

Binding to Human Extracellular Matrix by *Neisseria meningitidis*

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Adhesion of *Neisseria meningitidis* strains to extracellular matrix (ECM) and purified matrix components was examined. Most strains bound to subendothelial ECM as well as to immobilized fibronectin and types I, III, and V collagen. Strains from healthy carriers adhered significantly better than isolates from patients. The binding site was localized to the central 75-kDa cell-binding domain of the fibronectin molecule. This domain has not been described previously to interact with bacterial structures.

Neisseria meningitidis is a common colonizer of the nasopharyngeal mucosa. Colonization is usually asymptomatic, but occasionally this organism passes beyond the mucosal barriers and causes severe infection leading to sepsis or meningitis. Together with *Haemophilus influenzae* and *Streptococcus pneumoniae*, *N. meningitidis* accounts for more than three-quarters of all cases of bacterial meningitis (2).

The potential of *N. meningitidis* to cause systemic disease is dependent both on its ability to attach to nasopharyngeal tissue and on its capacity to penetrate the different layers of the tissue. Most *N. meningitidis* strains express pili responsible for interaction with and adherence to epithelial cells in the nasopharynx (10, 16, 19). Following attachment, meningococci can be transferred through the epithelial cells in phagocytic vacuoles as a result of endocytosis (8, 17). This is in contrast to *H. influenzae*, which causes a breakdown of tight junctions between epithelial cells and passes the epithelium via an intercellular route (19, 22). In a later step, the bacterium has to penetrate submucosal tissue to reach the vascular system. This phase, which has been poorly investigated, involves both attachment to extracellular matrix (ECM) and a subsequent degradation process. Also, during penetration from blood to cerebrospinal fluid, the adhesiveness to ECM may be important, as the matrix in the fenestrated endothelium of the choroid plexus is open to circulation and thus should be accessible to bacteria that have invaded the bloodstream (7, 22). Many different organisms have been demonstrated to react with ECM proteins. Recently, both *H. influenzae* (29) and *S. pneumoniae* (24) have been shown to adhere to ECM, suggesting a possible role for such interactions in the pathogenesis of meningitis.

To further elucidate meningococcal interaction with ECM, selected strains were tested for adhesion to preparations of endothelial ECM as well as to isolated matrix proteins. The tested strains included both isolates from diseased patients collected during an outbreak in Norway in 1982 (all from group B) (3) and isolates from healthy carriers (four were nongroupable, one was from group A, one was from group B, and one was from group C) (5). Strains were cultivated overnight in brain heart infusion broth supplemented with 1% Isovitalex (BBL, Cockeysville, Md.) and 40 mg of hemin (Difco, Detroit, Mich.) per liter. All strains were demonstrated to express fim-

briae by a hemagglutination assay (15). All strains also expressed the opacity proteins Opa and Opc, as detected by an immunoblotting technique using the monoclonal antibodies 4B12C11 (Opa) and B306 (Opc), kindly provided by M. Achtman (1).

Preparations of ECM on glass slides were obtained by culture of the endothelial cell line EA.hy926 and subsequent removal of the cell layer by trypsin digestion. The number of bacteria adhering to the remaining ECM layer after 2 h of sedimentation was estimated by computerized image analysis, as described previously (21, 29). Ten *N. meningitidis* strains were screened for the ability to adhere to these ECM preparations. All tested strains demonstrated dose-dependent adherence when bacterial concentrations in the range of 5×10^7 to 1×10^9 organisms/ml were used (Fig. 1). Adhesion in control experiments using glass slides coated with bovine serum albumin (BSA) was significantly lower and lacked dose dependence.

Meningococcal adherence to immobilized matrix proteins (laminin, fibronectin, and types I, III, IV, and V collagen) on glass slides was tested with a computerized image analysis system similar to that used for ECM (29, 30). Human fetuin (glycosylated) and BSA (unglycosylated) (both purchased from Sigma) were used as control proteins. Binding was measured at four different bacterial concentrations, ranging from 5×10^7 to 1×10^9 organisms/ml. Representative results with nongroupable strain BT 162 are presented as an example in Fig. 2. Dose-dependent bacterial binding to fibronectin (Fibrogenex Inc., Chicago Ill.) and to types I, III, and V collagen (all from Sigma except for type III, from Collaborative Research) were observed. However, binding to laminin (Upstate Biotechnology Inc.) and type IV collagen (Sigma) lacked dose dependence and was of the same magnitude as the binding to BSA and fetuin. Matrix protein binding by strains isolated from patients as well as from healthy carriers was also compared. In Fig. 3, binding values at a bacterial concentration of 10^9 /ml are given for five strains in each group. Overall, the strains showed patterns similar to that shown by strain BT 162, with adhesion to fibronectin and to types I, III, and V collagen. Strains from healthy carriers, however, exhibited significantly higher adhesiveness than those from patients toward all ECM proteins except collagens I and IV and laminin (*P* values obtained by the Mann-Whitney two-tailed test: fibronectin, <0.01 ; collagen III, <0.05 ; collagen V, <0.01).

