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ELECTRON MICROSCOPICAL EVIDENCE FOR A DIRECT CONTACT BETWEEN NERVE FIBRES AND MAST CELLS

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Abstract. A subungual solitary glomus tumour was examined by both light and electron microscopy. By light microscopy, typical vascularized tumour tissue was seen, surrounded by connective tissue. By electron microscopy, a very close morphological relationship was noted between non-myelinated nerve fibres and mast cells. These mast cells could be divided into two groups: the mast cells of the first group contain numerous mature granules and show few lamellopodia. The distance to the nerve fibre bundles ranged between 2 000 and 20 nm. In the second group, the mast cells always showed direct contact with nerve fibres. They had many lamellopodia and contained almost exclusively immature granules. In some cases, invaginations of lamellopodia and broader cytoplasmatic processes into the axon bundles could be observed. Our findings support the view that mast cells may play an important part in the function of neurons.

Key words: Nerve fibres and mast cells; Solitary glomus tumour; Electron microscopy; Ultrastructure of mast cells

A close morphological relationship between nerve fibres and mast cells has been described repeatedly (6, 11, 16, 17), and it has been suggested that mast cells may play an important role in the generation of pain in solitary glomus tumours (11). The association of mastocytes and nerves might be of interest with regard to a variety of dermatological diseases, especially to those caused by immediate-type skin reactions.

A glomus tumour was selected for this investigation of the relationship between nerve fibres and mast cells, for two reasons: It has a well-known rich nerve supply, and it contains numerous mastocytes (5, 12, 13, 14, 18). In the present study, we have obtained data suggesting a functional relationship between nerve fibres and mast cells which is closer than has hitherto been supposed.

MATERIALS AND METHODS

A painful subungual solitary glomus tumour was excised from the right thumb of a 23-year-old woman and examined by light and electron microscopy. For light microscopic studies, one-half of the specimen was fixed in 4% formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin, methylene blue, and toluidine blue (pH 4.1). For electron microscopic studies, the other half of the specimen was fixed for 2 hours in 2% glutaraldehyde in 1/15 M Sørensen buffer at pH 7.35, rinsed in the same buffer for 20 hours and post-fixed for 2 hours in 1% osmic acid. The specimen was then dehydrated in graded ethanol and embedded in Epon. Ultrathin serial sections were stained with uranyl acetate and lead citrate and examined with a Philips EM 200.

