Am J Physiol Gastrointest Liver Physiol
280: G1043–G1048, 2001.

Human biliary mucin binds to E-selectin: a possible role in modulation of inflammation

NAIR SREEJAYAN,[1] BIANCA M. WITTIG,[2] NIKO VON STILLFRIED,[1] MATTHIAS S. HENNICKE,[1] GUNTHER MEYER,[3] PETRA STIEBER,[4] ROLF LAMERZ,[1] AND CHRISTOPH VON RITTER[1]
[1]*Departments of Medicine II,* [4]*Clinical Chemistry, and* [3]*Surgery, Ludwig Maximilians University, Klinikum Grosshadern, D 81377 Munich; and* [4]*Deutsche Krebsforschungszentrum, Heidelberg, Germany*

Received 6 January 1999; accepted in final form 28 November 2000

Sreejayan, Nair, Bianca M. Wittig, Niko von Stillfried, Matthias S. Hennicke, Gunther Meyer, Petra Stieber, Rolf Lamerz, and Christoph von Ritter. Human biliary mucin binds to E-selectin: a possible role in modulation of inflammation. *Am J Physiol Gastrointest Liver Physiol* 280: G1043–G1048, 2001.—E-selectin, expressed on endothelial cells, mediates adhesion of leukocytes and tumor cells to endothelium. CA19-9 (sialyl-Lewis^a) and sialyl-Lewis^x are specific ligands for E-selectin. We have recently shown that mucin-rich culture media from human gallbladder epithelial cells contains CA19-9. In this study, we have tested whether human biliary mucin binds to E-selectin. The ability of mucins to inhibit the adhesion of HL-60 cells to immobilized E-selectin was taken as an index for E-selectin binding. Gallbladder bile, hepatic bile, and culture medium from human gallbladder epithelial cells completely inhibited the adhesion of HL-60 cells to E-selectin. The mucin-rich fractions of human bile exhibited strong inhibition, whereas mucin-free fractions had little effect. In contrast to human bile samples, CA19-9-free medium from cultured dog gallbladder epithelial cells failed to inhibit HL-60 binding. Furthermore, after CA19-9 immunoaffinity chromatography, which selectively extracted CA19-9 from bile, bile samples showed poor inhibition of HL-60 adhesion to immobilized E-selectin. A good correlation was observed between E-selectin binding and CA 19-9 concentrations in bile. Our results show that human bile has E-selectin binding activity that is mediated by the CA19-9 side chain of biliary mucin.

adhesion; CA19-9; sialyl-Lewis; cholestasis

CELL ADHESION AND RECOGNITION mechanisms are basic requirements for the development and homeostasis of all tissues. The adhesion to endothelial cells regulates the trafficking and recruitment of leukocytes to lymphoid tissues and sites of inflammation (7, 14, 18). At the inflammatory sites, adhesion molecules mediate the binding of immune cells to vascular endothelium and their migration into the foci of ongoing inflammation (12, 14). The specificity of the leukocyte-endothelial cell interaction is controlled, in part, by selective participation of adhesion molecules, which has recently been reviewed by Panés and Granger (15). E-selectin, expressed on endothelial cells following induction by proinflammatory mediators such as interleukin-1 and tumor necrosis factor-α, mediates the first specific contact of leukocytes to the endothelial cells (4, 16). E-selectin is a member of the selectin family of cell adhesion molecules, which is known to recognize carbohydrate ligands (5, 19). The major E-selectin ligands are the tetrasaccharides sialyl-Lewis^x and sialyl-Lewis^a (also referred to as carbohydrate antigen 19-9 or CA19-9) (26). These ligands are known to be contained in a mucin-type glycoprotein (9).