To further investigate meningococcal interaction with fibronectin, adhesion to two different forms of fibronectin, as well as to three fragments of the fibronectin molecule, was

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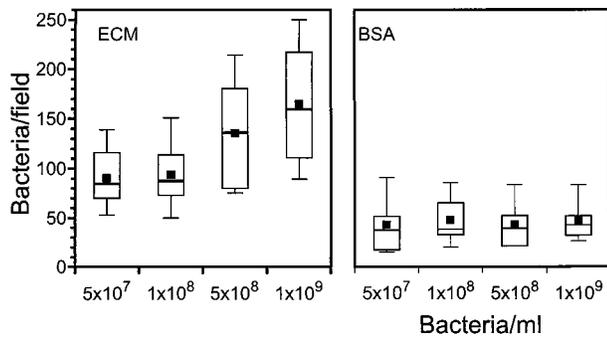


FIG. 1. Quantification of the adherence of 10 *N. meningitidis* strains to sub-endothelial ECM prepared by detergent extraction from cultured EA.hy926 cells. BSA was used as a negative control. Means (black squares), medians (horizontal lines), and 25th and 75th percentiles (boxes) and ranges (error bars) are indicated.

tested. The fragments were obtained by trypsin digestion and included the N-terminal 31-kDa segment, the adjacent 40-kDa segment, and the central 75-kDa segment (Bional, Tartu, Estonia). When bacterial binding to immobilized plasma fibronectin (Collaborative Research) and to cellular fibronectin were compared, similar values were obtained (Fig. 4). For comparative reasons, bacterial uptake of radiolabeled (23) plasma fibronectin in the soluble form was also tested. Uptake values with soluble fibronectin were generally low (<5%), suggesting that immobilization of the molecule is essential for bacterial binding (data not shown). When bacterial adhesion to immobilized fibronectin fragments was tested (4), no binding was scored with the N-terminal 31-kDa fragment. Also, 9 of 10 strains failed to bind with the neighboring 40-kDa fragment. However, when the 75-kDa fragment, representing the central cell-binding domain, was used, adhesion levels similar to those obtained with the complete plasma and cellular fibronectin molecules were obtained.

The interaction between fibronectin and *N. meningitidis* strains was further analyzed by blocking experiments with heparin and RGD peptides. Fibronectin-coated glass plates were preincubated with heparin (Pharmacia, Stockholm, Sweden) diluted in phosphate-buffered saline at concentrations of 0.01 to 2,500 IU/ml for 2 h prior to the addition of bacteria. Heparin

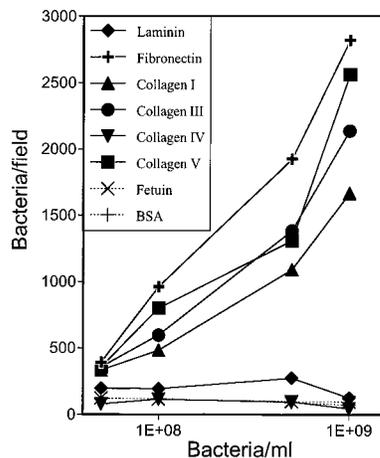


FIG. 2. Adherence of *N. meningitidis* BT 162 to immobilized ECM proteins.

