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$\alpha_6\beta_4$ Integrin and Newly Deposited Laminin-1 and Laminin-5 Form the Adhesion Mechanism of Gastric Carcinoma

Continuous Expression of Laminins but Not That of Collagen VII Is Preserved in Invasive Parts of the Carcinomas: Implications for Acquisition of the Invading Phenotype

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We studied the expression and distribution of different laminin chains, the $\alpha_6\beta_4$ integrin and type VII collagen, ie, components of the epithelial adhesion complex, in gastric carcinomas and in suggested preneoplastic stages of this malignancy. Intestinal-type gastric carcinomas showed strong reactivity for laminin α1, α3, β1, and β3 chains, the components of laminin-1 and -5, at the interface between malignant cells and tumor stroma. The reactivities were continuous throughout the carcinomas, even in structures invading through the smooth muscle layers of the gastric wall. The expression of different laminin chains was accompanied by strong polarized reactivity for the $\alpha_6\beta_4$ integrin, which is a receptor for both laminin-1 and laminin-5. Collagen type VII was only occasionally present at sites showing reactivity for laminin-5 and was totally absent from the cell islands invading through the gastric wall. Intestinalized gastric epithelium showed a similar expression pattern of laminins and the $\alpha_6\beta_4$ integrin as the gastric carcinomas. Our results suggest that gastric carcinomas use the $\alpha_6\beta_4$ integrin and newly deposited laminin-1 and -5, accompanied by the disappearance of type VII collagen, as their mechanism of adhesion during the invasion through surrounding tissues. Unlike in previous studies, the reactivity for the laminin-5 protein was not restricted to the invading cells but surrounded the malignant glandular structures throughout the tumor. Our results also show that both intestinal-type gastric carcinoma and intestinal metaplasia mimic the gastric surface epithelium in the expression pattern of laminins and the β_4 integrin subunit. This supports previous studies proposing a pathogenetic sequence from intestinal metaplasia to gastric carcinoma. (Am J Pathol 1996, 149:781–793)

Basement membranes (BMs) are structures that are located at the interface between epithelium and connective tissue.[1] Both epithelial and connective tissue cells have been shown to produce BM components.[2,3] Laminins are important because they show tissue-specific expression and thus produce hetero-

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geneity in BMs. Laminin-1 was the first laminin isoform to be characterized and is composed of $\alpha 1$, $\beta 1$, and $\gamma 1$ chains.[4] Laminin-5 ($\alpha 3\beta 3\gamma 2$) is considered to be an epithelial laminin as it is expressed in the BMs of many epithelial but not mesenchymal tissues.[5,6]

The adhesion complex of epithelial cells is a complicated structure formed by intracellular proteins, constituents of the epithelial BM and the $\alpha_6\beta_4$ integrin as a transmembrane link between the interior and exterior of the epithelial cell.[6] Hemidesmosome is a morphological concept that corresponds to the unique membrane-associated structure formed by the $\alpha_6\beta_4$ integrin and associated proteins.[7] Anchoring filaments are the morphological equivalent of type VII collagen, which physically interacts with laminin-5 (P. Rousselle, D.R. Keene, M.-F. Champliaud, M. van der Rest, and R.E. Burgeson, unpublished data). Both type VII collagen and laminin-5 are epithelium-specific BM constituents. Hence, the epithelial adhesion complex is an adhesive machinery that directly links the epithelial cytoskeleton through the $\alpha_6\beta_4$ integrin, laminin-5, and type VII collagen molecules to the surrounding connective tissue.[6,7] However, previous studies have suggested that only stratified and compound epithelia would express all components of the above-mentioned adhesion complex. The epithelia of the gastrointestinal tract, for example, have been reported to be almost devoid of collagen type VII[8,9] and hemidesmosomes.[6,7]

The existence of BMs in malignant tissues has been extensively studied both by electron microscopy and immunohistochemistry. Early morphological studies proposed that BMs would function as a barrier that the malignant cells have to transverse before they can invade surrounding tissues.[10,11] Later on, the malignant cells were found to be capable of producing proteolytic enzymes, which led to the widely accepted theory that production of these enzymes and subsequent breakdown of the epithelial BM is an essential prerequisite for invasion.[12,13] However, some investigators have argued that the breakdown of the epithelial BM in invasive carcinomas is merely coincidental and most likely caused by the inflammatory cells.[14] In addition, more recent studies have shown that both malignant cells and cells of the tumor stroma are capable of depositing BM components, suggesting a more dynamic role for these molecules (for Review see Refs. 10 and 11). In a study on colorectal carcinomas, the expression of laminin was considerably more often preserved in primary tumors that had metastasized to liver than in those that had not and, in addition, 95% of the liver metastases had intact BMs.[15] The expression of laminin-1 has been previously studied in gastric carcinomas,[16–18] and the expression of laminin-1 in the periphery of gastric carcinoma has been proposed to be a risk factor for liver metastasis.[17]

The $\alpha_6\beta_4$ integrin has a special role in cell-matrix adhesion as it is associated with cytokeratin filaments on the cytoplasmic side.[19] The $\alpha_6\beta_4$ integrin is a receptor for at least laminin-1 and laminin-5.[20] In some studies, overexpression and inappropriate localization of this integrin in malignant cells has been proposed to correlate with the invasive phenotype, whereas in some other studies, complete loss of this receptor has been suggested to have a similar effect.[21–27]

We have previously reported that laminin isoforms show a distinct distribution in the BMs of gastric epithelia.[28] Laminin-5 is localized in the BMs of surface epithelium and gastric pits, whereas laminin-2 is localized in the BM of gastric glands. The areas of distribution for laminin-2 and laminin-5 overlap in the foveolar area, the site of proliferative cells. Laminin-1 is found in the BMs of gastric epithelium throughout the thickness of gastric mucosa, thus differing from that reported on intestine.[29,30] Previous studies have suggested different pathogenetic mechanisms for intestinal- and diffuse-type carcinomas (IGCAs and DGCAs).[31] IGCAs have been suggested to be derived from the intestinalized epithelium in atrophic gastritis, whereas DGCAs have been proposed to be associated with the early non-atrophic stages of gastritis (reviewed in Refs. 32 and 33). In this study we wanted to investigate the expression pattern and localization of laminins and the $\alpha_6\beta_4$ integrin in different types of gastric carcinomas. Special attention was given to the possible differences in the expression of these molecules between the mucosal part of the tumor and the cell nests invading through the gastric wall as well as for the similarities between gastric carcinomas and the suggested precursor of this malignancy.

