INFECTION AND IMMUNITY, July 1992, p. 2893–2899
0019-9567/92/072893-07$02.00/0

Vol. 60, No. 7

Inhibition of Adhesion of S-Fimbriated *Escherichia coli* to Buccal Epithelial Cells by Human Milk Fat Globule Membrane Components: a Novel Aspect of the Protective Function of Mucins in the Nonimmunoglobulin Fraction

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Received 16 September 1991/Accepted 14 April 1992

We investigated the presence of factors in human milk that inhibit invasion of pathogenic bacteria. The effect of human milk fat globule membrane (HMFGM) components on adhesion of cloned S-fimbriated *Escherichia coli* to human buccal epithelial cells was analyzed. S fimbriae are a common feature of *E. coli* strains causing sepsis and meningitis in newborns and are bound to epithelia via sialyl-(α-2-3)galactoside structures. Human milk fat globules (HMFG) could be agglutinated by the above-mentioned bacteria. Agglutination could be inhibited by fetuin, human glycophorin, and α_1-acid glycoprotein. In addition, pretreatment of HMFG with *Vibrio cholerae* neuraminidase markedly reduced bacterium-induced agglutinations, indicating the involvement of neuraminic acid-containing glycoproteins. In contrast, lipid droplets of infant formula or artificial lipid emulsions (Intralipid) could not be agglutinated. HMFG were present in stools of breast-fed neonates as shown by indirect immunofluorescence staining with a monoclonal antibody directed against carbohydrate residues present on HMFGM. These HMFG could be agglutinated by bacteria. HMFG inhibited *E. coli* adhesion to buccal epithelial cells. To further characterize relevant *E. coli* binding structures, HMFGM components were separated by gel chromatography. The mucin fraction showed the most pronounced inhibitory effect on adhesion of S-fimbriated *E. coli* to human buccal epithelial cells. Our data suggest that HMFG inhibit bacterial adhesion in the entire intestine and thereby may provide protection against bacterial infection.

Human milk provides protection against different types of infections (9). This protective function is based on a complex immunologic system. The importance of immunoglobulins, predominantly secretory immunoglobulin A and other factors such as lysozyme, lactoferrin, the lactoperoxidase system, and cellular components (macrophages, lymphocytes) has been assessed. Further protective properties can be ascribed to nonimmunoglobulin components such as glycolipids, glycoproteins, and free oligosaccharides that are capable of inhibiting bacterial adhesion to epithelia, thus preventing bacterial infection at the stage of bacterial colonization (1, 2, 14, 20).

Adhesion of bacteria to target cells is usually mediated by well-defined glycoproteins on bacterial fimbriae (5, 21, 27). S-fimbriated *Escherichia coli* organisms used in our studies are of major importance in newborns as pathogens causing sepsis and meningitis (23). Adhesins, lectinlike molecules of S fimbriae, recognize receptor structures present in a variety of membrane glycoproteins expressing NeuAc α-2-3 residues (17, 28, 33).

Milk fat in human milk is concentrated in globules with diameters of 1 to 10 μm (29, 35). The membrane surrounding the lipid core is derived from the apical plasma membrane of mammary epithelial cells by apocrine secretion (11, 12). High-molecular-mass glycoproteins with a high content of O-glycosidic-linked carbohydrates (termed mucins) are a major constituent of these membranes (7, 13, 41). As these human milk fat globule membranes (HMFGM) are of epithelial origin, we expected them to express carbohydrate structures that might serve as receptor analogs for certain pathogenic bacteria. For porcine milk fat globule membranes, binding of porcine enteropathogenic K88-positive *E. coli* and inhibition of guinea pig hemagglutination by K88-positive *E. coli* has been described previously (3, 4).

Therefore, we addressed two major questions: (i) can human milk fat globules (HMFG) inhibit binding of S-fimbriated *E. coli* to epithelial cells, and (ii) what are the relevant membrane components mediating inhibition of bacterial adhesion?