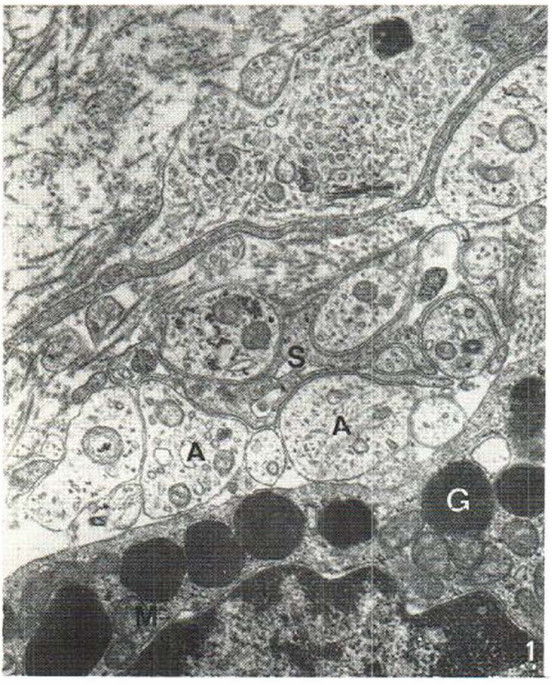
RESULTS

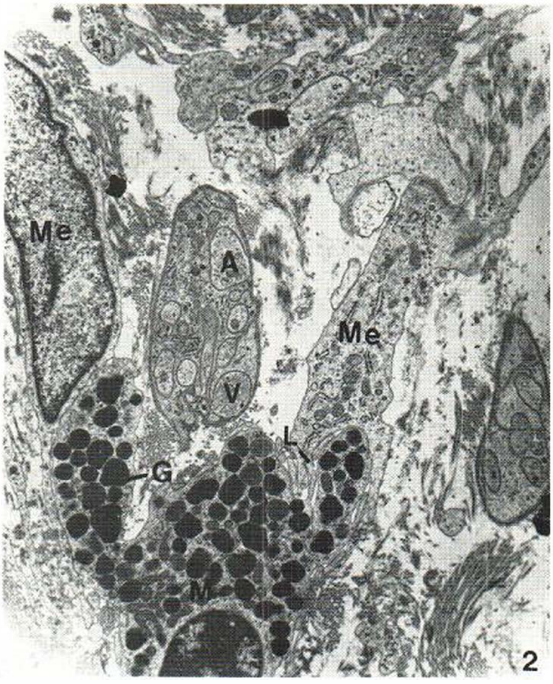
Light microscopy

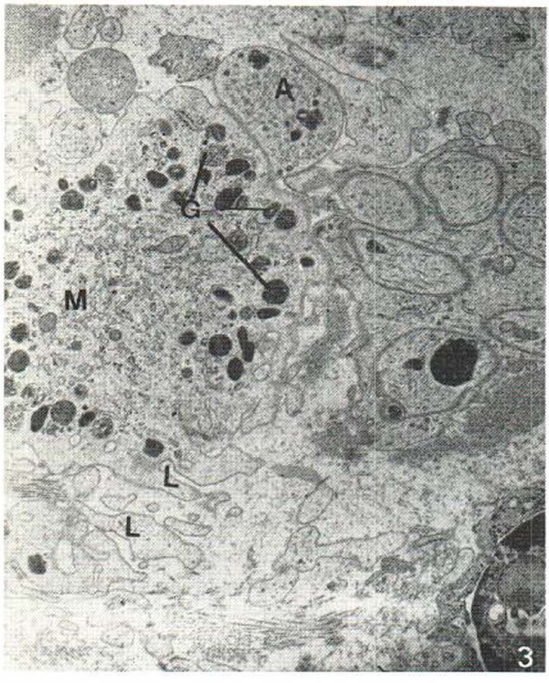
The tumour consisted of clusters of proliferating cells and some vascular channels. The tumour cells surrounding the blood vessels had elongated nuclei, while those in the periphery of the tumour had round nuclei and showed a slightly basophilic staining with hematoxylin-eosin. Numerous mast cells, as demonstrated by their metachromatic staining characteristics with toluidine blue, were localized mainly in the connective tissue adjacent to the tumour. A few mast cells were found in connective tissue strands at the periphery of the tumour. Many mastocytes were arranged in a strikingly linear, beaded manner.

Electron microscopy

Most of the tumour cells showed similarities to smooth muscle cells. The vascular channels were lined by endothelial cells. In the border region between the tumour and the surrounding connective







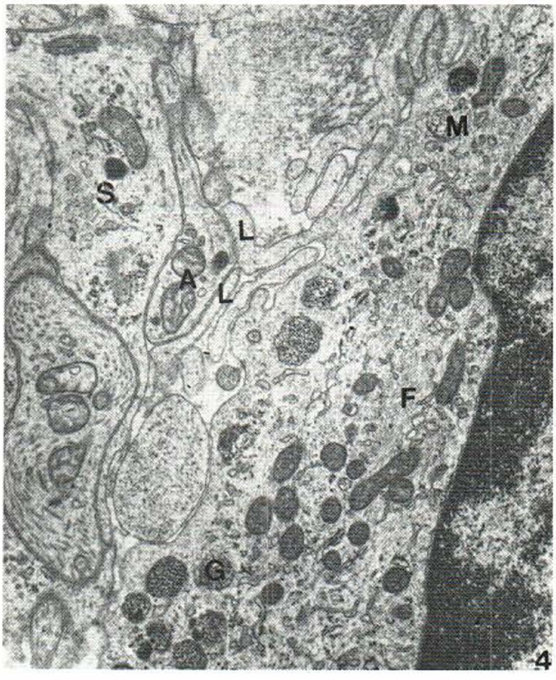


Fig. 1. A mast cell (*M*) of the 1st group in direct contact with some axons (*A*) which are not covered by the Schwann cell (*S*). *G*, granules. ×16465.

