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Comparison of various basement membrane components in benign and malignant peripheral nerve tumours

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Summary. Immunohistochemical methods were used to analyse benign and malignant tumours of peripheral nerve tissue. We tested for the distribution of basement membrane (BM) components collagen IV, laminin, heparan sulphate proteoglycan, fibronectin, for S100 protein and for the presence of interstitial collagens III and V. Laminin was generally noted in association with Schwann cells, but collagen IV occurred with perineural cells. When tested for BM components, fibroblasts were notably non-reactive except for fibronectin. Three specific area-dependent BM patterns were observed in the benign tumours: (a) Schwann cell-like, in fascicular areas (Antoni A areas of schwannoma, central fibrous bundles of plexiform neurofibromas and central areas of cutaneous neurofibroma), (b) perineural cell-like (capsular structures of schwannoma) and (c) fibroblast-like (myxoid and fibrously transformed areas). Most malignant tissues showed a variably fragmentary focal deposition of laminin. Other BM components were present only in well-differentiated areas. Poorly differentiated tumours demonstrated fibronectin reactivity alone. Our results provide evidence that the specific staining pattern for BM components helps to differentiate the various cellular proliferations in neurogenic tumours. Schwann cells are not only distinguishable from perineural cells by S100 protein staining, but also by their specific BM staining. In additon, perineural cells can be separated from fibroblasts, which do not express BM material. The "tropism" of laminin in normal nerves and benign neural tumours – which persists in neurogenic sarcomas – indicates preferential Schwann cell differentiation in these cells.

Key words: Basement membrane – Schwannoma – Neurofibroma – Malignant Schwannoma – Collagen IV

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Introduction

The connective tissue in large peripheral nerves is composed of an epineurium which surrounds large nerve trunks, the perineurium enclosing separate bundles or fascicles of nerve fibres, and the endoneurium which surrounds individual nerve fibres. Small nerves differ in that they lack a distinct epineurium (Mei Lin 1988; Shellswell et al. 1979). Previous immunohistochemical and biochemical studies have shown that peripheral nerve tissue contains various types of collagen, as well as certain basement membrane (BM) components (Schleicher et al. 1989; Shellswell et al. 1979). At the ultrastructural level, it has been shown that basement membranes are accompanied by Schwann and perineural cells (Erlandson and Woodruff 1982).

BMs are complex structures composed of diverse proteins. The most important constituent is collagen IV, which is an essential BM component responsible for mechanical stability. Laminin provides cell adhesion, while the BM-associated heparan sulphate proteoglycan (HSPG) is presumed to regulate transmembrane flux. Fibronectin can also be found associated with various other structures in connective tissue (Timpl 1989). Recent studies have demonstrated that certain specific variations exist in the qualitative and quantitative composition of these components in different basement membranes (Schleicher et al. 1989).

Our study was designed to analyse immunohistochemically the pattern of BM components in normal peripheral nerve tissue and to find out whether histogenetic similarities exist between benign and malignant tumours.

Materials and methods

Routinely formalin-fixed and paraffin-embedded tissue was collected for study from 75 patients with various types of benign and malignant peripheral nerve tumours. In an additional 5 cases, normal peripheral nerves from different locations (ischial nerve, femo-

ral nerve and multiple small nerves in adipose tissue) were obtained at autopsy (within 12 h of death) from young patients who had died of unrelated causes.

In this study we used type-specific antibodies obtained as follows: antihuman placental collagen IV and anti-EHS (Engelbreth-Holm-Swarm) sarcoma laminin were obtained from Eurodiagnostics (Apeldoorn, NL). Antihuman fibronectin and anti-S100 protein came from Dako (Hamburg, FRG). The antibody against BM HSPG has recently been characterized in detail showing specific immunostaining of various basement membranes (Schleicher et al. 1989). Antibodies specific for collagens III and V were prepared according to Timpl et al. (1977).

