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Neurotized nevi of the oral mucosa: an immunohistochemical and ultrastructural analysis of nevic corpuscles

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BACKGROUND: Nevic corpuscle (NC), a stacked lamellar structure reminiscent of Meissner corpuscle, is frequently observed in dermal melanocytic nevi. Although the heading 'neurotized' is classically used for these nevi, the exact neural nature of NC has been a topic of considerable debate. Neurotized nevi have received little attention in the dental literature, and there was no information on NC in oral melanocytic nevi.

METHODS: Six cases of oral intramucosal nevi with a significant number of NC (two completely and four partially neurotized nevi) were examined immunohistochemically and ultrastructurally.

RESULTS: NC was composed of closely piled laminar cells devoid of visible melanin. NC and associated spindle nevus cells were immunopositive for S-100 protein but negative for HMB-45, myelin basic protein and epithelial membrane antigen. Within NC, no reactivity for neurofilament protein, protein gene product 9.5 or peripherin was evident. Numerous CD34-positive dendritic cells were located between nevus cells and often encircled NC. Ultrastructurally, NC consisted of concentrically layered elongated cells with a slender lamellated cytoplasm rich in thin filaments and pinocytotic vesicles. Their cytoplasmic processes were focally covered by external basal lamina and continuous to spindle nevus cells. Occasional NC cells contained a few melanosomes. There was no interposed axon in NC.

CONCLUSIONS: Despite the close resemblance to Meissner corpuscle, NC showed no axonal supply. NC cells lacked terminal Schwannian differentiation and appeared to be modified melanocytes with some perineurial ultrastructural characteristics. The presence of CD34-positive cells, presumably corresponding to endoneurial fibroblasts, further supports an organizational relationship of NC and peripheral nerve sheath elements.

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Introduction

Masson (1) first described a laminated organoid structure in melanocytic nevi under the name of nevic corpuscle (NC). Because of its morphologic resemblance to Meissner corpuscle, he concluded that NC was a neuroid element, suggesting a Schwann cell origin. Since then, NC-containing lesions are often labelled neurotized nevi. According to Misago (2), dermal nevi with features of Schwannian differentiation were divided into three types; type I comprises the most common neurotized nevi, type II consists of nerve fascicle-like structures without NC and type III demonstrates Verocay-like bodies. However, it is a consensus of opinion that neurotized nevus cells are not identical to mature Schwann cells (3). Because NC may be the predominant component in certain long-standing nevi (4, 5), other investigators promoted the theory that so-called neurotization likely represents simple maturation sequence of nevus cells (4–6).

The oral mucosal involvement is relatively common in melanocytic nevi (7). Nevertheless, oral neurotized nevi have been underrecognized, and NC was not the subject of investigation in oral pathology field. With this construct in hand, we undertook the present study to clarify some of the controversial issue surrounding NC, especially trying to suggest its possible link to peripheral nerve sheath elements.

Material and methods

Melanocytic nevi of the oral mucosa between 1979 and 2006 were retrieved from our pathology files. Lip lesions of the skin surface were all excluded. Thirty-seven cases of oral nevi [intramucosal (n = 28), compound (n = 2), junctional and combined (n = 1 each), and common blue (n = 5) type] were identified. From this group, six lesions containing multiple NC (more

than 20% of tumor area) are the basis of the present report.

Immunohistochemical studies were performed in all cases by avidin–biotin complex technique using antibodies as follows: S-100 protein (1:2000, Dakocytomation, Glostrup, Denmark), HMB-45 (gp100, 1:2000, Dakocytomation), myelin basic protein (1:2000, Dakocytomation), epithelial membrane antigen (EMA; E29, 1:100, Dakocytomation), neurofilament protein (NF; 2 F11, 1:50, Dakocytomation), protein gene product 9.5 (PGP 9.5, 1:1000, Dakocytomation), peripherin (1:500, Chemicon, Temecula, CA, USA) and CD34 (My10, 1:100, Becton Dickinson, San Jose, CA, USA).