Serum CA19-9 levels have frequently been employed as leading tumor markers for pancreatic, gallbladder, and biliary tract cancers (25). The specificity of this marker is, however, reduced by the fact that elevated serum CA19-9 levels are observed in benign pancreatohepatobiliary diseases such as acute pancreatitis, obstructive jaundice, cholecysto- or choledocholithiasis, liver cirrhosis, or hepatitis. Particularly high values are observed in choledocholithiasis aggravated by cholangitis (1, 10). Although it is well known that tumor cells, owing to the loss of the basal-apical polarity typical of normal epithelial cells, are able to secrete CA19-9-containing mucins into the bloodstream (3), the source of CA19-9 in benign hepatobiliary diseases has been a matter of debate. Recently, we have shown (22) that biliary mucin secreted by cultured human gallbladder epithelial cells is rich in CA19-9. We found that there is a good correlation between CA19-9 levels and the concentration of mucin purified from human gallbladder bile (22). We confirmed these findings by subjecting bile samples to concanavalin-A affinity chromatography and CA19-9 immunoaffinity chromatography (22). Together, these experiments demonstrate that biliary mucins carry the epitope of CA19-9. Leakage of CA19-9-containing mucin across an impaired epithelial barrier from bile into the vasculature may be the mechanism for the serum CA19-9 levels in benign hepatobiliary diseases.

Address for reprint requests and other correspondence: C. von Ritter, Dept. of Medicine, Kreiskrankenhaus Prien, Harrasser Straße 61-63, 83209 Prien, Germany.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

A high incidence of septic complications has been documented in patients with obstructive jaundice (2, 6). In a recent study, Swain et al. (20) have shown that bile duct resection in experimental animals results in attenuation of adherence and migration of activated neutrophils to endothelial cells. Furthermore, these authors have shown that plasma samples from cholestatic patients inhibit neutrophil adherence to endothelial cells. However, the factor in the plasma of cholestatic patients that modulates neutrophil function has not been identified. In the present study, we show that human bile, owing to the presence of mucins that carry the CA19-9 side chain, have strong affinity to E-selectin receptor and may, thereby, modulate leukocyte adherence to endothelial cells.

MATERIALS AND METHODS

Materials. The promyelocytic leukemia HL-60 cells were supplied by M. Heike (Mainz, Germany). Vitrogen was purchased from Celtrix Laboratories (Palo Alto, CA). Tissue culture plates and petri dishes for adhesion experiments (Falcon 1008) were from Falcon (Lincoln Park, NJ). Gentamicin (10 g/ml), L-glutamine, trypsin-EDTA (10×), and HEPES buffer (1 mM) were obtained from GIBCO (Eggenstein, Germany). Eagle's MEM, DMEM, RPMI-1640 medium, bovine serum albumin, collagenase type I, goat anti-human IgG, NaCl, $MgCl_2$ hexahydrate, anhydrous $CaCl_2$, $MnCl_2$ tetrahydrate, sodium azide, phenylmethylsulfonyl fluoride, EDTA disodium salt, and 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) were from Sigma Chemical (St. Louis, MO). E-selectin monoclonal antibody was obtained from Becton Dickinson (San Jose, CA). Other materials included peroxidase (DAKO, Hamburg, Germany) and Sepharose CL-4B (35 × 1 cm column; Pharmacia, Uppsala, Sweden).

Bile samples. Gallbladder bile was obtained from patients with cholesterol gallstone disease who were undergoing elective cholecystectomy. During cholecystectomy, bile was completely aspirated by gallbladder puncture. Hepatic bile samples were obtained from by nasobiliary drainage from patients with obstructive jaundice due to common bile duct stones. The bile samples were aliquoted and stop frozen in liquid nitrogen and stored at −70°C until they were used for the experiments. Plasma samples from the blood bank (Clinical Chemistry Department, Klinikum Grosshadern) were screened for CA19-9, and representative samples were selected for the adhesion experiments. Informed consent was obtained from all patients from whom bile samples were obtained for this study, in conformation with the ethical guidelines of the Ludwig Maximilians University, Munich.

Gel filtration chromatography. Bile samples were fractionated on a Sepharose 4B-CL (35 × 1 cm) gel chromatography column at a constant flow rate of 0.5 ml/min using Tris·HCl (pH 8.0, 20 mM) buffer. Eluant fractions of 1 ml were collected and analyzed for E-selectin binding and CA19-9 activity.

Medium from cultured human gallbladder epithelial cells. To culture human gallbladder epithelial cells, gallbladders were obtained from gallstone-free patients during hemihepatectomy. The epithelial mucosa was sharply dissected from the muscle layer and incubated for 30 min at 37°C in collagenase type I. This was followed by centrifugation (200 *g* for 5 min) in Eagle's MEM containing 10% fetal calf serum, 2 mM L-glutamine, 2.5 mM HEPES, and 50 mg/ml gentamicin. The pellet was then layered on collagen I-coated Falcon plates and incubated with Eagle's MEM until the cells were confluent. The medium was changed every other day. On the day of confluence, the medium was harvested.