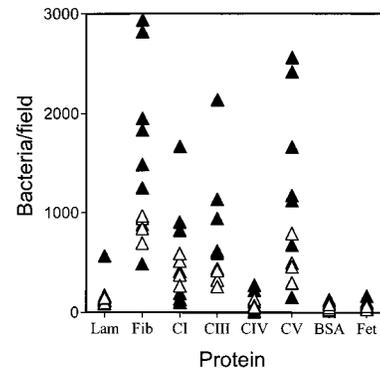


FIG. 3. Adherence of *N. meningitidis* strains to immobilized ECM proteins. Strains were isolated either from healthy carriers (▲) or from patients with meningococcal disease (△). Abbreviations: Lam, laminin; Fib, fibronectin; CI, collagen I; CIII, collagen III; CIV, collagen IV; CV, collagen V; Fet, fetuin.

at equal concentrations was added to the bacterial suspensions immediately prior to addition to the wells. No blocking was observed with 2,500-IU/ml concentrations of heparin (data not shown), suggesting that the N-terminal and C-terminal heparin-binding regions are not essential for bacterial binding. We also tested the influence of RGD peptides, which are analogs to the cell-binding site of fibronectin and are known to block binding to integrin receptors on cells. Bacteria were preincubated with synthetic peptide A6677 (RGDSPA [6, 31]) or G4391 (GRGDSPK [13]) at a concentration of 1 mM for 1 h before transfer to the fibronectin-coated wells. The unrelated peptide S5151 (AQNYPIV) from the human immunodeficiency virus *gag* gene product was used as a negative control. (All peptides were purchased from Sigma.) No blocking effect was observed, suggesting that binding of the 75-kDa fragment to *N. meningitidis* involves a different site (data not shown).

Neisserial opacity proteins have been demonstrated to contribute to cellular adhesion under certain conditions (26–28). To examine whether interaction with fibronectin and collagens is dependent on the expression of opacity proteins, adhesion experiments were performed with the Opa⁻ *N. meningitidis* strain 2c4.3Opa⁻ (kindly provided by Xavier Nassif) as well as *Neisseria gonorrhoeae* MS11(wt) (Opc⁻). The results are summarized in Table 1. The Opa⁻ strain 2c4.3Opa⁻ demonstrated a binding pattern very similar to the corresponding Opa⁺ isogenic variant. Also, *N. gonorrhoeae* MS11(wt) adhered to fi-

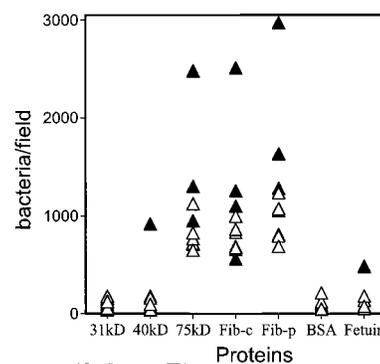


FIG. 4. Adherence of *N. meningitidis* strains to immobilized cellular (Fib-c) and plasma (Fib-p)-derived fibronectin and fibronectin fragments. Strains were isolated either from healthy carriers (▲) or from patients with meningococcal disease (△).

TABLE 1. Bacterial adherence to immobilized ECM proteins

Strain	Opa	Opc	Adherence (bacteria/field) to:				
			Collagen I	Collagen IV	Collagen V	Fibronectin	IgG ^a
<i>N. meningitidis</i> 2c4.3Opa+	+	+	169	9	113	1,163	9
<i>N. meningitidis</i> 2c4.3Opa-	-	+	98	12	151	1,948	16
<i>N. gonorrhoeae</i> MS11(wt)	+	-	633	40	526	607	32
<i>Escherichia coli</i> ATCC 25922	-	-	11	17	47	18	31

^a IgG, immunoglobulin G.

bronectin and collagens I and V but to neither collagen IV nor the control protein (immunoglobulin G). The results imply that neither Opa nor Opc is essential for the adhesion of *Neisseria* spp. to fibronectin and collagens.

Taken together, our data demonstrate that *N. meningitidis* strains can adhere to components of subendothelial ECM, especially fibronectin and collagens I, III, and V. Interaction with fibronectin requires surface association and can be attributed to the central 75-kDa cell-binding domain. This domain contains a cell-binding site that interacts with cellular integrin receptors partly via an Arg-Gly-Asp (RGD) sequence (12). However, this motif does not seem to be involved in meningococcal binding, as we were not able to block binding by the addition of RGD analogs.

In earlier investigations with other bacterial species, interaction with fibronectin has in most cases been attributed to the N-terminal or C-terminal domain. Staphylococci and group A streptococci have been demonstrated to react with the N-terminal heparin-binding domain (9, 11), whereas pneumococci seem to react mainly with the C-terminal heparin-binding site (24). Group B streptococci, on the other hand, interact with the collagen-binding domain adjacent to the N-terminal region (20). Our association of the central fibronectin domain with meningococcal binding thus contrasts earlier findings with other bacteria and suggests a different mechanism for this interaction.

