The Mucin Epiglycanin on TA3/Ha Carcinoma Cells Prevents $\alpha_6\beta_4$-mediated Adhesion to Laminin and Kalinin and E-cadherin-mediated Cell-Cell Interaction

Hans Kemperman, Yvonne Wijnands, Jelle Wesseling, Carien M. Niessen, Arnoud Sonnenberg, and Ed Roos

Divisions of Cell Biology and Tumor Biology, The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands

Abstract. TA3/Ha murine mammary carcinoma cells grow in suspension, do not adhere to extracellular matrix molecules, but do adhere to hepatocytes and form liver metastases upon intraportal injection. Recently we showed that the integrin $\alpha_6\beta_4$ on the TA3/Ha cells is involved in adhesion to hepatocytes. However, despite high cell surface levels of $\alpha_6\beta_4$, TA3/Ha cells do not adhere to the $\alpha_6\beta_4$ ligands laminin and kalinin. Here we show that this is due to the mucin epiglycanin that is highly expressed on TA3/Ha cells. Some monoclonal antibodies generated against epiglycanin induced capping of most of the epiglycanin molecules. TA3/Ha cells treated with these mAb did adhere to laminin and kalinin, and an epithelial monolayer was formed on kalinin, with $\alpha_6\beta_4$ localized in HD1-containing hemidesmosome-like structures and E-cadherin at the cell-cell contact sites. Similar results were obtained after treatment of TA3/Ha cells with *O*-sialoglycoprotein endopeptidase which removes all epiglycanin. In addition, the enzyme induced E-cadherin-mediated cell-cell aggregation. Both treatments also enhanced the adhesion to hepatocytes, but given the potent anti-adhesive effect of epiglycanin it is remarkable that nontreated TA3/Ha cells adhere to hepatocytes at all. We found that during this interaction, epiglycanin was redistributed. We conclude that epiglycanin can completely prevent both intercellular and matrix adhesion, but that this effect can be overcome in certain intercellular interactions because of the induced redistribution of the mucin.

STRINGENT regulation of cell adhesion is crucial for normal development and maintenance of tissue architecture, as well as for the control of processes that involve migration of cells (Edelman and Crossin, 1991; Hynes and Lander, 1992; Hyndes, 1992). This regulation is achieved by changes in the expression of a large variety of adhesion molecules and splice variants, and in addition by modulation of the avidity of these molecules for their ligands, due to altered molecular conformation or to changes in surface distribution. In addition, adhesion can be impeded by molecules that mask binding sites. For surface molecules, this was first shown for embryonic neuronal cell adhesion molecule (NCAM),[1] which contains a large amount of negatively charged sialic acid. This hinders both the adhesion mediated by NCAM itself and interactions between other surface molecules (Hoffman and Edelman, 1983; Rutishauser et al., 1988). More recently, it was shown that cell surface mucins and proteoglycans can have similar effects and cause substantial reduction of cell adhesion (Ligtenberg et al., 1992; Hilkens et al., 1992; Manjunath et al., 1993; Vleminckx et al. 1994).

We have studied the interaction between carcinoma cells and hepatocytes, which is likely to play a major role in the formation of liver metastases (Roos, 1991). One of the cell lines studied was the TA3/Ha murine mammary carcinoma. TA3/Ha cells grow as single cells in suspension and do not adhere to several different extracellular matrix proteins (Kemperman et al., 1994). Yet, the cells do adhere to hepatocytes and form metastases in the liver after intraportal injection, and also in the liver the cells interact closely with hepatocytes (Roos et al., 1978). TA3/Ha cells express very low levels of β_1 integrins, but do express high levels of the integrin $\alpha_6\beta_4$. We have demonstrated that $\alpha_6\beta_4$ is involved in the interaction with hepatocytes (Kemperman et al., 1993). This integrin is known to bind to laminin (laminin-1) and kalinin (laminin-5) (Lee et al., 1992; Niessen et al., 1994; for the new nomenclature for the laminins see Burgeson et al., 1994), but these proteins appear not to be present at substantial amounts in hepatocyte cultures and the protein on the hepatocyte surface to which the TA3/Ha cells bind therefore remains to be identified. The lack of adhesion of

Address all correspondence to E. Roos, Division of Cell Biology (H1), The Netherlands Cancer Institute, 121 Plesmanlaan, 1066 CX Amsterdam, The Netherlands. Ph: (31) 20-512-1931. Fax: (31) 20-512-1944.

1. *Abbreviation used in this paper*: NCAM, neuronal cell adhesion molecule.

TA3/Ha cells to extracellular matrix components might be explained by the low levels of β_1 integrins. However, we were surprised to find that the cells also do not adhere to the $\alpha_6\beta_4$ ligands laminin (Kemperman, et al., 1994) and kalinin, as shown here.

TA3/Ha cells express high levels of a mucin, termed epiglycanin. This mucin has been suggested to mask surface molecules, in particular H2 histocompatibility antigens, and thus, to be responsible for the ability of the cells to grow in allogeneic mouse strains, despite the expression of the H2 antigens (Codington et al., 1978; Miller et al., 1982). This is in line with findings by others that surface mucins can interfere with host immune responses (Sherblom and Moody, 1986; Bharathan et al., 1990; Van de Wiel-van Kemenade et al., 1993). Epiglycanin expression has so far not been associated with the lack of adhesive capacity of the TA3/Ha cells. We show here that these cells express an intact E-cadherin/catenin complex, despite their lack of intercellular adhesion. Upon capping of epiglycanin, the cells formed an epithelial monolayer on kalinin with $\alpha_6\beta_4$ in hemidesmosome-like structures and E-cadherin concentrated in intercellular contact areas. After complete removal of epiglycanin, the cells were also capable of E-cadherin-mediated aggregation in suspension. This demonstrates that epiglycanin is extremely potent in the prevention of cell adhesion. However, we also show that this effect can be overcome in certain interactions, e.g., between TA3/Ha cells and hepatocytes, apparently because epiglycanin is redistributed.

Materials and Methods

Cells, Antibodies, and Extracellular Matrix Proteins

Mouse TA3/Ha mammary carcinoma cells were maintained as described (Hauschka et al., 1971). Metastasis formation in vivo and adhesion to hepatocyte cultures in vitro by these cells have been described (Roos et al., 1978).

Mouse and rat fibronectin were from Telios Pharmaceuticals Inc. (San Deigo, CA) and GIBCO BRL (Gaithersburg, MD), respectively. Mouse laminin was from Boehringer Mannheim GmbH (Mannheim, Germany), and mouse type I and type IV collagen were from Sigma Chemical Co. (St. Louis, MO) and GIBCO BRL, respectively. Kalinin-rich matrix was prepared as described by Sonnenberg et al. (1993). Purified epiglycanin (Codington et al., 1979) was kindly supplied by Dr. Codington.

The rat mAb GoH3 against mouse α_6 was described by Sonnenberg et al. (1988). FITC-labeled GoH3 was prepared according to the instructions of the supplier of the fluorescent dye (Molecular Probes, Eugene, OR). Mouse mAb 121 reacting with mouse HD1 was kindly supplied by Dr. Owaribe (Hieda et al., 1992). Polyclonal antibodies and mAb DECMA-1 against mouse E-cadherin were kindly supplied by Dr. Kemler (Vestweber and Kemler, 1984). Rat mAb KM 201 directed against mouse CD44 was kindly supplied by Dr. Kincade (Miyake et al., 1990). Rabbit antiserum 67 reacting with mouse β_4 was obtained as follows. A cDNA fragment encoding the complete cytoplasmic domain of the human β_4 integrin subunit was cloned into pQE-11 (Qiagen Inc., Chatsworth, CA). Production of the 6×His-β_4 fusion protein by transformed bacteria was induced according to the instructions of the supplier. Bacteria were lysed in 1% Triton X-100, and centrifuged at 3,000 *g* for 10 min. The pellet was resuspended in Laemmli sample buffer and proteins were separated by 7% reduced SDS-PAGE. The gel was stained in 0.02% Coomassie brilliant blue, and the band containing the 120-kD fusion protein was excised, crushed, and used to immunize a rabbit. Subsequent booster immunizations were given at 4-wk intervals.

Hamster hybridomas producing mAb against TA3/Ha cells were generated by fusion of mouse Sp2/0 cells with spleen cells of Armenian hamsters immunized with TA3/Ha plasma membranes, using standard procedures (Bright et al., 1990). The immunization schedule was as follows. Hamsters were injected subcutaneously with 400 μg TA3/Ha plasma membrane protein in Freund's complete adjuvant, followed by two booster injections in Freund's incomplete adjuvant after 2 and 4 wk. A final booster injection was given i.v. without adjuvant after eight weeks. 4 d later the fusion was performed. Fab fragments were prepared as described (Kemperman et al., 1993).