Medium from cultured dog gallbladder epithelial cells. Dog gallbladder epithelial cells were isolated from dog gallbladders as reported elsewhere (13) and were grown to confluence on 60-mm petri dishes precoated with 1 ml vitrogen gel (1:1 mixture of vitrogen and media). The cell culture media were aliquoted, stop frozen in liquid nitrogen, and stored at −70°C until they were used for the experiments.

HL-60 cell culture. The promyelocytic leukemia cell line HL-60 was cultured in 50-ml culture flasks in RPMI-1640 medium supplemented with 2.5 mM L-glutamine, 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin.

CA19-9 immunoaffinity chromatography. For CA19-9 immunoaffinity chromatography, CA19-9 antibodies (gift from Centocor, Malvern, PA) were coupled to cyanogen bromide-activated Sepharose 4B (2 × 15 cm column; Pharmacia). Starting buffer contained $NaPO_4$ (0.02 M, pH 7.3), NaCl (0.15 M), and NaN_3 (0.02%). Eluting buffer was the starting buffer + KSCN (3 M). The bound and unbound fractions were collected separately and were concentrated to the original volume of bile used in the assay.

Determination of CA19-9 activity. CA19-9 activity of samples was determined by using a commercial sandwich enzyme immunoassay (Enzymun; Boehringer Mannheim; methodological sensitivity 1.8 U/ml).

Preparation and purification of soluble E-selectin. The construction of the chimeric receptor globulins has been described by Walz et al. (23). A chimeric protein containing the extracellular domain of E-selectin fused to the hinge region of human IgG1 was expressed in COS cells. COS cells were therefore seeded onto 10-cm plates, grown to 50% confluence, and transfected with 3 μg plasmid DNA using the DEAE/dextran method. Seven days after transfection, supernatants were collected and stored at 4°C. The concentration of the receptor-globulin chimeras was determined by ELISA with a rat anti-human IgG monoclonal antibody (MAb) conjugated to peroxidase (1 μg/ml PBS, pH 7.4). The ELISA was developed with 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), and absorbance was determined at 405 nm.

Spot adhesion assay. The E-selectin binding property of the test samples was assessed by studying the ability of these samples to inhibit the binding of the sialyl-Lewis^a-carrying promyelotic cell HL-60 to the receptor-globulin chimera by using a spot adhesion assay, as reported earlier (23, 24). In brief, Falcon 1008 dishes were incubated with a 50-μl spot of goat anti-human IgG at a concentration of 10 μg/ml in 50 mM Tris, pH 9.5, for 90 min. Dishes were washed three times with PBS and blocked with 1% bovine serum albumin at 4°C overnight. Subsequently, the dishes were incubated with 1 ml of cell culture supernatant containing 5 μg/ml E-selectin-IgG fusion protein and incubated for 30 min at room temperature. Following the binding of E-selectin-IgG, 500 μl of the test samples were added and incubated for 30 min at 37°C. The samples tested included *1*) native human gallbladder bile, *2*) native human hepatic bile, *3*) fractions of human gallbladder bile, *4*) fractions of human hepatic bile, *5*) culture medium from human gallbladder epithelial cells, and *6*) culture medium from dog gallbladder epithelial cells. Following incubation, the spot was washed with binding buffer (1% bovine serum albumin, 50 mM HEPES, pH 7.5, 100 mM sodium chloride, 2 mM magnesium chloride, 1 mM calcium chloride, 3 mM manganese chloride, 0.02% sodium azide, and 0.2 mM phenylmethylsulfonyl fluoride). Subsequently, 10^5 tumor cells/ml (HL-60 cells) in binding buffer were added to the spot and allowed to bind for 30 min followed by three

washes with the buffer described above. Adherent cells were independently counted under a microscope by two investigators in a blinded manner. Plasma samples from normal and cholestatic patients were tested in an identical manner. The specificity of the binding assay was demonstrated with MAb against E-selectin. The Falcon dishes coated with E-selectin IgG fusion protein were incubated with anti-E-selectin MAb for 30 min at room temperature. Following three washes with PBS, the adhesion experiment was performed as described above. The dishes treated with the antibody retained only 2–3% of the cells compared with that retained by the control dishes without antibody treatment, demonstrating the specificity of the assay.

