INFECTION AND IMMUNITY, Dec. 1991, p. 4524–4530
0019-9567/91/124524-07$02.00/0

Vol. 59, No. 12

Identification of the Leukocyte Adhesion Molecules CD11 and CD18 as Receptors for Type 1-Fimbriated (Mannose-Specific) *Escherichia coli*

AWNI GBARAH,[1] CARL G. GAHMBERG,[2] ITZHAK OFEK,[3] URI JACOBI,[3] AND NATHAN SHARON[1]*

Department of Biophysics, The Weizmann Institute of Science, Rehovot,[1] and Department of Human Microbiology, Sackler School of Medicine, Tel-Aviv University, Ramat Aviv, Tel-Aviv,[3] Israel, and Department of Biochemistry, University of Helsinki, Helsinki, Finland[2]

Received 10 June 1991/Accepted 4 September 1991

Attachment of bacteria to phagocytic cells may be mediated by lectin-carbohydrate interactions, resulting in lectinophagocytosis. The best-studied system is the interaction of type 1-fimbriated (mannose-specific) *Escherichia coli* with human phagocytic cells. Here we demonstrate that the leukocyte integrins CD11 and CD18 (CD11/CD18) constitute the major receptors for type 1-fimbriated *E. coli*. Bacteria were bound in a dose-dependent and saturable manner to CD11/CD18, which was immobilized to microwells, whereas nonfimbriated *E. coli* cells failed to bind. The binding was efficiently inhibited (82 to 85%) by methyl-α-mannoside but not by galactose, and it was reduced by treatment of the immobilized CD11/CD18 with sodium metaperiodate, endoglycosidase H, or a mixture of endoglycosidase F and *N*-glycosidase. The fimbriated bacteria also bound to CD11a,b,c and CD18 separated by polyacrylamide gel electrophoresis with sodium dodecyl sulfate and blotted onto nitrocellulose paper. This binding was inhibited specifically by methyl-α-mannoside and was significantly diminished by treatment of the blots with sodium metaperiodate. Only minimal binding to the blotted CD11/CD18 that had been deglycosylated enzymatically prior to electrophoresis was observed. On blots of granulocyte lysates, specific binding to two glycoproteins (M_rs, 90,000 to 100,000 and 165,000) with mobilities similar to that of CD11/CD18 was observed. Monoclonal antibodies to CD11a, CD11b, or CD18 inhibited the binding of the bacteria to intact human granulocytes by 55 to 80%, whereas antibodies against other leukocyte surface antigens were not inhibitory. We conclude that type 1-fimbriated *E. coli* binds to human granulocytes via the oligomannose and hybrid N-linked units of CD11/CD18. Since CD11b/CD18 and CD11c/CD18 are known to serve as receptors for complement fragment iC3b, this study provides a link between opsonophagocytosis and lectinophagocytosis.

Opsonophagocytosis of bacteria is an extensively investigated process in which recognition between phagocytes and their target cells is mediated by antibody, complement, or both (18, 35). Evidence obtained during the past few years has shown that in the absence of opsonins, phagocytes may recognize bacteria by lectin-carbohydrate interactions, with resultant activation of the phagocytes and uptake and killing of the bacteria (15, 27). This process has been named lectinophagocytosis (26), in analogy to opsonophagocytosis. The lectins can be located either on the bacteria or on the phagocytic cells, and they react with the carbohydrates on the surfaces of the apposing cells. The best-characterized system of lectinophagocytosis is that of bacteria carrying mannose-specific lectins in the form of type 1 fimbriae that bind mannose residues on human or mouse phagocytes (6, 7, 9, 15, 27).

Little is known, however, about the nature of the receptors for the bacteria on the phagocytes. In a previous study (31), which used affinity chromatography on immobilized fimbriae, three glycoproteins were isolated from human granulocytes, two of which migrated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as the leukocyte adhesion molecules or antigens CD11 and CD18 (CD11/CD18). These antigens, which belong to the integrin receptor superfamily, consist of three surface membrane heterodimeric glycoproteins, each of which is composed of a different α subunit (CD11a [M_r, 160,000 to 180,000], CD11b [M_r, 155,000 to 165,000], and CD11c [M_r, 130,000 to 150,000]) and the same β subunit (CD18 [M_r 90,000 to 95,000]) (2, 28, 36). The heterodimers thus formed are CD11a/CD18 (also known as LFA-1), CD11b/CD18 (Mac-1 or Mol), and CD11c/CD18 (Leu M5 or gp150,95). CD11/CD18 antigens contain only N-linked oligosaccharides, 38% of which are of the oligomannose type and 5% of which are of the hybrid type (3). Here we present data showing that type 1-fimbriated *Escherichia coli* binds specifically to CD11/CD18 and that the binding occurs via the oligomannose and hybrid units of the glycoproteins. It is known that CD11b/CD18 and CD11c/CD18 function in opsonophagocytosis, since they serve as receptors for iC3b, a fragment of complement C3 generated by cleavage of C3b (2, 28, 36). Our findings thus provide a link between opsono- and lectinophagocytosis.