MATERIALS AND METHODS

Bacteria. Adhesion experiments were carried out with *E. coli* HB101 with the recombinant plasmid (pANN 801-4) which encodes S fimbriae (17). In controls, *E. coli* HB101 organisms without plasmid and therefore without S fimbriae were used. Only in a few preliminary experiments, because of the instability of S fimbria expression in vitro, strain IH 3084 (kindly provided by T. Korhonen, University of Helsinki, Helsinki, Finland), which was isolated from a patient with neonatal meningitis and which also carries S fimbriae, was used. Bacteria were grown on NB agar (Difco Laboratories, Detroit, Mich.) supplemented with 0.1% glucose and tetracycline (30 μg/ml). After an 18-h incubation period at 37°C, bacteria were harvested with ice-cold 20 mM sodium borate buffer, pH 9.0, and washed twice with the same buffer. Following adjustment to 10^{10} bacteria per ml, 1 ml of the suspension was labelled with 100 μg of fluorescein isothiocyanate (FITC) (Sigma Chemical Co., St. Louis,

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MO.) with constant shaking for 30 min at 23°C. To remove excess FITC, the reaction mixture was layered on top of 4 ml of phosphate-buffered saline (PBS) containing 6% (wt/vol) bovine serum albumin (BSA) and centrifuged (15 min, 1,500 × *g*). The pellet containing labelled bacteria was washed once or twice with PBS (containing 0.1% BSA and 0.5% methyl-α-D-mannoside), pH 7.4, until the supernatant was clear (32).

Isolation of buccal epithelial cells. Buccal epithelial cells were obtained from healthy adult nonsmokers by scraping buccal mucosa with a spoon-shaped spatula several times. Cells were washed off the spatula with PBS, washed three more times, and were microscopically adjusted to 10^5 cells per ml.

Binding of bacteria to buccal epithelial cells and preincubation of bacteria with inhibitors. Bacteria in buffer (2×10^8/ml) or the bacterium-inhibitor mixture was added to 250 μl of epithelial cell suspension (ratio of cells to bacteria, 1:1,000) and incubated for 60 min in a shaking ice bath. Epithelial cells were washed three times with PBS (centrifugation for 5 min at 4°C and 120 × *g*) before microscopic evaluation.

For inhibition studies, 125 μl of the bacterial suspension (2×10^8 bacteria per ml) was incubated with 125 μl of one of the following inhibitors for 15 min in an ice bath: human glycophorin (HGP) (1 mg/ml), α_1-acid glycoprotein (10 mg/ml), fetuin (5 mg/ml), HMFG, HMFGM fractions (1 mg/ml), asialo-HGP (1 and 8 mg/ml), and NeuAc (1 to 20 mg/ml, adjusted to pH 7.4). By using the viable count technique, it was shown that inhibitors did not influence the bacteria's viability. All chemicals were obtained from Sigma.

In order to better define receptor structure, 2×10^5 epithelial cells were treated with *Vibrio cholerae* neuraminidase (1 U/ml; Behring, Marburg, Germany) for 1 h at 37°C and washed twice with PBS. The number of bacteria binding to the cell surface was analyzed by fluorescence microscopy. Fifty epithelial cells were analyzed for each experiment. All experiments were done in duplicate.

Isolation of milk fat globules from human colostrum and stools of breast-fed infants. Fifty milliliters of fresh milk collected on the fourth day of lactation was used for agglutination experiments. The milk was centrifuged at 1,000 × *g* at room temperature for 30 min to obtain cream. The cream was washed four times in 10 volumes of sucrose-PBS (0.25 M sucrose, to which was added 10% PBS, pH 7.4). HMFG in sucrose-PBS were used at a concentration 20 times higher than the concentration resulting in an optical density of 1.8 at 605 nm. Freshly obtained cow's milk was treated in an identical manner. For control experiments, an adapted infant formula (Pre-Aptamil; Milupa) and Intralipid 20% (Kabi Vitrum, Stockholm, Sweden) were used, the latter as a control for artificial phospholipid membranes. Stools of five breast-fed and five formula-fed infants (day 4) as well as the ileostomy secretions of a 10-day-old breast-fed infant with intestinal atresia were mixed with 4 volumes of sucrose-PBS and centrifuged in the same manner as milk. The lipid layer on top was taken off and washed six times with sucrose-PBS. Major particles were removed mechanically between centrifugation steps.

Demonstration of HMFG in stools of breast-fed infants. Fifty microliters of the washed lipid fraction isolated from stools of breast-fed and formula-fed newborns was added to a 1:50,000 dilution of ascitic fluid containing monoclonal antibody directed against HMFGM, 115 G 3 (kindly provided by J. Hilkens, University of Amsterdam, Amsterdam, The Netherlands). The antibody recognizes the carbohydrate structure Galβ-1-3[Fucα-1-4]-GlcNAcβ-1-3-Galβ-1-4-[Fucα-1-3]-GlcNAcβ (Lewis^a and Lewis^x sequence) (15). The mixture of antibody and lipid fraction was incubated for 10 min in an ice bath with gentle shaking. Cross-reaction has been shown for mucins in various body fluids. Excess antibody was removed by washing before 20 μl of a phycoerythrin-labelled goat anti-mouse antibody (Southern Biotechnology Associates, Birmingham, Ala.) diluted 1:10 in PBS was added. Following another incubation period of 10 min at 4°C in the dark, 200 μl of sucrose-PBS was added for fluorescence microscopy. Nonspecific mouse immunoglobulin (Sigma) was used in a control experiment instead of the monoclonal antibody.

