Pergamon

Oral Oncol, Eur J Cancer, Vol. 30B, No. 2, pp. 132–137, 1994

0964–1955/94 $6.00+0.00

Enhanced Tenascin Immunoreactivity in Leukoplakia and Squamous Cell Carcinoma of the Oral Cavity: An Immunohistochemical Study

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Tenascin is an extracellular matrix glycoprotein that shows a site restricted expression especially in areas of cell proliferation, cell motility, and tissue modeling at the epithelial–mesenchymal junction during embryogenesis. Tissue specimens obtained from surgery and/or biopsy for oral leukoplakia ($n=22$) and squamous cells carcinoma ($n=36$) were examined for the presence of tenascin by using monoclonal antibody. In normal tissue specimens ($n=5$), tenascin immunoreaction appeared as a linear continuous lining at the immediate vicinity of basement membrane ($n=3$). Hyperplastic epithelia in leuoplakia showed a distinct increase in tenascin immunoreactivity in the submucosa correlating with the degree of hyperplasis and/or dysplasia. In squamous cell carcinoma (SCC), the reactivity was most intense extending deeply into the underlying stroma with marked reaction around large tumour cell nests and the infiltrating tumour margin. The connective tissue stroma, however, in undifferentiated carcinoma showed traces of immunoreactivity. Positive immunoreactivity was seen around metastatic squamous cell carcinoma masses in regional lymph nodes. The stromal tissues infiltrated by inflammatory cells were usually unreactive while those with desmoplastic changes were positive for tenascin. The authors conclude that an enhanced expression of tenascin may play an important role during active phases of tumour cell proliferation and stromal changes in the premalignant and malignant lesions of the oral mucosa.

Keywords: Tenascin, leukoplakia, squamous cell carcinoma, oral, immunohistochemistry.

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INTRODUCTION

TENASCIN IS a large glycoprotein component of extracellular matrix with a molecular weight of 190–250 kD [1] also termed as cytotactin [2], hexabrachion [3], myotendinous antigen [4], GP250 [5] and J-1 protein [6]. Tenascin is expressed in a spatially and temporarily restricted manner particularly in areas of epithelial–mesenchymal interaction during embryogenesis [7]. In adult tissues it has been found during wound healing [8, 9] and in stroma of benign and malignant tumours [7, 10].

Tenascin has been suggested as a multifunctional glycoprotein that participates in cell adhesion and motility, guidance along cell migration pathways, shedding of epithelial cells from surfaces, promotion of cell growth, demarcation of tissue boundaries and tissue modeling [1, 11]. These findings suggest that tenascin plays an important role in embryonic morphogenesis, wound healing and tumour cell proliferation, invasion and metastasis by altering cell-to-cell and cell–extracellular matrix communication.

An enhanced expression of tenascin has been found in *in situ* carcinoma of breast [12] and uterine cervix [13]. It has been demonstrated in stromal tissue of epithelial tumours including brest carcinomas [12], salivary gland tumours [14], lung carcinomas [15], squamous cell carcinomas of skin [16]; and in nonepithelial tumours including melanomas [17, 18] and gliomas [19]. In the present study, we demonstrate expression of tenascin in normal mucosa, leukoplakia and squamous cell carcinoma of the oral cavity.

MATERIALS AND METHODS

Tissue specimens were obtained following biopsy procedures or following surgical resection of oral squamous cell carcinoma. Specimens of normal oral mucosa ($n=5$), leukoplakia ($n=22$) and squamous cell carcinoma ($n=36$) were studied. The specimens were fixed in 10% neutral formalin for 12 h and embedded in paraffin and processed for conventional histopathological evaluation. Four μm sections were used to detect tenascin by immunohistochemical methods.

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Received 5 July 1993, provisionally accepted 21 July 1993; revised manuscript received 27 Aug. 1993.