Appropriate paraffin sections were deparaffinized in xylol and enzymatically pretreated (0.4% pepsin, Sigma, Deisenhofen, FRG) for enhancement of immunostaining (Barsky et al. 1984). Following incubation with the specific primary antibody and several washing steps with PBS buffer, a streptavidin-biotin complexed specific secondary antibody was applied to the sections (Dako) for the visualization of the antigen (Hsu et al. 1981). Subsequently, the reaction products were stained using aminoethylcarbazole or diaminobenzidine (Dako). All control experiments obtained by omission of the primary antibody yielded negative results.

Results

In normal nerve tissue and post-traumatic neuromas there were no significant qualitative differences in the staining patterns demonstrated by nerves obtained from different sites. The periaxonal basement membrane around Schwann cells was found to consist predominantly of laminin, and, to a far lesser degree, of collagen IV, HSPG and fibronectin. The Schwann cells demonstrated S100 protein reactivity, while the perineural cells surrounding the Schwann cell bundles were distinctly non-reactive. Interestingly, the perineural cells showed a circumcellular BM material consisting mainly of collagen IV, with laminin and HSPG being present in minor amounts. The adjacent connective tissue fibroblasts did not react for both S100 protein and BM components (Fig. 1, Table 1). The only tissue components demonstrable in the vicinity of fibroblasts were fibronectin, collagen III and collagen V, which were also readily evident in regions adjacent to perineural cells.

All post-traumatic neuromas analysed showed a qualitative and quantitative distribution of BM components comparable to that seen in normal nerve tissue (data not shown). Moreover, the perineurium of the small regenerative nerve bundles revealed a staining pattern similar to that of the inner perineural layer of large nerves.

Our series of 44 benign neurogenic tumours was divided into those areas of fascicular, reticular or myxoid type of nerve cell proliferation. Thus, this tumour group was composed of both schwannomas and neurofibromas with variable histomorphological features (Enzinger and Weiss 1988). We found the characteristic distribution of fascicular and reticular areas (Antoni A/B areas) typical for schwannomas, in 15 cases. The Antoni A fields contained significantly more cells that were both BM- and S100-protein positive than the Antoni B areas. Antoni A areas could be further distinguished from the B areas by the pattern of diverse BM components, reacting strongly with anti-laminin and to lesser degree with anti-collagen IV (Fig. 2). The distribution of HSPG reactivity was only moderate and inconstant in both regions, except for areas with typical cell palisading (Verocay bodies), that were strongly HSPG positive (Fig. 2). Fibronectin and collagens III and V were occasionally sharply manifest in the Antoni B fields, but not in the Antoni A areas. All 15 tumours were surrounded by a more or less well delineated, partly fibrotic capsule. Capsular cells of the inner sheath demonstrated a pericellular BM composed mainly of collagen IV, with little laminin and HSPG, while the cells of the outer layer of the capsule contained no BM material (Fig. 2). Fibronectin was evenly distributed throughout the inner and outer part of the capsule (Table 1).

Another 12 cases showed typical morphological features of cutaneous neurofibroma with poorly defined border, a central hypercellular area and a peripheral well-vascularized hypocellular part. In these cases the central hypercellular area showed a strong laminin positive reaction. The staining intensity for collagen IV appeared reduced. HSPG reacted unevenly (Table 1). In a further 8 cases of neurofibroma we found a strongly fibrosed central bundle and surrounding perineural-like structures with interspersed myxoid matrix. This central fibrous bundle also revealed intense laminin staining. Collagen IV, HSPG and fibronectin reacted less intensly. In the surrounding myxoid matrix very few BM positive and S100 protein positive cells were seen (Table 1). The perineural-like structures tested mainly positive for collagen IV and fibronectin, less for laminin and HSPG, and totally negative for S100 protein.

The remaining 9 cases were not attributable to any one of the three groups mentioned, showing variable degrees of fascicular-myxoid or fasicular-reticular areas. Their staining patterns, however, corresponded to those indicated above.

