Virchows Arch [Pathol Anat] (1985) 408:49–59

Virchows Archiv A

Myofibroblastic stromal reaction in carcinoma of the breast: variations of collagenous matrix and structural glycoproteins

Réal Lagacé [1], Jean-Alexis Grimaud [2], Walter Schürch [3], and Thomas A. Seemayer [4]

[1] Service de pathologie, Hôtel-Dieu de Québec et department de pathologie, Faculté de médecine, Université Laval, 11 Côte du Palais, Québec G1R 2J6, Canada
[2] Laboratoire de pathologie cellulaire du foie, CNRS, ERA 189, Institut Pasteur, Lyon, France
[3] Département de pathologie, Hôtel-Dieu de Montréal et département de pathologie, Université de Montréal, Montréal, Québec, Canada
[4] Department of Pathology, The Montreal Children's Hospital, McGill University, Montréal, Québec, Canada

Summary. The matrix of mammary dysplasia, noninvasive ductal carcinoma, and invasive lobular and ductal carcinoma was analyzed by indirect immunofluorescence using antibodies to types I, proIII, III, and IV collagens, and laminin and fibronectin. Types proIII and III collagens were present in increased amounts in invasive carcinomas and were most abundant in the "young" edematous mesenchyme, areas corresponding to the peripheral invasive cellular front. Type I collagen was distributed throughout the matrix of invasive carcinomas but was most prominent within the central sclerotic zone of the neoplasms. Mammary dysplasia and noninvasive ductal carcinomas showed a uniform fibrillar and granular distribution of all types of collagen. In all but two cases of invasive carcinoma, staining with anti-laminin and anti-type IV collagen demonstrated the loss of basement membranes around tumor cells. In contrast, fluorescence pattern in noninvasive ductal carcinoma and dysplasia revealed an intact basement membrane. The distribution of fibronectin was similar to types proIII and III collagen.

These findings support and extend our previous studies which suggested an analogy between the dynamics of matrix changes in granulation tissue and invasive carcinomas. These data also strengthen the concept that the myofibroblast could be a pivotal cell involved in the synthesis and redistribution of matricial proteins. The loss of basement membrane in invasive carcinomas appears to be an initial step for inducing the matricial alterations.

Key words: Matrix – Myofibroblast – Breast carcinoma – Collagen – Laminin – Fibronectin

Offprint request to: R. Lagacé at the above address

Ongoing studies by our group have addressed the matter of stromal alterations in carcinoma (Tremblay 1979; Seemayer et al. 1979; Schürch et al. 1981; Schürch et al. 1984). Considerable numbers of myofibroblasts (MF) were noted within the stroma of diverse primary invasive and metastatic carcinomas, especially those neoplasms which demonstrated desmoplasia and retraction. We suggested that this matrix alteration might represent a stromal response directed toward containment of invasive and/or metastatic carcinoma. Studies on the spatial distribution of MF in a series of scirrhous mammary carcinomas revealed a striking localization of MF within these neoplasms (Schürch et al. 1982). The abundance of MF in edematous peripheral regions led us to draw an analogy between the matrix alterations in granulation tissue (Gabbiani et al. 1976) and those observed in invasive carcinomas. Invasion of tumor cells beyond the basement membrane was posited to induce transformation of quiescent fibroblasts to contractile fibroblasts, i.e., myofibroblasts. The latter, endowed with synthetic properties, were thought likely to elaborate initially type III collagen. At some point in the evolution of the neoplasm, we reasoned that type I collagen synthesis was initiated. It was at this level of conceptualization that the matter rested in 1982.

This study was designed to investigate the matrix of mammary dysplasia, intraductal carcinoma, and invasive lobular and ductal carcinoma using indirect immunofluorescence (IF) microscopy. A semi-quantitative study of the distribution of types I, proIII, and III collagen by monospecific antibodies was performed in an attempt to demonstrate the dynamics of matrix changes in these pathologic states. This study was also directed to determine basement membrane alterations which occurred in these conditions, as judged by a semi-quantitative study of the distribution of laminin, type IV collagen, and fibronectin.

Material and methods

Tissues. Specimens were obtained fresh from surgical pathology material at the time of frozen section. Twenty-eight desmoplastic (scirrhous) ductal and six lobular carcinomas and two noninvasive intraductal carcinomas were examined. Four samples from normal breasts and seven cases of mammary dysplasia served as controls. Small portions of each were snap frozen in liquid isopentane stored at $-70°$ C.

Antisera. The antisera were kindly provided by L'Institut Pasteur de Lyon, France (Laboratoire de Páthologie cellulaire du foie, CNRS, ERA 189). Antibodies to type I, proIII, and IV collagens were raised in New Zealand rabbits, as were antibodies to laminin and fibronectin. The antiserum against type III collagen was prepared in the goat. The preparation and purification of these antibodies have been described elsewhere (Flejou et al. 1984). The specificity of the antisera was assessed by ELISA technique, according to Rennard et al. 1980.