Materials and Methods

Tissues

Samples of gastric tumors (6 IGCAs and 6 DGCAs) and the surrounding gastric mucosa (4 samples of corpus and 4 samples of antrum) were obtained from gastrectomies at the Jorvi Hospital or at the Second Department of Surgery, Helsinki University Central Hospital. Biopsies of normal and metaplastic gastric mucosa (14 samples of antrum and 13 samples of corpus) were obtained during gastroscopies at the Jorvi Hospital. Tissues were immediately frozen in liquid nitrogen and stored at −70°C. Diagnoses were

confirmed from standard hematoxylin-eosin-, Alcian blue-PAS-, and high-iron-diamine-stained sections by experienced pathologists.

Antibodies

The following monoclonal antibodies (MAbs) were used in this study. MAbs against laminin α chains were 4C7 against the α1 chain,[34] 2G9 against the α2 chain,[34] and BM-2 against the α3 chain.[5] The laminin β1 and β3 chains were detected by the MAbs 3E5[34] and 6F12,[35] respectively. MAbs AA3[36] and 3E1[37] (Biogenesis, Poole, UK) were used to detect the β_4 integrin subunit. Polyclonal rabbit antisera against laminin-1[38] and laminin-5[5] were used in double-immunofluorescence staining. The polyclonal antiserum against cytokeratin (CK)19[39] and the MAb against the collagen type VII[40] have been described previously.

Immunohistochemistry

The tissue specimens were sectioned at 6 μm and fixed in acetone precooled to −20°C. As some of the tissue samples showed endogenous alkaline phosphatase activity that could not be inhibited by levamisole, two methods were applied in light microscopy, one using the alkaline phosphatase enzyme and the other using horseradish peroxidase. Both antibodies against the β_4 integrin subunit were unreactive with samples treated with H_2O_2, which made it impossible to detect the β_4 integrin subunit by peroxidase techniques. In the alkaline phosphatase anti-alkaline phosphatase (APAAP) method, the sections were treated with non-immune rabbit serum for 15 minutes, after which they were incubated with the primary antibody for 30 minutes. The procedure was continued by incubating the sections with rabbit anti-mouse antibody (Dako, Glostrup, Denmark) for 30 minutes and with the APAAP complex (Dako) for another 30 minutes. The immunoreactivities were visualized by using a substrate solution containing naphthol AS-BI phosphate, levamisole, and new fuchsin (all from Sigma Chemical Co., St. Louis, MO) reacted with $NaNO_2$ (E. Merck, Darmstadt, Germany) and diluted in Tris-buffered saline, pH 8.7. Histostain SP reagents (Zymed Laboratories, San Francisco, CA) were used in the method using peroxidase enzyme. In this method, the fixed specimens were treated with 0.6% H_2O_2 in methanol for 15 minutes to block the endogenous peroxidase activity. The sections were then incubated with non-immune sheep serum for 15 minutes and with primary antibody for 30 minutes. This was followed by incubation with biotinylated goat anti-mouse antibody (Zymed) for 15 minutes and with streptavidin-peroxidase complex (Zymed) for 15 minutes. The immunoreactivities were detected by a substrate solution containing H_2O_2 and 3-amino-9-ethylcarbazole in 0.1 mol/L acetate buffer, pH 5.2. Both in the APAAP and peroxidase enzyme method, the sections were counterstained with Mayer's hematoxylin (E. Merck) diluted with distilled water 1:5 and embedded in water soluble GVA-Mount embedding agent (Zymed). Immunofluorescence microscopy was used for double-immunodetection purposes. In this method, the fixed sections were incubated with the primary antibody for 30 minutes followed by fluorescein-isothiocyanate- or tetraethylrhodamine-isothiocyanate-conjugated goat anti-mouse or anti-rabbit antibody (Jackson Laboratories, West Grove, PA) for another 30 minutes. The specimens were embedded in buffered glycerol and examined in a Leica Aristoplan microscope equipped with appropriate filters.

Results

Intestinal Metaplasia

We first studied the expression of laminin chains in intestinal metaplasia, the suggested precursor of IGCAs.[31–33] Laminin α1 and β1 chains were expressed in gastric epithelia showing intestinal metaplasia, but the reactivity was not as strong as in the normal gastric glands. However, the laminin α3 chain (Figure 1a) could be detected even in the deepest parts of metaplastic glandular structures in four of five biopsy samples of intestinalized antral mucosa. BMs of the glands showing intestinal metaplasia were devoid of the laminin α2 chain (Figure 1b) in all (five of five) biopsy samples, but some stromal reactivity could be seen (Figure 1b). The β_4 integrin could also be seen in the epithelia that had undergone intestinal metaplasia (Figure 1c). The expression pattern of these antigens was not completely uniform, because one of five biopsy samples showing intestinal metaplasia and some of the intestinalized glands in surgical specimens showed only a faint reactivity or no reactivity at all for the laminin α3 chain. However, even these samples were devoid of laminin α2 chain and showed reactivity for the β_4 integrin subunit.

Pseudopyloric Metaplasia and Antral and Fundic Glands in Atrophic Gastritis

As a control, we compared intestinal metaplasia with pseudopyloric metaplasia of fundic glands and non-