Preparation of HMFGM. Freshly secreted human breast milk (first 14 days of lactation) was obtained from volunteers, pooled, and defatted by centrifugation at 3,000 × *g* for 1 h at 4°C. The cream was washed several times in 5 volumes of 0.01 M Tris HCl, pH 7.2, by centrifugation under the same conditions. Washed cream from approximately 3 liters of human milk was suspended in a total volume of 500 ml of 0.01 M Tris HCl, pH 7.2, and kept in the cold (4°C) for 1 h. The suspension was then allowed to reach room temperature while it was shaken on a laboratory shaker until butter formed. The process of membrane release was completed by incubation of the mixture at 40°C for 30 min. The membranes were recovered as a yellow pellet after centrifugation at 35,000 × *g* (30 min) and washed twice with water prior to freeze-drying.

Agglutination of HMFG and HMFGM by bacteria. Agglutinations of HMFG from colostrum and stools and lipid droplets from infant formula and Intralipid suspended as described above were analyzed after mixture of 1 drop of suspension with 1 drop of bacterial (*E. coli* HB101 [pANN 801-4] and *E. coli* IH 3084) suspension (5×10^9/ml) on a glass slide at 4°C and room temperature with gentle shaking. Sonicated HMFGM were treated in an analogous manner. In control experiments, bacteria without plasmid were used.

In inhibition experiments, 20 μl of bacterial suspension was mixed with 20 μl of putative inhibitor at different concentrations for 15 min at 4°C. NeuAc served as a monovalent control, and asialo-HGP served as a polyvalent control. Agglutination intensity was read macroscopically and microscopically. To determine the role of NeuAc in HMFG residues responsible for bacterial binding, 40 μl of HMFG was incubated with 1 ml of *V. cholerae* neuraminidase (1 U/ml) at 37°C, washed, and then added to the bacteria.

Isolation and immunochemical characterization of HMFGM mucin. Solubilization of the membrane proteins and fractionation according to their sizes were performed as described by Shimizu and Yamauchi (41). Fractions HMFG-A to HMFG-D (see Fig. 2) were dialyzed against water at 4°C, freeze-dried, and delipidated by extraction with cold methanol-chloroform (1/2, vol/vol).

HMFG-A was immunochemically identified as the polymorphic epithelial mucin-containing fraction after chemical deglycosylation with trifluoromethanesulfonic acid according to the method of Hakimuddin and Bahl (18). Binding of monoclonal antibody SM-3 defining the peptide sequence Pro-Asp-Thr-Arg-Pro (6) was analyzed in a double-sandwich solid-phase enzyme immunoassay by using anti-mouse immunoglobulin (Z 259; Dako, Hamburg, Germany) and anti-alkaline phosphatase-alkaline phosphatase complex detection.

Isolation and purification of S fimbriae. S fimbriae were isolated essentially according to the procedure described by Salit and Gotschlich (37) as modified by Wevers et al. (43).

TABLE 1. Inhibition of bacterial adhesion (*E. coli* HB101 [pANN 801-4]) to human buccal epithelial cells[a]

Inhibitor	Concn (mg/ml)	Inhibition of adhesion[b] (%)
α_1-Acid glycoprotein[c]	10	43 ± 11
Fetuin[c]	5	42 ± 7
HGP[c]	1	49 ± 12
Asialo-HGP	1	9 ± 4
Asialo-HGP	8	28 ± 6
NeuAc	1–20	1 ± 2

[a] Specificity of bacterial adhesion has been confirmed in control experiments by neuraminidase treatment of buccal epithelial cells.
[b] Means ± standard deviations for five experiments.
[c] Inhibitors were used at concentrations of 1 to 20 mg/ml, and values corresponding to approximately 50% are given. The actual decrease of adhesion in percent is presented if 50% inhibition could not be reached.

The purity of the preparation was confirmed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and examination by electron microscopy.