Table 1. Distribution of S100 protein and various basement membrane components in benign neurogenic tumors

	S100	CIV	LM	HSPG	FN
Normal nerve					
periaxonal	+++	++	+++	+	+
perineural	−	+++	+	+	+++
Neurilemmoma (reticular-fascicular)					
Antoni A-areas	+++	++	+++	+/+++*	+
Antoni B-areas	++	+	+	+/−	++
capsule	−	+++	+/−	+/−	++
cutaneous neurofibroma					
central areas	++	+/++	+++	+	+
peripheral areas	+	+/++	++	+/−	+
plexiform neurofibroma					
central bundles	++	+/++	+++	+	+
myxoid areas	+/−	+	++	+/−	++
"capsule"	−	+++	+	+/−	++

− = absent; + = weakly positive; ++ = moderate positive; +++ = strongly positive;
* = depending on nuclear palisading
CIV = collagen IV LM = laminin FN = fibronectin

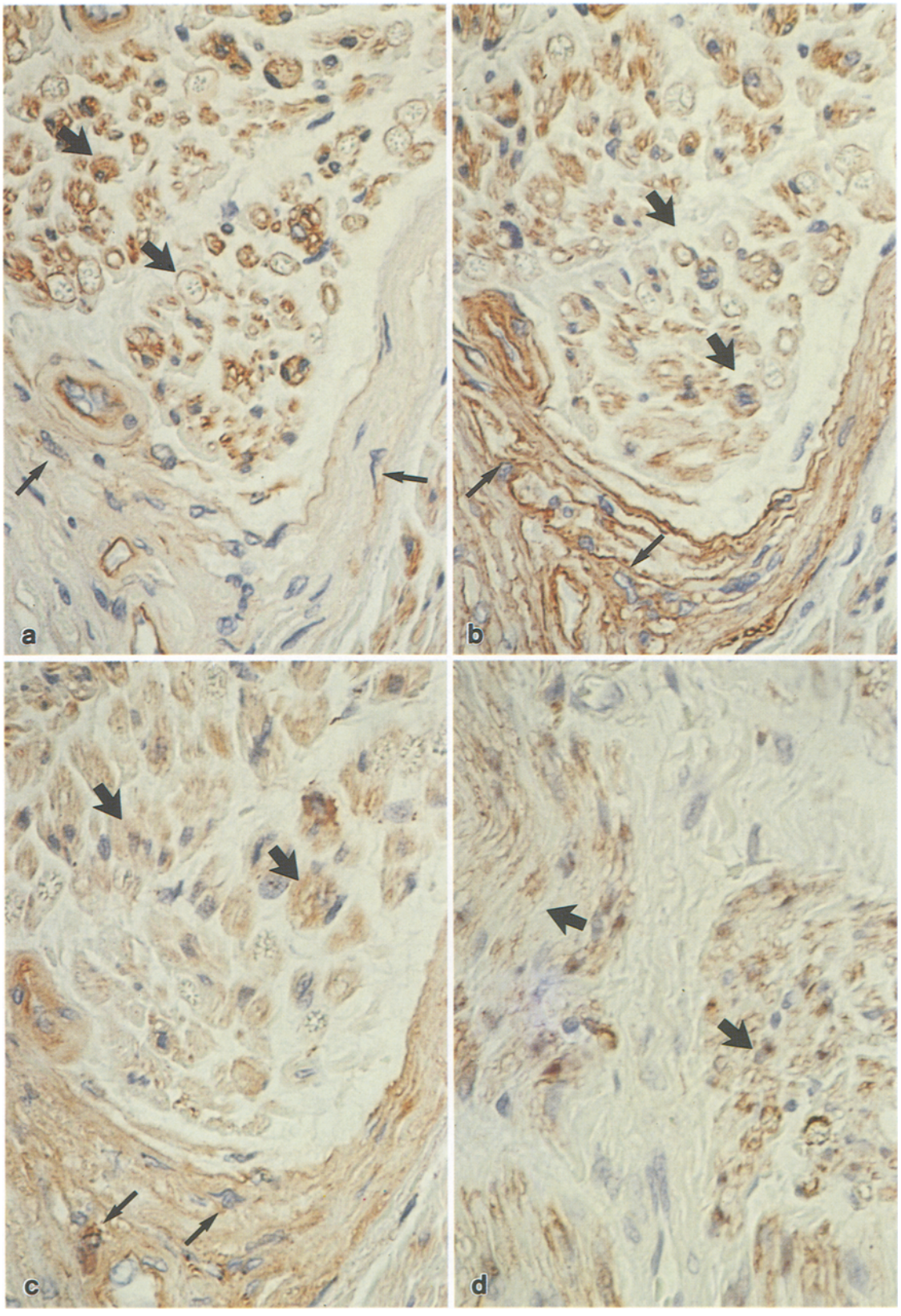

Fig. 1 a–d. Immunohistochemical localization of various basement membrane components in normal nerve tissue. **a** Laminin shows a prominent staining of the pericellular BM of Schwann cells (*thick arrows*), while perineural cells are only faintly pericellularly stained (*thin arrows*). **b** A corresponding section stained for collagen IV demonstrates a predominant staining pattern for perineural cells (*thin arrows*). The Schwann cell BM is much less positively labelled (*thick arrows*). **c** The staining for fibronectin is predominant in the perineural cell region (*thin arrows*), while it is less intense around Schwann cells (*thick arrows*). **d** Using antibodies against S100 protein only the cytoplasm of Schwann cells (*thick arrows*) is positively stained. Note in **a–c** the almost equally well labelled endothelial BM of small capillaries. × 600