In three cases (one completely and two partially neurotized nevi), 2.5% glutaraldehyde-fixed materials were available for ultrastructural analysis. After post-fixed in 1% osmium tetroxide, epon-embedded ultrathin sections were stained with uranyl acetate and lead citrate, and examined in a JEM 100CX electron microscopy (JEOL Ltd., Tokyo, Japan).

Results

Clinical findings

All the six patients were females, ranging in age from 27 to 63 (mean, 44) years. Each case presented with a solitary, asymptomatic, well-circumscribed, slightly raised or elevated nodular lesion involving the palate and lip ($n = 2$ each) and gingiva and buccal mucosa ($n = 1$ each). Three of 4 partially neurotized nevi were pigmented, but two completely neurotized lesions exhibited no clinical pigmentation. The size of the lesion ranged from 4 to 14 mm (mean, 8 mm). Because the patients were not aware of its existence, the information regarding the duration appeared to be speculative in all cases.

Microscopic findings

All neurotized nevi were classified into intramucosal type. NC was a round-to-ovoid structure with stacked internal lamellae containing a few peripherally situated spindle nuclei. Two completely neurotized nevi were composed mostly of clustered NC (Fig. 1a). They were

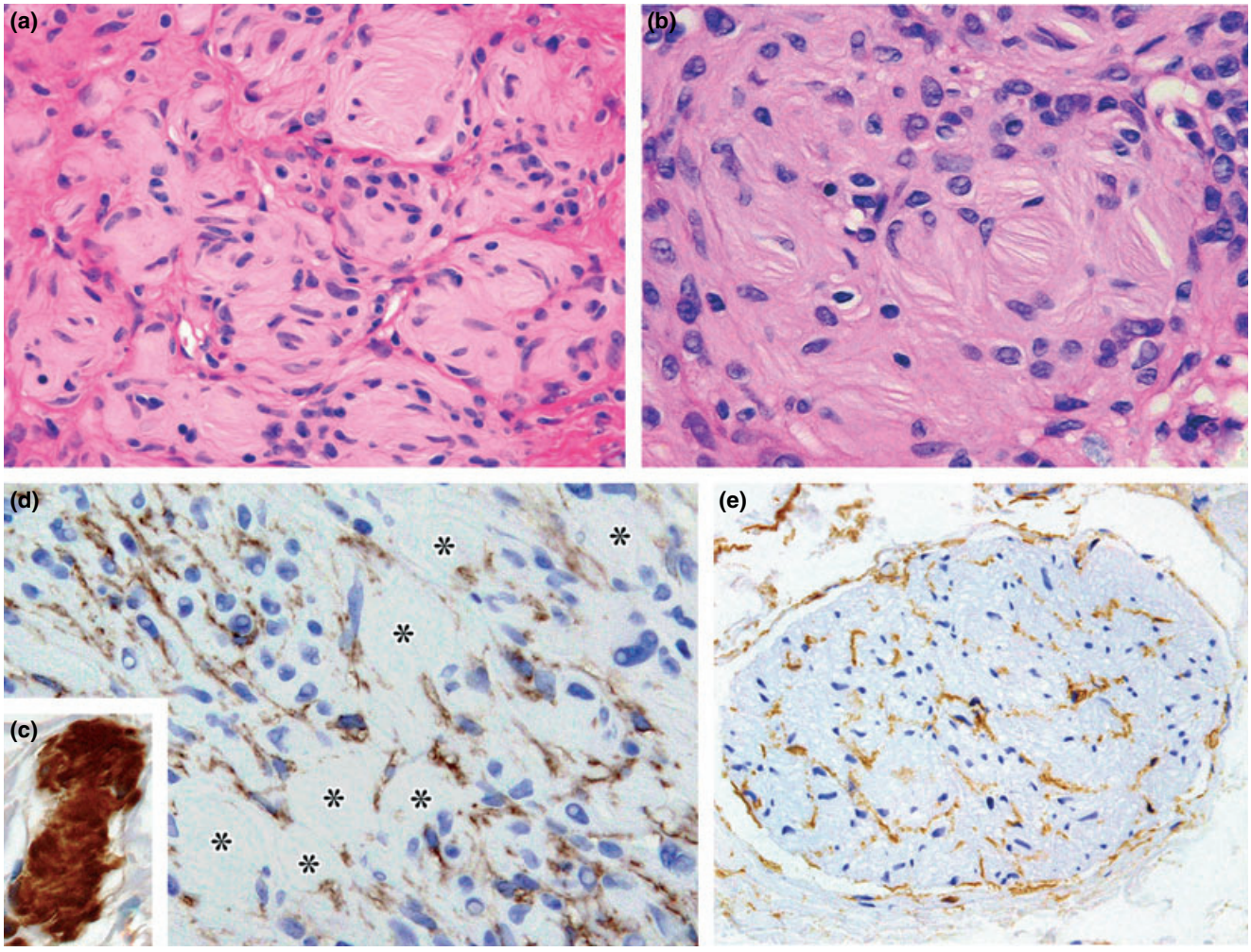

Figure 1 (a) Nevic corpuscle (NC) in completely neurotized nevus (H&E, ×400). (b) NC among spindle nevus cells (H&E, ×400). (c) S-100 protein in NC (Immunostain, ×400). (d) CD34-positive dendritic cells between nevus cells and around NC (asterisks) (Immunostain, ×400). (e) Normal nerve bundle contains CD34-positive endoneurial fibroblasts (Immunostain, ×400).

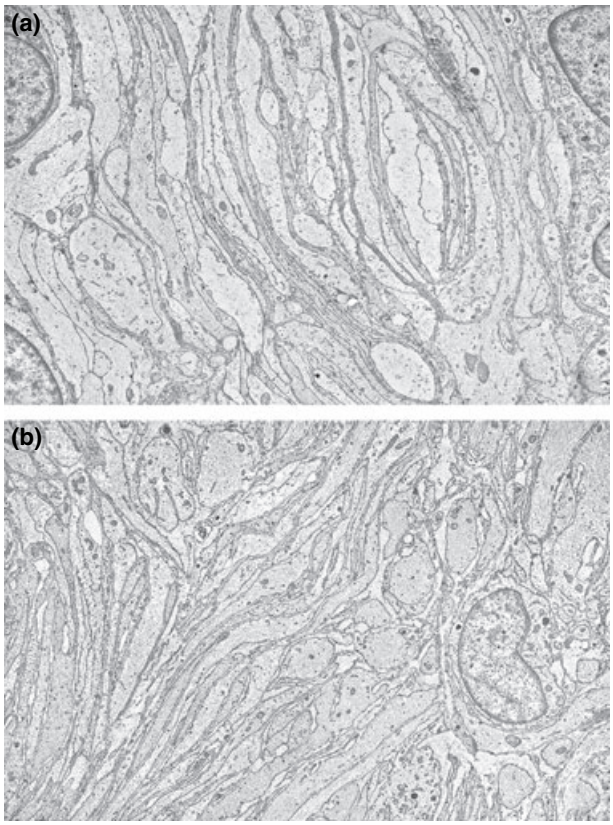

Figure 2 (a) Well-formed nevic corpuscle (NC; ×3900). (b) NC among spindle nevus cells (×3900).

nevi, the bulk of the lesion consisted of typical pigmented epithelioid nests; however, in the deeper submucosa, ill-defined NC were scattered within the spindle nevus cell foci (Fig. 1b). NC-containing areas in these four cases ranged from 20% to 35% (average, 25%) of the tumor area.

