Virchows Archiv A Pathol Anat (1989) 414:447–452

*Virchows Archiv A*
*Pathological Anatomy and Histopathology*


# The immunocytochemical demonstration of basement membrane deposition in transitional cell carcinoma of bladder

**R.J. Zuk, S.I. Baithun, J.E. Martin, E.L. Cox, and P.A. Revell**
Department of Morbid Anatomy, The London Hospital, Whitechapel, London, E1 1BB, UK

**Summary.** We assessed the staining characteristics of the basement membrane of transitional cell carcinoma of bladder using a monoclonal antibody to type IV collagen. Basement membranes were clearly stained at the stromal/carcinoma interface. As transitional cell carcinoma became less well differentiated and the depth of invasion increased interruptions to basement membrane staining became more extensive and these findings are comparable to those described in similar series of transitional cell carcinoma using polyclonal antibodies to type IV collagen. The defects in basement membrane staining may be related to the degree and direction of tumour cell differentiation or may be explained by increased degradation compared to synthesis of basement membrane components. Demonstration of the basement membrane may be of value in diagnostic histopathology as a marker of the biological behaviour of transitional cell carcinoma of bladder.

**Key words:** Urinary bladder – Transitional cell carcinoma – Basement membrane – Type IV collagen

## Introduction

Basement membranes (BM) are complex extracellular matrices that surround and support capillaries, adipocytes, skeletal and smooth muscle cells, cardiac cells and Schwann cells and are attached to the basal layer of all epithelial cells. Visualised by electron microscopy BM have 2 distinct zones: (a) the lamina lucida (lamina rara), an electron-lucent layer immediately beneath the cell that the membrane is supporting; (b) the lamina densa (basal lamina), the central electron dense layer. A third zone, the lamina fibroreticularis (sublamina densa), is present in most BM but is absent from renal glomeruli and lung alveoli (Abrahamson 1986).

Biochemically the components of BM are of intrinsic and extrinsic origin. Type IV collagen and laminin are considered intrinsic structures as they are produced by adjacent epithelial cells and only occur in BM. Components such as type V collagen and fibronectin are considered extrinsic structures as they are also found in other structures and are not wholly synthesised by the surrounding epithelial cells (Martinez-Hernandez and Amenta 1983). Type IV collagen is one of the most extensively studied components and is localised to the lamina lucida and lamina densa (Roll et al. 1980).

The BM of epithelium may represent a structural barrier to early infiltration of malignant cells into the underlying tissues. Knowledge of the state of BM structure may therefore be useful in diagnostic histopathology to distinguish between non-invasive and invasive cancers.

Classically BM may be visualised on light microscopy using silver impregnation techniques or the periodic acid-Schiff stain (PAS) but more sensitive and reliable immunohistochemical techniques are now available to stain BM components (Barsky et al. 1984). These techniques have shown that benign tumours and in situ lesions are surrounded by intact BM and that many invasive tumours lack BM or contain fragmented BM (Barsky et al. 1983; Bosman et al. 1985).

In certain cases of transitional cell carcinoma (TCC) of bladder great difficulty is encountered in predicting the biological behaviour of the tumour. The identification of superficial invasion is an important factor in patient management as staging remains the single most important therapeutic and prognostic factor. There is an excellent

*Offprint requests to:* R.J. Zuk

correlation between both depth of invasion and vascular invasion and a greatly decreased survival rate but other indicators are still needed.

To date only two studies have been published on the immunohistochemical staining characteristics of the urothelial BM in non-invasive and invasive (TCC) of the bladder (Conn et al. 1987; Daher et al. 1987). Both these studies used polyclonal antisera to type IV collagen and laminin.

We performed a study using a monoclonal antibody to type IV collagen to assess the staining characteristics of BM in non-invasive and invasive TCC of bladder and to determine if the results were comparable to those using polyclonal antisera.

## Materials and methods

Fresh tissue specimens from 20 bladder tumours were obtained at cystoscopy. The specimens were snap-frozen immediately in liquid nitrogen with an isopentane stage to preserve cellular morphology and were stored at −70° C prior to sectioning. Cryostat sections were cut at 5 μm and stained by the indirect immunoperoxidase method (Sternberger 1979) with a monoclonal antibody to type IV collagen. (This antibody was supplied by Dr. Stephan Gay, WHO Collagen and Collagen Antibody Reference Centre, Birmingham, Alabama, USA). The peroxidase activity was visualised using diaminobenzidine. Sections were also stained with haematoxylin and eosin (H & E), a silver impregnation technique (Gordon and Sweets' stain for reticulin fibres) and PAS.