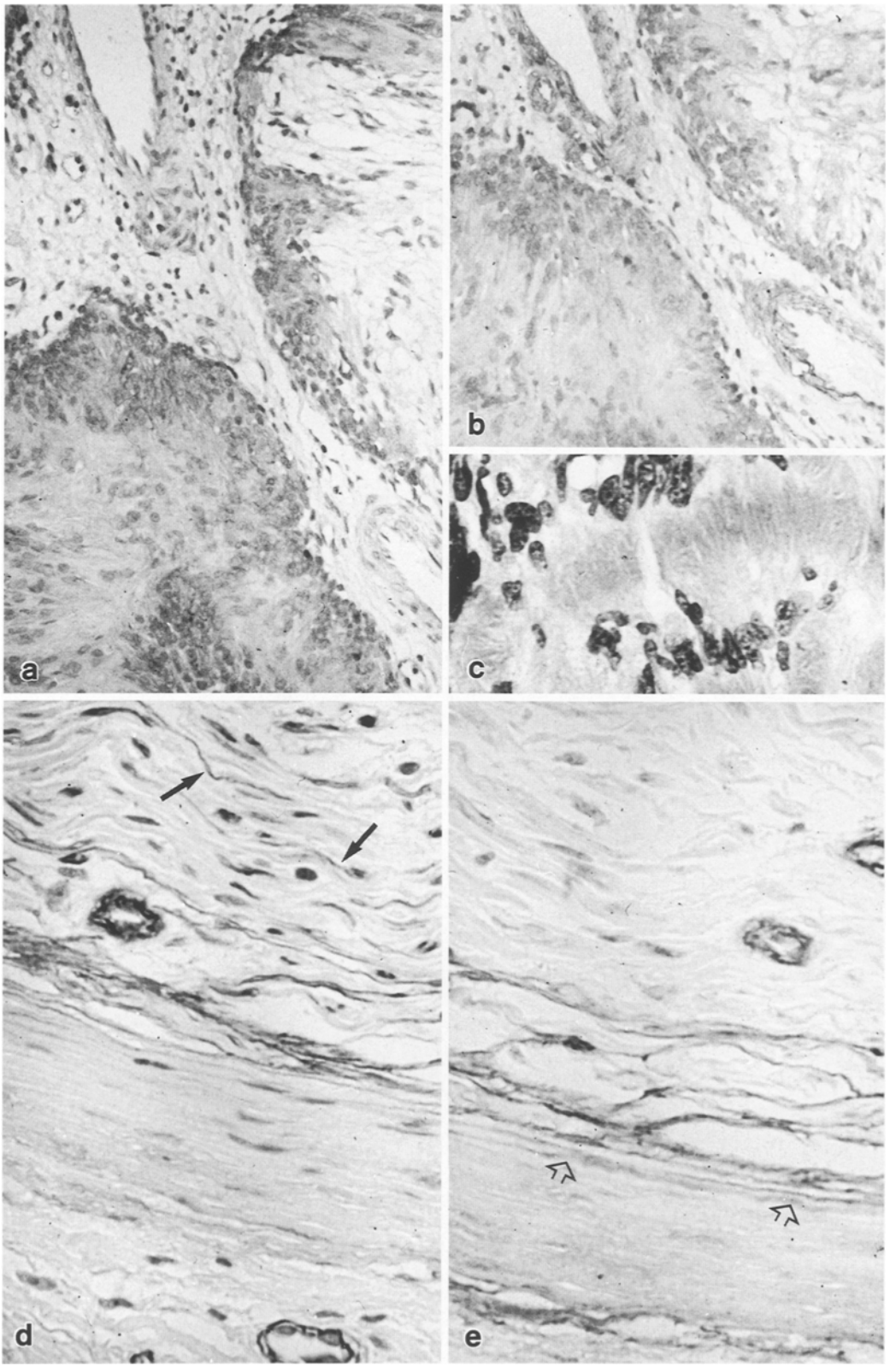

Fig. 2a–e. Staining pattern of BM material in benign tumors of peripheral nerve. **a, b** In benign nerve tumours with typical fascicular-reticular pattern (schwannoma) the Antoni A areas are more intensely stained for laminin (**a**) than collagen IV (**b**). **c** Note the strong staining of extracellular matrix in Verocay bodies for the HSPG. **d, e** While tumour cells in fascicular areas (*solid arrows*) are strongly positively labelled for laminin **d** and only weakly for collagen IV **e**, the adjacent capsular structure (*open arrows*) shows a small rim of collagen IV positive cells which appear only weakly positive for laminin. Note the endothelial BM staining pattern as internal control. **a, b** ×160; **c** ×600; **d, e** ×400

We divided our 16 cases of neurogenic sarcoma into three different groups based arbitrarily on the pattern of BM material. The amount of S100 protein positive cells was variable between these groups, but remained somewhat constant within the groups. Group I contained those cases with the lowest degree of cytological and structural differentiation and group III that of highest degree. There was, however, no strict correlation between the amount of S100 protein positive cells and cellular differentiation.