Adhesion to ECM was observed irrespective of the serological group (A, B, or C) and was even more pronounced for the nongroupable carrier isolates. The higher adhesion values for carrier strains suggest a possible role for this property in colonization. According to the literature, adhesion of meningococci to nasopharyngeal cells and endothelial cells is highly correlated to pilus expression (14, 18, 25). However, opacity proteins can also contribute to cellular binding, as demonstrated by Virji et al. (26–28). Our data with the Opa⁻ strain demonstrate that Opa is not essential for the ECM binding studied here. The finding that *N. gonorrhoeae* strains, which are naturally Opc⁻, bind matrix proteins with a pattern similar to that of *N. meningitidis* suggests that neither bacterial Opc is required for this interaction. Future investigations with isogenic strains will be needed to further address the nature of ECM binding.

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REFERENCES

- Achtman, M., M. Neibert, B. A. Crowe, W. Strittmatter, B. Kusecek, E. Weyse, M. J. Walsh, B. Slawig, G. Morelli, A. Moll, and M. Blakes. 1988. Purification and characterization of eight class 5 outer membrane protein variants from a clone of *Neisseria meningitidis* serogroup A. *J. Exp. Med.* **168**:507–525.
- Cartwright, K. (ed.). 1995. Meningococcal disease. John Wiley & Sons, Chichester, United Kingdom.
- Gedde-Dahl, T. W., A. E. Højby, A. Schillinger, A. Lystad, and K. Bövre. 1983. An epidemiological, clinical and microbiological follow-up study of incident meningococcal disease cases in Norway, winter 1981–1982. *NIH Ann.* **6**:155–168.
- Hayashi, M., and K. M. Yamada. 1983. Domain structure of the carboxyl-terminal half of human plasminogen activator. *J. Biol. Chem.* **258**:3332–3340.
- Kristiansen, B. E., K. W. Lind, K. Mevold, B. Sørensen, L. O. Frøholm, K. Bryn, T. Tjade, and K. Bövre. 1988. Meningococcal phenotypic and genotypic characteristics and human antibody levels. *J. Clin. Microbiol.* **26**:1988–1992.
- Levesque, J. P., A. Hatzfeld, and J. Hatzfeld. 1986. Fibrinogen mitogenic effect on hemopoietic cell lines: control via receptor modulation. *Proc. Natl. Acad. Sci. USA* **83**:6494–6498.
- Levine, J. 1987. Choroid plexus: target for systemic disease and pathway to the brain. *Lab. Invest.* **56**:231–233.
- McGee, Z. A., S. A. Stephens, L. H. Hoffman, W. F. Schlech III, and R. G. Horn. 1983. Mechanisms of mucosal invasion by pathogenic *Neisseria*. *Rev. Infect. Dis.* **5**(Suppl. 4):S708–S714.
- Müller, H.-P., and M. Lindberg. 1992. Fibronectin-binding proteins of staphylococci and streptococci, p. 57–64. *In* T. K. Korhonen (ed.), *Molecular recognition in host-parasite interaction*. Plenum Press, New York, N.Y.
- Nassif, X., and M. So. 1995. Interaction of *Neisseria* with nonphagocytic cells. *Clin. Microbiol. Rev.* **8**:376–388.
- Patti, J. M., B. L. Allen, M. J. McGavin, and M. Hook. 1994. MSCRAMM-mediated adherence of microorganisms to host tissues. *Annu. Rev. Microbiol.* **48**:585–617.
- Potts, J. R., and I. D. Campbell. 1994. Fibronectin structure and assembly. *Curr. Opin. Cell Biol.* **6**:648–655.
- Pytela, R., M. D. Pierschbacher, M. H. Ginsberg, E. F. Plow, and E. Ruoslahti. 1986. Platelet membrane glycoprotein IIb/IIIa: member of a family of Arg-Gly-Asp-specific adhesion receptors. *Science* **231**:1559–1562.
- Rayner, C. F., A. Dewar, E. R. Moxon, M. Virji, and R. Wilson. 1995. The effect of variations in the expression of pili on the interaction of *Neisseria meningitidis* with human nasopharyngeal epithelium. *J. Infect. Dis.* **171**:113–121.
- Salit, I. E. Hemagglutination by *Neisseria meningitidis*. 1981. *Can. J. Microbiol.* **27**:586–593.
- Stephens, D. S., and Z. A. McGee. 1981. Attachment of *Neisseria meningitidis* to human mucosal surfaces: influence of pili and type of receptor cell. *J. Infect. Dis.* **143**:525–532.
- Stephens, D. S., L. H. Hoffman, and Z. A. McGee. 1983. Interaction of *Neisseria meningitidis* with the human nasopharyngeal mucosa: attachment and entry into columnar epithelial cells. *J. Infect. Dis.* **148**:369–376.
- Stephens, D. S. 1989. Gonococcal and meningococcal pathogenesis as defined by human cell, cell culture, and organ culture assays. *Clin. Microbiol. Rev.* **2**:S104–S111.
- Stephens, D. S., and M. M. Farley. 1991. Pathogenic events during infection of the human nasopharynx with *Neisseria meningitidis* and *Haemophilus influenzae*. *Rev. Infect. Dis.* **12**:22–33.
- Tamura, G. S., and C. E. Rubens. 1994. Group B streptococci (GSB) adhere to a variant of fibronectin attached to a solid phase. *Mol. Microbiol.* **15**:581–589.
- Toba, T., R. Virkola, B. Westerlund, Y. Björkman, J. Sillanpää, T. Vartio, N. Kalkkinen, and T. K. Korhonen. 1995. A collagen-binding S-layer protein in *Lactobacillus crispatus*. *Appl. Environ. Microbiol.* **61**:2467–2471.
- Tunkel, A. R., and W. M. Scheld. 1993. Pathogenesis and pathophysiology of bacterial meningitis. *Clin. Microbiol. Rev.* **6**:118–136.
- Ullberg, M., G. Kronvall, and B. Wiman. 1989. New receptors for human plasminogen on gram positive cocci. *APMIS* **97**:996–1002.
- van der Flier, M., N. Chhun, T. M. Wizemann, J. Min, J. B. McCarthy, and E. I. Tuomanen. 1995. Adherence of *Streptococcus pneumoniae* to immobilized fibronectin. *Infect. Immun.* **63**:4317–4322.
- Virji, M., H. Kayhty, D. J. Ferguson, C. Alexandrescu, J. E. Heckels, and E. R. Moxon. 1991. The role of pili in the interactions of pathogenic *Neisseria* with cultured human endothelial cells. *Mol. Microbiol.* **5**:1831–1841.
- Virji, M., K. Makepeace, D. J. Ferguson, M. Achtman, J. Sarkari, and E. R. Moxon. 1992. Expression of the Opc protein correlates with invasion of