*Histological examination.* The sections were assessed by three different pathologists for the following features:
(I) The H & E stained sections were used to grade ($G_1$–$G_3$) and stage pTa-PT2) the tumours according to the standardised international classification of the UICC (International Union against Cancer) 1978.
(II) The quality of staining of BM with PAS and silver stains were compared with that obtained by immunoperoxidase staining.
(III) The completeness of staining of the BM with type IV collagen was determined for all grades of non-invasive and invasive tumours.
(IV) The intensity of BM staining in tumours with a marked inflammatory cell infiltrate in the lamina propria was assessed.
(V) The sections were examined of evidence for vascular invasion.

## Results

The results of the histological classification and immunocytochemistry with the type IV collagen antibody are summarised in Table 1. The sections stained with the immunoperoxidase method revealed clearly detectable BM and this was far superior to conventional techniques i.e. silver impregnation technique or PAS stains.

BM were strongly stained at the stromal/epithelial interface and around blood vessels and muscle fibres (Fig. 1). In several specimens, adjacent normal urothelium showed continuous BM staining. Lymphatic vessels were not stained.

**Table 1.** Histological and immunocytochemical results

| Case No. | Grade of tumour | Stage of tumour | BM staining with type IV collagen antibody* |
|---|---|---|---|
| 1 | $G_1$ | pTa | ++++ |
| 2 | $G_1$ | pTa | ++++ |
| 3 | $G_1$ | pTa | +++ |
| 4 | $G_2$ | pTa | +++ |
| 5 | $G_2$ | pTa | ++ |
| 6 | $G_2$ | pTa | +++** |
| 7 | $G_2$ | pTa | +++ |
| 8 | $G_2$ | pTa | +++ |
| 9 | $G_2$ | pTa | ++++ |
| 10 | $G_2$ | pTa | +++ |
| 11 | $G_2$ | pTa | +++ |
| 12 | $G_3$ | pTa | ++ |
| 13 | $G_3$ | pTa | ++ |
| 14 | $G_2$ | pT1 | ++++ |
| 15 | $G_3$ | pT1 | ++ |
| 16 | $G_3$ | pT1 | + |
| 17 | $G_3$ | pT1 | ++ |
| 18 | $G_2$ | pT2 | ++ |
| 19 | $G_3$ | pT2 | + |
| 20 | $G_3$ | pT2 | + |

* Completeness of staining: ++++ = intact; +++ = focal interruptions; ++ = large interruptions; + = minimal staining
** This case had a marked mixed inflammatory cell infiltrate in the lamina propria. The staining was indistinct and showed interruptions in areas where the inflammation involved the stromal/carcinoma interface

There were 13 non-invasive and 7 invasive tumours as assessed by conventional histology. The results with type IV antibody on non-invasive tumours (pTa) showed small focal interruptions to BM as the tumour became less well differentiated. Complete staining was identified most often in papillary well differentiated ($G_1$) neoplasms (Fig. 1). Invasive tumours demonstrated larger interruptions of staining compared with the non-invasive group (Fig. 2) and only one invasive tumour (case 14) showed intact BM staining. Discontinuity of staining was most marked in pT2 tumours and the most extensive interruptions were demonstrable in the $G_3$ pT2 lesions. None of the cases examined showed total loss of BM staining, there was no evidence of intracytoplasmic staining within tumour cells and there was no evidence of vascular invasion.

Single and small groups of invasive tumour cells within the lamina propria showed no type IV collagen staining in the majority of cases of