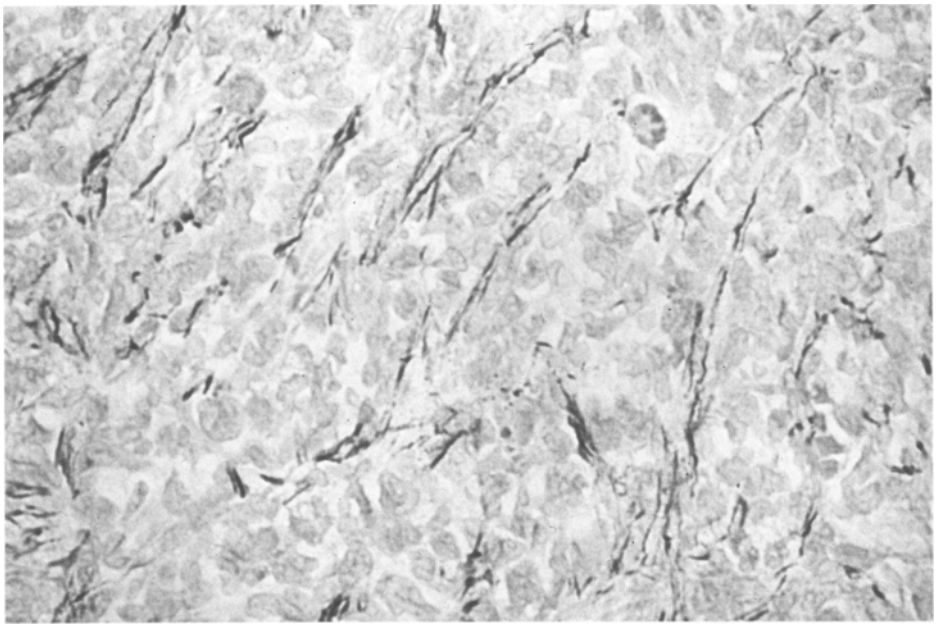

Fig. 3. Distribution of fibronectin in a dedifferentiated malignant nerve tumour (group I), demonstrating short bands of positive material between the tumour cell groups. ×250

We classified three tumours in group I which were identified as neurogenic sarcomas only by a positive case history for this tumour type. These tumours consisted of "dedifferentiated" spindle-shaped polymorphous cells without typical morphological features of neurogenic origin. Very few isolated S100 protein positive cells were found in these cases. The only BM-associated component demonstrable here was fibronectin (Fig. 3), while collagen IV, laminin and HSPG remained absent.

Group II contained nine tumours which showed a moderate number of cells positive for S100 protein. Almost all displayed a clear but fragmented pericellularly positive reaction for laminin and fibronectin, while only very few isolated pericellular fragments were visible for collagen IV and HSPG (Fig. 4).

Group III, which showed the highest degree of differentiation, comprised 4 cases with large amounts of S100 protein-containing cells. In these cases all BM components could be found as variably large pericellular fragments (Fig. 4). Usually all BM components tested were found in a pattern as in benign tumours occasionally showing a Schwann cell-like, a perineural cell-like or a fibroblast-like BM pattern. The interstitial collagens III and V were present in variable amounts in the stroma of most tumours in groups II and III, however, very little material could be found in the cases of group I.

The blastemic parts of neuroblastomas analysed were negative for S100 protein and all BM components tested. In ganglioneuromas the encapsulating cells around the ganglion cells showed a Schwann cell-like BM pattern with a predominance of laminin expression.

Discussion

Although reports on the localization of isolated BM components in small series of schwannomas and neurofibromas and in a few neurogenic sarcomas exist (Fleishmajer et al. 1985; Gay et al. 1983; Oda et al. 1988; Peltonen et al. 1986; Reibel et al. 1985; Weber and Krieg 1984), no extensive and comparative analysis of the BM pattern of nerve tumours has been published to our knowledge. We have provided semiquantitative data for the first time on the distribution of the various matrix components using "internal marker structures", such as endothelial BM, allowing a reproducible and comparable semiquantitative estimation of the most important BM components (Nerlich and Schleicher 1991). This approach has given insight into the mechanisms of cell and tissue differentiation, in both benign and some malignant neurogenic tumours.

In normal peripheral nerve tissue we found specific differences in the distribution of the BM components in certain anatomical structures. Based on their composition, Schwann cell-associated BM and that of perineural cells varied by the predominant expression of laminin or collagen IV, respectively. Moreover, BM material provided a good criterion for the distinction of Schwann and perineural cells from nerve-associated fibroblasts, which lack BM. Our findings correlated well with in situ hybridization data from Jaakkola et al. (1989) who analysed extracellular matrix components obtained from nerve cells in vitro. Our results also agreed well with the biochemical findings of Dziadek et al. (1986).

