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Immunohistochemical characterization of capsular cells in neuromuscular spindles of the neck

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BACKGROUND: To identify capsular components of neuromuscular spindles in man by means of immunohistochemistry.
METHODS: Investigation of histologically observed neuromuscular spindles in surgical specimens with the use of markers for sheath cells and basement membranes.
RESULTS: Epithelial membrane antigen and CD34 immunoreactivities were found in the outer and inner capsular layers, respectively. S-100 protein was not expressed in the capsules and there was more collagen type IV than laminin.
CONCLUSIONS: Cells resembling perineurial cells and endoneurial fibroblasts, and basement membrane rich in collagen type IV comprise the capsules of neuromuscular spindles.
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Keywords: basement membrane; fibroblast; immunohistochemistry; neuromuscular spindle; perineurium

Introduction

Although the multilayered capsule that surrounds the neuromuscular spindles is affected by various pathological processes and experimental manipulations (1–5) the nature of its components is controversial. Rhodin suggested that the capsule is composed of cells similar to Schwann cells rather than fibroblasts (6). Subsequent ultrastructural investigations of neuromuscular spindles in mammals and birds suggested that the cells in the outer and inner capsular layers resemble perineurial cells and endoneurial fibroblasts, respectively (7–10). It is desirable to be able to ascertain whether these findings apply in man by means of immunohistochemistry. As this has not been previously explored, the present communication concerns material from a block dissection and a biopsy in which histochemical and immunohistochemical analysis of neuromuscular spindles has been effected.

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Materials and methods

Two archival specimens in which neuromuscular spindles had been observed fortuitously by the author in routine histopathological sections were investigated. The specimens had been obtained from two patients (Table 1), fixed in buffered formalin and routinely processed.

Sections for histology and histochemistry were stained with haematoxylin and eosin, and alcian blue (AB) at pH 2.5 followed by periodic acid-Schiff (PAS) to demonstrate neutral glycoproteins staining red (periodate-reactive) and glycosaminoglycans staining sky blue (alcianophilic; 11).

Sections for immunohistochemistry were treated with biotinylated antibodies that are of value in identifying components of the nerve sheaths (Table 2; 12–15). A streptavidin–horseradish peroxidase complex was used for the amplification and visualization of immune complexes. Details of the immunohistochemical procedures are described in Triantafyllou and Coulter (15).

In accordance with ethical requirements, throughout reporting the results, all patient details have been anonymized. In addition, the investigation had no implications for the diagnosis or the management of the patients.

Results

The spindles showed typical histology (Fig. 1; 6, 16). The capsules were moderately stained by PAS, whereas intrafusal stroma contained alcianophilic glycosaminoglycan that was concentrated in the subcapsular regions (Fig. 2).

Weak epithelial membrane antigen (EMA) immunoreactivity was localized in the outermost capsular layer (Fig. 3). Multilayered wrappings of cells exhibiting CD34 positivity were present (Fig. 4). No S-100 protein immunoreactivity was observed at the capsules (Fig. 5).

Table 1 Clinical details

Specimen	*Age (years)*	*Sex*	*Features*
1	65	Male	Suprahyoid dissection of the left neck for a T2 squamous cell carcinoma of the lateral border of the tongue. No irradiation had been given
2	10	Female	Biopsy of left neck for suppurative granulomatous inflammation

Immunostaining for constituents of basement membranes was heterogeneous. Collagen type IV was abundant in the capsules (Fig. 6). Laminin antigenicity was weaker and principally associated with the innermost layers (Fig. 7).

Discussion

The neuromuscular spindles examined in the present investigation were from specimens received in a routine diagnostic oral and maxillofacial histopathology service. The spindles were deemed normal by conventional histology, and so the consideration of the capsular immunoreactivities described here as normal appears justified. That only two specimens were investigated reflects the fortuitous observation of the spindles. Skeletal muscle from neck dissections is not routinely processed for histology nor is often contained in diagnostic biopsies from that region.

Because of its clear appearance in routine histological sections (Fig. 1), the space between the intrafusal muscle cells and the capsule of the spindle has been regarded as containing tissue fluid or lymph (6, 16). The findings of the present investigation indicate the presence of alcianophilic glycosaminoglycan therein. An investigation of feline neuromuscular spindles has also demonstrated the presence of intrafusal glycosaminoglycan that has been further characterized as hyaluronic acid (17). The glycosaminoglycan appears necessary for maintaining impulses (17) and stretching of the spindle.