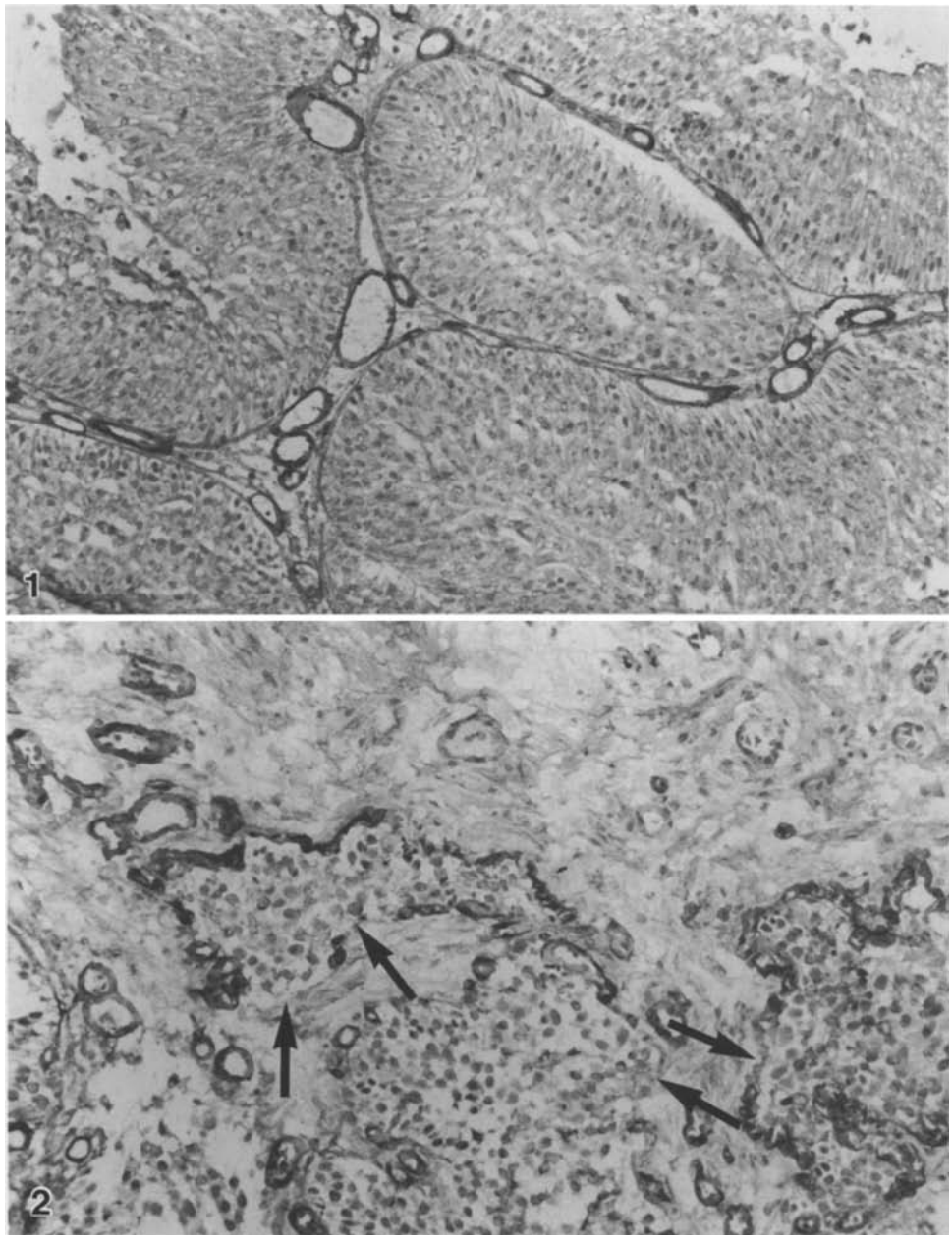

**Fig. 1.** Immunocytochemical staining using monoclonal antibody to type IV collagen on non-invasive TCC of bladder ($G_1$pTa). Intact BM staining at the stromal/carcinoma interface. Blood vessels within the lamina propria show prominent BM staining. Original magnification ×40

**Fig. 2.** Immunocytochemical staining using monoclonal antibody to type IV collagen on invasive TCC of bladder ($G_2$) within the lamina propria. The tumour islands are largely surrounded by a band of type IV collagen which varies in thickness. Large interruptions (*arrows*) are seen within the BM. Original magnification ×40

invasive tumours no matter what the tumour grade (Fig. 3).

A marked inflammatory cell infiltrate within tumour was present in one case. Staining of BM in this case was indistinct and showed interruptions in areas where the inflammation involved the stromal/carcinoma interface (Fig. 4).

## Discussion

Penetration of the BM matrix by tumour will lead eventually to lymphatic and blood vessel invasion and ultimately to metastases. Research has centred on the mechanisms of invasive growth and on methods to distinguish non-invasive from invasive tumours. The BM is a natural barrier to early invasion therefore investigation has centred on this structure.