Indirect immunofluorescence. Cryostat section were cut at 3–5 μ, air dried at room temperature for 30–45 minutes, washed in phosphate buffered saline (PBS) and fixed in 95% ethanol for 30 minutes. After several washings in PBS, the sections were incubated for 45 minutes with antisera at room temperature at the following dilutions:

anti-collagen I	1:4
anti-proIII	1:8

anti-collagen III	1:4
anti-collagen IV	1:8
anti-laminin	1:8
anti-fibronectin	1:10

Sections were washed in PBS and incubated with a 1:30 dilution of FITC-conjugated sheep anti-rabbit or rabbit anti-goat IgG globulin for 45 minutes. The sections were mounted in buffered glycerine. The intensity of fluorescence was compared with sections treated with normal rabbit serum in lieu of the primary antibodies. Photographs were taken with a Leitz microscope equipped with epiillumination and specific filters for fluorescein using Daylight Ektachrome High Speed Color Film. Black and white prints were prepared from color slides.

Histology. Frozen sections of all tissues were stained with toluidine blue for purposes of comparison with fluorescence sections.

Results

A. Collagenous matrix

1. *Invasive lobular and ductular scirrhous carcinoma.* Immunofluorescence staining for types I, proIII, and III collagen demonstrated diffuse deposits in the desmoplastic stroma in both forms of invasive carcinomas. The pattern of staining was distinctive: type I collagen was most prominent in the central hyalinized region and appeared as thick regular strands, either separating lobules of tumour cells in ductal carcinomas (Fig. 1 a, e) or dissociating small nests of tumor cells in lobular carcinomas (Fig. 1 b). Antibodies to anti-proIII and III collagen stained bundles of irregular fibrillar fibers, and showed a strong tendency to dissociate individual tumor cells and stromal spindle cells (Fig. 1 c). The intensity of immunoreactivity for proIII and III type collagen was most intense in the peripheral invasive cellular front (Fig. 1 d, f). The tumor cell cytoplasm failed to react with all three sera, hence, staining was confined to the extracellular matrix.

2. *Noninvasive ductal carcinoma, dysplasia and normal breast.* The pattern of staining was similar with all three anti-sera in intraductal carcinoma. The three collagens stained as fibrillar bundles surrounding nests of tumor cells (Fig. 2 a–c). Comparatively, there was no difference in staining pattern in sections from normal or dysplastic breast tissues. The single exception, a case of sclerosing adenosis, demonstrated strong staining with anti-collagen I.

B. Basement membrane

1. *Invasive lobular and ductular scirrhous carcinoma.* All but two cases of invasive carcinoma were devoid of laminin and collagen IV, even though positive staining was observed within vessel walls (Fig. 3 a) and peripheral nerve bundles. In one case of invasive lobular carcinoma, type IV collagen and laminin stained distinctively but irregularly at the periphery of nests of malignant epithelial cells (Fig. 3 b). In another case of invasive ductal