Electrophoresis and binding of fimbriae to transblots. Glycoprotein samples (1 mg preincubated in 100 μl of sample buffer containing 0.3% SDS and 3% 2-mercaptoethanol for 10 min at 90°C) were separated on polyacrylamide gels (7.5% or 3 to 13%) in the presence of SDS (0.1%) at 40 mA. Gels were fixed and stained with Coomassie blue or periodic acid Schiff's (PAS) reagent. Electroblotting onto nitrocellulose (0.45 μm) was performed at 200 mA in Tris (25 mM)–glycine (192 mM)–methanol (20%).

The nitrocellulose sheets were immersed in PBS, pH 7.2, containing 5% (wt/vol) BSA to block residual surface activity and overlaid with a solution of lectin peroxidase conjugate diluted in 0.5% BSA-PBS (5 to 10 μg/ml). As lectins, peroxidase-labelled lectins from *Triticum vulgaris* (WGA) and from *Arachis hypogeae* (PNA) were used. After incubation for 1 h at room temperature and three successive washing steps (0.5% BSA-PBS), the membranes were developed in PBS-methanol (17% [vol/vol]) containing 1-chloro-4-naphthol (0.055%) and hydrogen peroxide (0.008%). Maximum staining was obtained after 30 min in the dark. For binding of purified fimbriae, membranes were overlaid for 17 h with fimbriae suspended in PBS (125 μg of protein per ml) or with fimbriae and HGP (1 mg/ml) at 4°C.

For immunostaining, membranes were incubated for 1 h at 4°C with monoclonal antibody A 21356 A1 (1:1,000), which recognizes S fimbriae (28) (kindly provided by K. Jann). Alkaline phosphatase-conjugated anti-mouse immunoglobulin (1:1,000) (Dako) was added for 30 min at room temperature and washed four times with PBS. Membranes were developed with 5-bromo-4-chloro-3-indoxyl-phosphate (Na_2 salt) and Nitro Blue Tetrazolium chloride (Serva, Heidelberg, Germany) for 15 min at 37°C.

RESULTS

Adhesion of S-fimbriated *E. coli* to human buccal cells. Fluorescence microscopy of buccal epithelial cells after incubation with FITC-labelled S-fimbriated *E. coli* showed that 75 to 95% of epithelial cells from 10 different donors had bound bacteria. On average, 1,103 (±293) bacteria had bound to 50 cells. Characteristics of binding of the clinical isolate IH 3084 to buccal epithelial cells did not differ from those for the cloned strain HB101 (pANN 801-4) (data not shown). The abilities of human glycophorin, fetuin, and α_1-acid glycoprotein to inhibit binding were markedly different. For quantitative comparison of the efficacy of inhibition, concentrations resulting in approximately 50% inhibition were compared with each other (Table 1). If inhibition was less than 50%, individual values are given. NeuAc as a monovalent control showed no inhibition; asialo-HGP as a polyvalent control substance was slightly able to inhibit binding. Pretreatment of epithelial cells with neuraminidase markedly reduced the number of binding bacteria to 27% of the control, indicating the relevance of NeuAc for binding of S fimbriae.