Immunohistochemical methods

Anti-tensacin monoclonal antibody, mouse IgG1 raised by using purified human tenascin, was obtained from Chemicon International Inc. (California, U.S.A.) as lyophilised, unpurified ascitic fluid. A three stage avidin–biotin immunoperoxidase staining kit from Dakopatts, Denmark was used. In brief, after deparaffinization and rehydration through graded alcohol, the sections were treated with 0.1% protease solution for 10 min at room temperature. Endogenous peroxidase activity was blocked by treating the sections with methanol containing 0.3% H_2O_2 for 30 min. After washing in phosphate buffered saline (PBS), normal rabbit serum (1:20) was overlaid on the tissue sections for 30 min to reduce nonspecific background staining. The sections were then treated with diluted anti-tenascin antibody (1:500) for 1 h at room temperature. After three consecutive washes in PBS, biotinylated anti-mouse IgG was applied at a dilution of 1:200 and incubated for 30 min at room teperature. Following washing in PBS, AB complex (1:500) was applied for 30 min at room temperature. The reaction was visualized by using 3,3′diaminobenzidine hydrochloride in 0.1 ml/l Tris buffer solution containing 0.05% H_2O_2, pH 7.6 for 5 min.

Sections of breast carcinoma and experimentally induced carcinoma on hamster cheek pouch were used as positive controls. The primary antibody was omitted for negative controls.

RESULTS

In normal oral mucosa, tenascin immunoreactivity at the epithelial–connective tissue interface was either negative ($n=2$) or visualized as a delicate band ($n=3$) at the immediate vicinity of basement membrane (Fig. 1). The tenascin immunoreactivity was also seen in the connective tissue around the large blood vessels and perineural region. The minor salivary glands obtained in the tissue specimen, with histologically normal features had characteristic immunoreactivity for tenascin in peripheral connective tissue of large excretory ducts or interlobular duct, while staining was negative in striated or intralobular ducts.

In leukoplakia showing hyperplasia and hyperkeratosis of the oral mucosa, an enhanced immunoreactivity was seen in all cases at the epithelial–mesenchymal interface which correlated with the degree of increasing hyperplasia and/or dysplasia (Fig. 2A and B). Expression of tenascin was also detected between the hyperplastic rete pegs and adjacent stromal connective tissue.

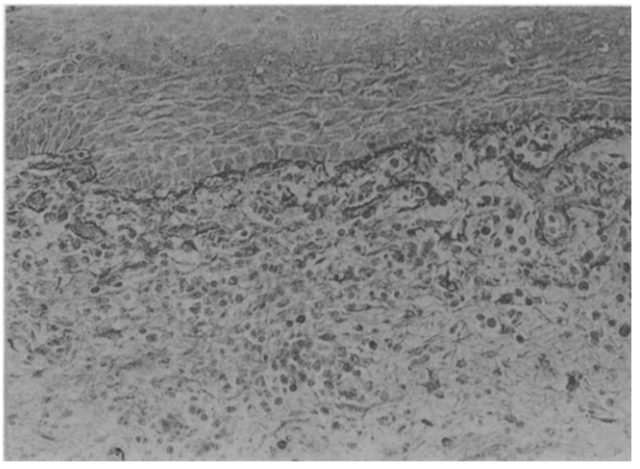

Fig. 1. Normal oral mucosa of the floor of the mouth: tenascin immunostraining is positive as a delicate band at the epithelial–mesenchymal interface (×100).