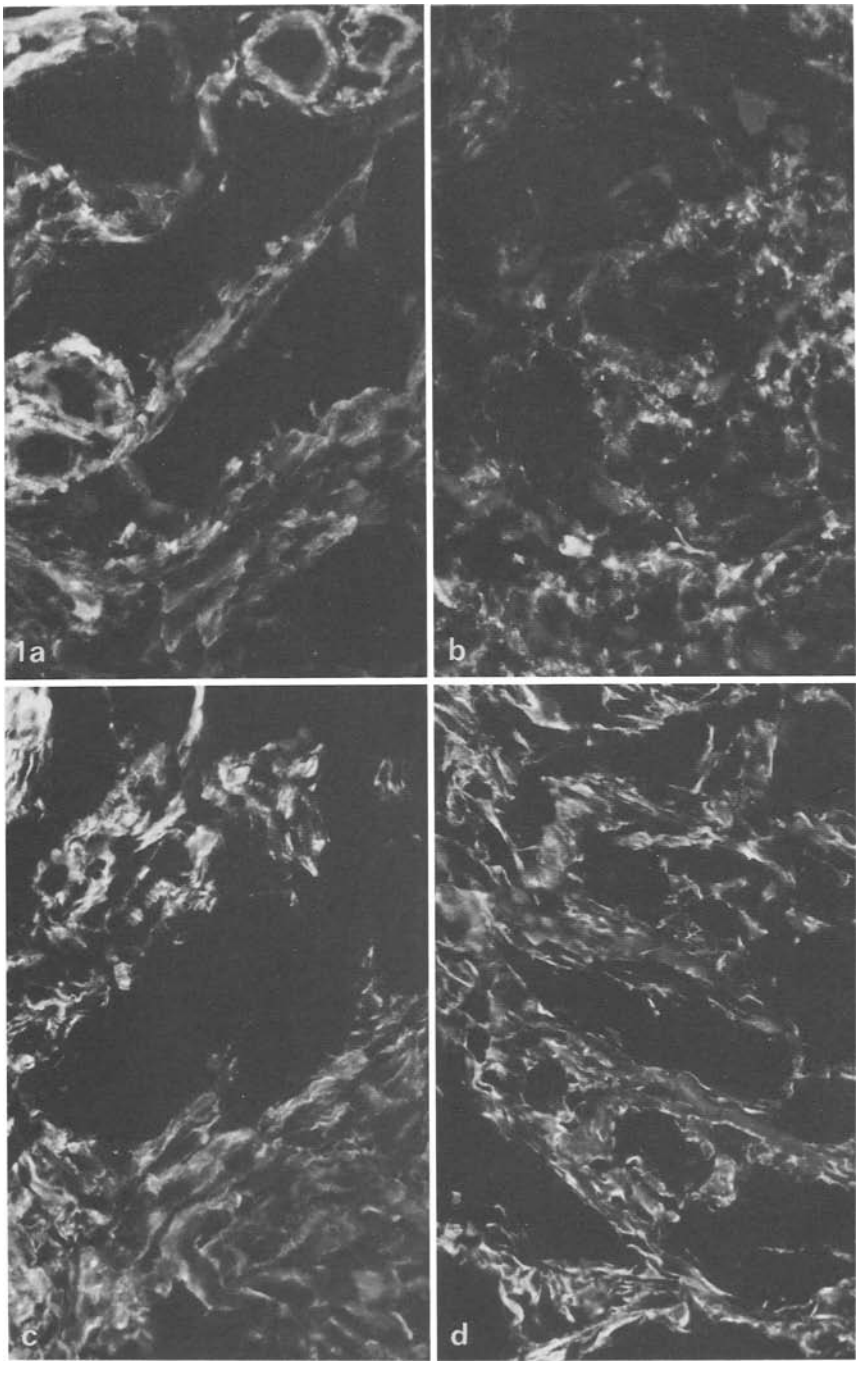
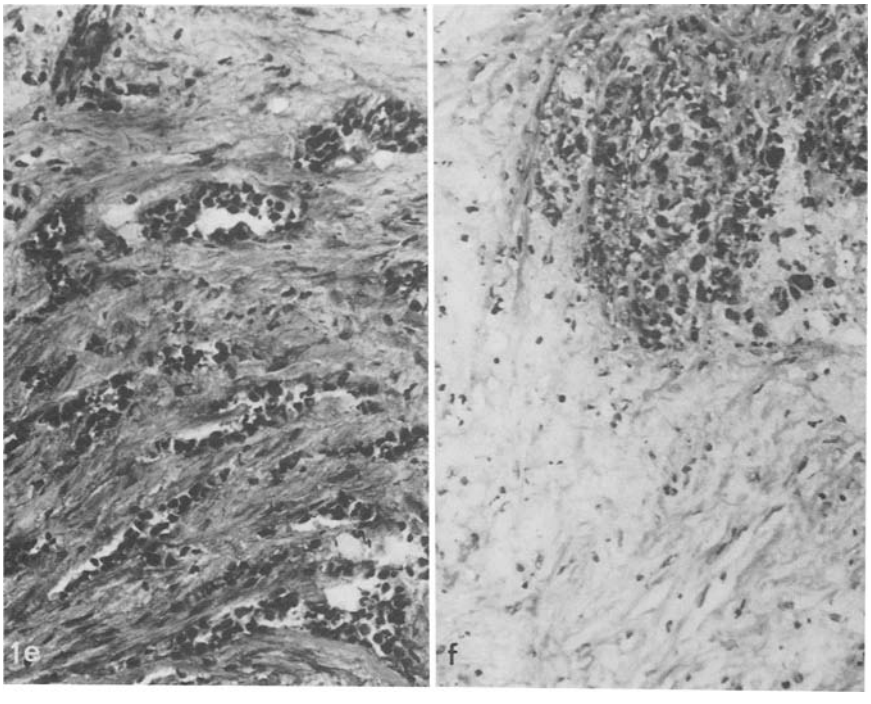


Fig. 1. **a** Invasive ductular carcinoma. Immunofluorescence staining of type I collagen shows thick regular fibers separating tumor nests. × 250. **b** Invasive lobular carcinoma. Type I collagen is distributed diffusely as fibrillar structures dissociating small nests of tumor cells. × 250. **c** Invasive ductular carcinoma. Fibrillar deposits of proIII collagen are seen within the stroma. × 250. **d** Peripheral portion of ductular carcinoma showing a diffuse immunostaining with anti-proIII collagen. × 250. **e** Invasive ductular carcinoma. Photomicrograph of the central portion of the tumor showing nests of tumor cells lying within dense hyalin collagen (cryostat section stained by toluidine blue × 160). **f** Invasive ductular carcinoma. Peripheral portion of the same tumor whose matrix is characterized by a loose young mesenchyme (cryostat section stained by toluidine blue × 160)



carcinoma, the staining reaction was regular, delicate, and linear surrounding lobules of tumor cells (Fig. 3c).