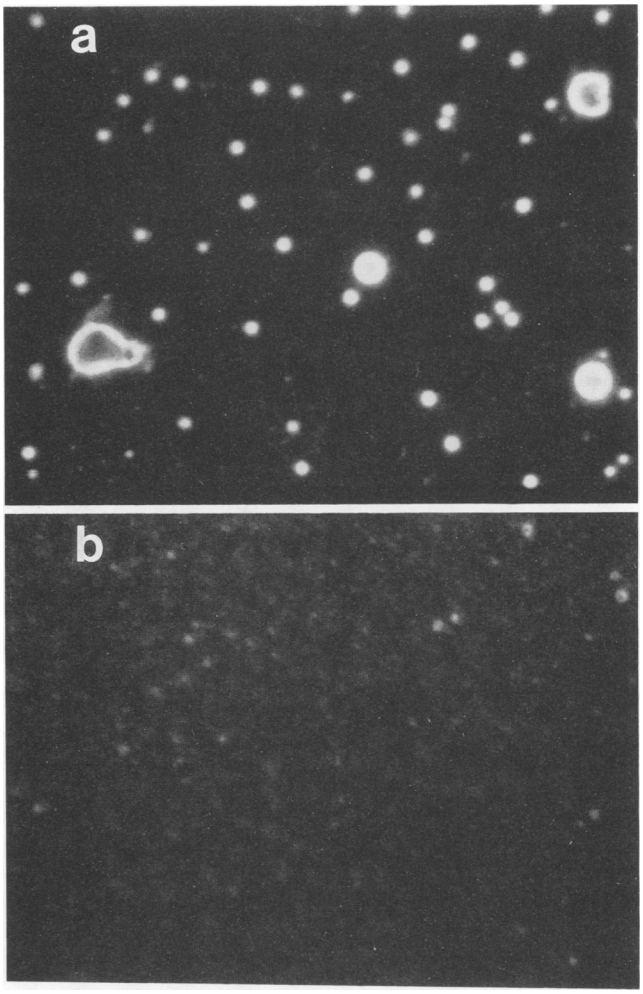

FIG. 1. (a) Demonstration of HMFG in stool of a breast-fed newborn by indirect immunofluorescence with monoclonal antibody 115 G 3. Magnification, ×600. (b) Stool of a formula-fed newborn prepared as described for panel a.

Agglutination of HMFG and HMFGM by bacteria. HMFG isolated from colostrum were agglutinated by S-fimbriated *E. coli* HB101 (pANN 801-4) and IH 3084 after 10 min, as shown macroscopically and microscopically (data not shown). HB101 without plasmid failed to agglutinate HMFG. Raising the temperature from 4°C to room temperature markedly accelerated the reaction. This agglutination was probably due to specific interaction of bacteria with HMFGM because isolated membranes could also be aggregated (data not shown).

By using FITC-labelled monoclonal antibody (115 G 3) directed against HMFGM (15), undigested HMFG could be demonstrated in both normal stools of breast-fed infants and

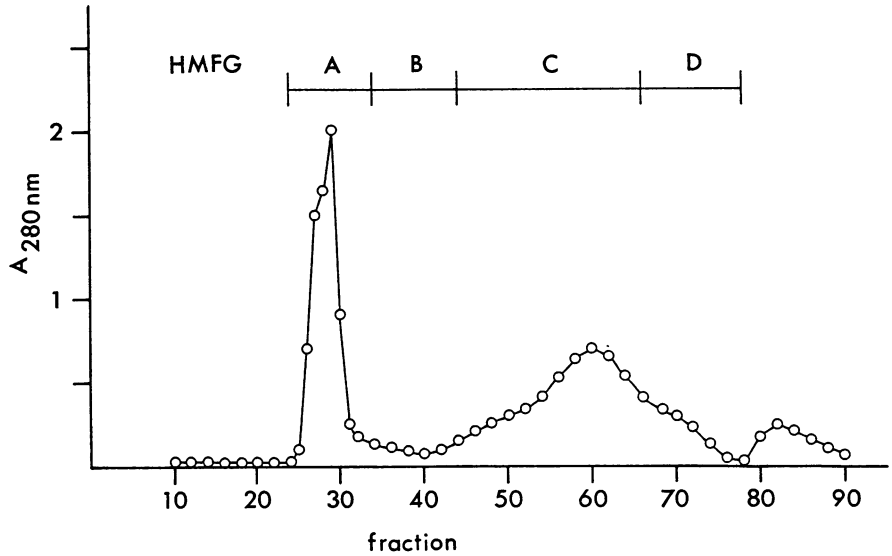

FIG. 2. Sepharose CL-4B column chromatography of membrane proteins from HMFG. Gel exclusion chromatography of extracted membranes was performed on a Sepharose CL-4B column (1.5 by 60 cm), equilibrated, and run in 15 mM Tris-HCl buffer (pH 8.0) containing 100 mM NaCl and 10 mM sodium deoxycholate (flow rate, 6 ml/h). The eluate was monitored at 280 nm and pooled in subfractions HMFG-A to HMFG-D as indicated. Fractions HMFG-A and HMFG-B contain the majority of HMFGM mucins (41).

intestinal fluid from one breast-fed infant with ileostomy. In contrast, stools of formula-fed infants were devoid of HMFG (Fig. 1a and b).

Nonspecific mouse immunoglobulin G did not react with HMFG. Globules isolated from stools could still be agglutinated by bacteria. Agglutination was not altered in slightly acidic conditions (stool of breast-fed infants, pH 5). In contrast, lipid droplets isolated from adapted infant formula or Intralipid showed no reactions with bacteria either macroscopically or microscopically. Milk fat globules isolated from fresh cow's milk reacted, but the agglutination was weaker compared with that of HMFG.

Pretreatment of HMFG with neuraminidase almost completely abrogated agglutinability. Agglutination could be inhibited with concentrations of inhibitors identical to those used in the buccal epithelial cell binding assay. Inhibition by all effective inhibitors was dose dependent from 0.15 to 20 mg/ml.