The BM composition of post-traumatic neuromas was close to that of the normal nerve. In benign nerve tumours (schwannoma and neurofibroma) a pattern of BM staining was found that resembled either that of normal Schwann cells or normal perineural cells. In general, we found that a fascicular type of morphology (usually known as Antoni A areas of schwannoma) coincided with the highest amounts of S100 protein positive and BM positive cells with a preferential expression of laminin (Schwann-cell type). Similar findings were observed

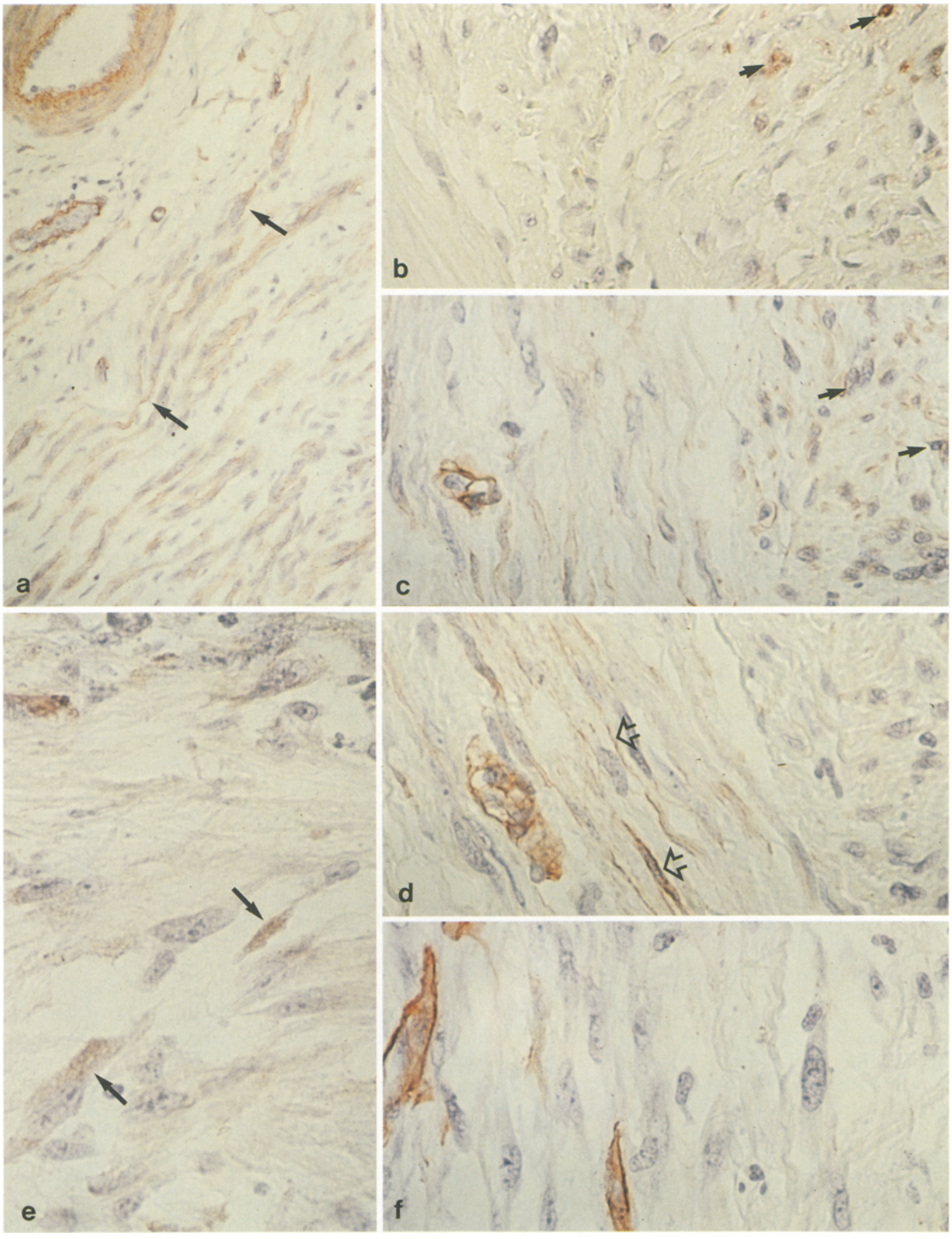

Fig. 4a–f. BM pattern in malignant peripheral nerve tumours. **a** In a well-differentiated malignant schwannoma a distinct pericellular deposition of laminin (*arrows*) can be found (group III). **b–d** Closer detail of a neurogenic sarcoma (group II) displays focal positive cytoplasmic staining for S100 protein in a few tumour cells (*arrows*) (**b**). **c** A serial section stained for laminin demonstrates the pericellular positive reaction of BM material which is more prominent on the right side (Schwann cell-like area) (*arrows*) than on the left side (perineural cell-like area). **d** A further serial section shows the distribution of collagen IV, which is somewhat more predominent on the left side (perineural cell-like area) (*arrows*) than on the right side. **e, f** In poorly differentiated areas only a very faint cell-associated staining for laminin is present (*arrows*) **e**, while collagen IV is negative (**f**). **a** ×400; **b–d** ×600; **e, f** ×1000

in the central areas of cutaneous neurofibromas and the central fibrous bundles of plexiform neurofibromas, indicating some histogenetic relationship between these structures. In addition, we found that cellular palisading in the so-called Verocay bodies was associated with the expression of large amounts of laminin and HSPG. This may indicate a rather high degree of tissue organization.

Certain other structures in peripheral nerve tumours were characterized by enhanced staining for collagen IV and were lacking S100 protein expression. Thus, it can be assumed that these cells were most probably perineural cells. We found evidence that the capsular structures of schwannomas and the perineural-like structures of plexiform neurofibromas (called "capsule", see Table 1) may have a common histogenetic origin as well. In schwannomas we observed moreover a certain capsular zoning phenomenon. On the basis of the BM staining we concluded that an inner layer may have perineural cell differentiation while the outer represented a fibroblastic cell layer. Finally, we observed in myxoid areas of benign tumours a lack of pericellular BM material (as well as a lack of reactivity for S100 protein), so that these cells were most obviously attributed to fibroblasts of the nerve tissue. This type of cell was also found in the reticularly arranged areas of schwannomas (Antoni B areas).