RESULTS

We have assessed the E-selectin binding property of human bile and the medium from cultured gallbladder cells by studying the ability of these samples to interfere with the adherence of the sialyl-Lewis^x-carrying HL-60 leukemia cell line to immobilized E-selectin. Figure 1 gives the concentrations of CA19-9 in fractionated human gallbladder bile and the effect of these fractions on the binding of HL-60 cells to immobilized E-selectin. Fractionation of human gallbladder bile with Sepharose 4B-Cl gel chromatography showed CA19-9 activity in one main peak in the void volume (excluded fraction); only low activity was found in the included fractions. Earlier, we showed (22) that this void volume corresponds to the mucin-rich fraction of bile. Furthermore, the CA19-9-carrying mucin-rich fraction of human gallbladder bile exhibited maximal inhibition of the binding of HL-60 cells to immobilized E-selectin (89.4 ± 7%), whereas the low-molecular-weight (mucin-free) fractions had little effect (10.5 ± 5%). Figure 2 gives the data obtained using various fractions of human hepatic bile. Hepatic bile behaved in an identical manner to the mucin-rich void (excluded) volume fraction containing maximum CA19-9 activity and expressing maximal inhibition of the binding of HL-60 cells to immobilized E-selectin.

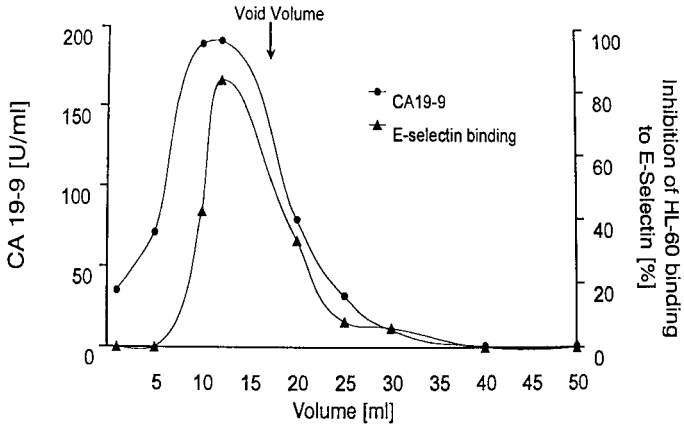

Fig. 2. CA19-9 concentrations and E-selectin binding activity of fractionated human hepatic bile. CA19-9 levels were determined by using a commercial sandwich enzyme immunoassay. E-selectin binding was assessed by studying the effect of different fractions of human hepatic bile on the binding of HL-60 cells to immobilized E-selectin IgG. The mucin-rich (void volume) fraction of hepatic bile completely inhibited adherence of HL-60 cells to E-selectin, whereas the mucin-free (excluded) fraction showed no inhibition, which correlates well with the CA19-9 levels. Each point on the graph is the mean of triplicate determinations.

To further ascertain the specific role of CA19-9 as the mediator of inhibition of HL-60 binding to E-selectin, we separated CA19-9 from bile by using immunoaffinity chromatography. Figure 3 gives the CA19-9 content and inhibition of HL-60 binding to E-selectin in the native human gallbladder bile and the unbound and