Electrophoresis and Immunoblotting

Proteins were solubilized in Laemmli sample buffer, separated by reduced SDS-PAGE, and subsequently electrophoretically transferred to a nitrocellulose membrane using the Tris-glycine buffer system. To visualize protein transfer, blots were reversibly stained with 0.4% Ponceau-S in 3% trichloroacetic acid. All subsequent incubations were done on a rotary platform at 20°C. Blots were treated for 1 h in block buffer (1% low fat milk powder, 0.1% Triton X-100 in PBS), and then incubated with first antibodies in block buffer for 1 h. Next, biotin-conjugated second antibodies were applied, followed by streptavidin–alkaline phosphatase in block buffer. After each incubation, blots were washed three times with block buffer, and also once with PBS before the color reaction. The alkaline phosphatase was detected by incubating the blot in substrate buffer (100 mM Tris, pH 9.5, 100 mM NaCl, and 5 mM $MgCl_2$) containing nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate (both from Pierce Chemical Co., Rockford, IL).

Hepatocyte Isolation and Culture

Rat hepatocytes were isolated as described previously (Roos and Van de Pavert, 1982). Cells were seeded in 96-well Primaria microtiter plates (Falcon Labware, Oxnard, CA) at 4×10^4 cells per well in DME supplemented with 5 μg/ml bovine insulin, 2 mM glutamine, 20 mM Hepes. Before seeding, wells had been coated with 50 μl of a 10 μg/ml rat fibronectin solution. 2 h after seeding, the hepatocytes were washed twice and cultured overnight in the same medium containing 10% fetal calf serum.

In Vitro Adhesion to Hepatocytes and Extracellular Matrix Molecules

After overnight culture, hepatocytes were washed twice with DME. Adhesion of tumor cells to hepatocytes was quantitated as described (Middelkoop et al., 1985). Briefly, 3×10^4 ^{51}Cr-labeled (Amersham International, Amersham, UK) TA3/Ha cells were preincubated in 15 μl DME with or without antibodies for 30 min at 37°C. Subsequently, the cells were added to a microtiter well containing hepatocytes in 50 μl DME. The nonadherent cells were washed away, cultures were lysed in 100 μl 1 N NaOH, and radioactivity was counted.

For adhesion to extracellular matrix molecules, microtiter plates (96-well round-bottom; Greiner GmBH, Trickenhausen, Germany) were coated for 2 h at 37°C with 50 μl/well of a 10 μg/ml extracellular matrix protein solution in PBS, washed with DME, and incubated with 100 μl of 0.5% BSA in DME for 30 min at 20°C to block remaining protein binding sites. Adhesion of TA3/Ha cells to the various extracellular components was assayed similarly as described above for the adhesion to hepatocytes.

Immunoprecipitation

TA3/Ha cells (2×10^6 per precipitation) were surface-labeled with ^{125}I (Amersham International) using the lactoperoxidase method, or metabolically for 4 h with [^{35}S]methionine/cysteine (Amersham International). Cells were lysed in lysis buffer (1% Triton X-100, 100 mM NaCl, 4 mM EDTA and 25 mM Tris, pH 7.5) for 1 h at 4°C. Lysates were cleared by spinning at 14,000 *g* for 10 min. Rabbit antibodies were precipitated with protein A–Sepharose beads overnight, added to the lysate and centrifuged after 2 h. Rat, mouse, and hamster monoclonal antibodies were precipitated with protein A–Sepharose beads that had been preincubated with rabbit anti-rat, anti-mouse, or anti-hamster IgG antibodies, respectively (all from Nordic, Tilburg, The Netherlands). 2 μl from polyclonal sera or 50 μl hybridoma supernatant was used per precipitation. The immunoprecipitates were boiled in Laemmli sample buffer and analyzed by reduced SDS-PAGE.

O-Sialoglycoprotein Endopeptidase, Neuraminidase, and O-Glycosidase Digestions

O-sialoglycoprotein endopeptidase was purchased from Cedarlane Laboratories (Ontario, Canada). TA3/Ha cells (5×10^6/ml) were incubated with the glycoprotease (12 μg/ml) in PBS without Mg^{2+} and Ca^{2+} at 37°C for 45 min. If cells were to be used for aggregation assays, the digestion was performed in PBS containing 1 mM Mg^{2+} and Ca^{2+}.

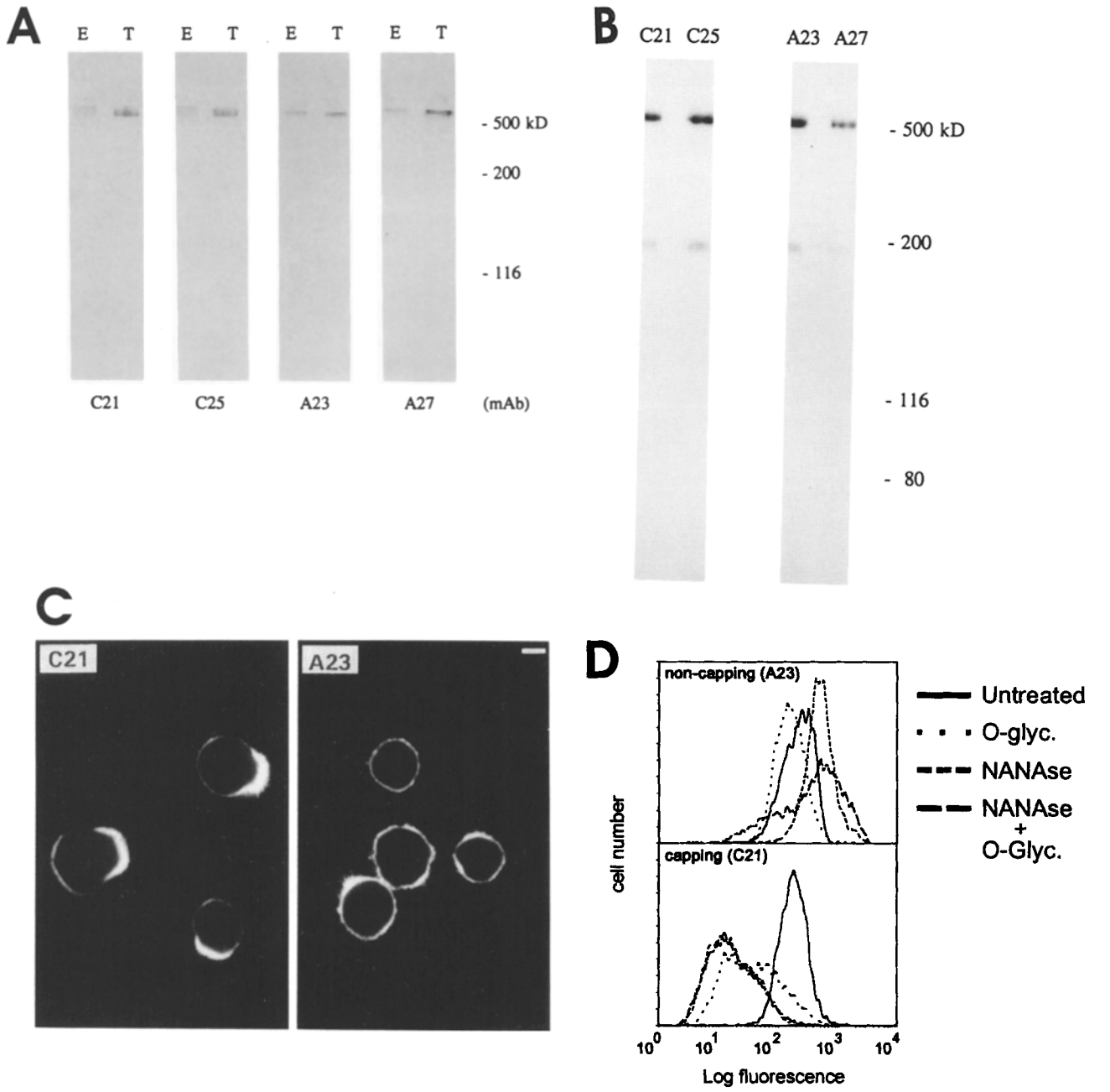

Figure 1. Characterization of anti-epiglycanin mAb. (*A*) Western blotting. Four identical blots (reduced 6% SDS-PAGE) with purified epiglycanin (*E*) and proteins of a TA3/Ha cell lysate (*T*), probed with the anti-epiglycanin antibodies C21, C25, A23, and A27. (*B*) Immunoprecipitation. Antigens were precipitated from ^{125}I-surface-labeled TA3/Ha cells (reduced 6% SDS-PAGE). (*C*) Capping capacity. Cell surface distribution of epiglycanin after incubation of TA3/Ha cells with the anti-epiglycanin mAb C21 (*left*) and A23 (*right*) at 20°C for 45 min. Hereafter cells were fixed in 2% paraformaldehyde in PBS and incubated with FITC-conjugated secondary antibodies, and viewed with a confocal laser scanning microscope. The majority of epiglycanin molecules is capped by C21 (*left*), but not by A23 (*right*). (*D*) Epitope. Cell surface expression of anti-epiglycanin mAb epitopes after treatment of TA3/Ha cells with neuraminidase, *O*-glycosidase, or a combination of both, examined by FACScan®. The epitope of the capping mAb C21 is susceptible to the digestions, but not that of the noncapping mAb A23. Bar, 4.4 μm.