- epithelial and endothelial cells by *Neisseria meningitidis*. *Mol. Microbiol.* **6**:2785–2795.
27. **Virji, M., K. Makepeace, D. J. Ferguson, M. Achtman, and E. R. Moxon.** 1993. Meningococcal Opa and Opc proteins: their role in colonization and invasion of human epithelial and endothelial cells. *Mol. Microbiol.* **10**:499–510.
28. **Virji, M., K. Makepeace, I. R. Peak, D. J. Ferguson, M. P. Jennings, and E. R. Moxon.** 1995. Opc- and pilus-dependent interactions of meningococci with human endothelial cells: molecular mechanisms and modulation by surface polysaccharides. *Mol. Microbiol.* **18**:741–754.
29. **Virkola, R., K. Lähteenmäki, T. Eberhard, P. Kuusela, L. van Alphen, M. Ullberg, and T. K. Korhonen.** 1996. Interaction of *Haemophilus influenzae* with the mammalian extracellular matrix. *J. Infect. Dis.* **173**:1137–1147.
30. **Westerlund, B., P. Kuusela, J. Risteli, L. Risteli, T. Vartio, H. Rauvala, R. Virkola, and T. K. Korhonen.** 1989. The O75X adhesin of uropathogenic *Escherichia coli* is a type IV collagen-binding protein. *Mol. Microbiol.* **3**:329–337.
31. **Yamada, K. M., and D. W. Kennedy.** 1984. Dualistic nature of adhesive protein function: fibronectin and its biologically active peptide fragments can autoinhibit fibronectin function. *J. Cell Biol.* **99**:29–36.

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