Top Curr Chem (2009) 288: 1–15
DOI: 10.1007/128_2008_15

Published online: 01 October 2008

Ins and Outs of Microbial Adhesion

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Abstract Microbial adhesion is generally a complex process, involving multiple adhesins on a single microbe and their respective target receptors on host cells. In some situations, various adhesins of a microbe may co-operate in an apparently hierarchical and sequential manner whereby the first adhesive event triggers the target cell to express receptors for additional microbial adhesins. In other instances, adhesins may act in concert leading to high avidity interactions, often a prelude to cellular invasion and tissue penetration. Mechanisms used to target the host include both lectin-like interactions and protein–protein interactions; the latter are often highly specific for the host or a tissue within the host. This reflective chapter aims to offer a point of view on microbial adhesion by presenting some experiences and thoughts especially related to respiratory pathogens and explore if there can be any future hope of controlling bacterial infections via preventing adhesion or invasion stages of microbial pathogenesis.

Contents

1 Background

Adhesion to mucosal epithelial cells is generally regarded as an essential process in the life cycle of microbes such as *Neisseria meningitidis* (meningococci), typable *Haemophilus influenzae* and *Streptococcus pneumoniae*. These normally capsulate

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strains are noteworthy as common causative agents of bacterial meningitis, whereas non-typable *H. influenzae* and *Moraxella catarrhalis* that are devoid of the protective surface capsular polysaccharides tend to cause localized infections such as otitis media, sinusitis and pneumonia. However, all these species fairly frequently colonise their exclusive human niche, the upper respiratory tract, without infection. The duration of their asymptomatic carriage varies but may last for several months [5, 8, 34]. Thus, whilst in healthy individuals these bacteria remain confined to mucosal surfaces, they have the capacity to invade the epithelial barrier to disseminate further in susceptible individuals. As such, adhesion to mucosal epithelial cells can be regarded as an important first step in their pathogenic process. In addition, *ex viv*o investigations on biopsies from tonsils of subjects with no apparent disease have shown the presence of *N. meningitidis* within intact epithelial cells [37]; such a location generally results from high avidity adhesion to surface receptors. Intracellular location of nontypable *H. influenzae* has also been observed and has been linked to the persistence of the organisms despite antibiotic treatments in patients [43]. As an introduction, several aspects of cellular adhesion are described below which may lead to persistence in the host and/or disease. The aim is to present some common perceptible microbial strategies of host targeting and the diversity of mechanisms evident from studies predominantly conducted in vitro; their verification in vivo is often lacking as few good animal models are available for human specific bacteria. The paradigm of *N. meningitidis* is used frequently, but notably, similar mechanisms are also found in enteric and urogenital bacteria. Finally, the question of whether we can expect to be able to control microbial colonisation or infection by interfering with adhesion using glycans or other analogues of ligands/receptors is addressed.

2 Some Facets of Bacterial Adhesion

Jumping the first hurdle: The mucosal epithelial barrier consists of ciliated and non-ciliated cells with overlying mucus layers that are wafted around by the movement of cilia creating an unstable environment for adhesion. Attachment at such sites requires fast and effective adhesion strategies to overcome this 'mucociliary escalator'. In addition, at physiological *p*H, the net negative charge of the host cell surface creates a charge barrier against predominantly negative charge on most bacterial surfaces. As such, bacterial adhesion necessitates initial penetration of this barrier. For *N. meningitidis*, pili are considered important in charge penetration but their targeted sites on the host tissues are not random. In the nasopharynx, meningococcal pili bring about specific interactions with non-ciliated but not ciliated cells of the epithelium [38]. They are also regarded as primary determinants of bacterial specificity for human epithelial and endothelial cells. In addition, *N. meningitidis* integral outer-membrane adhesins, such as Opa and Opc proteins, are basic in nature and this property may enable better targeting of the host receptors. However,

both these adhesins are also known to bind to host glycans and proteins with specific interactions that are not entirely dependent on electrostatic charge [24, 45].