Neuraminidase was purchased from Sigma. To 2 × 10^6 cells in 30 μl DME 8 μl neuraminidase (1 U/ml) was added, and cells were incubated at 37°C for 30 min. *O*-glycosidase was purchased from Boehringer Mannheim. TA3/Ha cells (2 × 10^6 in 20 μl DME) were incubated with 1 μl *O*-glycosidase (0.5 U/ml) at 37°C for 30 min.

Immunofluorescence

For immunofluorescence analysis, TA3/Ha cells were allowed to adhere to hepatocytes or matrix molecules on glass coverslips. The cells were fixed with 2% paraformaldehyde in PBS for 10 min, permeabilized with 0.5%

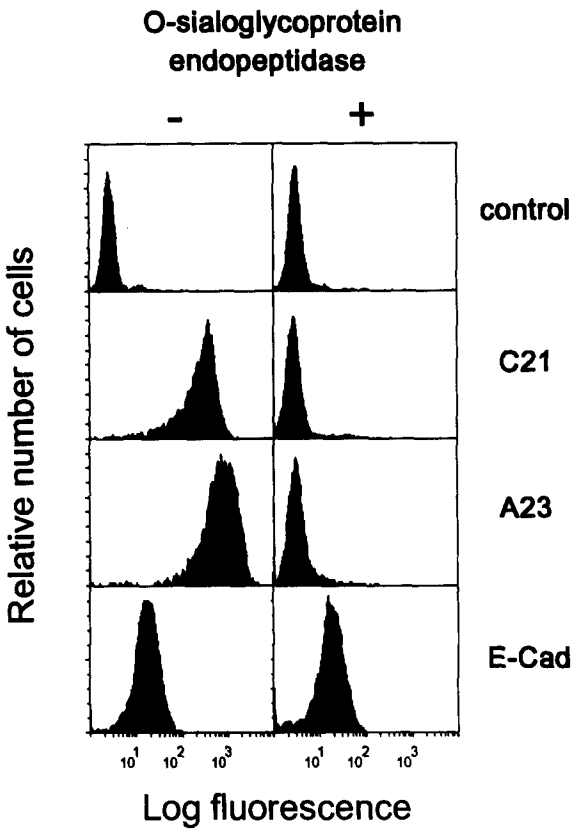

Figure 2. Effect of *O*-sialoglycoprotein endopeptidase treatment on epiglycanin cell surface expression. After treatment of TA3/Ha cells at 37°C for 45 min, cell surface expression of epiglycanin was assessed by FACScan® using the capping mAb C21 and noncapping mAb A23. Neither antibody detected any epiglycanin after the treatment. Cell surface E-cadherin expression using the DECMA-1 mAb was used as a control.

Triton X-100 followed by an incubation for 10 min in PBS containing 1% BSA. All immune incubations were performed at 37°C for 30 min, all antibodies were diluted in PBS containing 1% BSA. The secondary antibodies used were biotinylated and detected with extravidin-FITC or extravidin-TRITC. Actin was visualized with rhodamine-conjugated phalloidin. Hepatocytes were stained overnight with the lipophilic fluorescent probe DiI (octadecylindocarbocyanine; Molecular Probes) and washed three times before addition of TA3/Ha cells. The coverslips were washed, mounted with Vectashield (Vector Laboratories, Burlingame, CA), and viewed with a Bio-Rad MRC-600 confocal laser scanning microscope (Bio-Rad Laboratories, Hemel Hempstead, UK). To test the capping capacity of anti-epiglycanin mAb, cells were incubated in the presence of the different mAb at 20°C for 45 min. Hereafter, the cell suspension was spotted on coverslips, fixed, and further treated as described above.

Results

Antibodies That Cap Epiglycanin Are Directed against an O-linked Sugar Epitope

We have attempted to raise mAb blocking the adhesion of TA3/Ha cells to hepatocytes, but so far only obtained several mAb that enhanced adhesion. Two of these, C21 and C25, reacted with a large cell surface protein. However, we also obtained mAb that reacted with the same protein but did not enhance adhesion to hepatocytes, e.g., A23 and A27. We established that this protein is the mucin epiglycanin. All four mAb reacted on a Western blot with purified epiglycanin (E) (Codington et al., 1979) and with a protein of similar size in the TA3/Ha cell lysates (T), as shown in Fig. 1 *A*. Furthermore, as shown in Fig. 1 *B*, all four mAb precipitated a protein with an apparent M_r of 550 kD, comparable to that of purified epiglycanin, from lysates of surface-iodinated TA3/Ha cells. The faint band of 200 kD is a nonspecific band because it was also seen in control precipitations (not shown).

By immunofluorescence we observed a difference between the two types of antibodies: C21 and C25 induced capping of the epiglycanin molecules, whereas A23 and A27 did not. This is shown for C21 and A23 in Fig. 1 *C*. It should be noted that not all epiglycanin molecules were trapped in the cap, resulting in some staining on the remainder of the plasma membrane. This efficient cross-linking by C21 and C25, in the absence of secondary antibodies, might be explained if they were directed against O-linked carbohydrate epitopes, which are present in multiple copies within an epiglycanin molecule (Codington et al., 1975, 1986; Van den Eijnden et al., 1986). To test this, TA3/Ha cells were treated with O-glycosidase, neuraminidase or both. Cells were then incubated with the anti-epiglycanin antibodies and analyzed by FACScan®. Removal of sialic acid residues or O-linked sugar moieties completely abolished the reaction with the capping mAb C21 and C25, but not with the non-capping mAb A23 and A27, as shown for C21 and A23 in Fig. 1 *D*. This demonstrates that sialic acid–containing O-linked sugars form part of the epitopes of the capping but not of the noncapping mAb.

Epiglycanin Is Cleaved by the Glycoprotease O-Sialoglycoprotein Endopeptidase

O-sialoglycoprotein endopeptidase specifically cleaves heavily O-linked glycoproteins like glycophorin-A, CD34, CD43, CD44, and CD45 (Sutherland et al., 1992). To test whether epiglycanin was also cleaved by the glycoprotease, TA3/Ha cells were incubated with the enzyme, and subsequently the epiglycanin expression was analyzed by FACScan® using both capping and noncapping mAb. As can be seen in Fig. 2, for C21 and A23, neither type of mAb detected any epiglycanin after the treatment showing that epiglycanin was completely removed. As a control the cell surface expression of E-cadherin was analyzed and found not to be changed upon the glycoprotease treatment (Fig. 2).

Capping or Enzymatic Removal of Epiglycanin Causes Adhesion of TA3/Ha Cells to Laminin and Kalinin and Enhances Adhesion to Hepatocytes

TA3/Ha cells grow as single cells in suspension and do not adhere to various extracellular matrix components (Kemperman et al., 1994). This could be explained in part by the low levels of β_1-integrins on these cells, which are the main mediators of such interactions (Hemler, 1990; Hynes, 1992). However, the cells do express high levels of the integrin $\alpha_6\beta_4$ (Kemperman et al., 1993) which binds to laminin and kalinin (Lee et al., 1992; Niessen et al., 1994). Yet, the TA3/Ha cells did not adhere to either of these proteins. We have found that this is due to the presence of the mucin epiglycanin. After removal of epiglycanin from part of the