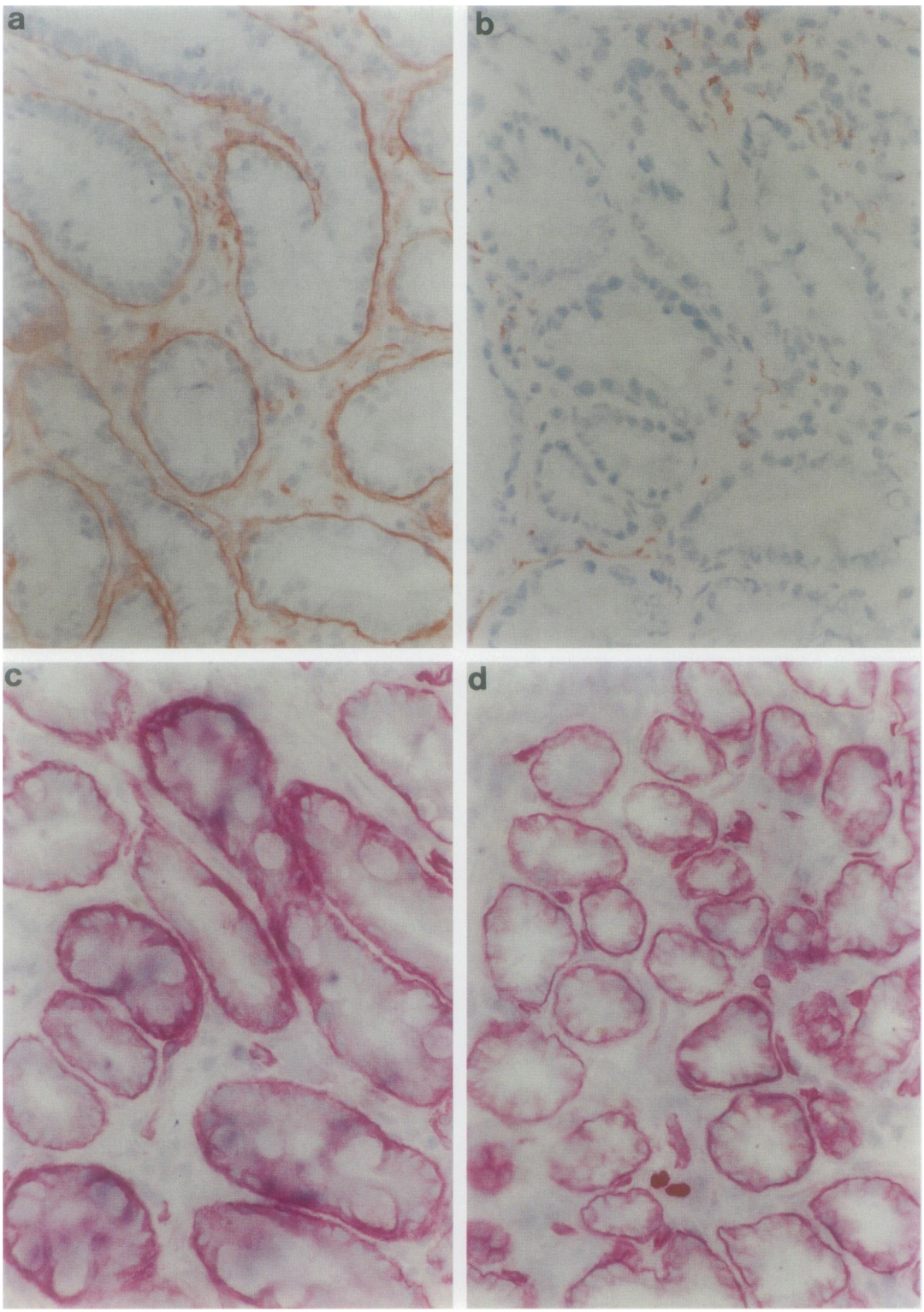

Figure 1. *Intestinal metaplasia and antral mucosa in atrophic gastritis.* a *to* c: *Different immunohistochemical stainings of intestinalized glands of an antral biopsy sample.* a: *There was a strong reactivity for the laminin α3 chain in the BMs of intestinalized glands. There is also some diffuse reactivity in the lamina propria.* b: *BMs of the intestinalized glands did not show any reactivity for the laminin α2 chain, whereas there is some reactivity in the surrounding connective tissue.* c: *The reactivity for the β_4 integrin subunit was localized in the basal aspects of the cells of the intestinalized epithelium. Unfortunately, there was some endogenous alkaline phosphatase activity in the tissue, causing some nonspecific cytoplasmic reactivity.* d: *Histologically normal antral glands in a biopsy sample of inflamed antral mucosa show strong reactivity for the β_4 integrin subunit. Histostain SP* (a *and* b)*; APAAP* (c *and* d)*; magnification, ×820.*

metaplastic antral and fundic glands in atrophic gastritis. In a surgical specimen of the fundic mucosa, nearly all glands showed typical morphological features of pseudopyloric metaplasia. These metaplastic glands showed reactivity for the laminin $\alpha 2$ chain in their BMs, but the reactivity was lower than in the normal fundic or antral glands. In all biopsy (13/13) and surgical (4/4) samples of fundic mucosa the laminin $\alpha 2$ chain could be detected in the BMs of the nonmetaplastic glands. The expression of the laminin $\alpha 2$ chain was considerably reduced or even below the level of detection in the glandular BMs in all biopsy (14/14) and surgical (4/4) specimens of antrum regardless of the presence and severity of gastritis. In addition, antral glands, although negative for the $\alpha 3$ laminin chain, showed an intense reactivity for the β_4 integrin subunit (Figure 1d) in 14/14 biopsy samples and 3/4 surgical specimens.

Intestinal-Type Gastric Carcinomas

All IGCAs studied (six of six) showed reactivity for the laminin $\alpha 1$ (Figure 2a) and $\beta 1$ (Figure 2b) chains at the interphase between malignant cells and tumor stroma. In most tumors the reactivities were somewhat discontinuous, showing abrupt changes in the intensity and at some locations total absence of reactivity (small arrows). These small discontinuities of the BM in the mucosal parts of the tumors did not seem to be associated with invasiveness and might be due to the processing of the tissue. Both chains could also be detected in blood vessels (large arrows). The $\alpha_6\beta_4$ integrin was also expressed in all IGCAs (Figure 2c, β_4 integrin subunit). In a double-immunofluorescence staining for the β_4 integrin subunit (Figure 2d) and laminin-1 (Figure 2e), these antigens showed exact co-localization, ie, the areas lacking laminin-1 were also devoid of $\alpha_6\beta_4$ integrin. Blood vessels showed reactivity for laminin-1 but not for the β_4 integrin (arrows). The expression of laminin-5 was more variable. Five of six intestinal-type tumors showed nearly continuous, intensive reactivity for this antigen, thus showing exact co-localization with laminin-1. One of six of these carcinomas showed reactivity for this antigen only at some sites of interaction between the malignant cells and tumor stroma. However, when the tumors showed reactivity for the laminin $\alpha 3$ chain, the laminin $\alpha 1$ chain (Figure 2f) could be detected in co-localization with laminin-5 (Figure 2g). None of the intestinal-type tumors studied showed reactivity for the laminin $\alpha 2$ chain in their BMs, but stromal reactivity could be seen in many samples.