MATERIALS AND METHODS

Materials. ^{125}I, 3-amino-8-ethylcarbazole, and *N*,*N*-dimethylformamide were purchased from the Radiochemical Centre (Amersham, Bucks, United Kingdom). Phenylmethylsulfonyl fluoride, Triton X-100, Tween 20, lactoperoxidase, dextran (average molecular weight, 488,000), sodium deoxycholate, bovine serum albumin (BSA), sodium fluoride, ATP, concanavalin A, horseradish peroxidase, and 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) were obtained from Sigma (St. Louis, Mo.). Cyanogen bromide-

* Corresponding author.

activated Sepharose 4B and Ficoll-Paque were obtained from Pharmacia (Uppsala, Sweden). Methyl-α-D-mannoside (MeαMan) and D-galactose were from Pfanstiehl (Waukegan, Ill.). Nitrocellulose paper (BA-8S, 0.45-μm pore size) was from Schleicher & Schuell (Dassel, Germany). *Saccharomyces cerevisiae* (baker's yeast) was a product of Standard Brands (New York, N.Y.). Rabbit polyclonal antibody to the CD11/CD18 complex was a gift of C. Kantor (Department of Biochemistry, University of Helsinki). Rabbit polyclonal antibody to *E. coli* 346 was prepared as described elsewhere (29). Aggregated human immunoglobulin G (IgG) was obtained by incubation of IgG (Travenol, Lessines, Belgium) at 63°C for 30 min (32). Anti-rabbit IgG developed in donkeys and linked to horseradish peroxidase was from BDH Chemicals (Poole, United Kingdom). Anti-rabbit IgG developed in goats and linked to horseradish peroxidase was from BioMakor (Rehovot, Israel). Anti-rabbit IgG (Fc) developed in goats and conjugated to alkaline phosphatase, as well as nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate, was from Promega (Madison, Wis.). Sodium metaperiodate was from E. Merck AG (Darmstadt, Germany). Endoglycosidase H (endo-H) was from Seikagaku Kogyo (Tokyo, Japan), and endoglycosidase F–*N*-glycosidase (endo-F/N) was from Boehringer GmbH (Mannheim, Germany). All other chemicals were of the highest purity available commercially.

Monoclonal antibodies. The following monoclonal antibodies, which react with intact human granulocytes, were used: 2E7, 7E4, 11D3 (23), and MAb59 (38) against CD18; MEM25 against CD11a (8); 3F4 against CD11b (prepared by P. Nortamo, Department of Biochemistry, University of Helsinki); SHCL3 against CD11c (34); and 84-3C1 against the major sialoglycoprotein (CD43) of leukocytes (10).

Bacteria. A uropathogenic isolate of *E. coli* (strain 346) was grown in brain heart infusion broth (Difco, Detroit, Mich.) without agitation for 24 h at 37°C. These conditions are optimal for expression of mannose-specific activity, as determined by yeast aggregation (24). Only bacteria exhibiting yeast aggregation activity of 5 to 10 U/min at a concentration of 10^9 bacteria per ml were used for further work. They were routinely stored at −70°C in phosphate-buffered saline (PBS) (0.15 M NaCl in 0.05 M phosphate buffer [pH 7.4]) containing 10% glycerol. To obtain the nonfimbriated phenotype, the bacteria were grown on agar plates, harvested, and stored, as described above (9).

Isolation of CD11/CD18. The CD11/CD18 glycoprotein complex was purified from 250 ml of packed human blood leukocytes (a gift of the Finnish Red Cross Blood Transfusion Service) by immunoaffinity chromatography on monoclonal antibody 11D3 (specific for CD18) coupled to Sepharose 4B (20), with a yield of 5.4 mg of protein. The isolation of human granulocytes has been previously described (15).

Binding assay in microwells. The ability of the isolated antigens to bind the bacteria was measured by enzyme-linked immunosorbent assay (ELISA) (4). For this purpose, the antigens or BSA (50 μl of a 64-μg/ml PBS solution) was dispensed into wells of a microtiter plate (Immulon 2; Dynatech, McLean, Va.), and the proteins were allowed to adsorb for 24 h at 4°C. The wells were washed four times with PBS containing 0.05% Tween 20 (PBS–0.05% Tween) and incubated overnight with 100 μl of BSA (5%, wt/vol) in PBS (PBS–5% BSA) at 4°C. After removal of the supernatant, fimbriated or nonfimbriated bacteria were added in a 50-μl solution of PBS containing 1% BSA (PBS–1% BSA), with or without inhibitors, and incubated for 1 h at 37°C. The wells were washed four times with PBS–0.05% Tween and were incubated for 1 h at 37°C with 50 μl of rabbit anti-*E. coli* polyclonal antibody diluted 1:210 in PBS–1% BSA, and then they were washed four times with PBS–0.05% Tween. Bound bacteria were visualized by incubation with peroxidase-conjugated goat anti-rabbit IgG for 1 h at 37°C followed by incubation with 0.25 mg of substrate 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) in 1 ml of 0.05 M citrate buffer (pH 4.0) containing hydrogen peroxide diluted 1:6,000 (from a stock solution of 30%). A_{405} was recorded after 15 min at 37°C with an ELISA apparatus (Bio-Tek Instruments, Burlington, Vt.). A standard curve giving the relation between the absorbance and the number of bacteria was generated as described elsewhere (4).