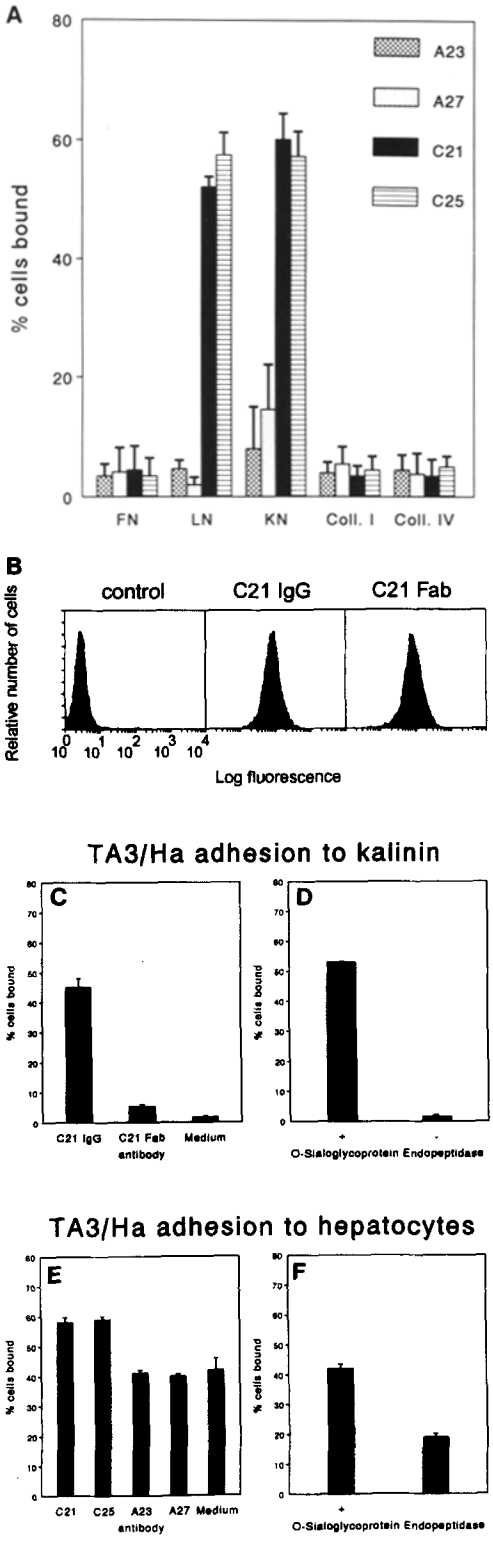

Figure 3. Induction of adhesion by antibodies and *O*-sialoglycoprotein endopeptidase. (*A*) Effect of anti-epiglycanin mAb on TA3/Ha adhesion to extracellular matrix molecules. The assay is described in Materials and Methods. Preincubation with the mAb was for 30 min and cells were allowed to adhere for 1 h. Fibronectin (*FN*), collagen I (*Coll. I*), collagen IV (*Coll. IV*), and laminin (*LN*) were coated at a concentration of 20 μg/ml, kalinin-rich matrix (*KN*) was prepared as described. Capping mAb C21 and C25 induced adhesion to kalinin and laminin, whereas the noncapping mAb A23 and A27 had no effect. (*B*) Reaction of C21 IgG and C21 Fab fragments with TA3/Ha cells, assessed by FACScan®. Both react to the same extent. (*C*) Effect of C21 IgG and C21 Fab fragments on adhesion of TA3/Ha cells to kalinin-rich matrix, assessed as in *A*. C21 IgG induced adhesion whereas C21 Fab fragments had no effect. (*D*) Effect of enzymatic removal of epiglycanin from the cell surface by *O*-sialoglycoprotein endopeptidase (see Fig. 2) on the adhesion of TA3/Ha cells to kalinin. Adhesion was quantitated as described for *A*. (*E*) Effect of anti-epiglycanin mAb on the adhesion of TA3/Ha cells to hepatocytes. (*F*) Effect of enzymatic removal of epiglycanin from the cell surface on the adhesion of TA3/Ha cells to hepatocytes. In *E* and *F* two different representative experiments are shown. Adhesion of TA3/Ha cells to hepatocytes varies between 20 and 40% of added cells between experiments.

cell surface by capping with mAb C21 and C25, up to 60% of added cells bound to kalinin and laminin, whereas the noncapping anti-epiglycanin mAb did not induce adhesion. No binding to collagen I and IV and fibronectin was observed (Fig. 3 *A*), probably because the cells express hardly any β_1 integrins. Fab fragments of C21, which bound to TA3/Ha cells to the same extent as whole antibody (Fig. 3 *B*), did not cap epiglycanin, and did not induce adhesion to kalinin as shown in Fig. 3 *C*. Furthermore, adhesion of TA3/Ha cells to hepatocytes was enhanced by C21 and C25, but not by the noncapping anti-epiglycanin antibodies, as can be seen in Fig. 3 *E*. Capping of another surface protein, CD44, by incubation of cells with rat anti-mouse CD44 mAb KM 201 followed by polyclonal anti-rat antibodies did not result in adhesion to kalinin, although large CD44 containing caps were seen by immunofluorescence analysis (not shown). Similarly, secondary antibody-induced capping of an as yet unidentified surface antigen, detected by another hamster anti-TA3/Ha cells mAb, 1C8.3, and highly expressed on TA3/Ha cells, did not induce adhesion (not shown).

Enzymatic removal of epiglycanin using *O*-sialoglycoprotein endopeptidase had the same effect. The treated TA3/Ha cells adhered to laminin and kalinin and their adhesion to hepatocytes was enhanced, as shown in Fig. 3, *D* and *F*; adhesion to kalinin was increased from virtually zero to 60% of added cells. Adhesion to hepatocytes was enhanced from 20 to 40% of added cells.

Antibody-induced Adhesion of TA3/Ha Cells to Kalinin Results in an Epithelial Morphology

Remarkably, the TA3/Ha carcinoma cells, which normally grow as single cells in suspension, not only adhered to kalinin upon capping of epiglycanin, but actually formed an epithelial monolayer. As shown in Fig. 4, most of the cells bound to kalinin in the presence of capping antibodies and spread within 2 h, whereas cells added in the absence of antibody or the presence of noncapping antibodies remained rounded and were easily removed by washing. After overnight incubation in the presence of capping antibodies, the cells had formed an epithelial monolayer. To study this epithelial morphology in more detail, the localization of $\alpha_6\beta_4$, epiglycanin, E-cadherin and the hemidesmosome-associated protein HD1 was examined. By immunoprecipitation we showed previously that on TA3/Ha cells the α_6 integrin subunit is associated only with β_4 and not with β_1 (Kemper-

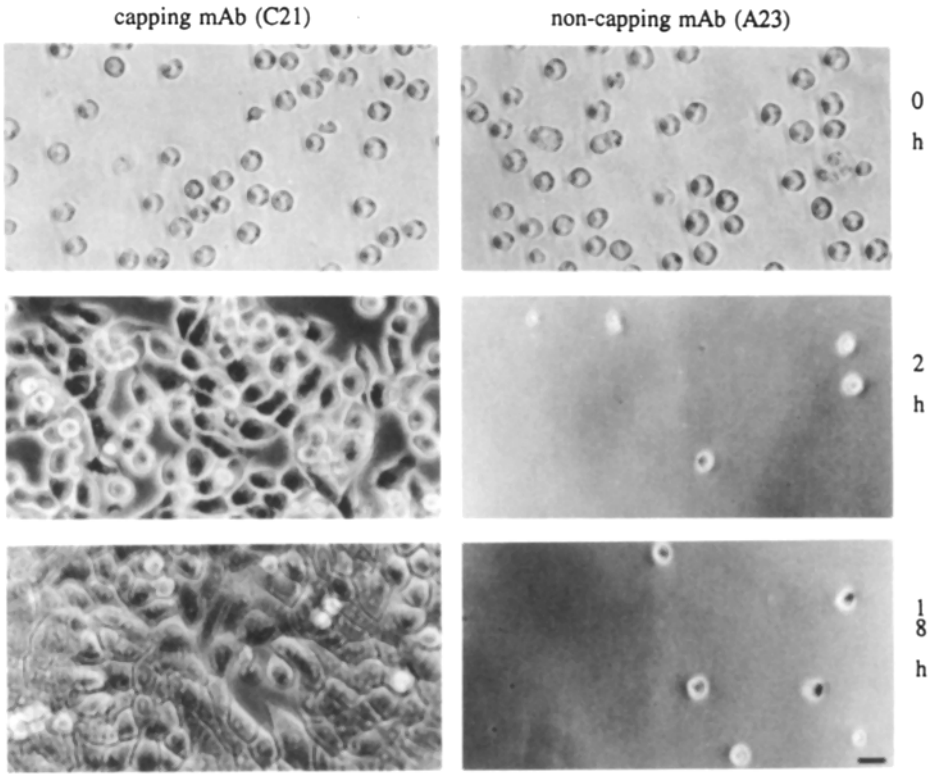

Figure 4. Effect of anti-epiglycanin mAb on the morphology of TA3/Ha cells that were allowed to adhere to kalinin. TA3/Ha cells were preincubated at 20°C for 30 min with the noncapping anti-epiglycanin mAb A23 or with the capping anti-epiglycanin mAb C21 and were then allowed to adhere to kalinin. After 2 h the plates were washed and incubated for another 16 h in the presence of the antibodies. Treatment with C21 led to the formation of an epithelial monolayer, whereas A23 did not induce adhesion. Bar, 11.7 μm.