Redundancy in adhesion: Redundancy or possession of multiple adhesins/mechanisms of attachment is common for bacteria, enabling binding to multiple targets on a single cell. This creates high affinity interactions required for effective cellular invasion. Distinct adhesins often co-operate to increase cellular entry as exemplified by *N. meningitidis* pili-assisted increased invasion mediated by outer-membrane proteins (Figs. 1 and 3f) [10, 48]. Redundancy also becomes important for bacteria with a single host as one of the strategies for immune evasion common in such pathogens is phase variation (on–off expression of surface structures). This requires the presence of several distinct adhesion mechanisms raising obvious problems for devising effective anti-adhesion control measures against such pathogens

Diversity of receptor targeting mechanisms: It would seem self evident that microbes exhibiting host specificity and tissue tropism must possess specific host targeting mechanisms, although, host-specific iron-acquisition mechanisms may add to the niche specificity. However, specificity at a molecular level exists in a wider context also for microbes with more than one host. From numerous investigations on the mechanisms by which microbes target host molecules, it has become evident that mimicry of the natural ligands of host receptors is often adopted by microbes to engage with them.

Some protein binding mechanisms: Taking integrins as examples of microbial receptors, several diverse mechanisms can be recognised with distinct levels of mimicry playing a noticeable role. In some cases, true ligand mimicry is apparent as bacterial adhesins possess structural components closely resembling the natural ligand for engaging with the receptor at the ligand binding site. This is exemplified by the adhesins FHA (filamentous haemagglutinin) and pertactin of *Bordetella pertussis* (responsible for whooping cough). Both of these proteins contain the RGD sequence and can target RGD-binding integrins, an event that results in host cell signalling [20, 35]. Alternately, bacteria may bind to receptors via 'functional mimicry' of the ligand whereby a somewhat variant but nonetheless effective strategy leads to the stimulation of receptor-associated signalling pathways. For example, the protein of *Yersinia pseudotuberculosis* (an enteric pathogen with the ability to cause systemic disease) termed 'invasin' binds to several β1 family of integrins via key conserved residues required for ligand–integrin interactions [19]. In addition, invasin crystal structure has revealed this to be a case of enhanced functional mimicry as an optimized binding region on invasin imparts higher affinity interactions with the integrin compared with the natural ligand [11]. Interestingly, in the absence of invasin, *Yersinia* utilises another ligand, YadA which binds to the fibronectin receptor α5β1 indirectly by initially targeting fibronectin [18]. Such a mechanism of 'Pseudo ligand mimicry', in which natural receptor ligands are used as accessory bridging molecules, is also observed in the case of *N. meningitidis* Opc protein that binds to vitronectin and fibronectin, and via these to αvβ3 and α5β1 integrins (Fig. 2a) [42, 46]. Finally, 'receptor mimicry' involving expression of host-like receptors by microbes has been described for *Mycobacterium avium–intracellulare*