Tenascin immunoreactivity in squamous cell carcinoma was varying showing a heterogeneous pattern of straining in the stroma with strongly reative to unreactive stromal areas in the same specimen. Enhanced immunoreactivity was predominantly seen in the submucosal stromal tissues between hyperplastic mucosa adjacent to the invading squamous cell carcinoma focus (Fig. 3A, B, C and D). The stromal immunoreactivity was mostly evident at the infiltrating tumour margin and around tumour cell nests (Fig. 3C and D). In well differentiated ($n=14$) and moderately differentiated ($n=10$) carcinoma, areas of liner positive immunoreactivity were frequently observed at the tumour–stromal interface (Fig. 4A, B and C). In squamous cell carcinoma foci scattered in the stromal tissue, the reaction product was found focally around the tumour cells but not all throughout the peripheral stromal fibres. However, small groups tumour cells proliferating and invading the most peripheral tissue showed only a trace or no tenascin immunoreactivity in the stroma. The

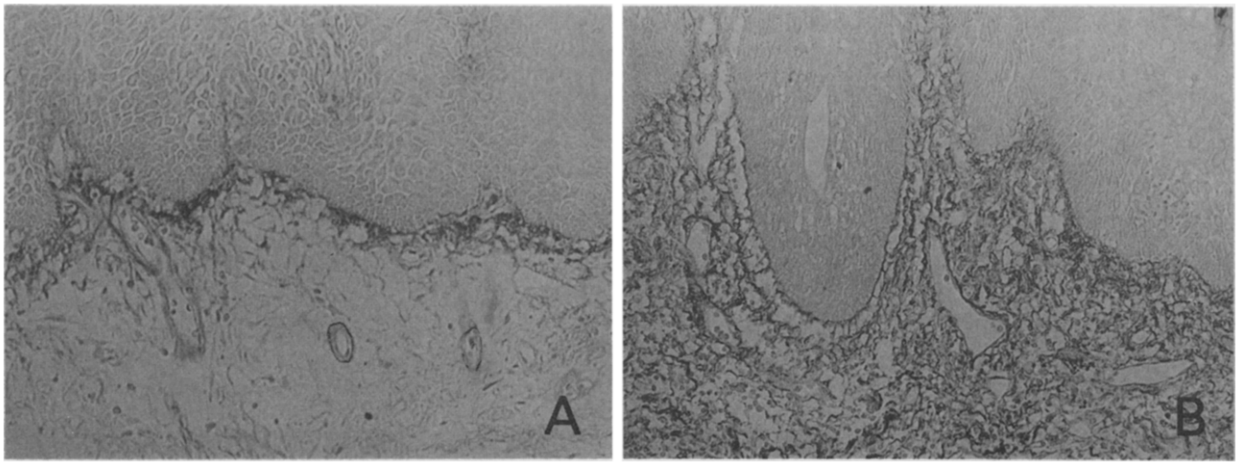

Fig. 2. Leukoplakia. (A) Tenascin immunoreactivity of the epithelial–connective tissue junction in leukoplakia with mild degree of hyperplasia. (B) The immunreactivity is enhanced with increased hyperplasia and dysplasia (×100).

reaction, on the other hand was traces but widespread with irregular stromal reaction (Fig. 4D) in poorly differentiated carcinoma ($n=12$). Areas adjacent to desmoplastic changes showed diffusely enhanced stromal immunoreactivity (Fig. 4C) however the reaction was negative or low with dense infiltration of inflammatory cells. Increased infiltration of inflammatory cells was accompanied by frequent breaks in the linear configuration of tenascin immunoractivity (Fig. 5A, B, C and D).

In metastatic lymph nodes (six lymph nodes from 3 cases), perivascular tissues showed a positive immunoreactivity for tenascin. Metastatic tumour cells infiltrating the lymph node showed a marked reactivity around relatively large masses of neoplastic cells (Fig. 6A and B), however, when a single or few tumour cells were present, the peripheral lymphoid stroma had trace to negative reaction.

DISCUSSION

Epithelial–mesenchymal interations are essential in the process of growth and differentiation, and extracellular matrix proteins act as substrate for cell attachment and motility in tumour proliferation and invasion. As tenascin has been found to alter the cell-to-cell and cell-extracellular matrix interactions at various levels, we carried out this immunohistochemical study in tissue specimens of normal mucosa, premalignant and malignant lesions of oral cavity. To date, there has been no report of tenascin in the premalignant and malignant lesions of oral mucosa.