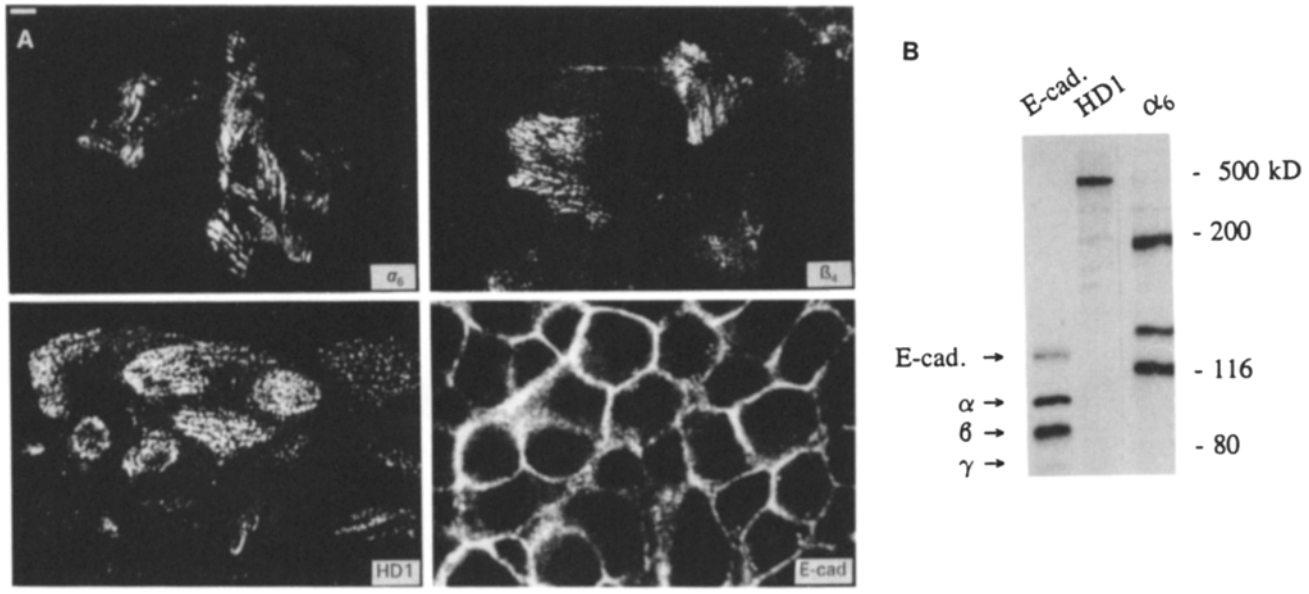

Figure 5. (*A*) Localization of α_6, β_4, HD1, and E-cadherin in TA3/Ha cells that had been allowed to adhere for 4 h to kalinin upon treatment with the capping mAb C21. Hereafter cells were fixed and probed with the α_6-specific mAb GoH3, rabbit polyclonal serum 67 directed against β_4, mAb 121 directed against HD1, and a rabbit polyclonal serum directed against E-cadherin (*E-cad*). Coverslips were viewed with a CLSM. (*B*) Immunoprecipitation of E-cadherin, HD1, and $\alpha_6\beta_4$. Antigens were precipitated from TA3/Ha cells that had been metabolically labeled with [^{35}S]methionine/cysteine, using a rabbit polyclonal serum against E-cadherin, mAb 121 against HD1, or mAb GoH3 directed against α_6, and analyzed by 6% reduced SDS-PAGE. Arrows indicate E-cadherin and the coprecipitated α, β, and γ catenins. Bar, 6.4 μm.

man et al., 1994). Using the mAb GoH3 against α_6 we showed that adhesion to laminin and kalinin is mediated by this $\alpha_6\beta_4$. Adhesion to laminin was completely blocked by GoH3, whereas adhesion to kalinin was partly blocked (result not shown), probably because kalinin is a higher affinity substrate for $\alpha_6\beta_4$ than laminin as shown previously (Sonnenberg et al., 1993). However, we can not exclude the presence of an additional kalinin receptor on TA3/Ha cells. To study the localization of $\alpha_6\beta_4$, we used polyclonal antibodies against the cytoplasmic domain of β_4 (antiserum 67) and the mAb GoH3 against the extracellular domain of α_6. The specificity of the polyclonal serum for β_4 was confirmed by immunofluorescence analysis of β_4-negative K562 cells and K562 cells transfected with both α_6 and β_4 cDNA (Niessen et al., 1994) (result not shown). As expected, we found α_6 and β_4 to be colocalized in structures at the substrate contact sites. These structures resembled hemidesmosomes, i.e., spots close to the substrate that are arranged in lines (Fig. 5 *A* and *6, A* and *B*) (Carter et al., 1990; Owaribe et al., 1990; Riddelle et al., 1991; Hieda et al., 1992; Sonnenberg et al., 1993). We determined by immunoprecipitation that the hemidesmosome-associated protein HD1 (Hieda et al., 1992) was expressed in TA3/Ha cells, as can be seen in Fig. 5 *B*. By immunofluorescence, HD1 was found to be localized in the same spots as $\alpha_6\beta_4$, confirming that these are hemidesmosome-like structures (Fig. 5 *A*).

The fact that TA3/Ha cells formed an epithelial monolayer after anti-epiglycanin antibody-induced adhesion to kalinin, indicated that also E-cadherin-mediated intercellular adhesion had occurred. In fact, immunoprecipitation results showed that TA3/Ha cells express E-cadherin. Comparable amounts were precipitated from suspended and adherent cells, showing that intercellular adhesion is not caused by induction of E-cadherin expression upon adhesion to kalinin. Also the other components of the cadherin/catenin complex were present in both adherent and suspended cells: in both cases α, β, and γ catenin were coprecipitated with E-cadherin as shown in Fig. 5 *B* for the suspended cells. In monolayers of TA3/Ha cells on kalinin, E-cadherin was found to be mainly localized at the cell–cell contact sites (Fig. 5 *A*). Apparently, the presence of the E-cadherin-catenin complex is not sufficient to prevent TA3/Ha cells from growing as single cells in suspension, suggesting that E-cadherin function is also impaired by epiglycanin.

If epiglycanin does hinder both cell–matrix and intercellular adhesion, it should be absent from the cell–substrate and cell–cell contact sites, and that is in fact what we observed: during initial attachment epiglycanin was mainly localized in a cap facing away from the substrate, and in smaller amounts on other parts of the membrane, but was absent from the substrate contact site. When the TA3/Ha cells had spread and had formed cell–cell contacts, epiglycanin was exclusively present at the apical plasma membrane and absent from both cell–substrate and cell–cell contact sites as shown in Fig. 6 *C*.

Enzymatic Removal of Epiglycanin from the Cell Surface Results in E-cadherin–mediated Cell Aggregation

Enzymatic removal of epiglycanin from the cell surface in the presence of Ca^{2+} and Mg^{2+} resulted in the formation of large cell aggregates. To study whether E-cadherin was involved, TA3/Ha cells were incubated with either the DECMA-1 mAb or polyclonal anti-E-cadherin antibodies prior to the glycoprotease treatment. Both prevented the aggregation showing that it is mediated by E-cadherin, as shown in Fig. 7 *B* for the mAb. In line with this finding, E-cadherin was found to be concentrated at the cell–cell contact sites in the aggregates, as shown in Fig. 7 *A*. Similar large aggregates were not formed after antibody-induced capping. This is probably due to the fact that not all epiglycanin molecules were capped, even though they did react with the capping mAb, as can be seen in Fig. 1 *C*. Epiglycanin is very heterogeneous with respect to its glycosylation state (Codington et al., 1975, 1986; Van den Eijnden et al., 1986). It is therefore conceivable that molecules containing fewer of the repeated carbohydrate epitopes are capped less efficiently. Our results shows that this limited amount of epiglycanin is sufficient to prevent E-cadherin–mediated cell aggregation completely.

Interaction of TA3/Ha Cells with Hepatocytes Results in a Redistribution of Epiglycanin

Given the complete prevention of both intercellular and matrix adhesion by epiglycanin, it is remarkable that the TA3/Ha cells adhere to hepatocytes at all. We have therefore studied the localization of epiglycanin on hepatocyte-bound nontreated TA3/Ha cells, and found that it was absent from the cell–cell contact sites (Fig. 8), indicating that epiglycanin was redistributed away from the interaction site. This redistribution occurs during the interaction with hepatocytes but not during contact with a kalinin-rich matrix.

Discussion

We have shown here that the mucin epiglycanin on the mouse mammary carcinoma cell line TA3/Ha completely prevents both $\alpha_6\beta_4$-mediated adhesion to extracellular matrix components and E-cadherin-mediated intercellular adhesion between the TA3/Ha cells. The cells express E-cadherin and $\alpha_6\beta_4$ at sufficient levels for rapid aggregation as well as adhesion to the $\alpha_6\beta_4$ ligands laminin and kalinin when epiglycanin has been removed, and on kalinin an epithelial monolayer is formed including the formation of HD1-containing hemisdesmosome-like structures. Yet, with epiglycanin on the surface, the cells do not adhere at all to these ligands and remain single cells in suspension.