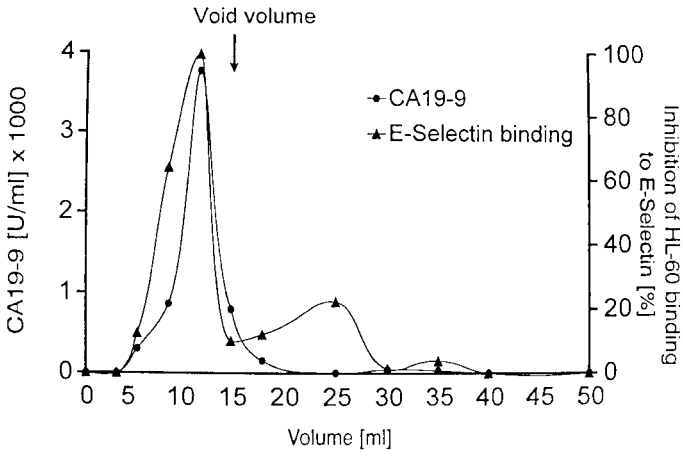

Fig. 1. CA19-9 concentrations and E-selectin binding activity of fractionated human gallbladder bile. CA19-9 levels were determined by using a commercial sandwich enzyme immunoassay. E-selectin binding was assessed by studying the effect of various fractions of human gallbladder bile on the binding of HL-60 cells to immobilized E-selectin IgG. The mucin-rich (void volume) fraction of human gallbladder bile completely inhibited adherence of HL-60 cells to E-selectin, whereas the mucin-free (excluded) fraction showed no inhibition, which correlates well with the CA19-9 levels. Each point on the graph is the mean of triplicate determinations.

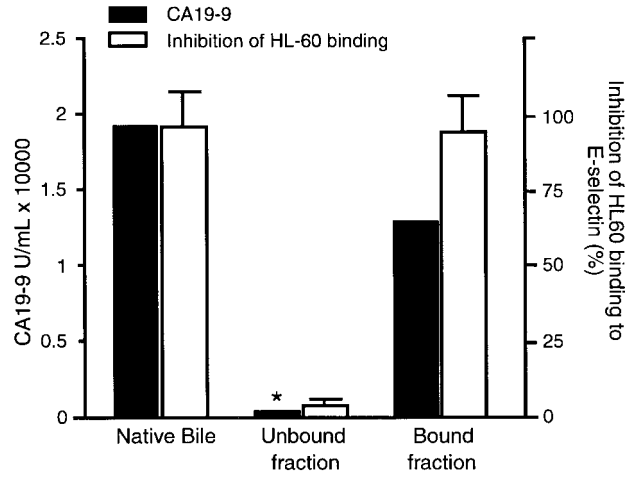

Fig. 3. CA19-9 concentrations and inhibition of HL-60 binding to E-selectin by gallbladder bile following immunoaffinity chromatography. The CA19-9 immunoaffinity column efficiently separated (~100%) CA19-9 from bile. The native bile and the bound fraction (eluted), which had large amounts of CA19-9, showed ~100% inhibition of HL-60 cell binding to immobilized E-selectin, whereas the unbound fraction, poor in CA19-9, failed to inhibit binding. The unbound fraction showed significantly less ($n = 5$, $^*P < 0.001$) inhibition of HL-60 binding compared with the native and bound fractions.

bound fractions of the same bile samples following separation over the immunoaffinity column. This figure shows that there was negligible CA19-9 activity in the unbound fraction and that the bound fraction and the native sample contained high CA19-9 activity. The E-selectin binding assay showed that the CA19-9-rich native and bound fractions caused almost complete inhibition of HL-60 binding to E-selectin, whereas the unbound fractions devoid of CA19-9 exhibited only negligible inhibition.

In further experiments, we studied the medium from cultured human gallbladder epithelial cells. In support of our findings with human bile samples, medium from cultured human gallbladder epithelial cells rich in CA19-9 (459 U/ml) completely blocked the binding of HL-60 cells to immobilized E-selectin (98.3 ± 2%) (Table 1). In contrast, the culture medium from the dog gallbladder epithelial cells almost devoid of CA19-9 (11 U/ml) poorly interfered with the binding of HL-60 cells to immobilized E-selectin (5.1%) (Table 1). The inability of the CA19-9-free culture medium from dog gallbladder epithelial cells to prevent the binding of HL-60 cells to immobilized E-selectin further substantiates the view that CA19-9 side chains of human biliary mucin mediate the binding of bile to E-selectin.

A good correlation ($r = 0.71$, $P < 0.05$) was found between the CA19-9 levels and the inhibition of the binding of HL-60 cells to immobilized E-selectin (Fig. 4). This positive correlation further strengthens the view that the CA19-9-carrying part of biliary mucin is a specific ligand for E-selectin.