Protein blotting and bacterial overlay. The purified antigens or the granulocyte lysates were separated by SDS-PAGE (21) and then blotted onto nitrocellulose sheets (11). To test binding of bacteria, the nitrocellulose sheets were cut into strips (5 by 1.8 cm) and incubated in PBS–5% BSA at 4°C for 12 h. Binding of the bacteria to blotted CD11/CD18 was measured by ELISA as follows. The strips were washed with PBS–0.05% Tween and incubated for 12 h at 4°C in 15 ml of a solution of PBS containing 10^8 bacteria per ml and BSA (2.5% wt/vol), in the absence or presence of sugar. The strips were washed four times with Tris buffer (pH 7.4) containing 0.05% Tween 20, and they were incubated for 1 h at 37°C with rabbit anti-*E. coli* polyclonal antibodies (diluted 1:210) in Tris buffer containing BSA (1%, wt/vol). Excess antibody was removed by washing the strips four times with Tris buffer containing 0.05% Tween 20. The strips were incubated for 1 h at 37°C with anti-rabbit IgG (Fc) antibodies conjugated with alkaline phosphatase (1:2,000 in Tris buffer containing 1% BSA). Bound bacteria were visualized by the addition of a substrate solution (0.33 mg of nitroblue tetrazolium and 0.16 mg of 5-bromo-4-chloro-3-indolylphosphate per ml of a buffer composed of 100 mM Tris, 100 mM NaCl, and 5 mM $MgCl_2$ [pH 9.5]). To test the binding of bacteria to human granulocyte lysates, the binding assay was performed as described above, but bound bacteria were detected by the addition of anti-rabbit IgG developed in donkeys and conjugated to horseradish peroxidase (1:1,000 in PBS–1% BSA). The bands were visualized by the addition of substrate solution (0.04% 3-amino-9-ethylcarbazole, 5% *N*,*N*-dimethylformamide, and 0.05% H_2O_2 in 0.05 M acetate buffer [pH 5]). When bands became clearly visible (usually after 2 to 3 min), the substrate was removed and the reaction was stopped by washing in water. The binding of concanavalin A to blots of granulocyte lysates was detected as described elsewhere (17).

Periodate oxidation. The nitrocellulose strips or the microwells containing the immobilized CD11/CD18 were washed once with PBS, incubated with 10 mM sodium metaperiodate in PBS at 37°C for 1 h (16, 25), washed four times with PBS, and then overlaid with bacteria as described above. To assay the binding of specific polyclonal anti-CD11/CD18 antibodies to the treated antigens, 50 μl of a 1:800 dilution of antiserum was added to each well and incubated for 1 h at 37°C. Binding of the antibodies was visualized as described above.

Labeling. Bacteria were labeled with ^{125}I by the lactoperoxidase method (22). Under these conditions, the radioactivity incorporated was 0.8 cpm per bacterium. CD11/CD18 (20 μg) was iodinated for 1 min at room temperature by the chloramine T method (19). The specific activity obtained was 2.3×10^6 cpm/μg of protein.

Enzymatic treatment of CD11/CD18. Endo-H was used in 0.15 M citrate phosphate buffer (pH 5), while endo-F/N was

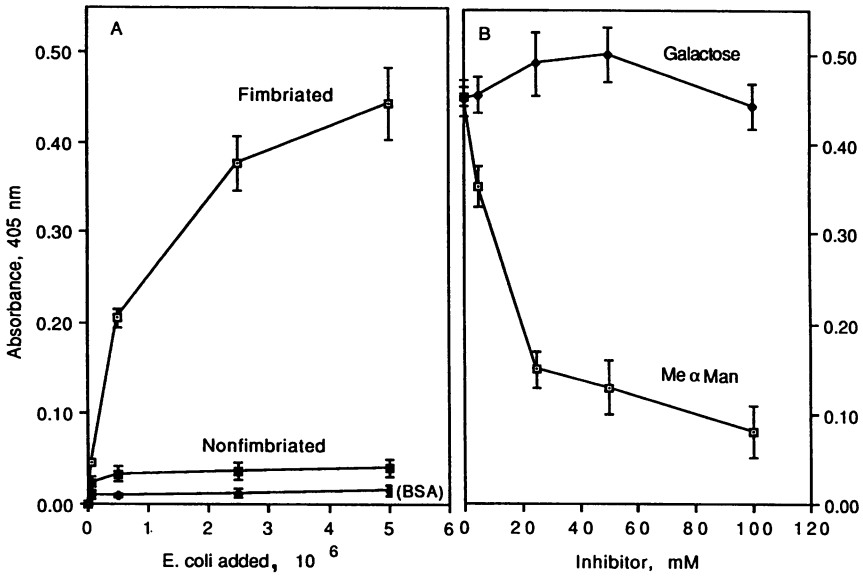

FIG. 1. (A) Binding of nonfimbriated and type 1-fimbriated *E. coli* to CD11/CD18 and of fimbriated *E. coli* to BSA adsorbed to microwells. The purified antigens or BSA (50 μl of a 64-μg/ml solution) was immobilized on microtiter plates and incubated with 50 μl of different concentrations of fimbriated or nonfimbriated bacteria at 37°C for 1 h. The binding of bacteria to the antigens or to BSA was visualized by ELISA, as described in the text. (B) Inhibition of binding of type 1-fimbriated *E. coli* to CD11/CD18 adsorbed to microwells. The CD11/CD18 complexes (50 μl of a 64-μg/ml solution) were adsorbed to microtiter wells, and the wells were incubated for 1 h at 37°C with 5×10^6 bacteria in 50 μl of PBS in the absence or presence of MeαMan or galactose at different concentrations.

used in 0.05 M potassium phosphate buffer (pH 7). To 20 μg of CD11/CD18 in 0.3 ml of the appropriate buffer, radioactively labeled CD11/CD18 (0.5×10^6 cpm) was added as marker for the mobility of the glycoproteins in SDS-PAGE by autoradiography. Endo-H or endo-F/N, at a final concentration of 0.015 or 0.075 U/ml, respectively, was added, and the mixture was incubated for 20 h at 37°C. Control samples were incubated in the respective buffers without enzyme. The ability of bacteria to bind to the treated CD11/CD18 was determined by ELISA.