The finding that EMA was expressed in the outermost layer of the capsules suggests the presence of cells exhibiting a perineurial phenotype, expression of EMA being a feature of normal perineurium (12, 14, 15). This is supported by ultrastructural investigations of spindles in other species, which demonstrated the presence of

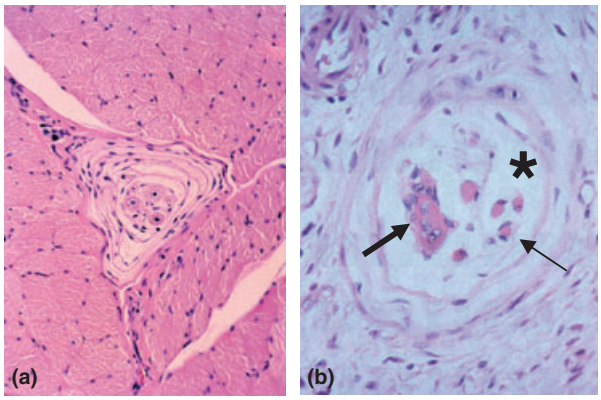

Figure 1 (a and b) Transverse-sectioned neuromuscular spindles in muscles of the neck. A number of concentric layers of flattened spindled capsular cells are inclosing nuclear bag muscle fibres (thick arrow) and nuclear chain muscle fibres (thin arrow) set in loose clear stroma (asterisk). The plane of the sections allows differences in the diameter of intrafusal and extrafusal fibres, and in the arrangement of nuclei of intrafusal fibres to be appreciated (haematoxylin and eosin).

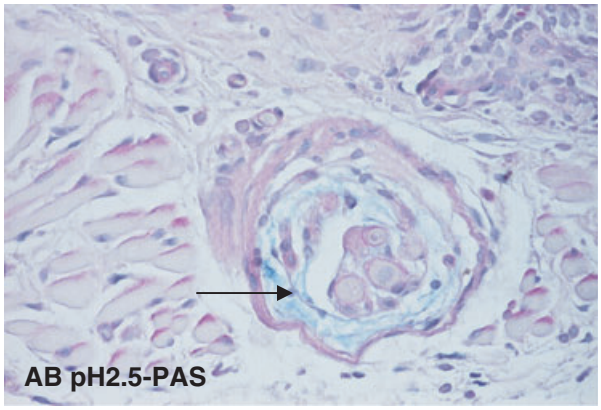

Figure 2 Alcianophilia of the stroma (arrow; AB at pH 2.5 – PAS).

pinocytic-like vesicles in cells of the outer capsule (7–9, 18), for similar vesicles feature in normal perineurial cells (12, 19). Nevertheless, pathways for any movement of injected marker solutes from blood to intrafusal space would need to be charted before establishing pinocytic activities in the outer capsular layer of the spindles.

Turning to the cells comprising the inner capsular layers, the present work showed that they express the CD34 antigen as do endoneurial fibroblasts (13, 15),

Table 2 Description of antibodies

Antibody clone	*Specificity*	*Pre-treatment*	*Dilution*	*Source*
E29	Epithelial membrane antigen (EMA)	None	1:400	Dako[a]
QBEnd 10	CD34	Trypsin (37°C; 30 min)	1:50	Dako
Polyclonal	S-100 protein	Trypsin (37°C; 30 min)	1:3000	Dako
COL-94	Collagen IV	Trypsin (37°C; 30 min)	1:200	Sigma[b]
Clone LAM-89	Laminin	Trypsin (37°C; 60 min)	1:30	NCL[c]

[a]Dako Ltd, Cambridge, UK.
[b]Sigma Chemical Co., Dorset, UK.
[c]Novocastra Laboratories Ltd, Newcastle upon-Tyne, UK.

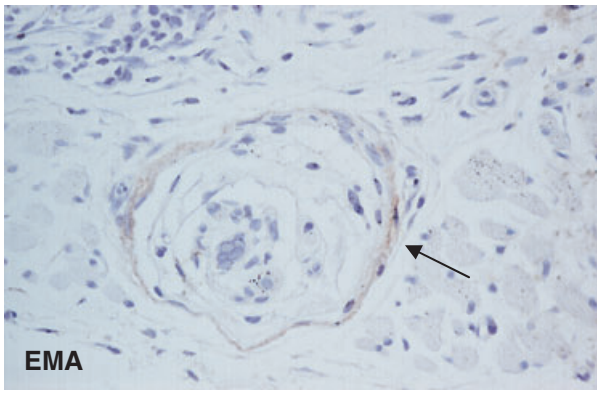

Figure 3 A single layer of epithelial membrane antigen-positive cells (arrow) covers the spindle (immunohistochemistry).

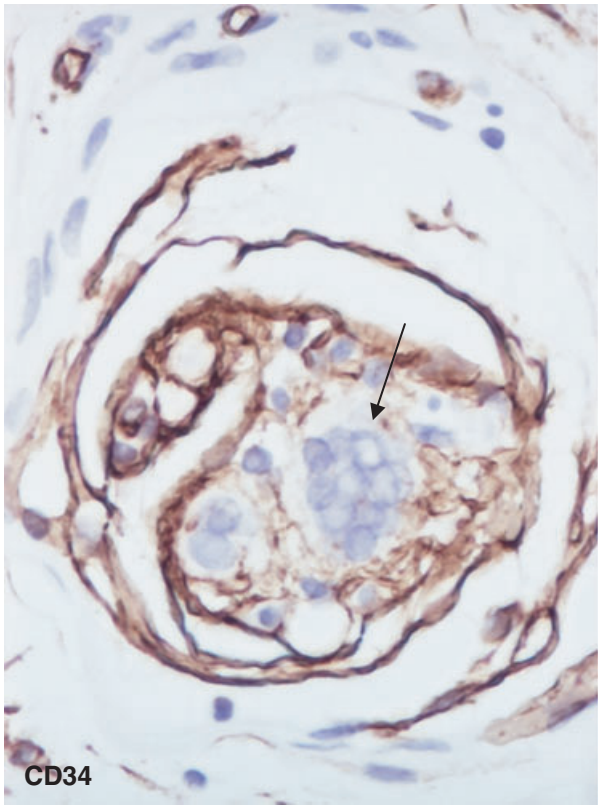

Figure 4 Lamellated sheaths of CD34-positive cells surround unstained intrafusal muscle fibres. The arrow indicates the equatorial region of a nuclear bag fibre where the nuclei form a cluster (immunohistochemistry).