2. *Intraductal carcinoma, dysplasia, and normal breast.* Antibodies to laminin and type IV collagen revealed a sharp delineation of intraductal carcinoma and small capillaries localized within the matrix (Fig. 4a). In no instance was discontinuity of the staining reaction found. Normal breast parenchyma and dysplastic breast lesions stained in like fashion. Staining was invariably negative within the cytoplasm of normal or neoplastic epithelial cells (Fig. 4b).

C. Fibronectin

1. *Invasive ductular and lobular carcinoma.* The distribution of fibronectin was variable. In most cases of ductular invasive carcinoma, the staining reaction revealed a somewhat fibrillar network which appeared brighter at the periphery of the tumors (Fig. 5a). In contrast, a weak, diffuse, sometimes granular reaction was observed in areas of dense hyaline sclerosis (Fig. 5b). In one case of invasive lobular carcinoma, the intensity of staining was striking, particularly in the stromal desmoplastic reaction between individual cell nests. Moreover, the pattern was condensed to resemble a basal lamina-like structure, similar to that seen in dysplastic epithelia. Fibronectin

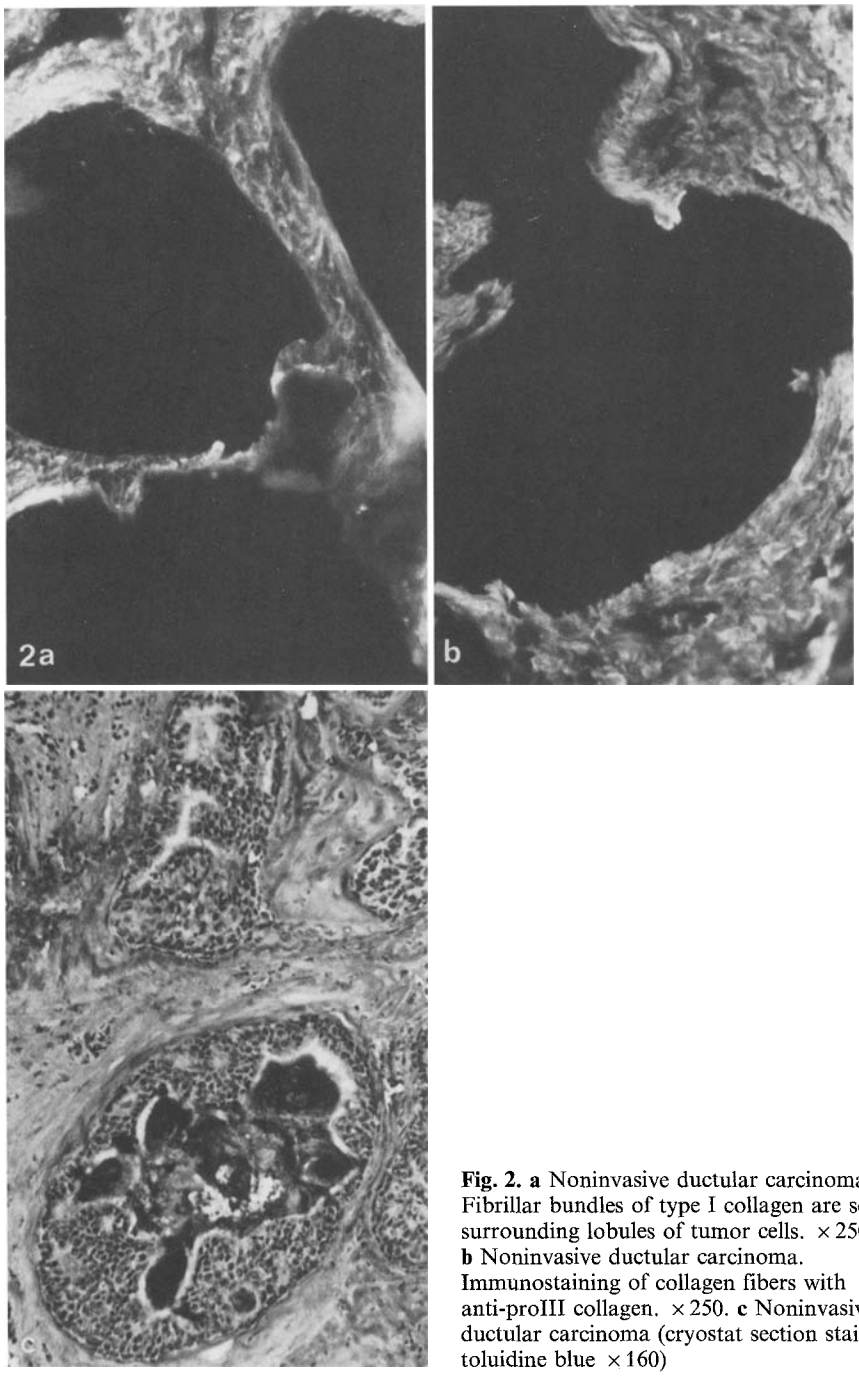


Fig. 2. a Noninvasive ductular carcinoma. Fibrillar bundles of type I collagen are seen surrounding lobules of tumor cells. ×250. **b** Noninvasive ductular carcinoma. Immunostaining of collagen fibers with anti-proIII collagen. ×250. **c** Noninvasive ductular carcinoma (cryostat section stained by toluidine blue ×160)