Effect of monoclonal antibodies on the binding of bacteria to granulocytes. A 0.05-ml suspension of granulocytes (10^7 cells per ml) was added to each well of a microtiter plate. Cells were allowed to adhere at 37°C for 45 min in an atmosphere containing 5% CO_2, and the nonadherent cells were removed by washing. The number of cells bound to each well was approximately 2×10^5, as determined by methylene blue staining and by using a standard curve to convert absorbance into number of bound cells (4, 5). The plates were incubated at 37°C for 30 min with PBS–5% BSA and then fixed with methanol as described previously (4, 5). The supernatant was removed, and the cells were incubated for 1 h at 4°C with 50 μl of aggregated human IgG (1 mg/ml) in order to block the Fc receptors. The cells were washed four times in the cold with PBS and were incubated for 1 h at 4°C with 50 μl (1 mg/ml) of different monoclonal antibodies against CD11a, CD11b, CD11c, or CD18 or against the major leukocyte sialoglycoprotein. Unbound antibodies were poured off, radiolabeled bacteria (50 μl of 1.7×10^8 bacteria per ml) were added for 1 h at 4°C, and the unbound bacteria were removed by four washings with PBS. The bound cells were lysed with Triton X-100 (1%), and the lysates were counted in a gamma counter. Parallel experiments without antibodies but in the presence of MeαMan or galactose at 0.1 M were carried out.

RESULTS

Binding of bacteria to the immobilized CD11/CD18 in microwells. The purified antigens bound type 1-fimbriated *E. coli* in a dose-dependent, saturable manner, as shown by ELISA (Fig. 1A). Binding was inhibited by MeαMan, whereas no inhibition was observed with galactose (Fig. 1B). The maximum number of bacteria bound per microgram of CD11/CD18 was 3.4×10^5. The bacteria of the nonfimbriated phenotype failed to bind to the antigens. Treatment of the antigens in the wells with periodate or with enzymes that cleave N-linked oligosaccharides of glycoproteins reduced almost completely the binding of type 1-fimbriated *E. coli* (Fig. 2). This treatment had little effect on the ability of the antigens to bind polyclonal anti-CD11/CD18 antibodies (data not shown), confirming the report of Giampapa et al. (16) that it did not significantly alter the protein moieties of receptors for type 1 fimbriae.

Binding of type 1-fimbriated *E. coli* to blots of CD11/CD18 or granulocyte lysates separated by electrophoresis. SDS-PAGE of the antigens, followed by staining with Coomassie blue, revealed three major bands with mobilities similar to those of CD11a plus CD11b, CD11c, and CD18, identified previously by monoclonal antibodies (20, 23) (Fig. 3). Blotting and overlay experiments showed that all the bands bound type 1-fimbriated *E. coli* and that this binding was inhibited by MeαMan but not by galactose (Fig. 3). The bacteria also bound to several low-molecular-weight proteins not detected by Coomassie blue. Sodium metaperiodate treatment of the blot before it was overlaid with the bacteria markedly diminished the bacterial binding (Fig. 4). Treatment of the purified CD11/CD18 complexes with endo-H or endo-F/N before electrophoresis reduced almost completely their abilities to bind the bacteria (Fig. 5). The enzyme-treated CD11/CD18 proteins migrated faster on

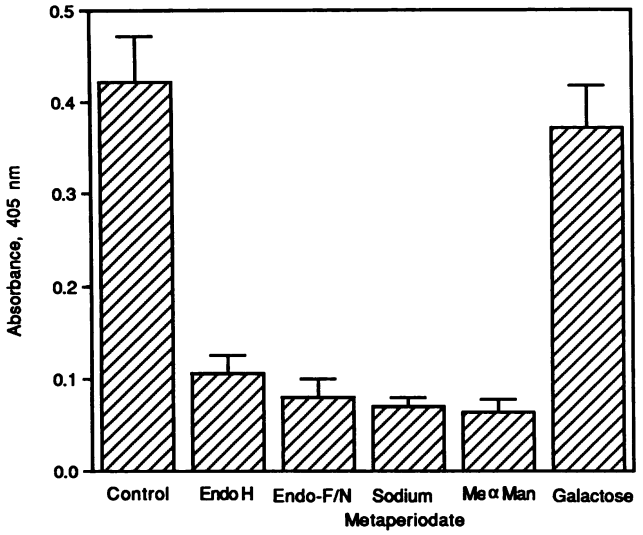

FIG. 2. Effect of enzyme and chemical treatments on binding of type 1-fimbriated *E. coli* to CD11/CD18. The antigens (50 μl of a 64-μg/ml solution) were immobilized on microtiter plates and incubated either for 60 min at 37°C with 100 μl of 10 mM sodium metaperiodate in PBS or for 24 h with 100 μl of a 0.05-U/ml endo-H solution or a 0.25-U/ml endo-F/N solution. The wells were washed and incubated with 5×10^6 bacteria in 50 μl of PBS, and binding of bacteria to the immobilized CD11/CD18 was visualized by ELISA, as described in the text. In parallel, the bacteria were incubated with untreated CD11/CD18 in the absence or presence of 100 mM MeαMan or galactose.

SDS-PAGE than the unmodified antigens did (data not shown). Overlay with type 1-fimbriated *E. coli* of blots from lysates of human granulocytes separated on SDS-PAGE showed that the bacteria bound to two glycoprotein bands corresponding to molecular weights in the ranges of 90,000 to 100,000 and 160,000 to 170,000, although as many as 10 to 12 glycoprotein bands were detected in blots overlaid with concanavalin A (Fig. 6). The binding of the bacteria to the granulocyte glycoproteins was inhibited by MeαMan and concanavalin A, suggesting that it was mannose specific.