The present study showed that tenascin, a component of the extracellular matrix is usually present in the stromal connective tissue immediately adjacent to basement membrane of oral epithelium and the expression is enhanced in premalignant and malignant lesions. The increase in the reactivity in leukoplakia correlated with the increasing degree of hyperplasia, hyperkeratosis and in particular, dysplasia in the lesion. A heterogeneity of enhanced expression was found in the well and poorly differentiated carcinoma. Tenascin was consistently present around the tumour cell focus in well differentiated tumours, however, the expression was low in poorly differentiated carcinoma suggesting a different level of stromal reaction according to the state of differentiation of tumour cells.

Sequencing of tenascin has shown regions homologous to epidermal growth factor [20] and purified tenascin stimulates the proliferation of breast carcinoma cells [21], suggesting a growth factor like function for tenascin.

Tissue specimens showing increased desmoplastic changes in the stroma had enhanced immunoreativity while those

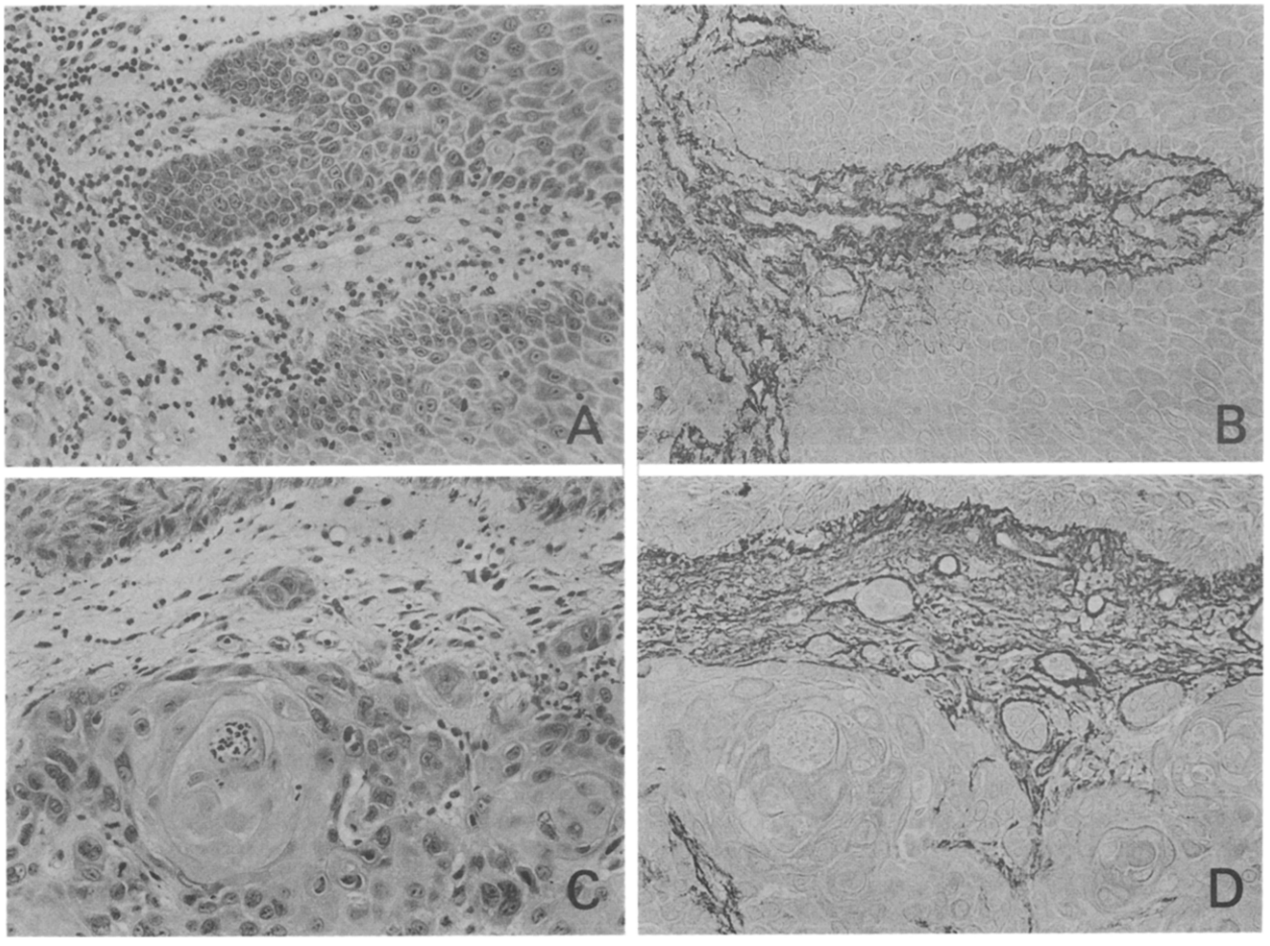

Fig. 3. (A) Haematoxylin and eosin stain showing hyperplastic oral mucosa with proliferating rete pegs adjacent to invading squamous cell carcinoma. (B) The reaction product is marked between the hyperplastic rete pegs and epithelial mesenchymal junction. (C and D). Well differentiated squamous cell carcinoma: (C) haematoxylin and eosin staining showing invasion of submucosal connective tissue by carcinoma. (D) Tenascin immunostaining is markedly present between the oral epithelium and submucosal SCC nests (× 100).

accompanied by dense inflammatory cell infiltration had no or trace reaction. We suggest that associated inflammatory cells and fibroblasts may affect the stromal tenascin expression in oral mucosa as also suggested in healing skin wounds [8] and fibrocystic disease of the breast [12]. Our experience of negative or trace immunoreactivity in tumour–mesenchymal interface densely infiltrated by inflammatory cells suggest the inflammatory cells may degrade or disorganize the tenascin with production of specific enzymes or tenascin inhibition factor, the detailed explanation of which is not yet well understood and needs further investigation.