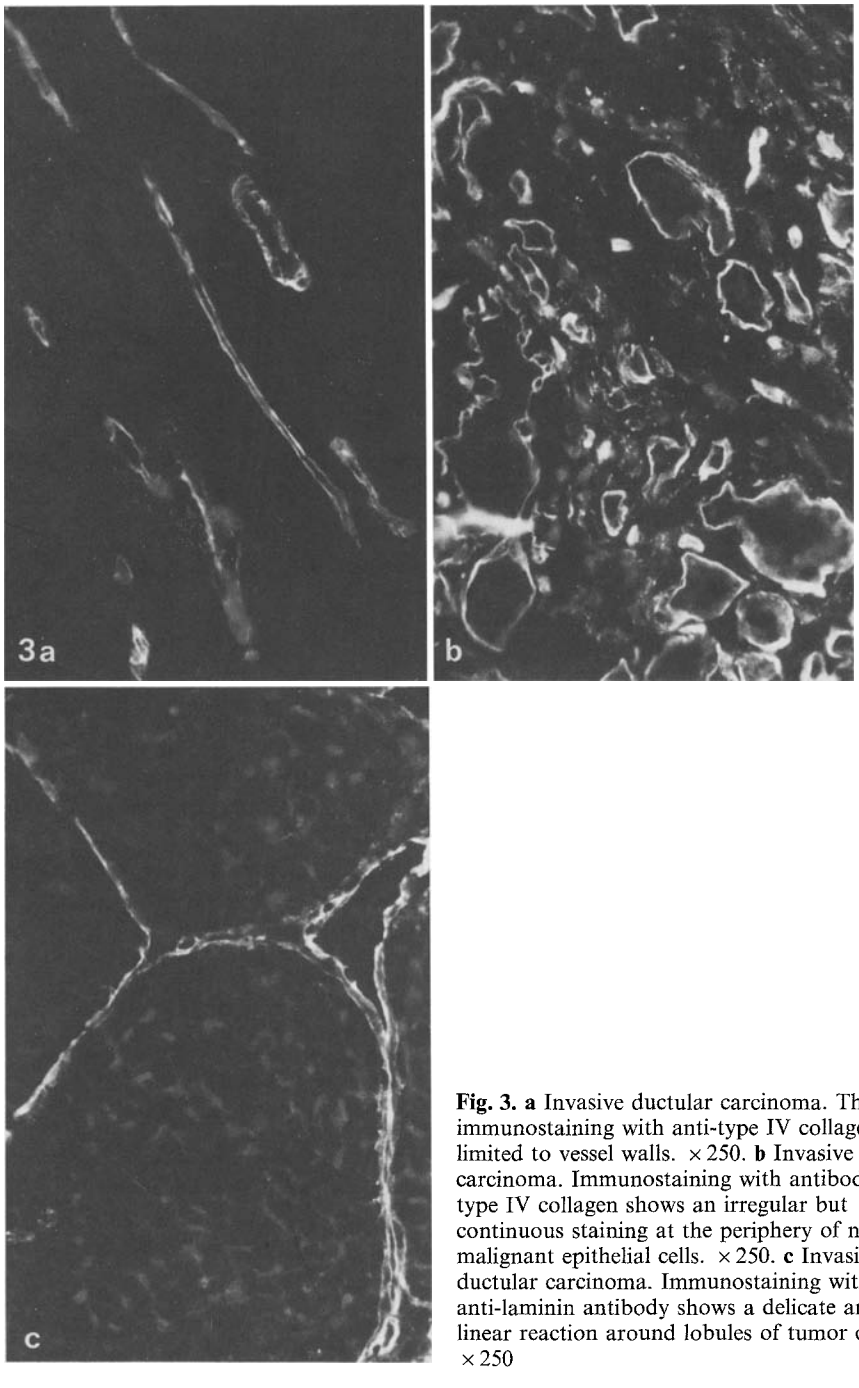


Fig. 3. a Invasive ductular carcinoma. The immunostaining with anti-type IV collagen is limited to vessel walls. ×250. **b** Invasive lobular carcinoma. Immunostaining with antibody to type IV collagen shows an irregular but continuous staining at the periphery of nests of malignant epithelial cells. ×250. **c** Invasive ductular carcinoma. Immunostaining with anti-laminin antibody shows a delicate and linear reaction around lobules of tumor cells. ×250