Effect of monoclonal antibodies to CD11a,b,c/CD18 on the binding of type 1-fimbriated *E. coli*. To further demonstrate that CD11/CD18 mediates the binding of type 1-fimbriated *E.*

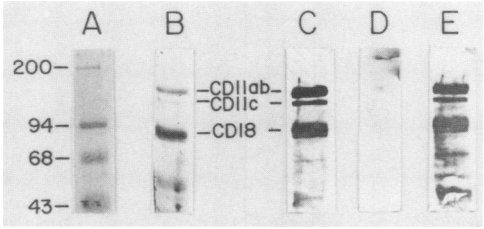

FIG. 3. Binding of type 1-fimbriated *E. coli* to blots of purified CD11/CD18. Purified CD11/CD18 (8 μg of protein per lane) was subjected to SDS–6% PAGE and either stained with Coomassie blue (lane B) or blotted to nitrocellulose. The nitrocellulose was overlaid with 15 ml of a suspension of 10^8 bacteria per ml alone (lane C) or in the presence of 100 mM MeαMan (lane D) or 100 mM galactose (lane E). Binding of bacteria to the blots was visualized by ELISA, as described in the text. Markers (lane A): myosin (M_r, 200,000), phosphorylase *b* (M_r, 94,000), BSA (M_r, 68,000), and ovalbumin (M_r, 43,000).

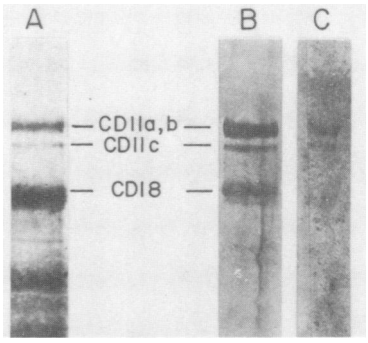

FIG. 4. Effect of sodium metaperiodate treatment on binding of bacteria to CD11/CD18. Purified CD11/CD18 (12 μg of protein per lane) was separated by SDS–6% PAGE and either stained with Coomassie blue (lane A) or blotted to nitrocellulose. The transferred proteins were treated without (lane B) or with (lane C) 10 mM sodium metaperiodate in PBS for 1 h at 37°C. The ability of the bacteria to bind to CD11/CD18 after this treatment was determined by ELISA, as described in the text.

coli to intact granulocytes, we examined the inhibitory effects of seven different anti-CD11/CD18 monoclonal antibodies on the binding of bacteria. The bacteria bound to the granulocyte monolayer in a mannose-specific manner, as evidenced by the parallel experiments showing that MeαMan, but not galactose, inhibited bacterial attachment to the phagocytes (Fig. 7). Efficient inhibition (55 to 80%) was observed with one monoclonal antibody against CD11a (MEM25), one monoclonal antibody against CD11b (3F4), and two monoclonal antibodies against CD18 (7E4 and MAb59). Only slight, but significant, inhibition (23% [$P < 0.01$]) was found with monoclonal antibodies against CD43, the major sialoglycoproteins of the granulocytes (10). Data from a typical experiment are shown in Fig. 7. Two other monoclonal antibodies against CD18 (2E7 and 11D3) also gave only slight inhibition (15 to 25%) (data not shown). Combinations of different monoclonal antibodies against

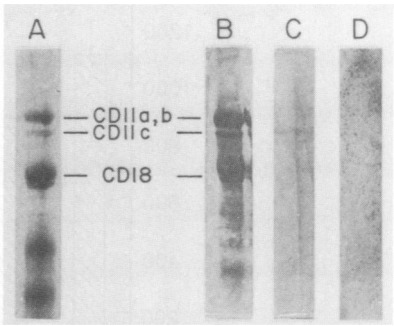

FIG. 5. Effect of endoglycosidase treatment of CD11/CD18 on the binding of the bacteria to the antigen transferred to nitrocellulose. Purified CD11/CD18 (20-μg aliquots of protein) was incubated with endo-H or endo-F/N for 20 h at 37°C and then electrophoresed, transferred to nitrocellulose, and stained with Coomassie blue (lane A) or overlaid with type 1-fimbriated *E. coli* (lanes B to D). Lane B, untreated CD11/CD18: lanes C and D, CD11/CD18 treated with endo-H (lane C) or endo-F/N (lane D). The ability of the bacteria to bind to CD11/CD18 after this treatment was determined by ELISA, as described in the text.

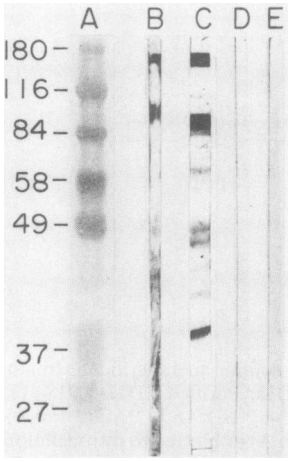

FIG. 6. Binding of type 1-fimbriated *E. coli* or concanavalin A to a lysate of human granulocytes. The lysate (20 μg of protein per lane) was subjected to SDS–8% PAGE and transferred to nitrocellulose. The nitrocellulose was overlaid with a suspension of 2×10^9 bacteria per ml alone (lane B), with concanavalin A alone (50 μg/ml) (lane C), or with the bacteria in the presence of 100 mM MeαMan (lane D) or 50 μg of concanavalin A per ml (lane E). The bound bacteria were visualized by ELISA, as described in the text. Markers (lane A): α-macroglobulin (M_r, 180,000), β-galactosidase (M_r, 116,000), fructose-6-phosphate kinase (M_r, 84,000) pyruvate kinase (M_r, 58,000), fumarase (M_r, 48,500), lactate dehydrogenase (M_r, 36,500), and triosphosphate isomerase (M_r, 26,500).