Mucins are large rodlike molecules that extend far above the cell surface, and contain large amounts of O-linked carbohydrate, including a substantial number of negatively charged sialic acid residues (Devine and McKenzie, 1992). Modulation of adhesion by mucins has been demonstrated before, in particular for episialin which is expressed by many human carcinomas (Hilkens et al., 1984; Zaretsky et al., 1990), and leukosialin (CD43) which is present on many types of leukocytes (Manjunath et al., 1993). For episialin it has been shown that the negative charge is not sufficient to cause the anti-adhesive effect. Episialin-expressing cells that had been treated with neuraminidase exhibited only a partial restoration of aggregation, suggesting that the extended structure of the molecule is as important as the negative charge (Ligtenberg et al., 1992).

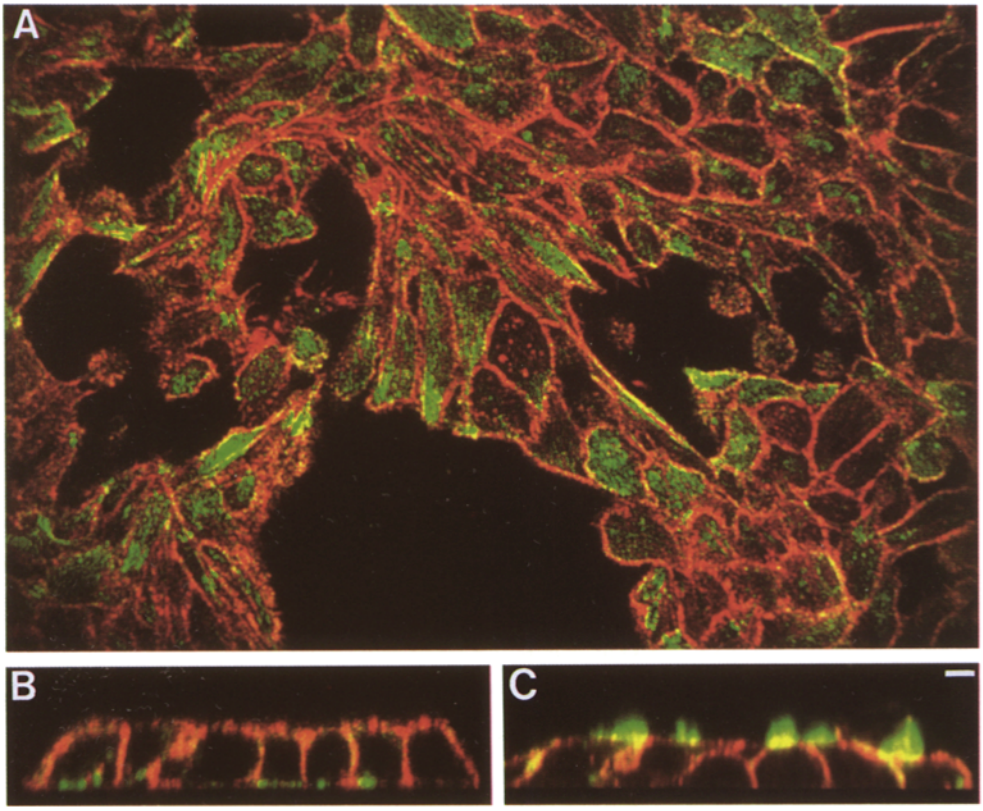

Figure 6. (*A*) Localization of $\alpha_6\beta_4$, actin, E-cadherin, and epiglycanin in TA3/Ha cells, allowed to adhere for 18 h to kalinin upon treatment with the capping mAb C21. Cells were probed with the α_6-specific mAb GoH3 (*green* in *A* and *B*), polyclonal antibodies directed against E-cadherin (*red* in *B* and *C*), mAb C21 directed against epiglycanin (*green* in *C*), and rhodamine-conjugated phalloidin to visualize actin (*red* in *A*). *A* is a section focused at the cell–substrate interface and parallel to the substrate. B and C are sections from the same coverslip, perpendicular to the substrate. Coverslips were viewed with a CLSM. Bar, 6.4 μm.

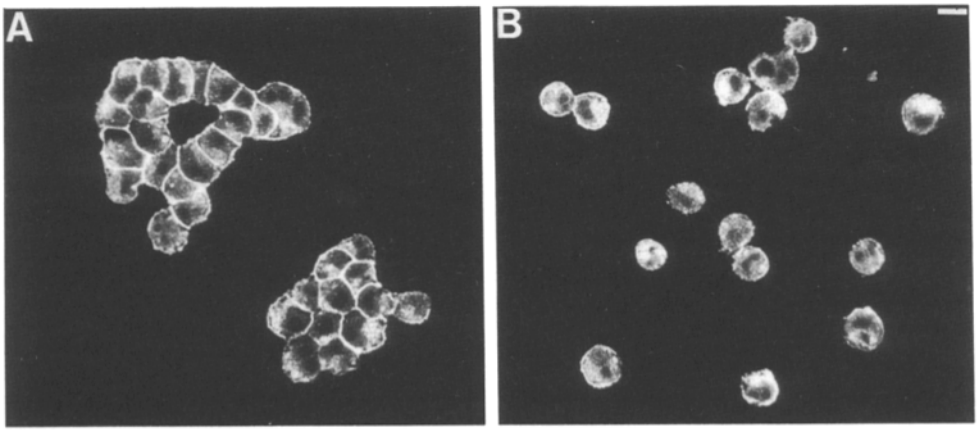

Figure 7. E-cadherin–mediated cell–cell aggregation after treatment of TA3/Ha cells with *O*-sialoglycoprotein endopeptidase in PBS. Cells were stained with polyclonal anti-E-cadherin antibodies and viewed with a confocal laser scanning microscope. In *B* TA3/Ha cells were preincubated with the DECMA-1 mAb, which blocks E-cadherin–mediated adhesion, prior to the treatment with the glycoprotease. Bar, 7 μm.

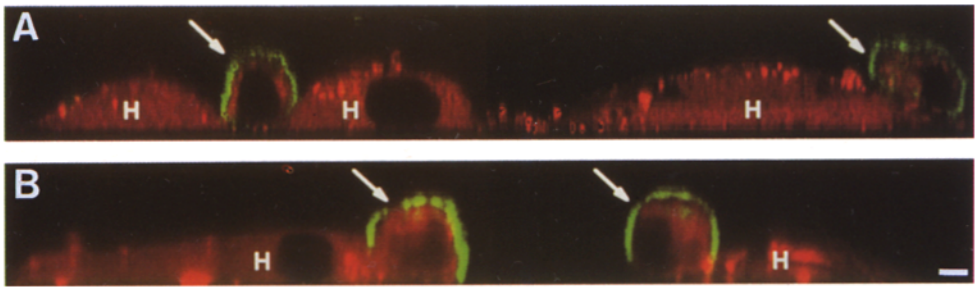

Figure 8. Redistribution of epiglycanin on TA3/Ha cells bound to hepatocytes. TA3/Ha cells were allowed to adhere for 1 h to hepatocytes. Cells were fixed, permeabilized and probed with anti-epiglycanin mAb C21 (*A*) and A23 (*B*). Hepatocytes were stained with the lipophilic probe DiI (*red*). Note that part of the DiI has leaked to the TA3/Ha cells. Coverslips were viewed with a CSLM. Shown are sections perpendicular to the substrate. Virtually no epiglycanin (*green*) is present at the site of interaction between the hepatocytes and the TA3/Ha cells. Hepatocytes are indicated with a (H), and arrows indicate TA3/Ha cells. Bar, 4.0 μm.

Although the effects of leukosialin and episialin are quite significant, they are relatively limited compared to the effect of epiglycanin. T cell mutants that had lost expression of the mucin leukosialin (CD43), showed a less than twofold increase in adhesion to fibronectin and HIV-1 glycoprotein 120 (Manjunath et al., 1993). Cell lines transfected with the cDNA encoding episialin exhibited reduced aggregation compared to revertants that had lost expression of episialin, but episialin did not block aggregation completely (Ligtenberg et al., 1992). It is not clear why epiglycanin is so effective. An obvious possibility is that epiglycanin is much longer, and this may in fact explain the difference with leukosialin, the length of which is only 45 nm. However, episialin has approximately the same length as epiglycanin, both ~450 nm, whereas other surface proteins like adhesion molecules do not extend further than 30 nm above the lipid bilayer (Calafat, J., and J. Hilkens, personal communication; Codington et al., 1979; Becker et al., 1989). Also the overall molecular weight and the proportion of carbohydrate of the two mucins are roughly comparable. The expression level of epiglycanin on TA3/Ha cells is extremely high (4×10^6 mol/cell, 1% of cell dry weight) (Codington et al., 1975), and this undoubtedly contributes to its effectiveness in preventing adhesion. However, E-cadherin–mediated aggregation was still prevented when a large proportion of the epiglycanin was removed by capping with the C21 and C25 antibodies. The small amount of epiglycanin, present on the remainder of the cell surface after capping, was sufficient to prevent E-cadherin function suggesting that the expression level is not the only reason for the effective adhesion suppression by epiglycanin.