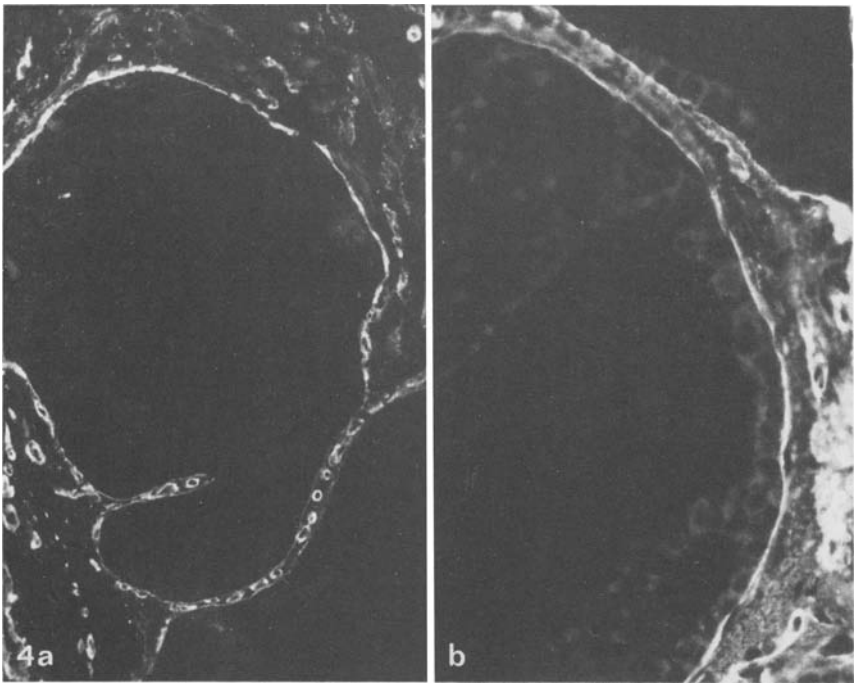


Fig. 4. **a** Intraductal carcinoma. Immunostaining with anti-type IV collagen delineates the areas of intraductal neoplastic proliferation and the small vessels within the matrix. × 100. **b** Intraductal carcinoma. The reaction with anti-laminin is restricted to the periphery of tumor cells nests and vessel walls. There is absence of immunostaining within epithelial cells. × 250

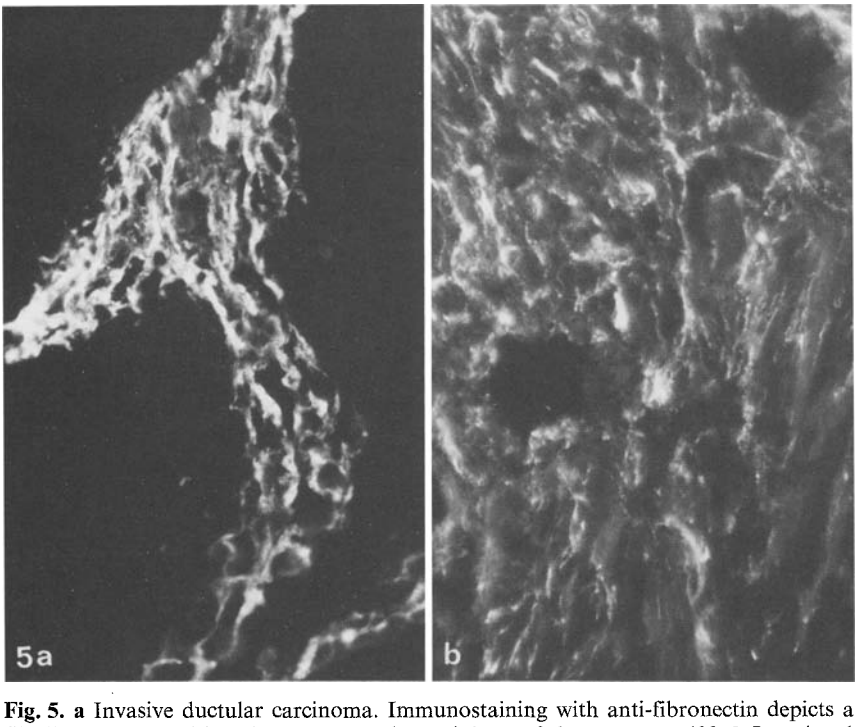


Fig. 5. **a** Invasive ductular carcinoma. Immunostaining with anti-fibronectin depicts a bright fibrillar network within the stroma at the periphery of the tumor. × 400. **b** Invasive ductular carcinoma. The pattern of reaction with anti-fibronectin serum appears diffuse and granular within dense hyalin areas of the tumor. × 250

was identified in vascular basement membranes but was absent around malignant cells.

2. *Intraductal carcinoma and dysplasia.* In noninvasive carcinoma and dysplasia, the distribution of fibronectin was identical. The staining reaction was intense within the basement membranes of ducts and acini.

