Penetration of fimbriate enteric bacteria through basement membranes: A hypothesis

Timo K. Korhonen, Ritva Virkola, Kaarina Lähteenmäki, Yvonne Björkman, Maini Kukkonen, Tiina Raunio, Ann-Mari Tarkkanen and Benita Westerlund

Department of General Microbiology, University of Helsinki, Helsinki, Finland

Received 10 June 1992
Accepted 18 June 1992

Key words: Basement membrane; *Escherichia coli*; Fimbriae; Laminin; Plasmin

1. SUMMARY

A mechanism for penetration of basement membranes by *Escherichia coli* is presented. The mechanism is based on the ability of the S fimbriae of meningitis-associated *E. coli* to bind to vascular endothelium and choroid plexuses in brain and to basement membranes. On the other hand, the S and the type 1 fimbriae of *E. coli* immobilize plasminogen and tissue-type plasminogen activator; this process generates proteolytic plasmin activity on the surface of fimbriate cells. Our hypothesis is that bacterium-bound plasmin activity, directed to basement membranes through fimbrial binding, promotes bacterial penetration through basement membranes.

2. INTRODUCTION

Bacterial adhesion at the site of infection is an important early phase of numerous infectious diseases. Mechanical protective factors at epithelial surfaces are an essential part of our defence against invading bacteria. Bacteria overcome the washing effect of these factors by firmly attaching to our tissue. This takes place through specific molecular interactions between the invading bacteria and receptor molecules in tissues (for recent reviews, see refs. 1, 2).

In enteric bacteria, adhesion is often mediated by bacterial surface appendages called fimbriae. Most fimbrial filaments consist of numerous copies of a major structural subunit, fimbrillin, which confers serological properties on the filament, and a few copies of minor subunits, one of which is an adhesin binding to tissue receptors [3]. A number of fimbrial types have been characterized on *Escherichia coli* strains associated with human urinary tract infections or neonatal meningitis. These fimbriae differ in serological properties and in molecular binding specificities and show distinct reactivity with tissue subcompartments [1]. Also the expression of the fimbrial types is differently regulated [4]. Important features of enterobacterial fimbriae are their multiplicity and phase variation [5–7]. The different fimbrial types of a strain are mostly expressed by separate cells that rapidly switch their fimbrial synthesis, which makes the infecting bacterial
population heterogeneous in regard to fimbria- tion.

3. ENTEROBACTERIAL FIMBRIAE BIND TO EPITHELIAL AND BASEMENT MEMBRANE RECEPTORS

Fimbrial functions have mainly been studied in regard to binding to epithelial glycoconjugates [2]. Normally, the invading bacteria first encounter epithelial surfaces, and it is not surprising that the virulence-associated fimbrial types of *E. coli* show specific binding to epithelial surfaces and glycoconjugates at the relevant tissue sites [1,2]. Moreover, the presence of soluble inhibitors of fimbrial binding determines in part the tissue tropism of bacterial adhesion [8]. On the other hand, many fimbrial types of *E. coli* and *Salmonella* exhibit a highly specific binding to proteins of the extracellular matrix or basement membranes [9–11]. Two mechanisms have been revealed: the interactions are based either on a protein–protein interaction or on a binding of the fimbrial adhesin to carbohydrate chains of the target protein. These interactions are seen also on frozen tissue sections and with reconstituted basement membranes, indicating that they represent true tissue adherence mechanisms of *E. coli* and *Salmonella*. Thus many fimbrial types bind to both epithelial and extracellular matrix receptors; the latter may be important in bacterial colonization of locally damaged tissue sites and in invasion through basement membranes to cause metastatic infections.

This article deals with two fimbrial types, the S fimbria and the type 1 fimbria. The S fimbriae are defined by their binding to sialyl α2–3 and α2–6 lactose structures of glycoproteins and occur on *E. coli* O18K1 strains associated with newborn meningitis [1]. The type 1 fimbriae bind to α-mannoside chains of glycoproteins and are common in most enterobacterial genera, including *Escherichia* and *Salmonella*. The chromosomal gene clusters encoding these fimbriae have been cloned and well characterized [4].

Both fimbrial types recognize epithelial and basement membrane receptors. On frozen sections of rat brain, the S fimbriae exhibit an efficient adherence to epithelial cells lining the choroid plexuses and brain ventricles; they bind also to vascular endothelium in brain [12]. The ability of S fimbriae to bind to vascular endothelium has also been observed using primary cultures of human umbilical vein endothelial cells [13] and frozen sections of human kidney [14]. The ability of the S fimbriae to bind to choroid plexus epithelium and subarachnoid endothelium in brain is thought to be important in localizing *E. coli* O18K1 infections to the brain. The type 1 fimbriae of *E. coli* bind to a number of epithelial cells, which include proximal tubular cells of human kidney [15] and human buccal [16] and neonatal rat oropharyngeal cells [17]. The pathogenetic functions of these interactions have remained unclear. It seems, however, that bacterial colonization of oral cavity is a prerequisite for bacteremia in neonatal rats [17,18].

