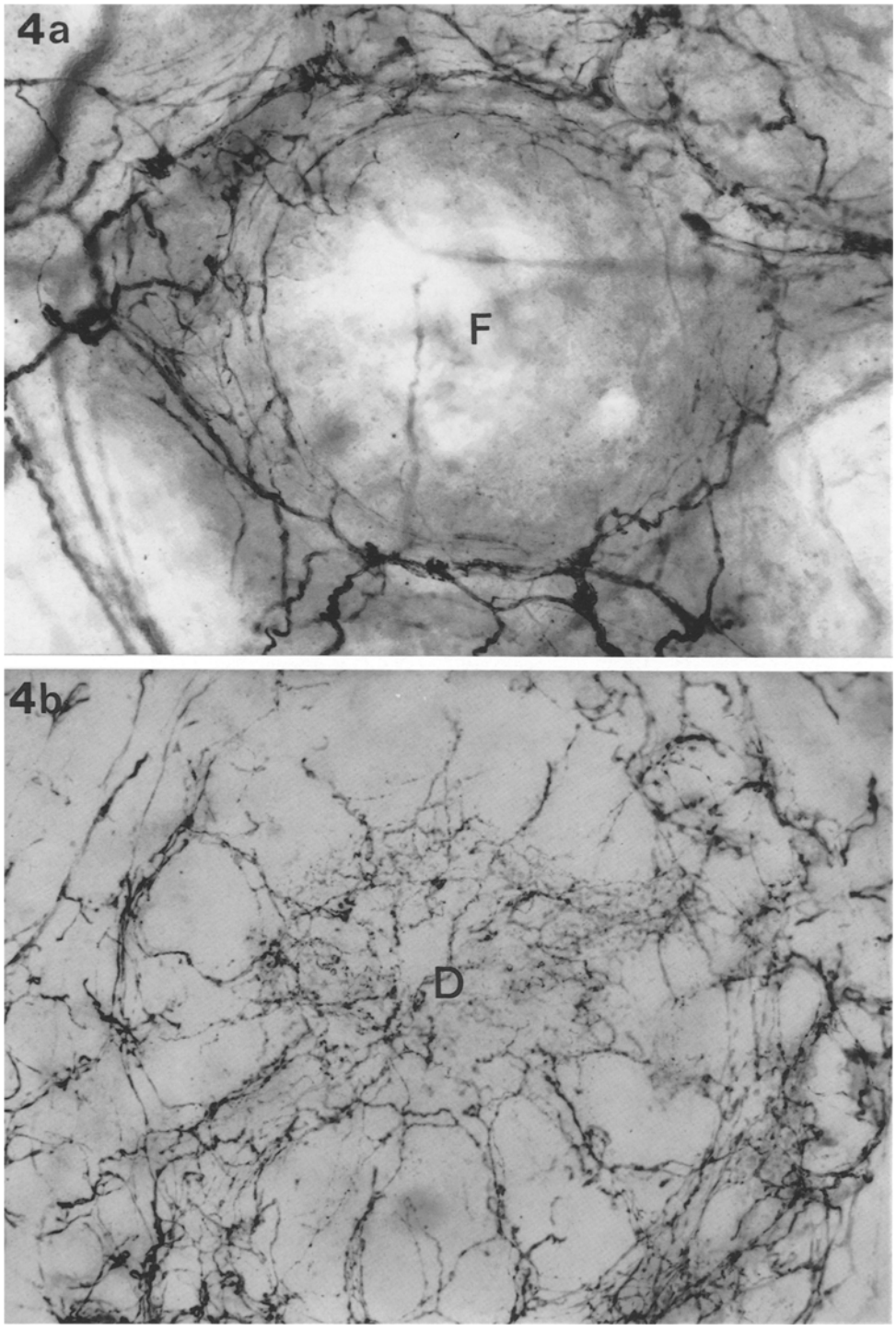

Fig. 4. Immunohistochemistry for GFAP (**a**) and PGP 9.5 (**b**) in the tunica mucosa. **a** In the lamina muscularis mucosae nerve fibers form an aganglionic plexus, which surrounds the follicle (*F*). **b** In the dome area (*D*) nerve fibers run together and form a dense plexus directly beneath the follicle-associated epithelium. **a** × 100; **b** × 160

vessels and the enteric nerves in the interfollicular region provides the basis for a neuronal influence on the immune function of these lymphoid aggregations. One of the most important properties of Peyer's patches concerns the overlying follicle-associated epithelium. Antigens reach Peyer's patches for processing by traversing through this epithelium (Bockman and Cooper 1973). The follicle-associated epithelium differs in two characteristics from the adjacent villus epithelium: (1) it contains fewer goblet cells (Bockman and Cooper 1973), and (2) specialized antigen-transport cells are interspersed among its columnar epithelial cells – the so called M-cells (Owen and Jones 1974; Wolf and Bye 1984). The presence of numerous nerves in close association with the follicle-associated epithelium at least circumstantially suggests a functional interrelationship.

The present study provides information on the structural organization of the enteric nervous system in Peyer's patches of the porcine small intestine, and provides a basis for further studies concerning the neu-

rotransmitters (e.g., galanin, vasoactive intestinal polypeptide, calcitonin gene- related peptide, substance P, neuromedin U, enkephalin, somatostatin and neuropeptide Y; see Timmermans et al. 1990) involved in the innervation of the different structural and functional compartments of Peyer's patches. Furthermore, description of this innervation pattern in healthy intestinal lymphoid tissue provides a basis for the investigation and comparison of alterations under experimental conditions and in disease.

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