In parallel, we observed that neurogenic sarcomas expressed BM material predominantly with a preferential synthesis of laminin. The first group of neurogenic sarcomas comprised dedifferentiated sarcomas which were regarded as neurogenic tumours on the basis of a previous diagnosis of neurosarcoma. No distinct BM expression could be found, indicating either a loss of differentiation or derivation from nerve-associated fibroblasts. The two other groups varied in the amount of S100 protein positive cells. In contrast to several previous reports (Matsunou et al. 1985; Weiss et al. 1983; Wick et al. 1987) the number of S100 protein positive tumours was relatively high in our series. This fact may be explained by differences in the scoring system. Thus, we regarded as positive even those cases where a few scattered tumour cells expressed S100 protein. In groups II and III we observed mainly an expression of laminin around individual tumour cells, which were found even when no other BM components were present.

Published studies involving BM staining in neurogenic sarcomas have been few. Ogawa et al. (1986) found a positive expression of collagen IV in all five malignant tumours analysed, while Miettinen et al. (1983) did not find laminin expression in the two cases they investigated. These conflicting data may be explained by our observations of heterogeneous staining depending on the degree of tumour differentiation. A direct comparison of our results with these previous reports is difficult, since in both previous studies neither the degree of differentiation nor the number of S100 protein positive cells present were reported.

In summary, we found predominant expression of laminin in most benign and malignant neurogenic tumours. This is assumed to be associated with a preferential Schwann cell-like type of differentiation. This neurotropism of laminin may have some impact on neural function and neural development. It is known that certain domains of the laminin molecule act as potent promotores for neurite outgrowth and are essential for the maintenance of the Schwann cell differentiation (Bunge et al. 1990). Our findings may have some diagnostic relevance in the differential diagnosis of various soft tissue tumours. We recently observed that myogenic sarcomas (unpublished observation) as well as spindle cells in Kaposi's sarcoma (Nerlich et al. 1991) possess a different configuration of BM matrix when compared with the neurogenic pattern. Further studies, however, are required to substantiate these findings with regard to other spindle-cell sarcomas, such as spindle-cell liposarcoma. In general, we feel that the application of differential staining for various BM components may be of significant value for the differential diagnosis of various soft tissue tumours.

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