Diffuse-Type Gastric Carcinomas

DGCAs showed an extremely heterogeneous expression pattern of the antigens studied. In one of the tumors, the malignant cells showed a strong reactivity for the β_4 integrin subunit (Figure 3a). Five of six DGCAs showed at least some reactivity for this antigen. Typically, only part of the malignant cells showed reactivity for a particular laminin chain and the reactivities were spot-like and failed to form any structures resembling BMs. $\alpha 1$, $\alpha 3$, $\beta 1$, and $\beta 3$ chains were found in the vicinity of the malignant cells in all DGCAs studied (six of six), but reactivity for the $\alpha 2$ chain associated with the malignant cells was detected in only four of six carcinomas. Mostly the reactivities were faint and could be demonstrated in double-immunofluorescence studies only at distinct areas. In a double-immunofluorescence staining for the laminin $\alpha 2$ chain (Figure 3b) and CK19 (Figure 3c), some of the CK19-positive tumor cells were occasionally surrounded by discontinuous reactivity. The $\alpha 1$ chain (Figure 3, d and e, CK19) in malignant cells invading through the smooth muscle showed a more even reactivity in the vicinity of the cell surface (arrows) although much weaker than in the surrounding smooth muscle cells. The laminin $\beta 3$ chain (Figure 3, f and g, CK19) was exceptionally strongly expressed around the malignant cells in this tumor.

Invasion through the Smooth Muscle Layers

The expression of laminin $\alpha 1$ to $\alpha 3$ chains and the $\alpha_6\beta_4$ integrin was studied specifically at sites showing invasion through the smooth muscle layers of the stomach. Both IGCAs and DGCAs were studied. Two of six IGCAs formed small tubular or glandular structures at the site of invasion through the smooth muscle layers of the gastric wall. These structures showed an intense BM-associated reactivity for the laminin $\alpha 1$ and $\alpha 3$ (Figure 4a) chains. In addition, the $\alpha 2$ chain was found diffusely around the malignant cells (Figure 4b), whereas the β_4 integrin subunit (Figure 4c) was strictly polarized in the tubular structures. Laminin $\alpha 1$ chain could be detected not only in the BMs of the malignant tubular structures but also in the BMs of the smooth muscle cells (Figure 4d). In a DGCA, on the other hand, occasional tubular/glandular structures were seen invading through the smooth muscle. Also, this tumor showed reactivity for the β_4 integrin subunit and the same laminin chains as the IGCA. In three of six DGCAs, malignant cells were diffusely infiltrating through the smooth muscle