Discussion

This study demonstrates that type proIII and III collagens are distributed throughout the matrix of desmoplastic breast carcinomas and are especially concentrated at the cellular periphery of the tumors. Such areas are characterized by a loose, edematous cellular "young" mesenchyme. The central portion of the tumors, histologically poorly cellular and characterized by dense fibrous retracted tissue, contains all three types of collagen, but predominantly type I. These observations extend our previous studies which suggested an analogy between the matrix alterations in granulation tissue (Gabbiani et al. 1976) and invasive carcinomas (Schürch et al. 1982). Since the pivotal role of the MF has been established in the genesis of matrix changes in granulation tissue, this unique cell could play an equally important role in the matrix changes of invasive carcinomas. Such has been proposed by others (Barsky et al. 1982) who, having described an increase of type V collagen in desmoplastic breast cancers, suggested that collagen could be produced by the MF. Another immunohistochemical study has also shown that the stroma of nonscirrhous gastric carcinoma is composed mainly of type I collagen with lesser amounts of type III collagen (Yamamoto et al. 1984). Our study reveals a uniform distribution of type I, proIII, and III collagens in dysplastic breast lesions and noninvasive ductular carcinomas. These findings are in agreement with other studies dealing with breast (Gordenne et al. 1982) and gastric lesions (Yamamoto et al. 1984).

The mechanism by which the matrix changes are induced in invasive carcinomas is poorly understood. It has been suggested that fibrin deposits in the periphery of tumors in man and animals might serve as a stimulus for angiogenesis and desmoplasia (Dvorak et al. 1981; Dvorak et al. 1981a). The accumulated electron microscopic and immunopathologic data suggest that myofibroblasts may represent an important element in these matrix changes.

Our study demonstrates that the loss of basement membrane components (laminin and type IV collagen) is associated with tumoral invasion. Dysplastic lesions, including sclerosing adenosis, and noninvasive ductular carcinomas show an intact basement membrane with linear staining of laminin and type IV collagen. In contrast, the majority of invasive lobular and ductular carcinomas are devoid of immunoreactivity for either basement membrane component. In only two instances, one case of invasive ductular and one case of invasive lobular carcinomas, did we observe staining with antisera against laminin and type IV collagen. Similar observations have been reported in various types of carcinomas (Burtin et al. 1982; Gusterson

et al. 1982; Barsky et al. 1983a; Birembaut et al. 1983; Berger et al. 1984; Cam et al. 1984). These studies showed that in situ carcinomas are invariably characterized by a linear staining pattern of laminin and type IV collagen, whereas microinvasive lesions show fragmentation and disruption of basement membrane components at the invasive front (Barsky et al. 1983a; Cam et al. 1984). These observations suggest that the lack of basement membrane components in invasive tumors could reflect either the impaired synthesis of basement membrane (Kefalides and Denduchis 1969) or enzymatic degradation of basement membrane by collagenase (Liotta et al. 1979; O'Grady et al. 1981; Barsky et al. 1983).

The role and modifications of fibronectin in mammary neoplasia have been addressed by others (Birembaut et al. 1981; Labat-Robert et al. 1981; Stenman and Vaheri 1981). It is interesting to note that greater amounts of fibronectin appear in parallel with types proIII and III collagen in our study. Wherever types proIII and III collagen demonstrate a striking immune staining in loose "young" stroma fibronectin depicts a similar pattern of reactivity.

This study provides support for the analogy between the dynamics of matrix changes in granulation tissue and invasive carcinoma. It has been demonstrated that fibronectin could function as a primary matrix for organization of connective tissue during tissue repair (Kurkinen et al. 1980). In granulation tissue, fibronectin appears early concomitant and types proIII, III and I collagen appear a few days later. There is growing evidence that alterations in the composition of tumor extracellular matrix stem from interactions between neoplastic and mesenchymal cells (Iozzo 1984). The data obtained in this study enhance the role of the myofibroblast as a host response to invasive neoplasms and its relationship to the matrix changes.

Acknowledgements. This work was realized to a great extent while one of us (RL) was on sabbatical leave at Le Centre hospitalier régional de Toulouse, CHU de Toulouse-Rangueil (directeur: Professeur H. Bouissou). The authors would like to express their thanks for professional and technical support at le CHU de Toulouse-Rangueil and le Laboratoire de pathologie cellulaire du foie, CNRS, ERA, 189, Institut Pasteur, Lyon, France.

We express our deep appreciation to Mrs J. Tremblay who typed the manuscript.