Recent immunohistochemical studies have shown that interruptions of the BM are identifiable in many invasive carcinomas. This has been demonstrated in numerous tumours: pancreatic, prostatic and endometrial carcinoma (Barsky et al. 1983); squamous cell carcinoma (Carter et al. 1985); breast carcinoma (Willebrand et al. 1986); laryngeal carcinoma (Visser et al. 1986). These

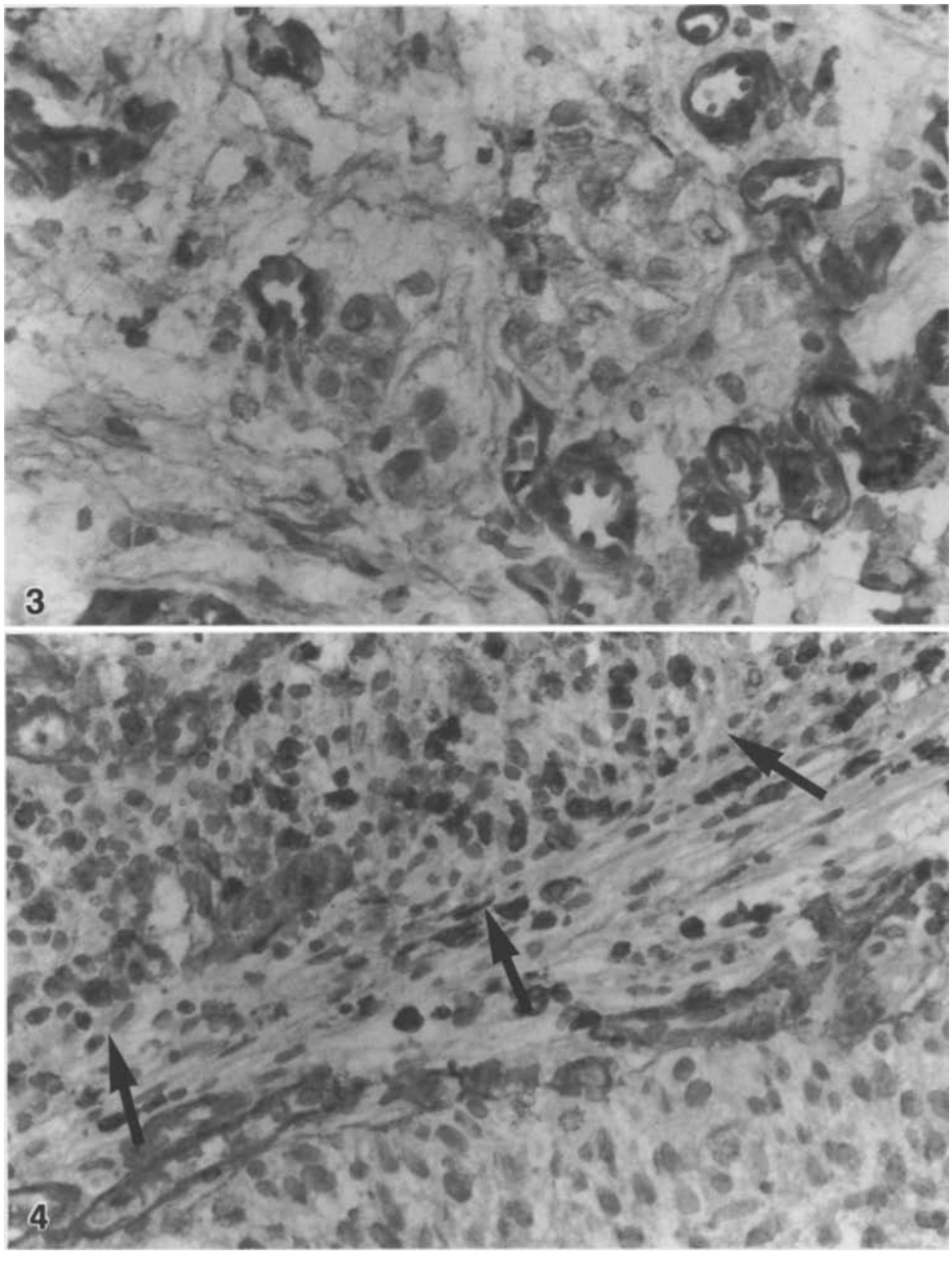


**Fig. 3.** Immunocytochemical staining using monoclonal antibody to type IV collagen on invasive ($G_3$) TCC of bladder. There is no staining of type IV collagen around small islands and individual tumour cells within the lamina propria. Small vascular channels show prominent BM staining. Original magnification $\times 100$