Fig. 2. A mast cell (*M*) with many dark and dense granules (*G*) and only few lamellopodia (*L*) in close proximity to a nerve fibre bundle and 2 mesenchymal cells (*Me*). One of the axons (*A*) is filled with vesicles (*V*). ×6010.

Fig. 3. A mastocyte (*M*) of the 2nd group, showing very few granules (*G*) in direct contact with an axon (*A*) which contains some vesicles. *L*, lamellopodia. ×8325.

Fig. 4. Two lamellopodia (*L*) of a mast cell (*M*) of the 2nd group are directly apposed to an uncovered axon (*A*). *S*, Schwann cell; *G*, granules; *F*, filaments. ×13875.

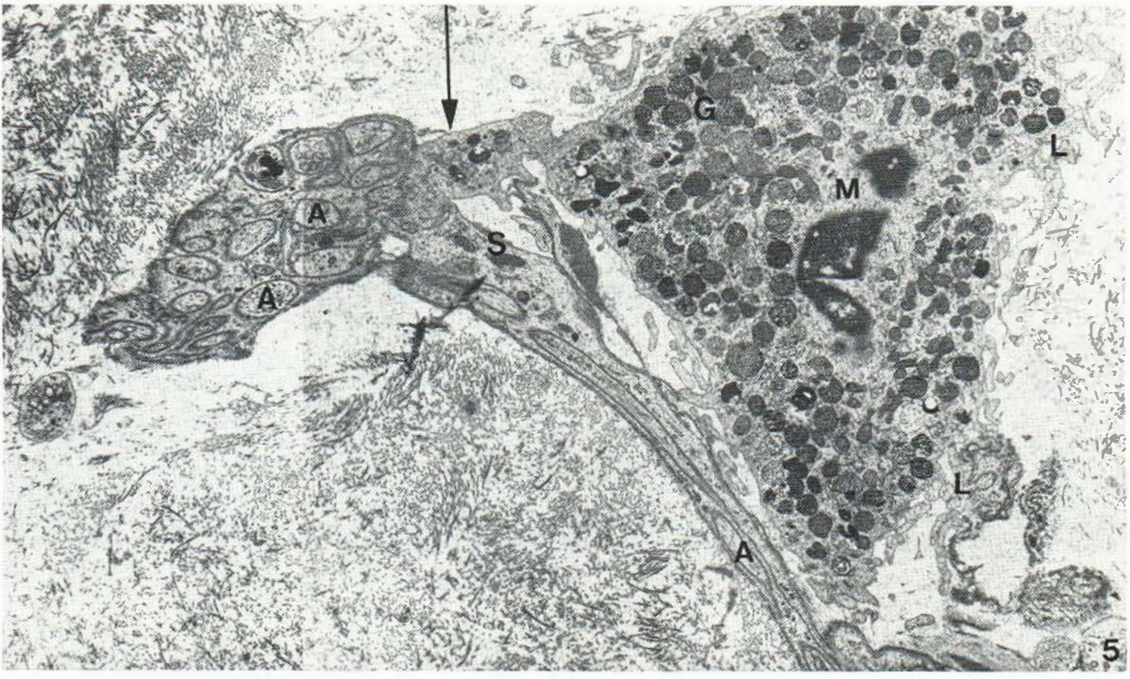


Fig. 5. A large cytoplasmatic process (*arrow*) of a mast cell (*M*) with mainly immature granules (*G*) is inserted into the nerve fibre bundle. *L*, lamellopodia; *A*, axons; *S*, Schwann cell. ×6 100.

tissue, numerous mast cells and bundles of non-myelinated nerve fibres were seen, with a conspicuously large number of close contacts with one another. The mast cells were situated among or adjacent to nerve fibre bundles which ran for the most part parallel to the tumour border. This explains the linear arrangement of many mastocytes, as observed by light microscopy.