CD11a, CD11b, CD11c, and CD18 were not more effective than each antibody alone (data not shown).

DISCUSSION

The results presented in this report show that the leukocyte surface antigens CD11/CD18 act as major receptors for type 1-fimbriated *E. coli*. This conclusion is supported by several lines of evidence. First, the functional activity of the purified CD11/CD18 is expressed by its ability to bind type 1-fimbriated *E. coli* in a dose-dependent and mannose-specific manner and by its inability to bind nonfimbriated *E. coli*. Second, the bacteria bound to CD11a,b,c and CD18 after the antigens were separated by SDS-PAGE and blotted onto nitrocellulose paper. Although the bacteria also bound specifically to other low-molecular-weight proteins present in the CD11/CD18 preparation, it is possible that these are degradation products of the antigens or contaminants of unknown origin. Third, treatment with agents that specifically remove N-linked carbohydrate units of glycoproteins or oxidize them diminished the binding of type 1-fimbriated *E. coli*. Since type 1 fimbriae exhibit a high affinity for oligomannose and hybrid N-linked oligosaccharides (13), but not for complex type N-linked oligosaccharides, our findings implicate the former two classes of carbohydrate units as the mediators of the interaction. The presence of substantial amounts of oligomannose units and smaller amounts of hybrid oligosaccharides in CD11/CD18 has recently been demonstrated (3). Fourth, binding of type 1-fimbriated *E. coli* to intact human granulocytes was efficiently inhibited by monoclonal antibodies to CD11/CD18, as well as by MeαMan. The inhibition by the antibodies was not complete, and combinations of monoclonal antibodies against CD11/CD18 did not result in greater inhibition (data not shown), possibly because the specificity of the antibodies is not directed against the carbohydrate moieties of the antigen. It is likely, however, that the monoclonal antibodies are directed against epitopes close to the sugar moieties. Fifth, among the dozen or so concanavalin A-binding glycoproteins in human granulocyte lysates, only two, with M_rs of 90,000 to 100,000 and 165,000, bound the type 1-fimbriated bacteria. Moreover, in preliminary experiments (performed with Alain Fischer at Hôpital Necker, Paris, France) with granulocytes of a patient deficient in leukocyte adhesion molecules (1), no specific binding of type 1-fimbriated *E. coli* was observed.

Several functions have been ascribed to the leukocyte adhesion molecules. Of special relevance to this discussion is that CD11b/CD18 and CD11c/CD18 act as receptors for

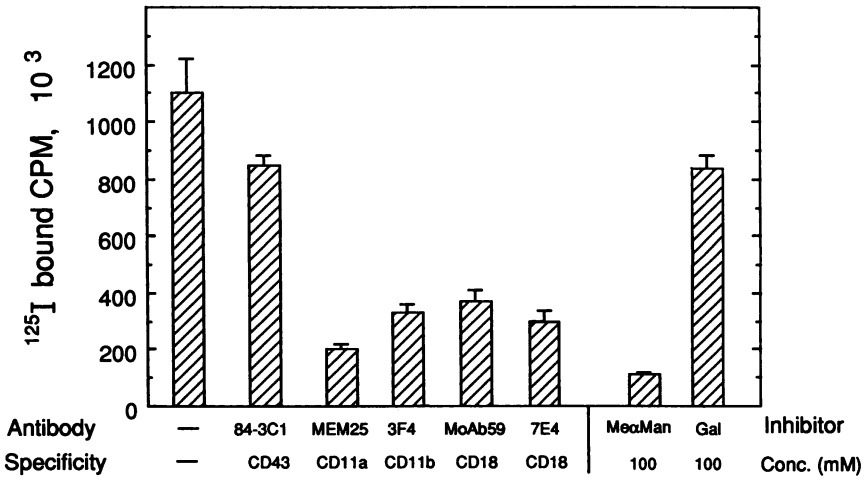

FIG. 7. Effect of monoclonal antibodies to CD11/CD18 on binding of type 1-fimbriated *E. coli* to granulocytes. The granulocytes (10^7 cells per ml; 50 μl per well) were treated with 50 μl of the different monoclonal antibodies (1 μg/ml) for 1 h at 4°C. The wells were rinsed, and 50 μl of a suspension of 1.7×10^8 radiolabeled bacteria per ml was added. In parallel, radiolabeled bacteria were also added to untreated cells in the absence or presence of 100 mM MeαMan or galactose for 1 h at 4°C. Bacterial binding was determined by counting the radioactivity, as described in the text.

complement fragment iC3b in opsonophagocytosis (2) and may serve, in the absence of opsonins, as receptors for the lectin-mediated phagocytosis of mannose-specific bacteria. Lectinophagocytosis via these receptors may be of clinical importance, as patients deficient in these antigens suffer from recurrent microbial infections and usually die at an early age (12, 37). Relman et al. (30) recently found that the CD11b/CD18 complex of human macrophages acts as a receptor for *Bordetella pertussis*.

In a previous study, three glycoproteins were isolated from membranes of human granulocytes by affinity chromatography on immobilized type 1 fimbriae (31). The present work strongly indicates that two of these, with M_rs of 90,000 to 100,000 and 150,000 are CD18 and CD11b or CD11a, respectively. The role of the third glycoprotein (M_r, 70,000 to 80,000) is not clear. It is premature to speculate on the roles of the individual components of the CD11/CD18 complex in the binding of bacteria.