**Fig. 4.** Immunocytochemical staining using monoclonal antibody to type IV collagen on non-invasive ($G_2pTa$) TCC of bladder. There is loss of BM staining in areas where the inflammatory infiltrate involves the stromal/carcinoma interface (*arrows*). Note many intraurothelial leucocytes. Original magnification $\times 100$

gaps within the BM may be due to an inability of tumour cells to manufacture BM components due to a fault in tumour differentiation (Gusterson et al. 1986) or an increased degradation of BM components compared to synthesis by the neoplastic cells. This degradation may be produced by enzymes formed by the tumour cells e.g. type IV collagenase (Barsky et al. 1983; Liotta et al. 1983) which may then attack the underlying BM.

Our results show that as TCC becomes less well differentiated and the depth of invasion increases BM staining shows more extensive discontinuities. However, these observations are purely morphological and can not be supported by statistical analysis as only 20 tumours were studied. The number of cases in our study were limited as the technique required fresh tissue. Our findings are similar to those of Conn et al. (1987) and Daher et al. (1987) who also found increasing discontinuities of BM staining in poorly differentiated TCC with invasion but these workers used polyclonal antibodies to type IV collagen on formalin and ethanol fixed tissue. Our study is the first reported using a monoclonal antibody. Other studies (Cam

et al. 1984; Gusterson et al. 1986) have also shown that BM staining is more clearly defined in well differentiated tumours as we have also found with well differentiated TCC.

A further interesting finding was that in one case of invasive TCC BM staining was still complete. This has also been described in the past in basal cell carcinomas (Van Cauwenberge et al. 1983; Gusterson et al. 1984) and may cast doubt on the requirement of a tumour in certain instances to destroy the BM as part of the invasive process.

In the case which showed marked inflammation, BM staining was indistinct and showed focal interruptions confined to areas of epithelial invasion by inflammatory cells. The appearance of defects in the BM in the presence of inflammation has been described previously (Visser et al. 1986; Conn et al. 1987) and may be due to the enzymatic degradation of the BM matrix by both inflammatory and neoplastic cells. This would make the determination of invasion difficult in those cases where a marked inflammatory cell infiltrate involves the stomal/carcinoma interface.

Great difficulty is encountered in predicting the biological behaviour of TCC therefore a search for markers of aggressiveness has been made. Recently, squamous metaplasia and chorionic gonadotrophin expression by invasive TCC of bladder has been shown to correlate with prognosis (Martin et al. 1989a, b). Furthermore, it has been found that tumours with a tendency to recur, invade and metastasise lack blood group antigens on their cell surfaces, induce angiogenesis, show alterations in junctional complexes on electron microscopy and have highly abnormal karyotypes (Koss 1982; Robbins et al. 1984). The completeness of BM staining may therefore be of use as a marker of the biologic behaviour of TCC. In breast carcinoma it has been suggested that the pattern of BM deposition is related to histological grade and may be of prognostic significance (Willebrand et al. 1986). Aggressive tumours with a high probability of metastasising could deposit less BM than neoplasms with low metastatic potential. A complete or almost totally complete BM may indicate noninvasiveness and larger defects or absence of BM might favour invasiveness.

The study by Conn et al. (1987) on 84 patients with TCC using polyclonal antisera to type IV collagen and laminin demonstrated that the incidence of progression of superficial tumours with patchy or absent BM was significantly greater than those with complete BM. Daher et al. (1987) studied 48 invasive TCC of bladder using a polyclonal antibody to type IV collagen and found that patients with minimal BM staining had a poor short term prognosis compared with those showing more complete staining. Therefore BM staining may be of value in diagnostic histopathology in predicting the subsequent biological behaviour of TCC of the bladder.

In conclusion we have demonstrated, using a monoclonal antibody specific for type IV collagen, that the epithelial BM in cases of TCC of bladder is prominently stained. Interruptions to staining increase and become more extensive as TCC becomes less well differentiated and the depth of invasion increases. These findings are similar to those observed in similar series of TCC using polyclonal antibodies. Several factors many explain these defects in BM deposition; the deposition of BM elements may be related to the degree and direction of tumour cell differentiation or there may be increased degradation compared to synthesis of BM components in TCC. Demonstration of BM may be of value in diagnostic histopathology as a marker of the biological behaviour of TCC.