On the other hand, we recently observed that the S fimbriae and the type 1 fimbriae of *E. coli* and *Salmonella* exhibit an efficient and specific binding to laminin, a major glycoprotein of basement membranes (R. Virkola et al., manuscript in preparation; M. Kukkonen et al., submitted). In both cases, the fimbriae bind to carbohydrate chains of laminin. This was inferred from three findings: (i) the interactions are specifically inhibited by receptor analogues, sialyl α2–3-lactose in the case of S fimbriae, α-methylmannoside in the case of type 1 fimbriae; (ii) the interactions are abolished by periodate oxidation of laminin carbohydrates; and (iii) no binding is seen with mutated fimbriae lacking the carbohydrate-binding minor subunit. Laminin is highly glycosylated and contains terminal sialyllactose oligosaccharides as well as high-mannose chains [19] that are probably recognized by the fimbriae. Both fimbriae exhibit a carbohydrate-dependent binding to reconstituted basement membranes, which indicates that the interactions take place also with the laminin network in basement membranes.

4. BINDING OF PLASMINOGEN TO BACTERIA

Plasminogen is a single-chain glycoprotein that occurs in relatively high concentrations in plasma
and extracellular fluids. Plasminogen is converted to its active, proteolytic form plasmin by eukaryotic activators urokinase and tissue-type plasminogen activator (tPA) as well as by prokaryotic activators staphylokinase and streptokinase. tPA is the principal plasminogen activator in plasma and intercellular fluid. Plasmin formation by tPA activity proceeds poorly in solution but is dramatically enhanced by immobilization of plasminogen, on fibrin (a natural substrate of plasmin) or on eukaryotic cells. Immobilization of plasminogen is mediated by its lysine-binding kringle domains and serves three purposes: (i) plasmin formation by tPA is greatly enhanced; (ii) plasmin is protected against its physiological inhibitors, α₂-antiplasmin and α₂-macroglobulin, which effectively inactivate soluble plasmin; and (iii) plasmin activity is localized (on fibrin or eukaryotic cells). Thus the activation mechanism generates targeted, localized and transient proteolytic activity. Plasmin is a trypsin-like serine protease of broad substrate specificity and degrades, in addition to fibrin, non-collagenous glycoproteins of extracellular matrices and basement membranes, such as laminin [20]. In addition to fibrinolysis, local plasminogen activation occurs in many cellular functions, such as penetration of basement membranes by metastatic cancer cells [21].

Several bacterial species have recently been observed to immobilize plasminogen and enhance plasmin formation. Plasminogen binds to staphylococcal species (including *Staphylococcus aureus*) as well as to group A, C and G streptococci [22,23] and several Gram-negative bacteria, including *E. coli* [24] and *Haemophilus influenzae* [25]. These interactions are inhibited by lysine analogues and obviously involve plasminogen receptors on bacterial cell walls.

5. BACTERIAL ADHERENCE AND PLASMIN FORMATION AT BASEMENT MEMBRANES

Meningitis caused by *E. coli* O18K1 involves penetration of the bacteria through epithelial layers to circulation and subsequently to the cerebrospinal fluid (CSF). The latter involves bacte-
(iii) On frozen sections of neonatal rat brain, the S fimbriae bind to choroid plexuses and ventricular epithelium [12]. This may be important in localizing the infection to the brain as bacteria are thought to invade into CSF through choroid plexuses [26]. This may in part be due to the ‘leaky’ fenestrated endothelium of choroid plexuses [30], where the subendothelial basement membrane is directly exposed, e.g. to circulating antibodies [26].

(iv) The S fimbriae bind in vitro to cultured human umbilical vein endothelial cells [13] and to vascular endothelium of human kidney [14]. Important for our hypothesis, the S fimbriae bind intensively to vascular endothelia of neonatal rat brain [12]. Sera obtained from patients with acute inflammation or from newborn infants inhibit the binding only poorly [13], suggesting that S-fimbrial binding to vascular endothelia may take place in circulation.