In regional lymph nodes containing metastatic deposits, as immunoreactivity of tenascin was characteristically present in stromal connective tissue adjacent to relativey large metastatic carcinoma focus while negative or trace reaction was seen around a single or few metastatic tumour cells, it is most likely that tumour cell masses produce tenascin or induce the connective tissues to produce the same. Numerous previous studies have suggested that tenascin is solely a mesenchymal product. However, recent studies using *in situ* hybridization have shown that epithelial cells may produce tenascin [12, 22]. In the present study, we could not conclude whether the tenascin is produced by normal oral epithelial, malignant or stromal cells in the primary tumours, however, the premalignant and malignant lesions had an enhanced stromal tenascin activity. In addition, secretion of tenascin is under growth factor regulation as transforming growth factor β from tumour cells have been found to stimulate production of tenascin by mesenchymal cells [23].

Absence of tenascin expression in most adult tissues in normal conditions have been suggested previously [24], however, numerous investigators have recently shown the presence of tenascin in adult tissues [10, 12, 25]. Our study showed that tenascin does exist at the epithelial mesenchymal interface of oral mucosa in normal conditions, if not in all instances. In addition, we have found reaction product of tenascin in wall of blood and lymph vessels and in perineural region as in normal adult breast and uterine cervix [12, 13].

In conclusion, tenascin may be present as a delicate band at the epithelial connective tissue interface in normal oral mucosa and its expression is enhanced in premalignant and malignant lesions of the oral cavity suggesting that it may play a role in induction and progression of the lesions, possibly by production and deposition which are affected by the state of differentiation of tumour cells, inflammatory and desmoplastic reactions, and alters the epithelial–mesenchymal interaction.