On quantitative evaluation, 58% of the 166 observed mastocytes were situated within a distance of 2 μm from the axon bundles. The other mast cells were in close contact with mesenchymal and tumour cells, or else they lay between collagen fibre bundles. Mast cells with a close topographical relationship to nerve fibres can be divided into two groups on the basis of the following criteria: 1) the morphological features of the mast cells; 2) the kind of contact between these cells and nerve fibres.

In the first group, the mast cells contained numerous mature granules and showed few lamellopodia. The distance to the axon bundles ranged between 2 000 and 20 nm. The mast cells of the second group were always in direct contact with nerve fibres. They showed many lamellopodia and almost exclusively immature granules.

The mast cells of the first group (Figs. 1, 2) were situated within a distance of 2 μm, with only a few of them in direct contact (20 nm) with nerve fibre bundles. The cell surface was mainly smooth, with only a few lamellopodia present. The cytoplasm contained many dark granules of even density, and very few other cell organelles could be found. The chromatin of the indented nuclei was closely apposed to the inner nuclear membrane, the central core of the nuclei exhibiting a diffuse structure, while nucleoli were frequently seen. Some of the adjacent axons were not enclosed by Schwann cells. In some parts, even a covering lamina basalis was absent.

The mast cells of the second group (Figs. 3–6) showed a type of contact to the nerve fibre bundles which, as far as we know, has not been described hitherto. The cells were always in direct contact with nerve fibres, either with their cell body (Fig. 3) or by means of lamellopodia or broader cytoplasmatic protrusions. Some of these cell processes were directly apposed to uncovered axons (Fig. 4) or were even inserted into the nerve fibre bundles (Figs. 5, 6) which may, in addition, be surrounded by other lamellopodia. Occasionally, accumulations

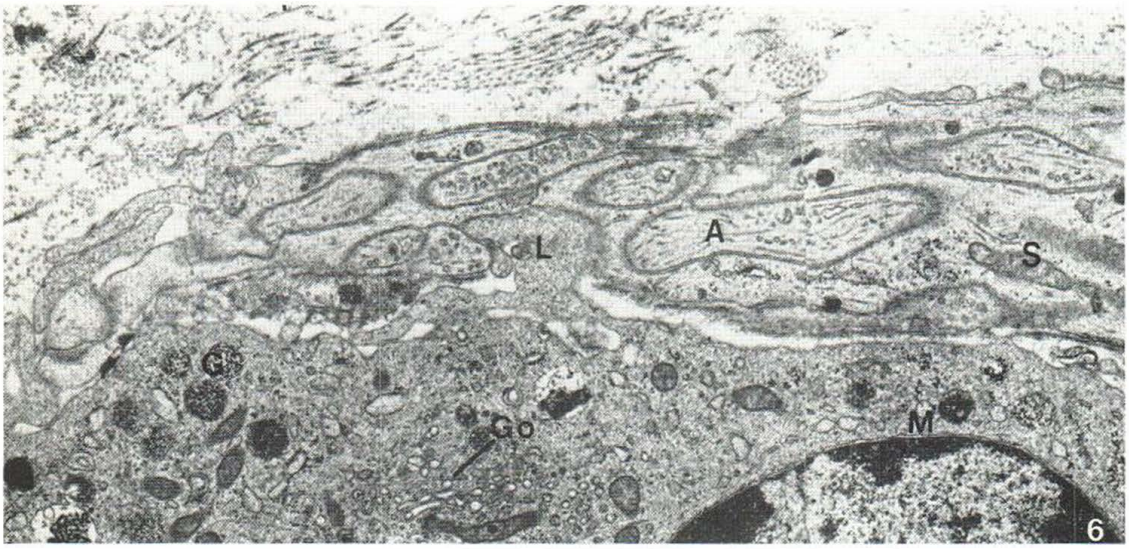


Fig. 6. One lamellopodium (*L*) of the mast cell (*M*) is invaginated into the nerve fibre bundle. *A*, axon; *S*, Schwann cell; *G*, granules; *Go*, Golgi apparatus. ×13 700.