References

Barsky SH, Rao CN, Grotendorst GR, Liotta LA (1982) Increased content of type V collagen in desmoplasia of human breast carcinoma. Am J Pathol 108:276–283

Barsky SH, Togo S, Garbisa S, Liotta LA (1983) Type IV collagenase immunoreactivity in invasive breast carcinoma. Lancet 1(8319):296–297

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

Berger G, Chevalier M, Grimaud JA, Feroldi J (1984) Les modifications du collagène IV et de la lamine dans les carcinomes intra-ductaux du sein. Étude en immunofluorescence. Arch Anat Cytol Pathol 32:160–161

Birembaut P, Gaillard D, Adnet JJ, Dousset H, Kalis B, Paynard JP, Leutenegger M, Labat-Robert J, Robert L (1981) La fibronectine: étude immuno-morphologique de sa distribution en pathologie. Ann Pathol 1:140–146

Birembaut P, Caron Y, Van Cauwenberge D, Foidart JM (1983) Distribution of Laminin,

a basement membrane glycoprotein in epithelial proliferations. A preliminary study in the breast, the lungs and uterine cervix. Collagen Rel Res 3:25–31

Burtin P, Chavanel G, Foidart JM, Martin E (1982) Antigens of the basement membrane and the peritumoral stroma in human colonic adenocarcinoma: an immunofluorescence study. Int J Cancer 30:13–20

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

Dvorak HF, Quay SC, Orenstein NS, Dvorak AM, Halm P, Bitzen AM (1981) Tumor shedding and coagulation. Science 212:923–924

Dvorak HF, Dickersin GR, Dvorak AM, Manseau EJ, Pyne K (1981a) Human breast carcinoma: Fibrin deposits and desmoplasia. Inflammatory cell type and distribution. Microvasculature and infarction. J Natl Cancer Inst 67:335–345

Flejou JF, Grimaud JA, Molas G, Baviera E, Potet F (1984) Collagenous colitis: Ultrastructural study and collagen immunotyping of four cases. Arch Pathol Lab Med 108:977–982

Gabbiani G, Lelous M, Bailey AJ, Bazin S, Delaunay A (1976) Collagen and myofibroblasts of granulation tissue. A chemical, ultrastructural and immunologic study. Virchows Arch [Cell Pathol] 21:133–145

Gordenne W, Foidart JM, Lapière CM (1982) Immunolocalisation de la laminine, de la fibronectine, et des collagènes I à V dans le sein normal et mastosique. J Gyn Obst Biol Repr 11:549–554

Gusterson BA, Warburton MJ, Mitchell D, Ellison M, Munro-Neville A, Rudland PS (1982) Distribution of myoepithelial cells and basement membrane proteins in the normal breast and in benign and malignant breast diseases. Cancer Res 42:4763–4770

Iozzo R (1984) Proteoglycans and Neoplastic – Mesenchymal cell interactions. Hum Pathol 15:2–10

Kefalides NA, Denduchis B (1969) Structural components of epithelial and endothelial basement membranes. Biochemistry 8:4613–4621

Kurkinen M, Vaheri A, Roberts PJ, Stenman S (1980) Sequential appearance of fibronectin and collagen in experimental granulation tissue. Lab Invest 43:47–51

Labat-Robert J, Birembaut P, Robert L, Adnet JJ (1981) Modification of fibronectin distribution pattern in solid human tumours. Diag Histopathol 4:299–306

Liotta LA, Abe S, Robey GP, Martin GR (1979) Preferential digestion of basement membrane collagen by an enzyme derived from a metastatic murine tumor. Proc Natl Acad Sci USA 76:2268–2272

O'Grady RL, Upfold LI, Stephens RW (1981) Rat mammary carcinoma cells secrete active collagenase and active latent enzyme in the stroma via plasminogen activator. Int J Cancer 50:509–515

Rennard SI, Berg R, Martin GR, Foidart JM, Gehron-Robey P (1980) Enzyme-linked immunoessay (ELISA) for connective tissue components. Anal Biochem 104:205–214

Schürch W, Seemayer TA, Lagace R (1981) Stromal Myofibroblasts in Primary Invasive and Metastatic Carcinomas. A combined Immunological, Light and Electron Microscopic Study. Virchows Arch [Pathol Anat] 391:125–139

Schürch W, Lagacé R, Seemayer TA (1982) Myofibroblastic stromal reaction in retracted scirrhous carcinoma of the breast. Surg Gynecol & Obst 154:351–358

Schürch W, Seemayer TA, Lagacé R, Gabbiani G (1984) The intermediate filament cytoskeleton of myofibroblasts: an immunofluorescence and ultrastructural study. Virchows Arch [Pathol Anat] 403:323–336

Seemayer TA, Schürch W, Lagacé R, Tremblay G (1979) Myofibroblasts in the Stroma of invasive and metastatic carcinoma. A host response to neoplasia. Am J Surg Pathol 3:525–533

Stenman S, Vaheri A (1981) Fibronectin in human solid tumors. Int J Cancer 27:427–435

Tremblay G (1979) Stromal aspects of breast carcinoma. Exp Mol Pathol 31:248–260

Yamamoto M, Sumiyoshi H, Nakagami K, Taniyama K, Tahora E (1984) Distribution of collagen types I and III and basal lamina in human gastric carcinoma: an immunohistochemical and electron microscopic study. Virchows Arch [Pathol Anat] 403:313–322

Accepted July 8, 1985