To investigate the pathophysiological relevance of these findings, we obtained plasma samples from a patient with a malignant cholestasis. The serum sample was fractionated over a CA19-9 immunoaffinity column, and the various fractions were used to perform the adhesion. Table 2 gives CA19-9 levels and the inhibition of HL-60 binding to immobilized E-selectin in the presence of these samples. The fraction rich in CA19-9 significantly inhibited the binding of HL-60 cells to immobilized E-selectin, whereas the fractions with low CA19-9 levels showed poor inhibition.

Table 1. *E-selectin binding and CA19-9 concentrations in culture media*

Samples	%Inhibition of Binding	CA19-9, U/ml
HGBEC	98.3 ± 4.9	459.1
DGBEC	5.1 ± 3.2	11.1

CA19-9 concentrations and inhibition of binding of HL-60 cells to immobilized E-selectin by culture media from human gallbladder epithelial cells (HGBEC) and culture media from dog gallbladder epithelial cells (DGBEC). CA19-9 concentrations and E-selectin binding were determined as given in Fig. 1. Culture media from HGBEC exhibited complete inhibition of binding of HL-60 cells to immobilized E-selectin, which was consistent with the presence of increased levels of CA19-9 in these samples. Medium from cultured DGBEC showed poor inhibition of the binding of HL-60 cells to E-selectin, consistent with low CA19-9 content.

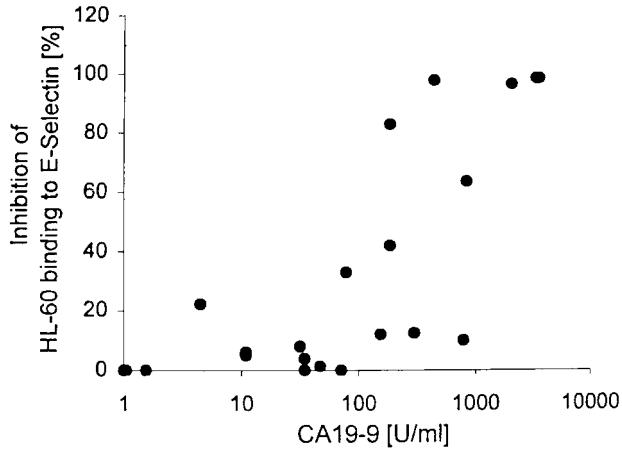

Fig. 4. Correlation between CA19-9 concentration and E-selectin binding property of various fractions of human gallbladder bile, human hepatic bile, and media from cultured human and dog gallbladder epithelial cells. A good correlation was observed ($r = 0.75$, $P < 0.05$) between CA19-9 levels and E-selectin binding property of the samples tested.

DISCUSSION

Recruitment of leukocytes to sites of inflammation is mediated in part by E-selectin, which is expressed on activated endothelial cells of the blood vessel walls (15). It has been shown that E-selectin recognizes the carbohydrate antigens sialyl-Lewis^a (CA19-9) and sialyl-Lewis^x. These antigens are present on the monocytes and granulocytes in adult blood, which mediate their adhesion to E-selectin (11). Elevated serum CA19-9 levels are also detectable in a variety of cancers and have been shown to bind to E-selectin (25). Besides carcinogenic conditions, CA19-9 is also expressed in the normal intraluminal secretions of salivary glands, pancreas, and bile. Since CA19-9 can also be detected within goblet cells of glandular tissues, it has been speculated that CA19-9 may be carried on mucins secreted into the ducts (26). Indeed, it has been shown that CA19-9 belongs to the group of compounds having a mucinlike structure (11, 21), and we recently reported that biliary mucins carry CA19-9 (22). Our present data show that human biliary mucins carrying CA19-9 block the binding of HL-60 cells to immobilized

Table 2. *E-selectin binding and CA19-9 concentrations of human cholestatic serum*

Samples	%Inhibition of Binding	CA19-9, U/ml
Native	98.3 ± 3.9	84,000.000
Fraction I	11.2 ± 3.1	28.28
Fraction II	35.5 ± 4.2	221.88
Fraction III	100	91,236.30

CA19-9 concentrations and inhibition of binding of HL-60 cells to immobilized E-selectin by cholestatic serum. The serum sample was fractionated using a CA19-9 immunoaffinity column, and 3 representative fractions were tested for E-selectin binding and CA19-9 content as described in Fig. 1. Fraction with high levels of CA19-9 exhibited maximum inhibition of binding of HL-60 cells to immobilized E-selectin.