Epiglycanin has not yet been cloned, and it is therefore difficult to speculate about possible structural differences with episialin. Epiglycanin is not the mouse homologue of episialin (Spicer et al., 1991; Vos et al., 1991), as suggested by preliminary results obtained by Northern blotting and staining with polyclonal anti-mouse episialin antibodies (Vos, H., and J. Hilkens, personal communication). This is in agreement with the earlier observation that the amino acid compositions are related but not identical (Codington and Haavik, 1992).

Given the effective adhesion suppression by epiglycanin, it is striking that TA3/Ha cells do adhere to hepatocytes, and in fact do form metastases in the liver upon intraportal injection (Roos et al., 1978), and also in the lungs after injection into a tail vein (Roos, E., unpublished results). Although removal of epiglycanin resulted in increased adhesion to hepatocytes, the level of adhesion of nontreated cells is still substantial: although levels of adhesion between 20 and 40% of added cells may seem low, it should be noted that the "monolayers" are not confluent but consist of cords and islands covering roughly 50% of the substrate, and many added cells therefore do not sediment onto a hepatocyte. Our results show that during this interaction, epiglycanin is redistributed to the surface facing away from the hepatocyte. It is not immediately obvious how this occurs. Conceivably, it is a due to a zipper-like process comparable to what has been proposed for phagocytosis (Shaw and Griffin, 1981), and that epiglycanin is simply pushed out of the interaction area. However, this depends on interaction with a cell surface, since it does not occur on a kalinin matrix. It should be noted that when the TA3/Ha cells had formed a monolayer on kalinin after capping with C21 or C25 mAb, the small amount of epiglycanin that was not capped and prevented aggregation in suspension, had also been redistributed to the apical plasma membrane, away from both cell–substrate and intercellular contact sites. This suggests that the redistribution is comparable to the normal polarization of epithelial cells, and depends upon contacts within an organized monolayer. In fact, mucins are located apically in normal epithelial cells, but often distributed over the entire cell surface in carcinomas (Zaretsky et al., 1990; Hilkens et al., 1992). The interaction with kalinin or normal epithelial cells like hepatocytes may induce polarization, in line with observations, e.g., at the invasive edge of a lung carcinoma in the lungs (Dingemans and Mooi, 1986), that carcinoma cells regain their epithelial morphology in contact with pre-existing basement membranes and normal epithelial cells.

The demonstrated effects of mucins on tumor cell behavior, and particularly metastasis, are limited to modulation of host defense. Cells transfected with episialin are to some extent protected against lysis by cytotoxic T-lymphocytes (Van de Wiel-van Kemenade et al., 1993). Furthermore, ASGP-1, a mucin present on the rat carcinoma cell line 13762 has been suggested to be responsible for the resistance of these cells to lysis by normal spleen lymphocytes (Sherblom and Moody, 1986). In addition, treatment of the same cells with tunicamycin led to a decreased level of ASGP-1 on their cell surface, and probably as a result of that to a higher susceptibility to lysis by natural killer cells (Bharathan et al., 1990; Moriarty et al., 1990). Also in this respect epiglycanin is extraordinarily effective, since TA3/Ha cells can not only be transplanted into allogeneic mice but even into rats (Codington et al., 1978; Miller et al., 1982). Whether epiglycanin, and mucins in general, influence metastasis by modulation of adhesion remains to be determined. However, it seems likely that epiglycanin can contribute to release from primary tumors, especially of E-cadherin–expressing carcinomas, but demonstration of this effect requires mutation and transfection of epiglycanin and therefore awaits cloning of its cDNA. We are currently investigating whether epiglycanin affects the invasion of bloodborne TA3/Ha cells into the liver, by pretreatment with *O*-sialoglycoprotein endopeptidase, in the absence of divalent cations to prevent aggregation. It is conceivable, that given the observed redistribution of epiglycanin upon contact with hepatocytes, this effect will be limited.

So far, the TA3/Ha cell line is the only one known to express epiglycanin, and also in normal tissues it has not been detected. In preliminary tests using immunohistology with the anti–epiglycanin mAb, we have not seen expression in neonatal mouse tissues. Given the very potent anti-adhesive effect of the molecule, it is likely to play a role in modulation of tissue architecture during development, and it will therefore be of interest to identify its normal site and timing of expression.

We thank Dr. H. Vos, J. Calafat and J. Hilkens for sharing unpublished information with us and for helpful discussions. We are grateful to T. Hamers for performing immunizations and collection of sera from rabbits, to A. J. Schrauwers for biotechnical assistance, to E. Noteboom for assistance with FACScan analysis, and to N. Ong and J. Lomecky for preparing photographs. We thank L. Oomen for excellent help with the confocal laser scanning microscope. We further wish to thank Dr. Kemler, Dr. Kin-

cade, and Dr. Owaribe for their generous gift of antibodies, and Dr. Codington for this generous gift of purified epiglycanin.

This research was supported by the Dutch Cancer Society grants NKI 92-55, NKI 91-61, and 91-260.

Received for publication 12 August 1994 and in revised form 28 September 1994.

References

Becker, J. W., H. P. Erickson, S. Hoffman, B. A. Cunningham, and G. M. Edelman. 1989. Topology of cell adhesion molecules. *Proc. Natl. Acad. Sci. USA.* 86:1088–1092.

Bharathan, S., J. Moriarty, C. E. Moody, and A. P. Sherblom. 1990. Effect of tunicamycin on sialomucin and natural killer susceptibility of rat mammary tumor ascites cells. *Cancer Res.* 50:5250–5256.

Bright, S. W., T. Y. Chen, L. M. Flebbe, M. G. Lei, and D. C. Morrison. 1990. Generation and characterization of hamster-mouse hybridomas secreting monoclonal antibodies with specificity for lipopolysaccharide receptor. *J. Immunol.* 145:1–7.

Burgeson, R. E., M. Chiquet, R. Deutzman, P. Ekblom, J. Engel, H. Kleinman, G. R. Martin, G. Meneguzzi, M. Paulsson, J. Sanes, R. Timpl, K. Tryggvason, Y. Yamada, and P. D. Yuchenco. 1994. A new nomenclature for the laminins. *Matrix Biol.* 14:209–211.

Carter, W. G., P. Kaur, S. G. Gil, P. J. Gahr, and E. A. Wayner. 1990. Distinct functions for integrins $\alpha 3\beta 1$ in focal adhesions and $\alpha 6\beta 4$/bullous pemphigoid antigen in a new stable anchoring contact (SAC) of keratinocytes: relation to hemidesmosomes. *J. Cell Biol.* 111:3141–3154.

Codington, J. F., A. G. Cooper, M. C. Brown, and R. W. Jeanloz. 1975. Evidence that the major cell surface glycoprotein of the TA3-Ha carcinoma contains the Vicia graminea receptor sites. *Biochemistry.* 14:855–859.

Codington, J. F., A. G. Cooper, D. K. Miller, H. S. Slayter, M. C. Brown, C. Silber, and R. W. Jeanloz. 1979. Isolation and partial characterization of an epiglycanin-like glycoprotein from a new non-strain-specific subline of TA3 murine mammary adenocarcinoma. *J. Natl. Cancer Inst.* 63:153–161.

Codington, J. F., M. R. Deak, D. M. Frim, and R. W. Jeanloz. 1986. Evidence for the presence of an N-acetyllactosamine-type chain in epiglycanin. *Arch. Biochem. Biophys.* 251:47–54.

Codington, J. F., and S. Haavik. 1992. Epiglycanin—a carcinoma-specific mucin-type glycoprotein of the mouse TA3 tumour. *Glycobiology.* 2:173–180.

Codington, J. G., G. Klein, A. G. Cooper, N. Lee, M. C. Brown, and R. W. Jeanloz. 1978. Further studies on the relationship between large glycoprotein molecules and allotransplantability in the TA3 tumor of the mouse: studies on segregating TA3-HA hybrids. *J. Natl. Cancer Inst.* 60:811–818.

Devine, P. L., and I. F. C. McKenzie. 1992. Mucins: structure, function, and associations with malignancy. *Bioessays.* 14:619–625.

Dingemans, K. P., and W. J. Mooi. 1986. Invasion of lung tissue by bronchogenic squamous-cell carcinomas: interaction of tumor cells and lung parenchyma in the tumor periphery. *Int. J. Cancer.* 37:11–19.

Edelman, G. M., and K. L. Crossin. 1991. Cell adhesion molecules: implications for a molecular histology. *Annu. Rev. Biochem.* 60:155–190.