Immunohistochemcal findings

S-100 protein decorated a distinct lamellar structure of NC (Fig. 1c). Other antibodies tested did not label NC in any case. Spindle nevus cells surrounding NC expressed for S-100 protein but not for HMB-45. There was no NF-, PGP 9.5- or peripherin-positive axon within NC. Additional interest was the presence of CD34-positive dendritic cells between nevus cells and around NC (Fig. 1d). Although a few mesenchymal cells outside the nevus nests also exhibited CD34 reactivity, this type of cells could not be detected inside NC. In normal nerve bundles, scattered stellate cells within the endoneurium were positive for CD34, whereas both Schwann and perineurial cells were negative (Fig. 1e).

Ultrastructural findings

Well-formed NC was composed of laminated cells with long slender cytoplasmic extensions arranged in parallel arrays (Fig. 2a). NC among spindle nevus cells was ill-defined, forming a more complicated labyrinth (Fig. 2b). Unmyelinated axons ensheathed by Schwann cell processes were scattered between nevus nests (Fig. 3a), but neither axonal structure nor mesaxon formation could

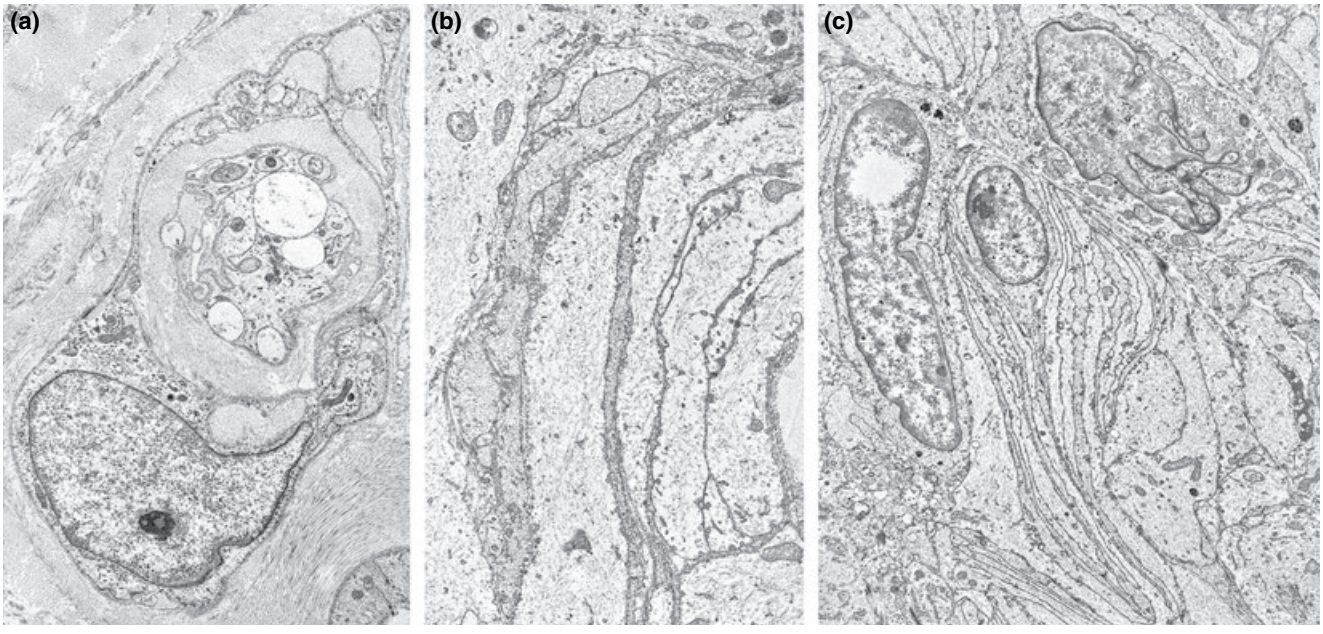

Figure 3 (a) Unmyelinated axon and mesaxon formation by Schwann cell (×5400). (b) Thin filaments and pinocytotic vesicles in nevic corpuscle (NC; ×9000). (c) Intimate relationship between NC and spindle nevus cells. Note nuclear electron-lucent area (×6000).