Inhibition of bacterial adhesion by HMFG and HMFGM fractions. Membranes prepared from milk fat globules of approximately 3 liters of human breast milk yielded 1 g of freeze-dried HMFGM. The solubilized membrane components were separated according to their size on a Sepharose CL-4B column into four major fractions corresponding to mucins (HMFG-A+B), sialoglycoproteins (HMFG-C), and glycolipids and phospholipids (HMFG-D) (41) (Fig. 2). The different fractions (HMFG-A to HMFG-D) obtained from column chromatography had extensively been characterized previously by Shimizu and Yamauchi (HMFG-A to HMFG-D corresponding to fractions F1 to F4 in their study) (41).

Patterns of SDS-polyacrylamide gel electrophoresis obtained from HMFG and their chromatographic subfractions are shown in Fig. 3. Staining with Coomassie blue revealed a series of 10 major bands, most of which were PAS-reactive glycoproteins. The prominent mucin band at the top of the gel was found exclusively in fractions HMFG-A and -B, which appeared to be homogenous on the basis of Coomassie blue and PAS staining. A Western blot (immunoblot) analysis of HMFGM glycoproteins by staining with WGA or PNA revealed strong binding activity by the HM FGM mucin for both lectins, while the membrane glycoproteins were stained only moderately or not at all (Fig. 3). The presence of PNA-reactive Thomsen-Friedenreich antigen (Galβ-1-3-GalNAc) on HMFGM mucin is a characteristic feature of this membrane component from lactating mammary epithelia (13). A high sialic acid content of the HMFGM mucin is indicated by its strong binding capacity for WGA on Western blots (41). In addition, polymorphic epithelial mucin was identified in fraction HMFG-A by using monoclonal antibody SM-3 (data not shown).

HMFG-A and -B contain the mucins, but fraction A is regarded to be contaminated by other proteins responsible for the strong absorption at 280 nm. The pure mucin in fraction B does not absorb at 280 nm because of its lack of aromatic amino acids. Accordingly, there is no relationship between antiadhesive effects and the protein profile registered at 280 nm.

HMFG and isolated fractions HMFG-A to -C were then functionally analyzed for the capacity to inhibit adhesion of our *E. coli* strain to buccal epithelial cells. The observed inhibition effects of HMFG (inhibition of adhesion, 46 to 55%) are probably due to a competition of HMFG versus buccal epithelial cells on a cellular level. On the other hand, in addition to reversible competition, irreversible entrapment of bacteria by HFMG agglutination is likely to contribute to the antiadhesive effect.

The strongest inhibitory effect of HMFGM fractions (55%) was located in mucin-containing fractions HMFG-A and HMFG-B (Table 2). The efficiency of inhibition by fraction HMFG-A+B was comparable to that of HGP. Equal concentrations of HMFG-C sialoglycoproteins had a markedly less pronounced inhibitory effect (18% inhibition).

Western blot analysis of HMFGM glycoproteins by immunostaining of bound S fimbriae revealed strong binding activity by high-molecular-mass glycoproteins of HMFGM (Fig. 4). Binding of fimbriae was inhibited by the receptor analog HGP (Fig. 4). The glycoproteins were positive on PAS and WGA staining and negative on Coomassie blue