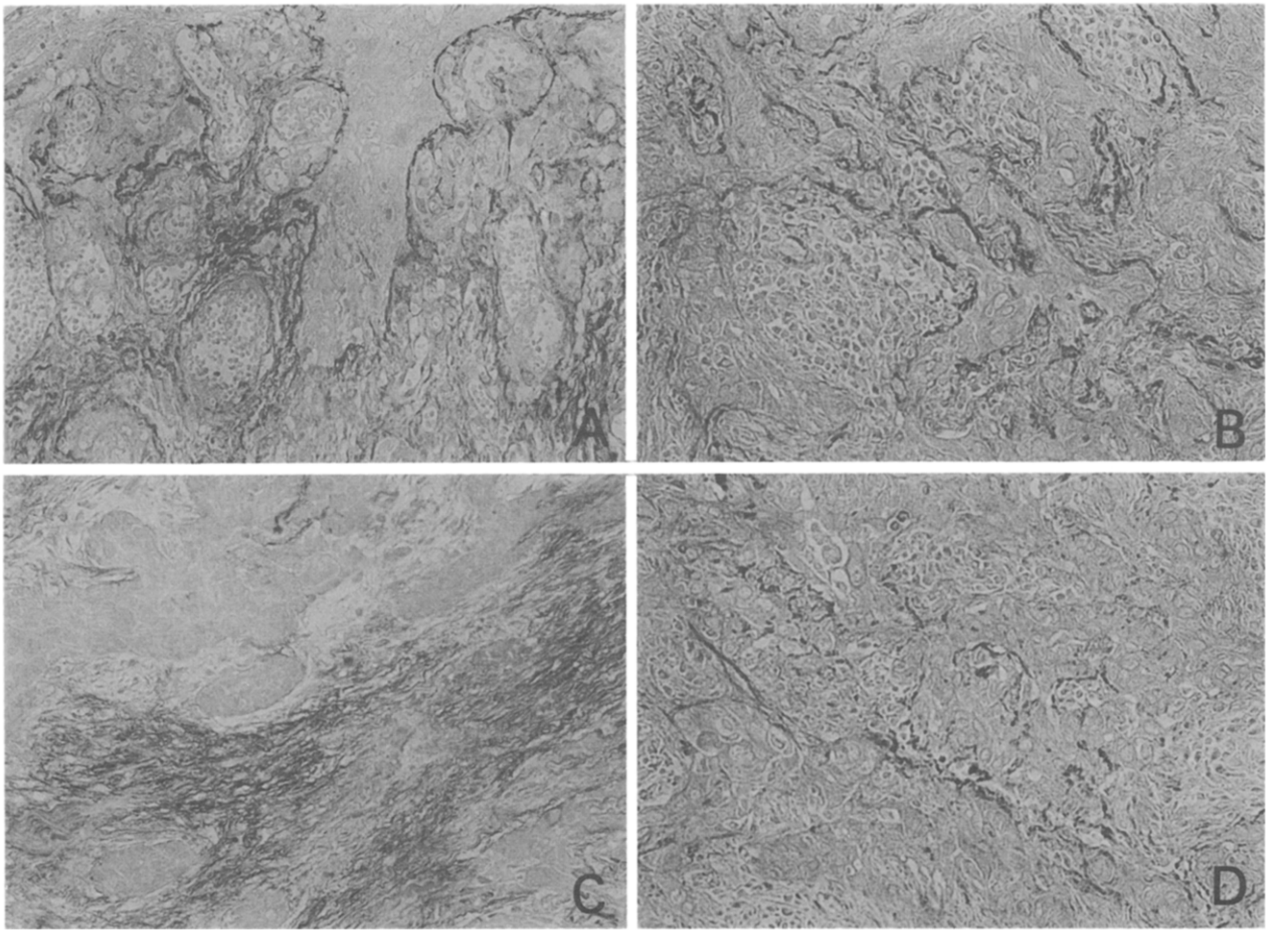

Fig. 4. Various patterns of tenascin immunostaining in SCC: (A) SCC focus reveal an intense immunostaining in stromal fibres adjacent to tumour cells as a basement membrane like appearance in well differentiated carcinoma. (B) Similar features are seen in the moderately differentiated carcinoma as in well differentiated tumour. (C) Moderately differentiated SCC with desmoplastic changes in the stromal tissue is associated with an enhanced expression of teanscin. (D) Poorly differentiated carcinoma: tenascin immunostaining is low and is seen focally in the stromal connective tissue adjacent to tumour cells or absent in the stromal fibres (× 100).