free of melanin pigments and frequently surrounded by spindle nevus cells. Both lesions also contained only a few melanin-laden epithelioid cells at the epithelial-connective tissue interface. In four partially neurotized

be detected within NC examined. Despite the paucity of organelles, thin filaments, plasmalemmal pinocytotic vesicles and dense patches, and discontinuous external basal lamina were conspicuous (Fig. 3b). In many areas,

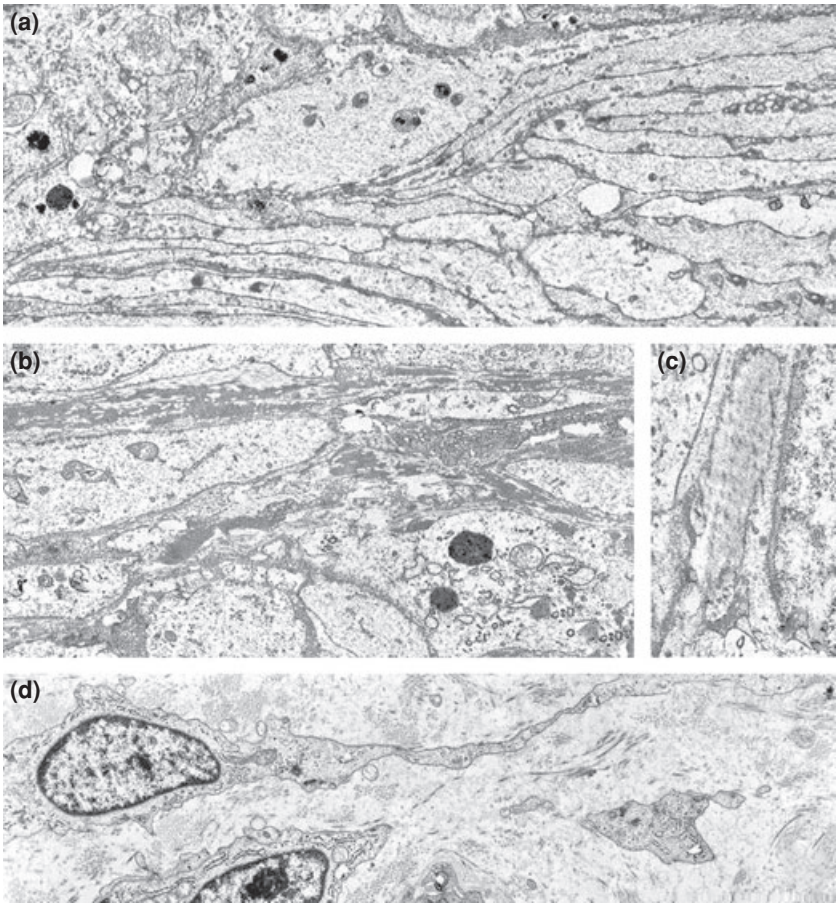

Figure 4 (a) Melanosomes in nevic corpuscle (NC; ×3500). (b) Basal lamina materials and melanosomes (×3500). (c) Long-spacing collagen (×4500). (d) Long slender fibroblasts around NC lacking external basal lamina (×3000).

NC and spindle nevus cells were imperceptibly blended with each other (Fig. 3c). These cells often showed non-membrane-limiting electron-lucent areas in the nuclear matrix. There were a few melanosomes in NC morphologically identical to those in the adjacent spindle nevus cells (Fig. 4a,b). Occasionally, the deposition of basement membrane material (Fig. 4b) and long-spacing collagen (Fig. 4c) was evident in the narrow intercellular spaces. Around NC were fibroblastic cells with elongated thin cytoplasmic processes (Fig. 4d). They characteristically lacked external basal lamina.