which accords with ultrastructural investigations in other species (10). Possibly the CD34-positive cells synthesize and secrete the subcapsular glycosaminoglycan of the spindles.

The absence of S-100 protein immunolocalized in the capsules does not support their perception as being composed of cells expressing a Schwannian phenotype (6), for S-100 protein has been widely accepted as marker of Schwann cells (12, 20). Annulospiral sensory and gamma motor nerve fibres (6, 16) could account

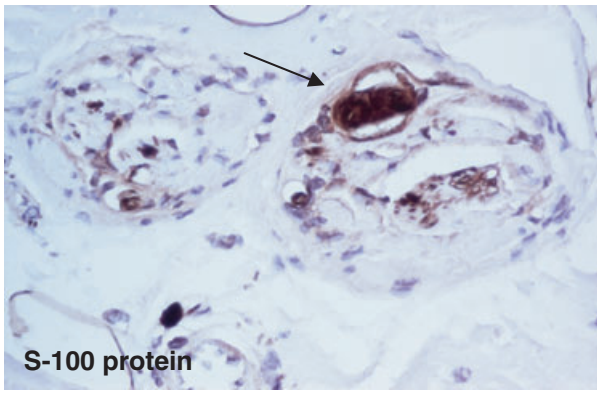

Figure 5 S-100 immunoreactivity appears restricted to intrafusal components (arrow; immunohistochemistry).

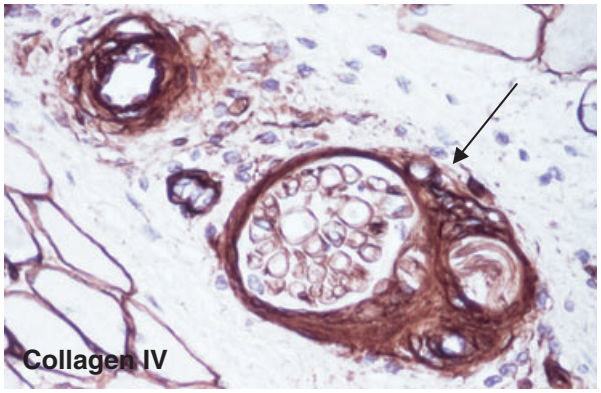

Figure 6 Collagen type IV positivity is condensated around a spindle (arrow) and in a vessel (on the left, above), and outlines intrafusal and extrafusal muscle fibres (immunohistochemistry).

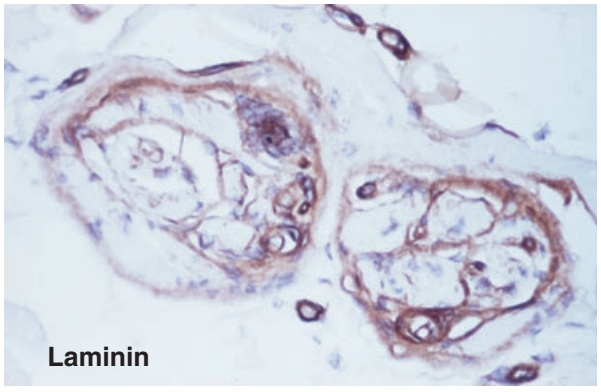

Figure 7 Laminin pattern in two spindles; outermost capsular layers show weak or no immunoreactivity (immunohistochemistry).

for the intrafusal immunoreactivities illustrated in Fig. 5.

The finding that there was more collagen type IV than laminin in the capsules supports preceding inferences on the identity of the cells therein. Collagen type IV and laminin are preferentially associated with the perineurium and Schwann cells, respectively (13–15). The particular composition of the capsular basement

membranes in neuromuscular spindles, which probably accounts for the periodate reactivities (Fig. 2), may be related to support and selective transport of fluid and small particulate matter. The latter would be consistent with the notion of the spindle capsule as barrier (8).

The conditions to create structural arrangements in the capsule of neuromuscular spindles are not understood. Possibly CD34-positive fibroblasts concentrate around intrafusal primordia, as do around skin appendages (20), and excretory ducts of minor salivary glands (A. Triantafyllou, unpublished observations), and are surrounded by a layer of perineurial-like cells in turn. It is wondered whether immunohistochemical investigations would support the notion of similarities between the capsule of neuromuscular spindles and stratum fibrosum externum of Chievitz's organ (21). It is unknown whether the capsular cells undergo tumorous proliferation.

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