Salmon et al. (33) suggested that the Fc receptor of human granulocytes is involved in the process of ingestion, but not attachment, of type 1-fimbriated *E. coli* by the phagocytes. They also suggested that the process is mediated by specific interaction between the oligosaccharide side chain of the Fc receptor and the type 1 fimbriae. As mentioned above, we found that on blots of SDS-PAGE-separated lysates of granulocytes, fimbriated bacteria recognized exclusively the CD11/CD18 glycoproteins. However, when the blots were overlaid with isolated cross-linked fimbriae, the main component recognized by the probe was a glycoprotein with an M_r of 55,000 (data not shown), which is in the reported range of the M_r of the Fc receptor of human granulocytes (50,000 to 70,000). The importance of multivalence for effective binding of glycoconjugates to lectins is well established, although the degree of multivalence required varies with the system. For example, the binding of various glycoproteins (e.g., fetuin, ovomucoid, and glycophorin) to wheat germ agglutinin immobilized on Sepharose beads was shown to be greatly influenced by the density of the lectin on the beads (14). The results of Salmon et al. (33) and our observations can best be explained by assuming that following the attachment of the fimbriated bacteria to granulocytes via the CD11/CD18 glycoproteins, the fimbrial lectin becomes concentrated in proximity to the Fc receptor, which may then interact with the lectin to trigger ingestion of the bacteria.

ACKNOWLEDGMENTS

We thank Halina Lis and Dvorah Ochert for their help in preparing the manuscript.

This study was supported in part by grants from the National Institutes of Health (AI-23165), the Wolfson Family Charity Trust (N.S.), the Academy of Finland, the Sigrid Jusélius Foundation, and the Finnish Cancer Society (C.G.G.) and by a FEBS Fellowship (A.G.).

REFERENCES

1. **Anderson, D. C., and T. A. Springer.** 1987. Leukocyte adhesion deficiency: an inherited defect in the Mac-1, LFA-1 and p150,95 glycoproteins. Annu. Rev. Med. **38**:175–194.
2. **Arnaout, M. A.** 1990. Structure and function of the leukocyte adhesion molecules CD11/CD18. Blood **75**:1037–1050.
3. **Asada, M., K. Furukawa, C. Kantor, C. G. Gahmberg, and A. Kobata.** 1991. Structural study of the sugar chains of human leukocyte cell adhesion molecules CD11/CD18. Biochemistry **30**:1561–1571.
4. **Athamna, A., and I. Ofek.** 1988. Enzyme-linked immunosorbent assay for quantitation of attachment and ingestion stages of bacterial phagocytosis. J. Clin. Microbiol. **26**:62–66.
5. **Athamna, A., I. Ofek, Y. Keisari, S. Markowitz, G. G. S. Dutton, and N. Sharon.** 1991. Lectinophagocytosis of encapsulated *Klebsiella pneumoniae* mediated by surface lectins of guinea pig alveolar macrophages and human monocyte-derived macrophages. Infect. Immun. **59**:1673–1682.
6. **Bar-Shavit, Z., R. Goldman, I. Ofek, N. Sharon, and D. Mirelman.** 1980. Mannose-binding activity of *Escherichia coli*: a determinant of attachment and ingestion of the bacteria by macrophages. Infect. Immun. **29**:417–424.
7. **Bar-Shavit, Z., I. Ofek, R. Goldman, D. Mirelman, and N. Sharon.** 1977. Mannose residues on phagocytes as receptors for the attachment of *Escherichia coli* and *Salmonella typhi*. Biochem. Biophys. Res. Commun. **78**:455–460.
8. **Bazil, V., I. Stefanova, I. Hilgert, H. Kristovana, and V. Horejsi.** 1990. Monoclonal antibodies against human leucocyte antigens. IV. Antibodies against subunits of the LFA-1 (CD11a/CD18) leucocyte adhesion glycoprotein. Folia Biol. (Prague) **36**:41–46.
9. **Boner, G., A. M. Mhashilkar, M. Rodriguez-Ortega, and N. Sharon.** 1989. Lectin-mediated, nonopsonic phagocytosis of type 1 *Escherichia coli* by human peritoneal macrophages of uremic patients treated by peritoneal dialysis. J. Leukocyte Biol. **46**:239–245.
10. **Borche, L., F. Lozano, R. Vilella, and J. Vives.** 1987. CD43 monoclonal antibodies recognize the large sialoglycoprotein of human leukocytes. Eur. J. Immunol. **17**:1523–1526.
11. **Burnette, W. N.** 1981. "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. **112**:195–203.
12. **Dana, N., J. Pitt, R. F. Todd, J. Melamed, H. R. Colten, and M. A. Arnaout.** 1983. Deficiency of a monocyte-granulocyte surface glycoprotein MO1 in man. Clin. Res. **31**:489–495.
13. **Firon, N., D. Duksin, and N. Sharon.** 1985. Mannose-specific adherence of *Escherichia coli* to BHK cells that differ in their glycosylation patterns. FEMS Microbiol. Lett. **27**:161–165.
14. **Furukawa, K., J. E. Minor, J. D. Hegarty, and V. P. Bhavanandan.** 1986. Interaction of sialoglycoproteins with wheat germ agglutinin-Sepharose of varying ratio of lectin to Sepharose. J. Biol. Chem. **261**:7755–7761.
15. **Gbarah, A., A. M. Mhashilkar, G. Boner, and N. Sharon.** 1989. Involvement of protein kinase C in activation of human granulocytes and peritoneal macrophages by type 1 fimbriated (mannose specific) *Escherichia coli*. Biochem. Biophys. Res. Commun. **165**:1243–1249.
16. **Giampapa, C. S., S. N. Abraham, T. M. Chiang, and E. H. Beachey.** 1988. Isolation and characterization of a receptor for type 1 fimbriae of *Escherichia coli* from guinea pig erythrocytes. J. Biol. Chem. **263**:5362–5367.
17. **Hawkes, R.** 1982. Identification of concanavalin A binding after sodium dodecyl sulfate-gel electrophoresis and protein blotting. Anal. Biochem. **132**:143–146.
18. **Horwitz, M. A.** 1982. Phagocytosis of microorganisms. Rev. Infect. Dis. **4**:104–123.
19. **Hunter, W. M., and F. C. Greenwood.** 1962. Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature (London) **194**:495–496.
20. **Kantor, C., H. Suomalainen-Nevanlinna, M. Patarroyo, K. Österlund, T. Bergman, H. Jörnvall, J. Schröder, and C. G. Gahmberg.** 1988. Purification in large scale and characterization of the human leukocyte adhesion glycoprotein gp90 (CD18). Eur. J. Biochem. **170**:653–659.
21. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
22. **Marchalonis, J. J., R. E. Cone, and V. Santer.** 1971. Enzymatic iodination: a probe for accessible surface proteins of normal and neoplastic lymphocytes. Biochem. J. **124**:921–927.
23. **Nortamo, P., M. Patarroyo, C. Kantor, J. Suopanki, and C. G. Gahmberg.** 1988. Immunological mapping of the human leucocyte adhesion glycoprotein gp90 (CD18) by monoclonal antibodies. Scand. J. Immunol. **28**:537–546.