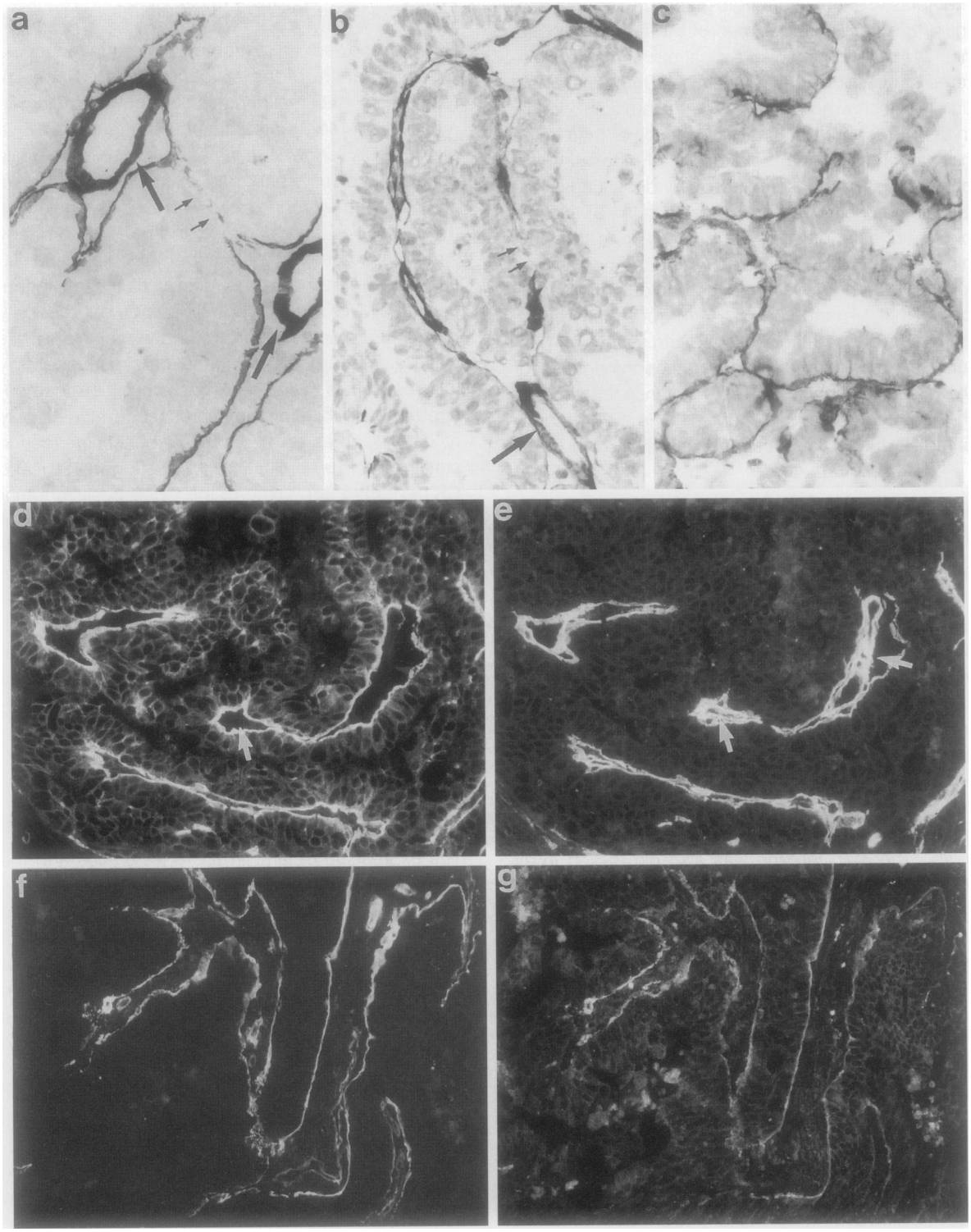

Figure 2. *Laminin chains and the β_4 integrin subunit in IGCAs. IGCAs showed strong reactivity for laminin $\alpha 1$* (a) *and $\beta 1$* (b) *chains in their BMs. At some points, the reactivities were slightly discontinuous* (small arrows). *Note also that vascular smooth muscle expressed these proteins* (large arrows). *The β_4 integrin subunit could be detected as a polarized reactivity in the basal aspects of cells forming the tubular and glandular structures* (c). *In a double-immunofluorescence staining, the β_4 integrin subunit* (d) *is seen in co-localization with laminin-1* (e). *Laminin-1 was demonstrated by a polyclonal antiserum. The blood vessels showed reactivity for laminin-1 but not for the β_4 integrin subunit* (arrows). *In a double-immunofluorescence staining for the laminin $\alpha 1$ chain* (f) *and laminin-5* (g; *polyclonal antiserum*), *these two antigens showed exact co-localization in the BMs of the malignant tubular structures. The laminin $\alpha 1$ chain but not laminin-5 is seen in vascular smooth muscle. Histostain SP* (a *and* b); *APAAP* (c); *immunofluorescence* (d *to* g); *magnification, $\times 610$* (a *to* c) *and $\times 480$* (d *to* g).

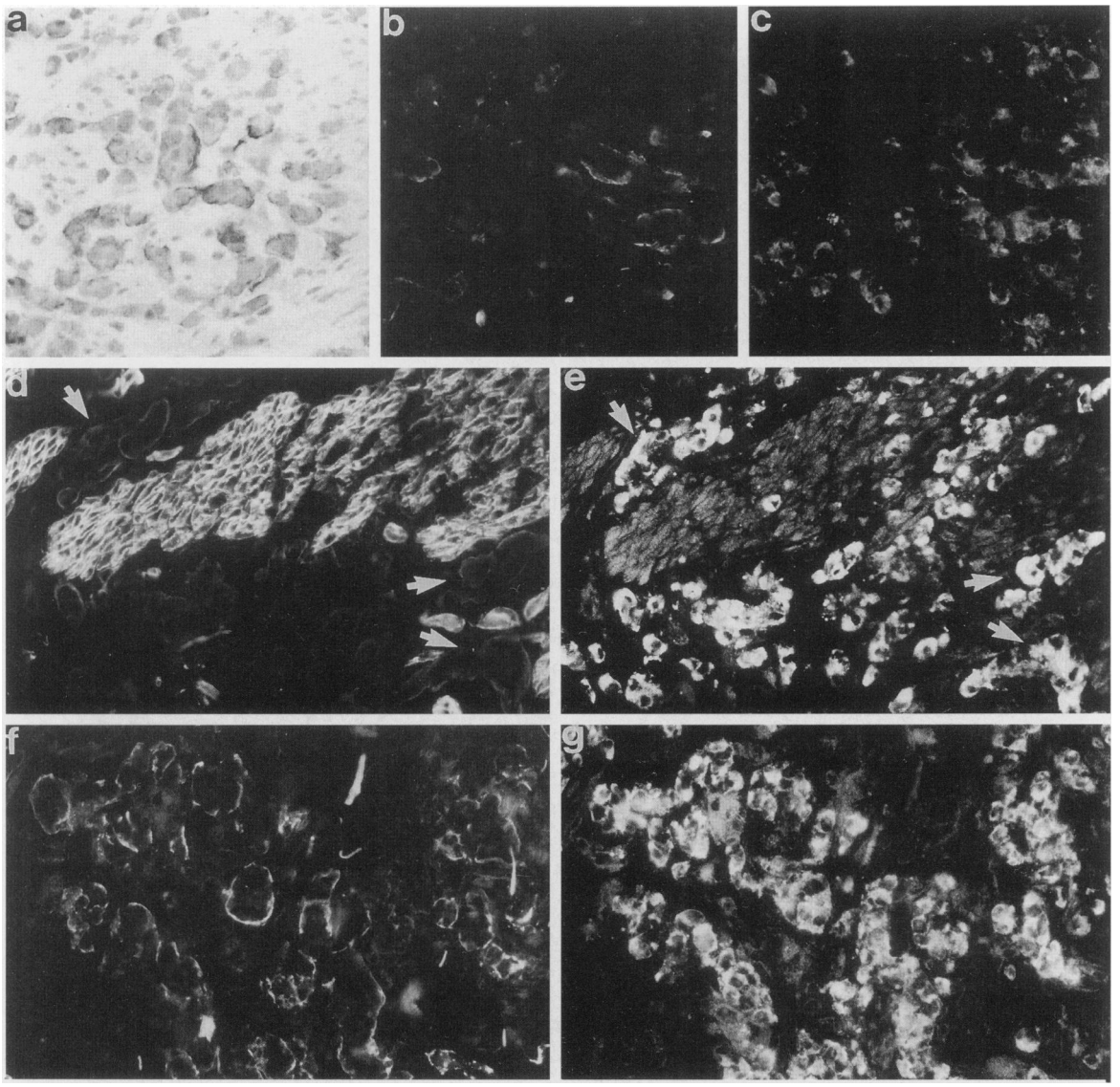

Figure 3. *Different laminin chains and the β_4 integrin subunit in a DGCA. Most malignant cells in this tumor showed cell-surface-associated reactivity for the β_4 integrin subunit* (a). *In a double-immunofluorescence staining for laminin $\alpha 2$ chain* (b) *and CK19* (c), *the CK19-positive tumor cells show faint reactivity for the $\alpha 2$ chain. In a double-immunofluorescence staining for the laminin $\alpha 1$ chain* (d) *and CK19* (e), *some of the malignant (CK19-positive) cells invading through smooth muscle showed reactivity for the laminin $\alpha 1$ chain* (arrows). *However, this reactivity was very low compared with that of the surrounding smooth muscle. In a double-immunofluorescence staining for the laminin $\beta 3$ chain* (f) *and CK19* (g), *the malignant cells showed intense reactivity for the $\beta 3$ chain. APAAP* (a); *immunofluorescence* (b *to* g); *magnification, ×610* (a) *and ×770* (b *to* g).

and the cells were surrounded by intense reactivity for the laminin $\alpha 3$ and $\beta 3$ chains.