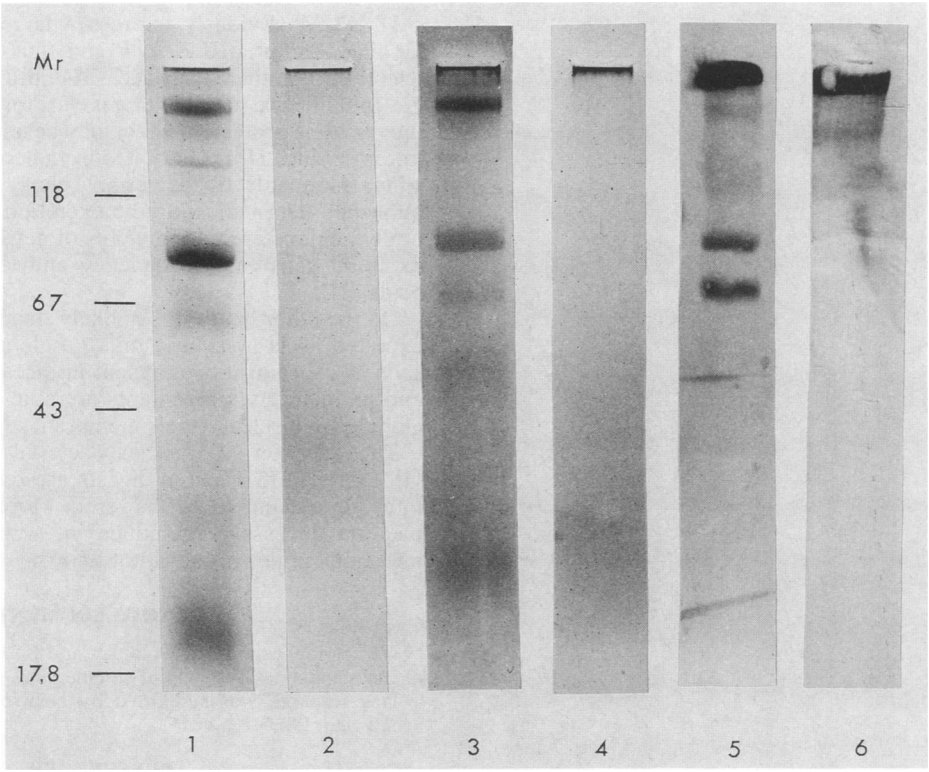

FIG. 3. Polyacrylamide gel electrophoresis and Western blot analysis of HMFGM proteins. Membrane proteins were separated in a 7.5% polyacrylamide gel in the presence of 0.1% SDS and stained with Coomassie blue (lanes 1 and 2) or PAS (lanes 3 and 4). Lanes: 1 and 3, HMFGM; 2 and 4, Sepharose CL-4B subfraction HMFG-A; 5 and 6, Western blots of HMFGM proteins stained with WGA (lane 5) or PNA (lane 6). The following proteins we used for calibration: β-galactosidase (118 kDa), BSA (67 kDa), ovalbumin (43 kDa), and horse myoglobin (17.8 kDa). Mr, molecular mass.

staining (data not shown). These high-molecular-mass glycoproteins should be regarded as mucins on the basis of several lines of evidence. The binding active components in Western blots are characterized by their molecular masses, which are higher than 200 kDa; a high carbohydrate content as demonstrated by staining with PAS and WGA; and, finally, by cochromatography with polymorphic epithelial mucin from human skim milk (Fig. 4).

DISCUSSION

In the present study, the ability of milk fat globules or membrane fractions to inhibit adhesion of S-fimbriated *E. coli* to epithelial cells has been analyzed. The most meaningful test system to study antiadhesive properties of milk fat globules present in stools is to use intestinal epithelia from newborns. Viable intestinal epithelia are difficult to obtain, and human intestinal cell lines as a putative alternative undergo morphological change with passage in tissue culture (10).

TABLE 2. Inhibition of bacterial adhesion (*E. coli* HB101 [pANN 801-4]) to human buccal epithelial cells

Inhibitor	Concn (mg/ml)	Inhibition of adhesion[a] (%)
HMFG	—[b]	55 ± 3
HMFG	—[c]	46 ± 13
HMFG-A+B	1	55 ± 6
HMFG-C	1	18 ± 14

[a] Means ± standard deviations for five experiments.
[b] One hundred twenty-five microliters of HMFG suspension corresponding to 7.5 μg of HMFGM containing 2.5 μg of Lowry protein.
[c] Sixty-three microliters of HMFG suspension corresponding to 3.8 μg of Lowry protein.

Although adhesive properties of a given bacterial strain vary depending on the tissues used (42), we utilized buccal epithelial cells for two reasons: first, oral epithelia are an easily accessible part of the gastrointestinal tract, and, second, the oropharynx together with the intestine serves as a natural entry route for invasive pathogenic *E. coli* (16, 39). Adhesion of S-fimbriated *E. coli* to buccal epithelial cells is, like that of type I fimbriae carrying *E. coli*, age independent (8, 40). Therefore, we used epithelial cells from adults as a model of bacterial adhesion.

The concept of bacterial adherence to milk fat globules has already been introduced in a variety of veterinary publications with reports of studies using *E. coli* K99 and K88 (3, 4, 25, 36). Our studies show that these observations may be relevant for humans since pathogenic *E. coli* bind to HMFG and cause their agglutination. Agglutination was specifically inhibited by soluble receptor analogs (fetuin and HGP) or treatment of HMFG with neuraminidase. This indicates a major role of NeuAc-containing glycoproteins as receptors for S-fimbriated bacteria. Lipid droplets derived from adapted infant formula or an intravenous fat emulsion are not surrounded by biomembranes and do not bind bacteria.