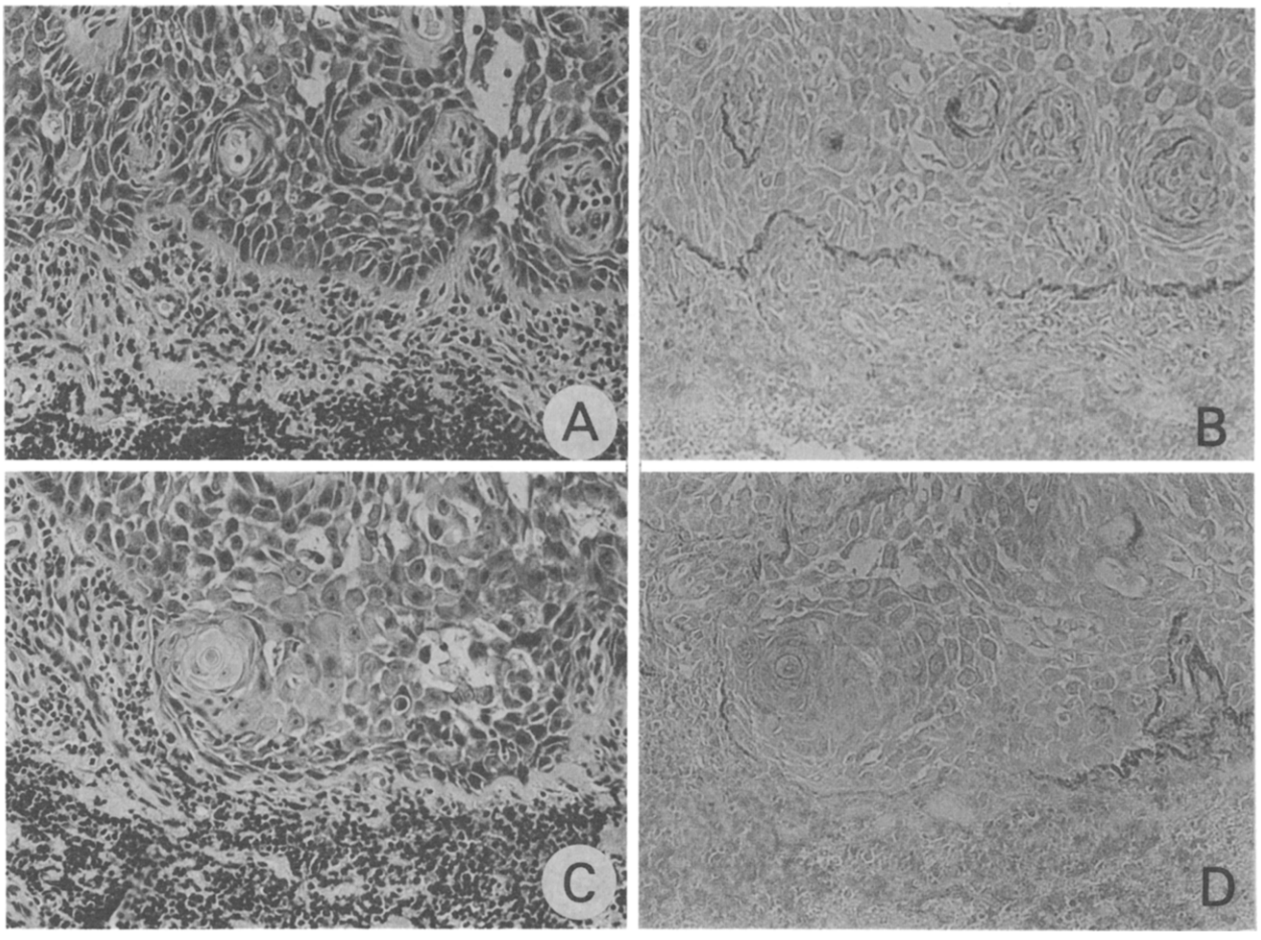

Fig. 5. Invading front of squamous cell carcinoma. (A) Haematoxylin and eosin staining showing carcinoma cells and adjacent tumour troma. The tumour nests are not infiltrated by inflammatory cells. (B) Tenascin immunoreactivity in this well differentiated carcinoma is positive as linear basement membrane like appearance. (C) Tumour cell nests infiltrated by inflammatory cells, haematoxylin and eosin straining. (D) A break in linear configuration of tenascin immunoreactivity is seen when inflammatory cells infiltrate the tumour focus (× 100).

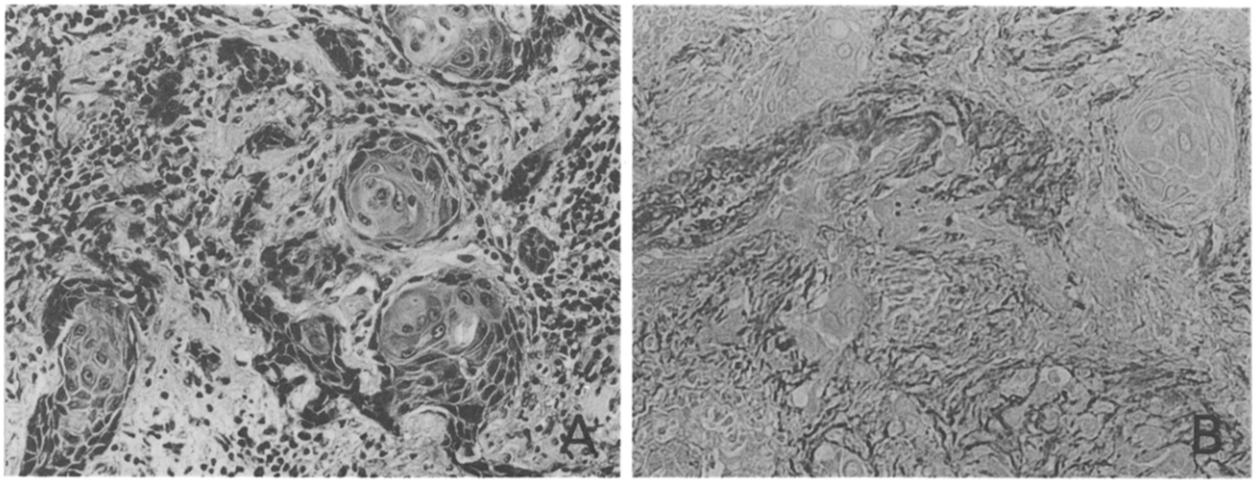

Fig. 6. Regional lymph node with metastatic carcinoma cells. (A) Metastatic SCC foci in lymph nodes, haematoxylin and eosin staining and (B) tenascin is positive in connective tissue around large masses of metastatic tumour cells in the lymph node (× 100).

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Notes added in proofs—Some epithelial cancer cells may synthesize and secrete tenascins *in vitro* (Kawakastsu *et al.*, *Jpn J Cancer Res* 1992, **83**, 1073–1080). A tongue carcinoma cell line LICR-LON-HN5 do not secrete tenascin *in vitro*, however, xenografts of the cells show positive tenascin immunostaining of tumour matrix and surrounding connective tissue (Anbazhaagan *et al.*, *Virchows Arch B* 1990, **59**, 59–63).

Acknowlegement—This study was supported in part by Grants-in-aid no. 004454458 from the Ministry of Education, Science and Culture of Japan.