Collagen Type VII in Normal Gastric Epithelium and Gastric Carcinomas

In normal gastric mucosa, collagen type VII was detected in the BM of the surface epithelium as a spot-like immunoreactivity. It was somewhat more restricted in its expression as compared with laminin-5, but mostly the reactivities for these proteins were detected in co-localization. In gastric carcinomas, there was occasional reactivity at the interface between the nests of malignant cells and the surrounding desmoplastic stroma. Laminin-5 was also seen at these sites (Figure 5a, collagen type VII; Figure 5b, laminin-5). However, in most places, the BMs of the tumors were devoid of collagen type VII. The same invasive front as shown in Figure 4 was stained by double-immunofluorescence technique for collagen type VII (Figure 5c) and laminin-5 (Figure 5d). Collagen type VII was totally absent from

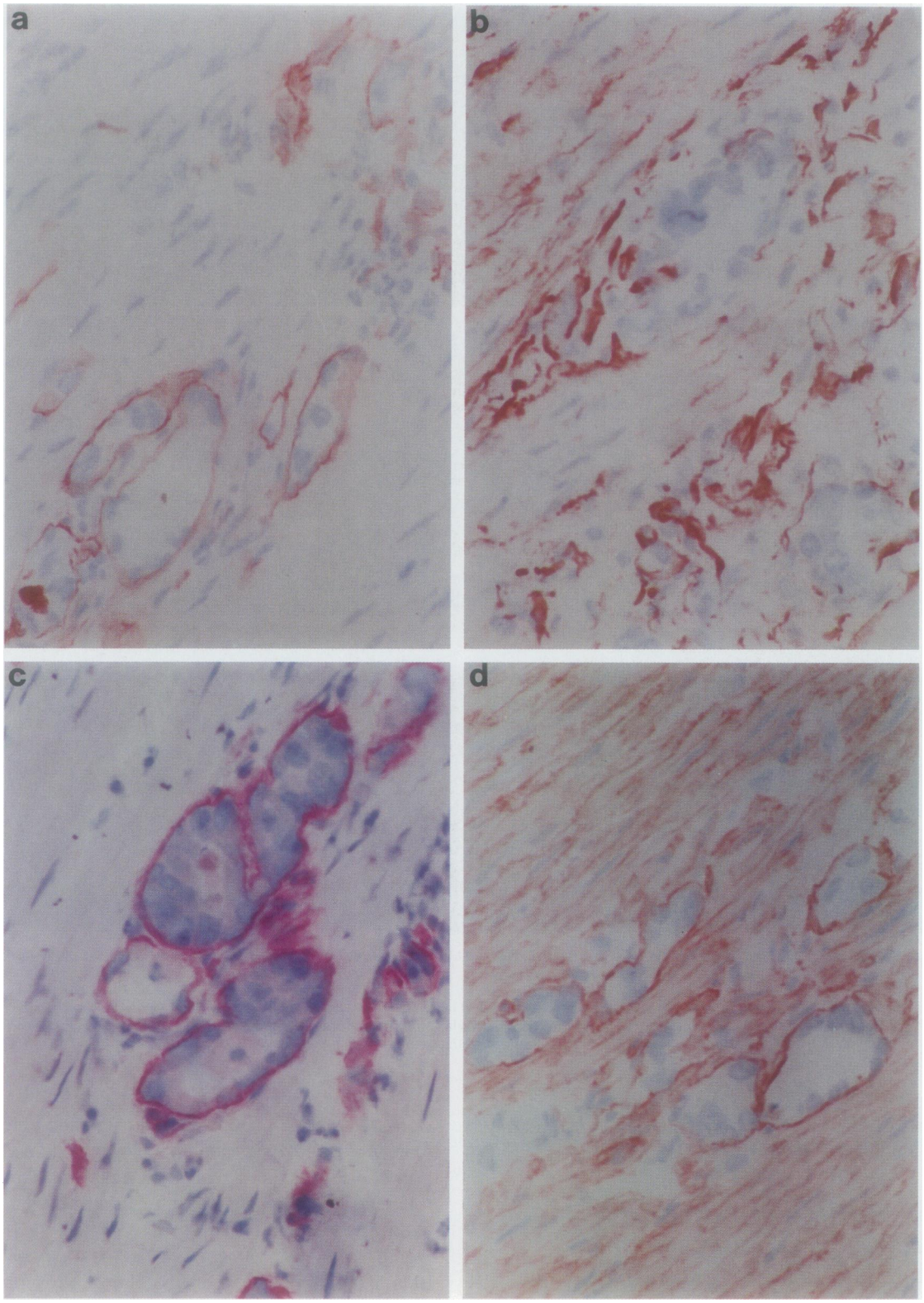

Figure 4. *Invasion of an IGCA through the smooth muscle layers of stomach. Laminin α3 chain was localized in the BMs of the tubular structures invading through the smooth muscle* (a). *The laminin α2 chain* (b) *was not incorporated in the BMs but was located diffusely around the invading malignant structures. The* β_1 *integrin subunit showed strictly polarized reactivity at the basal aspects of the malignant cells* (c). *Reactivity for the laminin α1 chain* (d) *was seen in smooth muscle but also in BMs of the invading structures. Histostain SP* (a, b, *and* d); *APAAP* c); *magnification, ×820.*