of empty and some granular vesicles, 70–120 nm in diameter, were found in an axon profile (Fig. 3). The mast cells showed a relatively small number of granules which exhibited a loose or "honeycomb-like" structure. In some cases, there were signs of exocytosis of granules. A well developed Golgi apparatus, some mitochondria, free ribosomes and numerous filaments were evident. The dense chromatin aggregations on the inner nuclear membrane seemed to be slightly smaller than those of the nuclei of the first mast cell group.

DISCUSSION

A close topographical relationship between peripheral nerve fibres and mast cells has been reported by several authors (6, 11, 16, 17). The morphological features of the mast cells and the type of contact with the axons as hitherto described in the literature, correspond to the findings with the first type of mast cells, as reported in this paper, i.e., cells with many dark and dense granules in close vicinity to nerve fibres. The second type of contact shows an even closer relationship between mast cells and nerve fibres.

The potential functional importance of mast cell–nerve fibre contacts may be indicated by the following findings: 1) release of histamine from rat mast cells is induced by acetylcholine (4) and ATP (10); 2) degranulation of mastocytes by axon reflexes and antidromic stimulation of cutaneous nerves has been demonstrated (8, 9); 3) histamine stimulates axons that conduct pain and also the sensation of pruritus (7). Therefore, in our opinion, the mast cell may be regarded as a target cell for nerve impulses as well as a stimulator of nerve fibres.

Mast cells with loosely structured granules similar to the second mast cell group described by us, have been demonstrated in relationship to peripheral nerves in the developing adrenergic innervation of the iris of neonatal rats (15). However, as far as we know, the occurrence of lamellopodia or broader cytoplasmatic protrusions which are invaginated into nerve fibre bundles, has not been reported hitherto.

The question arises whether the differences between the two mast cell groups also imply different functional activities in the interaction with nerve fibres. The mastocytes of the first group with their dense granules might be in a state of rest. The morphological features of the mast cells of the second group might be interpreted in the following way. Lamellopodia can occur as a result of exocytosis (2). Therefore, the mastocytes of the second group showing many lamellopodia and al-

most exclusively immature granules, might represent cells stimulated by nerve fibres which have been excited at the moment of excision. However, the insertion of some lamellopodia—and especially those of the broader cytoplasmatic processes—into a nerve fibre bundle cannot possibly have occurred at the moment of excision. The assumption that these connections between nerve fibres and mast cells might serve for an exchange of metabolic substances, seems more justified. The exact nature of this interaction remains to be elucidated, though.

Some findings in the literature indicate a relationship between mast cells and the proliferation of autonomic nerve tissue. Numerous mastocytes adjacent to or within proliferating autonomic nerve system elements of the gut have been found in Crohn's disease (3). Nerve growth factor induces a proliferation of sympathetic nerve tissue as well as an increase in the number of mast cells in the rat iris (1). Mast cells with loosely structured granules were noted to occur in the developing adrenergic innervation of the iris of neonatal rats (15). In animals older than 7 days, the number of mast cells had again decreased. The authors therefore suggest that the mast cells may regulate the development of the autonomic innervation of the iris.

Consequently, in our opinion, the mast cells of the second group, with cytoplasmatic protrusions which are inserted into nerve fibre bundles, may be involved in the development of autonomic nerve fibres in this glomus tumour. Alternatively, one should also consider the possibility that the mast cell development in the tumour is subject to neuronal influences.

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