E-selectin, thereby functioning as specific ligands for E-selectin.

Neutrophils play a central role in the host response to infection and tissue inflammation. The recruitment of neutrophils from the mainstream of blood to afflicted tissue is the key event mediating the inflammatory response. Among other factors, this event is believed to be mediated by E-selectin binding (15). A high incidence of septic complications has been documented in patients with obstructive jaundice, although the reason for this is unknown (2, 6). Plasma samples obtained from patients with obstructive cholestasis have been found to markedly inhibit the binding of activated neutrophils to endothelial cells, in contrast to plasma samples obtained from normal healthy individuals (20). Although heating did not affect this antiadhesive property of plasma samples obtained from cholestatic patients, treatment with the enzyme sialidase partially reduced the activity, suggesting that the antiadhesive factor requires, at least in part, a carbohydrate backbone for activity (20). The high levels of CA19-9 measured in the plasma of patients with cholestatic disease indicate that these patients have circulating mucins. The potent E-selectin binding activity of mucins may result in the inhibition of the rolling of leukocytes, an event that precedes leukocyte adhesion.

Under normal physiological conditions, most CA19-9-containing mucins are probably secreted into compartments in which they are degraded or excreted and are thus excluded from circulation (26). However, in the case of a breach of the barrier between the CA19-9-rich luminal content and the interstitial space, CA19-9 may leak into the bloodstream. Inflammatory destruction of the epithelial barrier is most probably the cause of increased serum levels of CA19-9 in cystic fibrosis (17) and rheumatoid arthritis (8). Similarly, an impaired epithelial barrier function during hepatobiliary diseases may lead to the diffusion of CA19-9 across the biliary epithelium. This diffusion may be driven by the high gradient between luminal and interstitial CA19-9 concentrations. Since neither active transcellular transport of CA19-9 nor secretion from the basolateral membrane of epithelial cells is a likely mechanism, passive diffusion is the most plausible pathway for the presence of increased CA19-9 in the serum of patients with benign hepatobiliary diseases (1, 10).

The leakage of CA19-9-carrying mucin into circulation during various hepatobiliary diseases may serve as one of the mechanisms for the feedback inhibition of granulocyte infiltration, which may precipitate inflammatory reactions associated with these conditions. According to this view, one could speculate that biliary mucin, because of its E-selectin binding activity, may be of therapeutic value in both acute and chronic pathological conditions. Testing different mucins or mucinlike glycoproteins for their capacity to inhibit the interaction between leukocytes and E-selectin could be an alternative strategy for modulation of inflammatory conditions.

We thank G. Paumgartner for critically reviewing the manuscript.

This study was supported by a grant from the Deutsche Forschungsgemeinschaft (Ri-584/3).