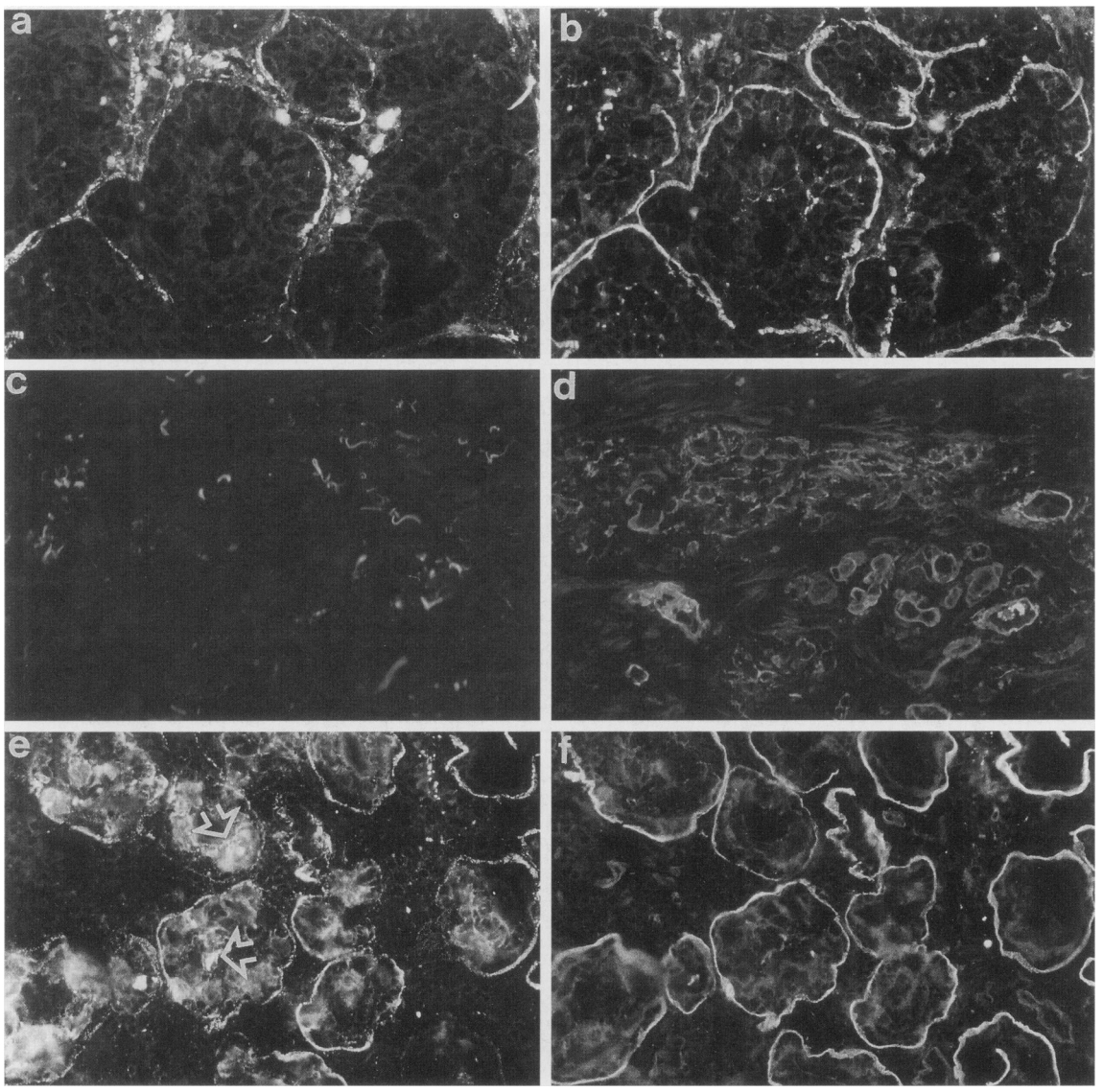

Figure 5. *Double-immunofluorescence staining of gastric carcinomas and intestinalized gastric epithelium for collagen type VII and laminin-5. In a non-invasive part of an intestinal-type gastric carcinoma, collagen VII* (a) *could be seen in co-localization with laminin-5* (b) *at many locations.* **Asterisks** *mark some nonspecific reactivity* (a). *In an invasive intestinal-type carcinoma, the invading nests of malignant cells did not show any reactivity for collagen type VII* (c), *even though there was a strong, continuous reactivity for laminin-5* (d). *The filamentous structures seen in* c *are due to the autofluorescence caused by elastin fibers. In intestinalized gastric glands, the collagen type VII* (e) *was seen in co-localization with laminin-5* (f), *much in the same way as in the normal gastric surface epithelium. Unfortunately, the FITC-conjugated secondary antibody showed nonspecific binding to metaplastic cells* (e). *Immunofluorescence; magnification, ×480.*

these invading glandular or nest-like structures despite the abundant expression of laminin-5. In intestinalized glands, type VII collagen (Figure 5e) and laminin-5 (Figure 5f) could be seen in co-localization.

Discussion

The results of this study suggest that IGCAs mimic the gastric surface epithelium in the constitution of their BM and adhesive structures and use the $\alpha_6\beta_4$ integrin and newly deposited laminin-1 and -5 as their adhesion and invasion machinery. Gastric epithelium with intestinal metaplasia showed a similar expression pattern of these antigens, whereas the DGCAs showed a more variable expression pattern.

The role of BM components in invasion has been an extremely controversial topic. Traditionally, the loss of integrity of the BM by a proteolytic activity has been considered as an absolute requirement for invasive growth (for reviews see Refs. 10–13). This assumption has had widespread consequences on our understanding of basic tumor biology and has

favored the division of the life history of epithelial tumors to carcinoma *in situ* in the beginning and to the time of invasive growth at later stages. However, recent studies have provided evidence for an active role for the expression of BM components in invasion.[41,42] In these studies the mRNA for laminin $\gamma 2$ chain was shown to be present in the carcinoma cells at the invasive front but absent from all other parts of the tumor. Earlier we have shown that the BM of the normal gastric epithelium expresses laminin-5 as detected by three different antibodies. In the present study we have provided evidence at the protein level that gastric carcinomas show a continuous reactivity for laminin-1 and laminin-5 in their BMs and that these continuous reactivities are preserved even in the structures invading through the smooth muscle layers of the gastric wall. In this respect, our data differ considerably from those of Pyke et al,[42] who reported that the expression of the laminin-5 is restricted to the invading cells also at the protein level. In addition, they proposed that the laminin $\gamma 2$ chain would preferentially be localized intracellularly and would not be deposited in the extracellular matrix. The most likely cause of this discrepancy is, in our opinion, in the differences between the antibodies used. The anti-$\gamma 2$ chain antiserum used by Pyke et al[42] was raised against a short peptide homologous to a fragment of the $\gamma 2$ chain, whereas the antibodies used in this study have been raised against native laminin-5. It may well be that the epitope recognized by the above-mentioned peptide antibody is masked in the native laminin-5, at least when deposited in the BM. Taken together, we propose a new model in which the laminin-5 is produced solely at the invasive front, as proposed by Pyke and co-workers.[41,42] However, the laminin chains are deposited in the BM of the tumor and preserved there in both the mucosal part of the tumor and at the invasive front.