24. **Ofek, I., and E. H. Beachey.** 1978. Mannose binding and epithelial cell adherence of *Escherichia coli*. Infect. Immun. **22**:247–254.
25. **Ofek, I., D. Mirelman, and N. Sharon.** 1977. Adherence of *Escherichia coli* to human mucosal cells mediated by mannose receptors. Nature (London) **265**:623–625.
26. **Ofek, I., and N. Sharon.** 1988. Lectinophagocytosis: a molecular mechanism of recognition between cell surface sugars and lectins in the phagocytosis of bacteria. Infect. Immun. **56**:539–547.
27. **Ohman, L., J. Hed, and O. Stendahl.** 1982. Interaction between human polymorphonuclear leukocytes and two different strains of type 1 fimbriae-bearing *Escherichia coli*. J. Infect. Dis. **146**:751–757.
28. **Patarroyo, M., J. Prieto, J. Rincon, T. Timonen, C. Lundberg, L. Lindbom, B. Asjo, and C. G. Gahmberg.** 1990. Leukocyte-cell adhesion: a molecular process fundamental in leukocyte physiology. Immunol. Rev. **114**:67–108.
29. **Perry, A., I. Ofek, and F. J. Silverblatt.** 1983. Enhancement of mannose-mediated stimulation of human granulocytes by type 1 fimbriae aggregated with antibodies on *Escherichia coli* surfaces. Infect. Immun. **39**:1334–1345.
30. **Relman, D., E. Tuomanen, S. Falkow, D. T. Golenbock, K. Saukkonen, and S. D. Wright.** 1990. Recognition of a bacteria adhesin by an integrin: macrophage CR3 (αMβ2, CD11b/CD18) binds filamentous hemagglutinin of *Bordetella pertussis*. Cell **61**:1375–1382.
31. **Rodriguez-Ortega, M., I. Ofek, and N. Sharon.** 1987. Membrane glycoproteins of human polymorphonuclear leukocytes that act as receptors for mannose-specific *Escherichia coli*. Infect. Immun. **55**:968–973.
32. **Ross, G. D., R. A. Thompson, M. J. Walport, T. A. Springer, J. V. Watson, R. H. R. Ward, J. Lida, S. L. Newman, R. A. Harrison, and P. J. Lachmann.** 1985. Characterization of patients with an increased susceptibility to bacterial infections and a genetic deficiency of leukocyte membrane complement receptor type 3 and the related membrane antigen LFA-1. Blood **66**:882–890.
33. **Salmon, J. E., S. Kapur, and R. P. Kimberly.** 1987. Opsonin-independent ligation of Fcγ receptors: the 3G8-bearing receptors on neutrophils mediate the phagocytosis of concanavalin A-treated erythrocytes and nonopsonized *Escherichia coli*. J. Exp. Med. **166**:1798–1813.
34. **Schwarting, R., H. Stein, and C. Y. Wang.** 1985. The monoclonal antibodies αS-HCL 1 (αLeu-14) and αS-HCL 3 (αLeu-M5) allow the diagnosis of hairy cell leukemia. Blood **65**:974–983.
35. **Silverstein, S. C., R. M. Steinman, and Z. A. Cohn.** 1977. Endocytosis. Annu. Rev. Biochem. **46**:669–722.
36. **Springer, T. A.** 1990. Adhesion receptors of the immune system. Nature (London) **346**:425–434.
37. **Springer, T. A., W. S. Thompson, L. J. Miller, F. C. Schmalstieg, and D. C. Anderson.** 1984. Inherited deficiency of the Mac-1, LFA-1, p150,95 glycoprotein family and its molecular basis. J. Exp. Med. **160**:1901–1918.
38. **Suomalainen, H. A., R. A. Goldsby, B. A. Osborne, and J. Schroder.** 1980. Mouse/human T-cell hybrids rosetting with sheep erythrocytes. Scand J. Immunol. **11**:163–168.