REFERENCES

1. **Albert MB, Steinberg WM, and Henry JP.** Elevated serum levels of tumor marker CA19-9 in acute cholangitis. *Dig Dis Sci* 33: 1223–1225, 1988.
2. **Armstrong CP, Dixon JM, Taylor TV, and Davies GC.** Surgical experience of deeply jaundiced patients with bile duct obstruction. *Br J Surg* 71: 234–238, 1984.
3. **Baeckstrom D, Karlsson N, and Hansson GC.** Purification and characterization of sialyl-Le(a)-carrying mucins of human bile; evidence for the presence of MUC1 and MUC3 apoproteins. *J Biol Chem* 269: 14430–14437, 1994.
4. **Bevilacqua MP, Pober JS, Mendrick DL, Cotran RS, and Gimbrone MAJ.** Identification of an inducible endothelial-leukocyte adhesion molecule. *Proc Natl Acad Sci USA* 84: 9238–9242, 1987.
5. **Bevilacqua MP, Stengelin S, Gimbrone MAJ, and Seed B.** Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science* 243: 1160–1165, 1989.
6. **Blenkharn JI, McPherson GA, and Blumgart LH.** Septic complications of percutaneous transhepatic biliary drainage. Evaluation of a new closed drainage system. *Am J Surg* 147: 318–321, 1984.
7. **Butcher EC.** Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. *Cell* 67: 1033–1036, 1991.
8. **Chia D, Terasaki PI, Suyama N, Galton J, Hirota M, and Katz D.** Use of monoclonal antibodies to sialylated Lewis^x and sialylated Lewis^a for serological tests of cancer. *Cancer Res* 45: 435–437, 1985.
9. **Foxall C, Watson SR, Dowbenko D, Fennie C, Lasky LA, Kiso M, Hasegawa A, Asa D, and Brandley BK.** The three members of the selectin receptor family recognize a common carbohydrate epitope, the sialyl Lewis(x) oligosaccharide. *J Cell Biol* 117: 895–902, 1992.
10. **Haglund C, Kuusela P, Jalanko H, and Roberts PJ.** Serum CA 50 as a tumor marker in pancreatic cancer: a comparison with CA 19-9. *Int J Cancer* 39: 477–481, 1987.
11. **Hilkens J, Ligtenberg MJ, Vos HL, and Litvinov SV.** Cell membrane-associated mucins and their adhesion-modulating property. *Trends Biochem Sci* 17: 359–363, 1992.
12. **Hynes RO and Lander AD.** Contact and adhesive specificities in the associations, migrations, and targeting of cells and axons. *Cell* 68: 303–322, 1992.
13. **Klinkspoor JH, Kuver R, Savard CE, Oda D, Azzouz H, Tytgat GN, Groen AK, and Lee SP.** Model bile and bile salts accelerate mucin secretion by cultured dog gallbladder epithelial cells. *Gastroenterology* 109: 264–274, 1995.
14. **Lawrence MB and Springer TA.** Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins. *Cell* 65: 859–873, 1991.
15. **Panés J and Granger DN.** Leukocyte-endothelial cell interactions: molecular mechanisms and implications in gastrointestinal disease. *Gastroenterology* 114: 1066–1090, 1998.
16. **Pober JS, Bevilacqua MP, Mendrick DL, Lapierre LA, Fiers W, and Gimbrone MAJ.** Two distinct monokines, interleukin 1 and tumor necrosis factor, each independently induce biosynthesis and transient expression of the same antigen on the surface of cultured human vascular endothelial cells. *J Immunol* 136: 1680–1687, 1986.
17. **Roberts DD, Monsein DL, Frates RCJ, Chernick MS, and Ginsburg V.** A serum test for cystic fibrosis using monoclonal antibody 19-9. *Arch Biochem Biophys* 245: 292–294, 1986.
18. **Springer TA.** Adhesion receptors of the immune system. *Nature* 346: 425–434, 1990.
19. **Stoolman LM.** Adhesion molecules controlling lymphocyte migration. *Cell* 56: 907–910, 1989.

20. **Swain MG, Tjandra K, Kanwar S, and Kubes P.** Neutrophil adhesion is impaired in rat model of cholestasis. *Gastroenterology* 109: 923–932, 1995.
21. **Tedder TF, Steeber DA, Chen A, and Engel P.** The selectins: vascular adhesion molecules. *FASEB J* 9: 866–873, 1995.
22. **Von Ritter C, Eder MI, Stieber P, Lamerz R, Jungst D, Strigl M, Meyer G, Reuter C, and Paumgartner G.** Biliary mucin secreted by cultured human gallbladder epithelial cells carries the epitope of CA 19-9. *Anticancer Res* 17: 2931–2934, 1997.
23. **Walz G, Aruffo A, Kolanus W, Bevilacqua M, and Seed B.** Recognition by ELAM-1 of the sialyl-Lex determinant on myeloid and tumor cells. *Science* 250: 1132–1135, 1990.
24. **Wittig BM, Thees R, Kaulen H, Gott K, Bartnik E, Schmitt C, Meyer zum Buschenfelde KH, and Dippold W.** alpha(1,3)Fucosyltransferase expression in E-selectin-mediated binding of gastrointestinal tumor cells. *Int J Cancer* 67: 80–85, 1996.
25. **Zhang K, Baeckstrom D, and Hansson GC.** A secreted mucin carrying sialyl-Lewis a from colon carcinoma cells binds to E-selectin and inhibits HL-60 cell adhesion. *Int J Cancer* 59: 823–829, 1994.
26. **Zopf D and Hansson GC.** The chemical basis for expression of the sialyl-Le(a) antigen. *Adv Exp Med Biol* 228: 657–676, 1988.