(v) Intravenously injected endotoxin causes a rapid and transient release of tPA from human endothelial cells [31]. The increase of tPA activity in plasma preceeds the increase in its inhibitor, plasminogen activator inhibitor-1, which suggests that bacterium-bound plasminogen may be activated by tPA during septicemia. In the brain, plasminogen activator levels are high compared with other organs [32] and are present in endothelial cells of vessels, in the meninges, in

![Graph A](image1)
![Graph B](image2)

Fig. 1. Binding of plasminogen and enhancement of plasmin formation on purified S fimbriae of E. coli. (A) Binding in increasing concentrations of plasminogen to S fimbriae (closed symbols) or bovine serum albumin (open circles) immobilized on microtiter plate wells. The binding was measured by time-resolved fluorometry using anti-plasminogen antibodies and Eu-labelled secondary antibodies. Dashed line: binding in the presence of 10 mM ε-aminocaproic acid, a lysine analogue. Solid line: binding in the absence of ε-aminocaproic acid. Note the inhibition of binding by the lysine analogue and the poor binding to albumin. (B) Formation of plasmin activity in the presence of purified S fimbriae (100 μg ml⁻¹; closed circles) or albumin (open circles). Plasminogen (20 μg ml⁻¹) and tPA (50 ng ml⁻¹) were incubated with the test protein and formation of plasmin activity was measured using the chromogenic substrate S-2251. Dashed line: plasmin formation in the presence of 1 mM ε-aminocaproic acid. Solid line: plasmin activity in the absence of ε-aminocaproic acid. Note enhancement of plasmin formation in the presence of S fimbriae. For further experimental details, see ref. 34.
choroid plexuses and in CSF [33]. By binding to endothelial cells, S fimbriae bring bacterial cells and endotoxin in close contact with endothelial cells and may thus promote release of tPA.

(vi) Purified S and the type 1 fimbriae of E. coli [24,34] as well as fimbriae of Salmonella enteritidis (K. Lähteenmäki, unpublished results) immobilize plasminogen and tPA and enhance plasmin formation by tPA activity (see Fig. 1 for an example). The interactions are inhibited by a lysine analogue, but not by receptor analogues of fimbrial adherence, and are thus probably mediated by the kringle domains of plasminogen and tPA. Plasminogen also binds to S- and type-1-fimbriate bacterial cells, which in the presence of tPA results in formation of bacterium-bound plasmin activity ([34]; K. Lähteenmäki, unpublished results).

(vii) The S and the type 1 fimbriae of E. coli bind in vitro to immobilized laminin as well as to reconstituted basement membranes (see above). This adhesion may bring bacterium-bound plasmin in close contact with basement membranes and promote degradation of laminin. Indeed, we have shown that plasmin generated on the surface of fimbriate S. enteritidis degrades immobilized $^{125}$I-labelled laminin (K. Lähteenmäki, unpublished results).

(viii) We have modified the tumor cell Matrigel invasion assay [35] to assess whether bacteria are able to penetrate through the reconstituted basement membrane from mouse sarcoma (Matrigel). This assay showed that the S-fimbriate E. coli recombinant strain HB101(pANN801-13) penetrates through Matrigel using cell-bound plasmin activity (Fig. 2). The penetration is inhibited by aprotinin, an inhibitor of plasmin activity, and is not seen with the non-fimbriate E. coli strain HB101 (R. Virkola, unpublished). The penetration of HB101(pANN801-13) through Matrigel evidently reflects degradation of basement membrane laminin by the bacterium-bound plasmin. A similar penetration through Matrigel has been observed with a fimbriate strain of S. enteritidis (K. Lähteenmäki, unpublished results).

The observations summarized above are compatible with the hypothesis that bacterium-bound plasmin is directed by bacterial adhesion to basement membranes, which are then degraded to a degree allowing bacterial penetration through the basement membrane. Our ongoing studies have suggested that both the adhesion to basement membranes and the plasmin formation are needed for S-fimbriate E. coli to penetrate Matrigel (R. Virkola, unpublished). It is obvious that many relevant points of the model need to be verified, e.g. the degradation of laminin and basement membranes needs to be physically demonstrated. We are currently localizing the plasminogen-binding sites in the S and the type 1 fimbriae, in part to construct mutants for testing in the rat meningitis model. Also, it remains open whether all variants of the S and the type 1 fimbriae, and whether also other fimbrial types and surface
molecules of *E. coli* and *Salmonella*, function as plasminogen receptors.

In essence, our model resembles the mechanism by which metastatic tumor cells invade basement membranes [20,21]. Tumor cells, like S- and type-1-fimbriate meningitis-associated *E. coli*, use laminin receptors and plasminogen activation in this process. Invasion of tumor cells also involves plasmin-catalysed activation of procollagenases; because of their small size, bacteria may not need to degrade the fairly loose type IV collagen network to penetrate through basement membranes. The findings that various invasive bacteria bind plasminogen suggest that generation of bacterium-bound plasmin may be a general mechanism to provide a powerful proteolytic mechanism for tissue penetration.

ACKNOWLEDGEMENTS

This study was supported by the Academy of Finland.

REFERENCES