The invading structures mostly showed reactivity for the β_4 integrin subunit and the same BM components as the surface epithelium, which suggests that the BM components are synthesized *de novo* at the invasive sites. It is possible that the newly deposited laminin chains and the $\alpha_6\beta_4$ integrin form a receptor-ligand system that enables the malignant cells to invade through the surrounding tissues. It has to be noted that in normal gastric epithelium the surface epithelial cells are continuously being renewed by cells migrating from the foveolar region.[43] These migrating cells express the $\alpha_6\beta_4$ integrin at their basal aspects,[28] which makes it tempting to speculate that gastric carcinomas use a similar adhesion mechanism for invasion. Additional support for this hypothesis is provided by the results suggesting that the expression of laminin-5[44] (T. Tani, R.E. Burgeson, and I. Virtanen, unpublished results) is associated with cell migration. In addition, in previous studies on gastric carcinoma[17] and colorectal carcinoma,[15] the expression of laminin-1 in the primary tumor was associated with increased risk of liver metastasis, which is in line with our present results on the deposition of BM components at sites of invasion through the smooth muscle. It is also interesting that the collagen type VII was lost, at least around the invading carcinoma cells. Collagen type VII is known to anchor epithelial cells through laminin-5 to connective tissue, and also in some other studies the loss of this anchoring molecule has been shown to correlate with the invasive phenotype,[22] suggesting that the carcinoma cells have to get apart from this anchoring mechanism before they can invade their surroundings. It is worth mentioning that our results on the expression of collagen type VII in normal gastric mucosa differ from previously published reports.[8,9] This may again be due to the different antibodies used. The antibody used in this study is directed against the NC-1 domain of collagen, which is known as a strong immunogen.[45]

Gastric carcinomas are classically divided into two main groups, namely, intestinal- and diffuse-type carcinomas.[31] The division into expanding and invading types has also been proposed,[46] but expanding and invading types are mostly equal to intestinal and diffuse types, respectively.[47] This division is important because these malignancies have been proposed to develop by different multistep pathogenetic ways. DGCAs have been suggested to be associated with early non-atrophic gastritis, whereas IGCAs have been proposed to be derived from later atrophic stages showing intestinal metaplasia.[32,33]

In the present study we found that IGCAs uniformly showed reactivity for the laminin $\alpha 1$ chain and the β_4 integrin subunit and, except for one tumor, also for the $\alpha 3$ chain of laminin. In normal gastric epithelium the reactivities for the $\alpha 3$ chain and the β_4 integrin subunit are strictly restricted to the surface and gastric pit epithelium,[28] which means that IGCAs mimic the surface epithelium at least in their cell-extracellular matrix interactions. The finding that gastric glands showing intestinal metaplasia expressed the β_4 integrin subunit and the laminin $\alpha 3$ chain instead of the $\alpha 2$ chain seen in the BM of normal gastric glands supports the theory of intestinal metaplasia as a preneoplastic stage of IGCAs. Previous reports have suggested that intestinal metaplasia originally derives from the proliferating

cells of the foveolar area.[48,49] Only as the intestinalization proceeds is the site of proliferating cells shifted toward the glandular region, thus resembling the situation in intestinal crypts.[48] The fact that intestinalized gastric epithelium expressed the same laminin chains as the surface epithelium may support the theory of the origin of this metaplasia in the foveolar area.[48] However, in normal intestinal epithelium, the reactivity for the laminin $\alpha 3$ chain is found in the BMs of the villi (I. Leivo, T. Tani, L. Laitinen, R. Bruns, E. Kivilaakso, V.-P. Lehto, R.E. Burgeson, and I. Virtanen, submitted for publication). This naturally provides evidence for structural and perhaps also functional similarities between the BMs of gastric surface epithelium, intestinal villous epithelium, and intestinalized gastric mucosa. Additional studies are required to estimate the role of BM components on the differentiation of gastric epithelial cells toward the phenotype of intestinal epithelium.

DGCAs variably expressed different laminin chains and the β_4 integrin subunit. There was considerable heterogeneity in the expression of these antigens in each tumor, different laminin chains showing a variable spatial distribution. Such a heterogeneity even between different parts of the same tumor is in line with the undifferentiated nature of these tumors. It has to be noted, however, that in epithelial BMs laminin $\alpha 1$ chain is known to be produced by both mesenchymal and epithelial cells,[2,50] whereas the $\alpha 2$ chain has been proposed to be produced solely by mesenchymal cells.[30,51] In four of six DGCAs and especially in invasive parts of IGCAs, the $\alpha 2$ chain was localized diffusely around the tumor cells and stromal cells. The expression of different extracellular matrix molecules may hence partly represent local responses to the invasive growth of the tumor cells. So-called pericryptal fibroblasts have been hypothesized to be responsible for the production of the laminin $\alpha 2$ chain in intestinal mucosa,[30] but *in situ* hybridization studies are required to elucidate the role of each cell type in the formation of BM components in normal gastric epithelium and in gastric tumors.

Taken together, our results suggest that gastric carcinoma cells use the $\alpha_6\beta_4$ integrin and newly deposited laminin-1 and -5 as the machinery of adhesion and invasion. Our results show that intestinal metaplasia and gastric carcinomas of the intestinal type show reactivity for the same laminin chains as the gastric surface epithelium and also express the $\alpha_6\beta_4$ integrin, which is a marker for surface epithelium. These results support previous studies suggesting intestinal metaplasia as a preneoplastic stage for IGCAs. In addition, the fact that intestinalized glands mimic the gastric surface or pit epithelium makes a contribution to the theory that intestinalized epithelium arises as an abnormal growth and differentiation of the foveolar epithelium.

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