The role of mucins in the prevention of bacterial adhesion to epithelial tissues has been documented in recent studies (12, 26, 38). Up to 200 carbohydrate chains are known to be

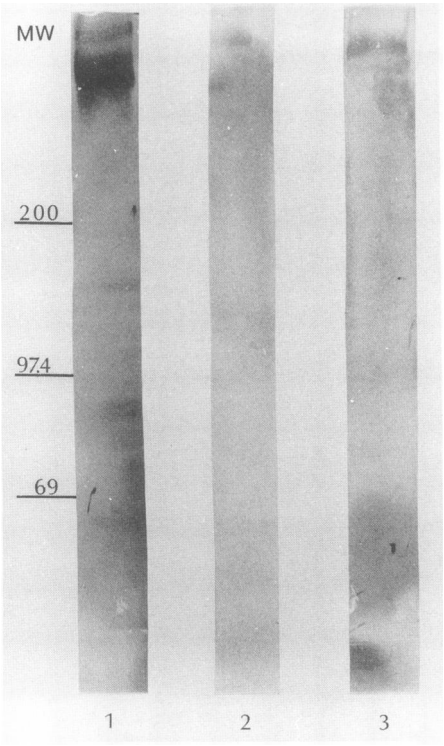

FIG. 4. Binding of isolated S fimbriae to immobilized glycoproteins on Western blots. Membrane proteins were separated on a 3 to 13% polyacrylamide gel in the presence of 0.1% SDS. Western blots of HMFGM proteins (lanes 1 and 2) and mucin isolated from human skim milk (lane 3) were stained immunochemically after being overlaid with purified S fimbriae as described in Materials and Methods. Skim milk mucin, which was available from previous work (19), was identified as the polymorphic epithelial mucin after chemical deglycosylation and immunochemical staining with monoclonal antibody SM-3. Lanes: 1, 0.5 mg of HMFG membranes overlaid with S fimbriae; 2, 0.5 mg of HMFG membranes overlaid with S fimbriae in the presence of HGP (1 mg/ml); 3, 100 μg of mucin isolated from human skim milk, overlaid with S fimbriae.

O-glycosidically linked to the peptide core of a mucin monomer exhibiting a high degree of structural diversity. According to this particular property, mucins which are found in human secretions or in epithelial membranes are suitable candidates for representing organ-characteristic glycosylation and thus express a variety of receptor-analog structures for bacterial adhesins. Mucins are regular constituents of human milk serum (19) and HMFGM (13). The common structural unit of all acidic glycans on secretory milk mucins is the terminal disaccharide unit NeuAcα-(2-3)-Galβ, which has been demonstrated to form a receptor analog for S-fimbriated *E. coli* (22).

The protective potential of membrane sialoglycoproteins and mucin on the milk fat globule against bacterial infections has not been demonstrated so far. But it is strongly suggested by the reported findings, in particular by the sialic acid-mediated binding of S-fimbriated *E. coli* to HMFGM and by the ability of HMFG and the isolated membrane mucins (fraction HMFG-A+B), to inhibit adhesion of these bacteria to buccal epithelial cells.

Recently, it has been reported that milk fat globule mucins in receptor binding assays have inhibitory activity (31) against human immunodeficiency virus and rotavirus (30).

HMFGM obviously express a large surface capable of binding S-fimbriated *E. coli* and thus decrease binding of bacteria to epithelial surfaces. As both buccal epithelia and gut epithelia are possible target cells for S-fimbriated *E. coli*, the resulting protective effect may be important for the entire intestine since HMFG isolated from ileostomy and stools are still agglutinable by bacteria. This is in accordance with published data indicating the excretion of up to 10% of the orally administered fat in stools of newborns due to relative exocrine pancreas insufficiency and low excretion of bile acids (24).

On the other hand, it is unlikely that the whole amount of excreted fat is present in milk fat globules: conjugated bile salts at concentrations present in digests of term newborns and prematurely born infants are able to break up milk fat globules and release their membranes (34).

The question of whether bacterial binding capacity of HMFG and HMFGM in digests are relevant in vivo in the intestine remains to be answered. Moreover, the number of bacteria necessary to induce intestinal or extraintestinal infections in newborns is not known.

ACKNOWLEDGMENTS

We acknowledge A. S. Goldman and K. Jann for critical reading of the manuscript and helpful comments.

This research was supported by Deutsche Forschungsgemeinschaft Schr 281/3-2.

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