Discussion

Non-pigmented spindle nevus cells (type C) in the deep dermis or submucosa have long suggested the likelihood of Schwannian characteristics (1–3). In addition to histochemical (8, 9) and immunohistochemical (3, 10–14) similarities, several lines of evidence support this assertion. First, these cells have a high affinity for axons and show an intimate relationship with NC (1, 10, 15–18). Second, dermal nevi composed of non-pigmented spindle cells with wavy delicate fascicular collagen [type II of Misago (2)] recapitulate morphological features of neurofibromas (2, 19). Finally, rare palisading nevi indistinguishable form Schwannomas [type III of Misago (2)] indeed exist (2, 20). Given the hypothesis that melanocyte-precursors may migrate from neural crest to the embryonic dermis among the immature peripheral nerve sheath cells (21), it is tempting to speculate that type C is morphologically and functionally nearer to peripheral nerve sheath elements, especially Schwann cells (2, 3).

Only a few reports have described the fine structure of NC (6, 9, 17, 22–24). NC was stacks of concentrically layered cells with elongated laminar cytoplasm containing thin filaments and pinocytotic vesicles. Although labyrinthine in appearance, complicated cytoplasmic extensions did not show mesaxon formation specific for terminally differentiated Schwann cells. In many areas, NC and type C spindle nevus cells were interdigitating with each other (9, 17, 22). Unlike the previous immunohistochemical data (16–18), axons were a neglected component at the ultrastructural level (23, 24). With rare exception (24), external basal lamina was discontinuous and incomplete. Niizuma (23) found, as we did, scattered melanosomes in occasional NC cells. Melanosome-like granules were also visible in Figure 6 of van Paesschen et al.'s paper (6). Long-spacing collagen was common in dermal nevi (17), but its presence by itself is not proof of, or even evidence for, neural differentiation (25).

Although NC are formed by intricate cytoplasmic extensions of type C spindle nevus cells, three kinds of cell lines have been proposed for the nature and origin of type C nevus/NC cells; Schwann cells (1–3, 8–14), melanocytes (19, 23, 26) and perineurial cells (17, 21, 27). Because of the lack of specific ultrastructural characteristics other than axon investment, Schwannian nature was based on the immunoprofile (3, 11–14) and histochemical demonstration of cholinesterase (8, 9). On the other hand, a melanocytic phenotype was suggested by the result of DOPA reaction of tyrosinase activity in melanosomes at the ultrastructural level (26). Sporadic immunoreactivity for EMA (21, 27) and fine structure as described above (17) raised another assumption of perineurial lineage. We confirmed, albeit in only six cases of neurotized nevi, the presence of melanosomes (23) and the absence of putative Schwannian differentiation (17) in NC cells, providing clear evidence of melanocytic nature. It must be borne in mind, however, that despite less than perfect, NC/spindle nevus cells adopt some peripheral nerve sheath phenotype as judged by certain ultrastructural characteristics of perineurial cells (17) as well as immunohistochemical aspect observed in Schwann cells at an early stage of differentiation (3).

To the best of our knowledge, there was no published account on the presence of CD34-positive stellate cells between nevus cells and around NC. Given that NC cells may be modified melanocytes sharing with their phenotype with peripheral nerve sheath lines (perineurial cells and probably Schwann cells in part), we have reason to believe that some of this specialized subpopulation may correspond to endoneurial fibroblasts (28, 29). As shown in the present and previous ultrastructural studies (24), dendritic fibroblastic cells lacked external basal lamina seen in pericytes. The absence of Weibel-Palade bodies as well as immunonegativity for CD31 and factor VIII-related antigen also preclude their endothelial nature (unpublished data). The possible participation of an additional nerve sheath cell, neural crest-derived endoneurial fibroblasts (29), in morphogenesis of NC is particularly appealing in consideration with the plausible kinship between NC and peripheral nerve sheath elements.

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