Hauschka, T. S., L. Weiss, B. A. Holdridge, T. L. Cudney, M. Zumpft, and J. A. Planinsek. 1971. Karyotypic and surface features of murine TA3 carcinoma cells during immunoselection in mice and rats. *J. Natl. Cancer Inst.* 47:343–359.

Hemler, M. E. 1990. VLA proteins in the integrin family: structures, functions, and their role on leukocytes. *Annu. Rev. Immunol.* 8:365–400.

Hieda, Y., Y. Nishizawa, J. Uematsu, and K. Owaribe. 1992. Identification of a new hemidesmosomal protein, HD1: a major, high molecular mass component of isolated hemidesmosomes. *J. Cell Biol.* 116:1497–1506.

Hilkens, J., F. Buijs, J. Hilgers, P. Hageman, J. Calafat, A. Sonnenberg, and M. van der Valk. 1984. Monoclonal antibodies against human milk-fat globule membranes detecting differentiation antigens of the mammary gland and its tumors. *Int. J. Cancer.* 34:197–206.

Hilkens, J., M. J. Ligtenberg, H. L. Vos, and S. V. Litvinov. 1992. Cell membrane-associated mucins and their adhesion-modulating property. *Trends Biochem. Sci.* 17:359–363.

Hoffman, S., and G. M. Edelman. 1983. Kinetics of homophilic binding by embryonic and adult forms of the neural cell adhesion molecule. *Proc. Natl. Acad. Sci. USA.* 80:5762–5766.

Hynes, R. O. 1992. Integrins: versatility, modulation, and signaling in cell adhesion. *Cell.* 69:11–25.

Hynes, R. O., and A. D. Lander. 1992. Contact and adhesive specificities in the associations, migrations, and targeting of cells and axons. *Cell.* 68: 303–322.

Kemperman, H., Y. Wijnands, D. de Rijk, and E. Roos. 1993. The integrin alpha 6 beta 4 on TA3/Ha mammary carcinoma cells is involved in adhesion to hepatocytes. *Cancer Res.* 53:3611–3617.

Kemperman, H., Y. Wijnands, A. M. L. Meijne, and E. Roos. 1994. TA3/St, but not TA3/Ha, mammary carcinoma cell adhesion to hepatocytes is mediated by alpha 5 beta 1 interacting with surface-associated fibronectin. *Cell Adhesion Commun.* 2:45–58.

Lee, E. C., M. M. Lotz, G. D. Steele, Jr., and A. M. Mercurio. 1992. The integrin $\alpha 6\beta 4$ is a laminin receptor. *J. Cell Biol.* 117:671–678.

Ligtenberg, M. J. L., F. Buijs, H. L. Vos, and J. Hilkens. 1992. Suppression of cellular aggregation by high levels of episialin. *Cancer Res.* 52:2318–2324.

Manjunath, N., R. S. Johnson, D. E. Staunton, R. Pasqualini, and B. Ardman. 1993. Targeted disruption of CD43 gene enhances T lymphocyte adhesion. *J. Immunol.* 151:1528–1534.

Middelkoop, O. P., P. van Bavel, J. Calafat, and E. Roos. 1985. Hepatocyte surface molecule involved in the adhesion of TA3 mammary carcinoma cells to rat hepatocyte cultures. *Cancer Res.* 45:3825–3835.

Miller, S. C., J. F. Codington, and G. Klein. 1982. Further studies on the relationship between allotransplantability and the presence of the cell surface glycoprotein epiglycanin in the TA3-MM mouse mammary carcinoma ascites cell. *J. Natl. Cancer Inst.* 68:981–988.

Miyake, K., K. L. Medina, S. Hayashi, S. Ono, T. Hamaoka, and P. W. Kincade. 1990. Monoclonal antibodies to Pgp-1/CD44 block lympho-hemopoiesis in long-term bone marrow cultures. *J. Exp. Med.* 171:477–488.

Moriarty, J., C. M. Skelly, S. Bharathan, C. E. Moody, and A. P. Sherblom. 1990. Sialomucin and lytic susceptibility of rat mammary tumor ascites cells. *Cancer Res.* 50:6800–6805.

Niessen, C. M., F. Hogervorst, L. H. Jaspers, A. A. de Melker, G. O. Delwel, E. H. M. Hulsman, I. Kuikman, and A. Sonnenberg. 1994. The alpha 6 beta 4 integrin is a receptor for both laminin and kalinin. *Exp. Cell Res.* 211:360–367.

Owaribe, K., J. Kartenbeck, S. Stumpp, T. M. Magin, T. Krieg, L. A. Diaz, and W. W. Franke. 1990. The hemidesmosomal plaque. I. Characterization of a major constituent protein as a differentiation marker for certain forms of epithelia. *Differentiation.* 45:207–220.

Riddelle, K. S., K. J. Green, and J. C. Jones. 1991. Formation of hemidesmosomes in vitro by a transformed rat bladder cell line. *J. Cell Biol.* 112:159–168.

Roos, E., 1991. Adhesion molecules in lymphoma metastasis. *Cancer Metastasis Rev.* 10:33–48.

Roos, E., K. P. Dingemans, I. V. Van de Pavert, and M. A. Van den Bergh-Weerman. 1978. Mammary carcinoma cells in the mouse liver: infiltration of liver tissue and interaction with Kupffer cells. *Br. J. Cancer.* 38:88–99.

Roos, E., and I. V. Van de Pavert. 1982. Effect of tubulin-binding agents on the infiltration of tumour cells into primary hepatocyte cultures. *J. Cell Sci.* 55:233–245.

Rutishauser, U., A. Acheson, A. K. Hall, D. M. Mann, and J. Sunshine. 1988. The neural cell adhesion molecule (NCAM) as a regulator of cell-cell interactions. *Science (Wash. DC).* 240:53–57.

Shaw, D. R., and F. M. Griffin, Jr. 1981. Phagocytosis requires repeated triggering of macrophage phagocytic receptors during particle ingestion. *Nature (Lond.).* 289:409–411.

Sherblom, A. P., and C. E. Moody. 1986. Cell surface sialomucin and resistance to natural cell-mediated cytotoxicity of rat mammary tumor ascites cells. *Cancer Res.* 46:4543–4546.

Sonnenberg, A., A. A. de Melker, A. M. Martinez de Velasco, H. Janssen, J. Calafat, and C. M. Niessen. 1993. Formation of hemidesmosomes in cells of a transformed murine mammary tumor cell line and mechanisms involved in adherence of these cells to laminin and kalinin. *J. Cell Sci.* 106:1083–1102.

Sonnenberg, A., P. W. Modderman, and F. Hogervorst. 1988. Laminin receptor on platelets is the integrin VLA-6. *Nature (Lond.).* 336:487–489.

Spicer, A. P., G. Parry, S. P. Patton, and S. J. Gendler. 1991. Molecular cloning and analysis of the mouse homologue of the tumor-associated mucin, MUC1, reveals conservation of potential O-glycosylation sites, transmembrane, and cytoplasmic domains and a loss of minisatellite-like polymorphism. *J. Biol. Chem.* 266:15099–15109.

Sutherland, D. R., K. M. Abdullah, P. Cyopick, and A. Mellors. 1992. Cleavage of the cell-surface O-sialoglycoproteins CD34, CD43, CD44, and CD45 by a novel glycoprotease from Pasteurella haemolytica. *J. Immunol.* 148: 1458–1464.

Van de Wiel-van Kemenade, E., M. J. Ligtenberg, A. J. de Boer, F. Buijs, H. L. Vos, C. J. Melief, J. Hilkens, and C. G. Figdor. 1993. Episialin (MUC1) inhibits cytotoxic lymphocyte-target cell interaction. *J. Immunol.* 151:767–776.

Van den Eijnden, D. H., W. M. Blanken, and A. van Vliet. 1986. Branch specificity of beta-D-galactosidase from Escherichia coli. *Carbohydr. Res.* 151: 329–335.

Vestweber, D., and R. Kemler. 1984. Some structural and functional aspects of the cell adhesion molecule uvomorulin. *Cell Differ.* 15:269–273.

Vleminckx, K. L., J. J. Deman, E. A. Bruyneel, G. M. Vandenbossche, A. A. Keirsebilck, M. M. Mareel, and F. M. van Roy. 1994. Enlarged cell-associated proteoglycans abolish E-cadherin functionality in invasive tumor cells. *Cancer Res.* 54:873–877.

Vos, H. L., Y. Vries, and J. Hilkens. 1991. The mouse episialin (MUC1) gene and its promoter: rapid evolution of the repetitive domain in the protein. *Biochem. Biophys. Res. Commun.* 181:121–130.

Zaretsky, J. Z., M. Weiss, I. Tsarfaty, M. Hareuveni, D. H. Wreschner, and I. Keydar. 1990. Expression of genes coding for pS2, c-erbB2, estrogen receptor and the H23 breast tumor-associated antigen. A comparative analysis in breast cancer. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 265:46–50.