## References

Abrahamson DR (1986) Recent studies on the structure and pathology of basement membranes. J Pathol 149:257–278

Barsky SH, Seigal GP, Jannotta F, Liotta LA (1983) Loss of basement membrane components by invasive tumours but not by their benign counterparts. Lab Invest 49:140–147

Barsky SH, Rao NC, Restrepo C, Liotta LA (1984) Immunocytochemical enhancement of basement membrane antigens by pepsin: applications in diagnostic pathology. Am J Clin Pathol 82:191–194

Bosman FT, Havenith M, Cleutjens JPM (1985) Basement membranes in cancer. Ultrastruct Pathol 8:291–304

Cam Y, Caulet T, Bellon G, Poulin G, Legros M, Pytlinska M (1984) Immunohistochemical localisation of macromolecules of the basement membrane and the peritumoral stroma in human laryngeal carcinomas. J Pathol 144:35–44

Carter RL, Burman JF, Barr L, Gusterson BA (1985) Immunohistochemical localisation of basement membrane type IV collagen in invasive and metastatic squamous cell carcinoma of the head and neck. J Pathol 147:159–164

Conn IG, Crocker J, Wallace DMA, Hughes MA, Hilton CJ (1987) Basement membranes in urothelial carcinoma. Br J Urol 60:536–542

Daher N, Abourachid H, Bove N, Petit J, Burtin P (1987) Collagen IV staining pattern in bladder carcinomas: Relationship to prognosis. Br J Cancer 55:665–671

Gusterson BA, Warburton MJ, Mitchell D, Kraft N, Hancock WW (1984) Invading squamous cell carcinoma can retain a basal lamina. An immunohistochemical study using a monoclonal antibody to type IV collagen. Lab Invest 51:82–87

Gusterson BA, Clinton S, Gough G (1986) Studies of early invasive and intraepithelial squamous cell carcinomas using an antibody to type IV collagen. Histopathology 10:161–169

Koss LG (1982) Evaluation of patients with carcinoma in situ of the bladder. Pathol Annu 17:353–359

Liotta LA, Rao CN, Barsky SH (1983) Tumour invasion and the extracellular matrix. Lab Invest 49:636–650

Martin JE, Jenkins BJ, Zuk RJ, Oliver RTD, Baithun SI (1989a) Human choronic gonadotrophin expression and histological findings as predictors of response to radiotherapy in carcinoma of the bladder. Virchows Archiv A 414:273–277

Martin JE, Jenkins BJ, Zuk RJ, Baithun SI (1989b) The clinical importance of squamous metaplasia within invasive transitional cell carcinoma of the bladder. J Clin Pathol (in press)

Martinez-Hernandez A, Amenta PS (1983) The basement membrane in pathology. Lab Invest 48:656–677

Robbins SL, Cotran RS, Kumar V (1984) (eds) Pathologic Basis of Disease. 3rd. ed., W.B. Saunders Co., Philadelphia USA, p 1076

Roll FJ, Madri JA, Albert J, Furthmayr H (1980) Codistribution of collagen types IV and $AB_2$ in basement membranes and mesangium of the kidney. An immunoferritin study on ultrathin frozen sections. J Cell Biol 85:597–616

Sternberger LA (1979) Immunocytochemistry. 2nd ed., John Wiley & Sons, New York

Uicc (1978) TNM Classifation of Malignant Tumours. 3rd. ed. Geneva, pg 113–117

Van Cauwenberge D, Pierard GE, Foidart JM, Lapiere CHM (1983) Immunohistochemical localisation of laminin, type IV and type V collagen in basal cell carcinomas. Br J Derm 108:163–170

Visser R, van der Beek JMH, Havenith MG, Cleutjens JPM, Bosman FT (1986) Immunocytochemical detection of basement membrane antigens in the histopathological evaluation of laryngeal dysplasia and neoplasia. Histopathology 10:171–180

Willebrand D, Bosman FT, De Goeij AFPM (1986) Patterns of basement membrane deposition in benign and malignant breast tumours. Histopathology 10:1231–1241

Received October 4, 1988 / Accepted December 27, 1988