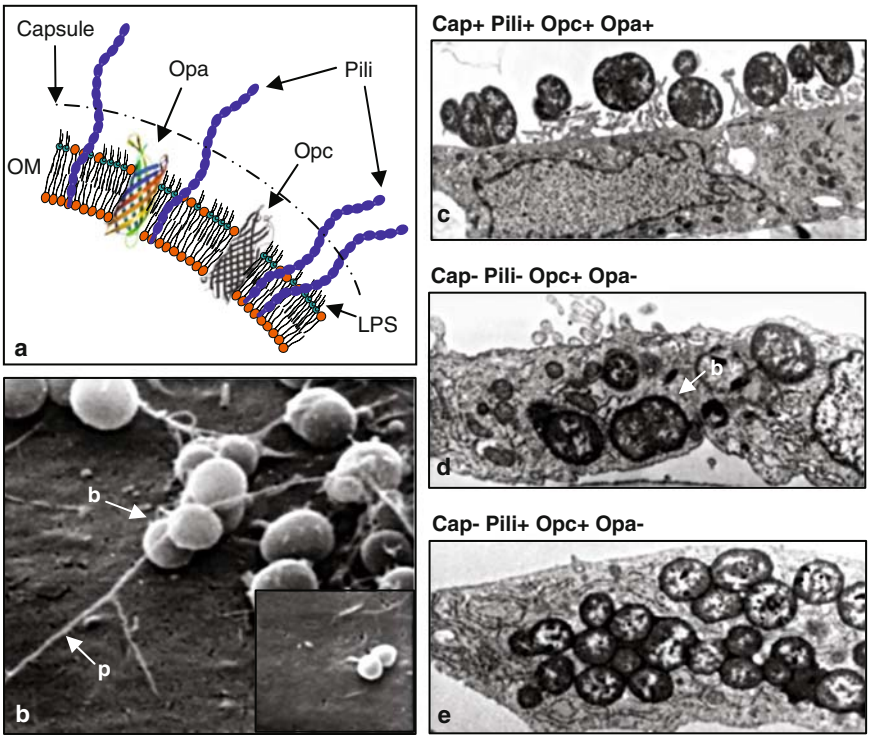

Fig. 1 Interplay between *Neisseria meningitidis* surface polysaccharides and adhesins in host cell targeting. **a** Some key common features of meningococcal disease and carriage strains that influence bacterial ability to adhere to and invade host tissues. OM: outer membrane. Meningococcal pili are long polymeric proteins which traverse the capsule and facilitate initial binding by overcoming the negative charge barriers at the cell–cell interface. They bind specifically to human cell receptors whose identity remains controversial to date. Opa and Opc are integral outer membrane proteins that have several surface exposed loops (four in Opa proteins and five in Opc) which participate in receptor binding. However, these adhesins are subcapsular and capsule can downmodulate their adhesion function in some settings. The ribbon diagrams are based on Opa-like *N. meningitidis* protein NspA (Neisseria surface protein A) [44] and the Opc structure as resolved by J. Derrick and others (adapted from [30]). (NspA diagram was kindly provided by Prof. Leo Brady, Department of Biochemistry, University of Bristol.) **b** The importance of pili (p) in mediating host cell interactions of capsulate bacteria (**B**) can be seen from the scanning electron micrographs (EM) of human endothelial cells infected with piliated meningococci. In contrast, very low binding of non-piliated bacteria is apparent in the inset of an equivalent field size at low magnification. In many cases, individual pili aggregate forming bundles that are often visible in EM (main picture, **b**) and can be seen making contact with the target cell surface. **c–e** *Evidence for interplay*. Transmission EM of the latter phenotype (capsulate, piliated and expressing the Opa and Opc adhesins) showing large numbers of adherent bacteria but none internalised (**c**). The cell entry inhibitory property of capsule is further apparent by comparison of **c** with **d** and **e** where capsule-deficient bacteria have been used to infect human endothelial cells. After a period of infection, a number of intracellular bacteria (**B**) were found in both cases. However, invasion potential of the phenotype in **e** (Opc+, Pili+) was much greater compared with **d** (Opc+, Pili−) [48]. (Note: Opa proteins were not expressed in the case of **d** and **e**.) As antibodies against Opc inhibit cellular invasion in both these cases, invasion is primarily driven by Opc.

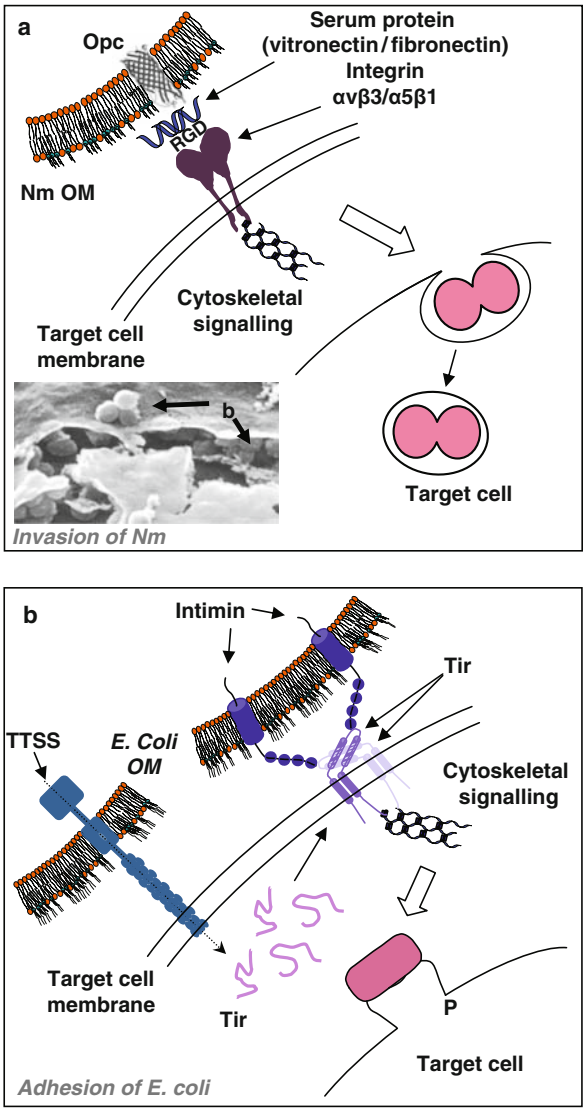

Fig. 2 Adhesion and invasion mechanisms of mucosal bacteria. **a** RGD-dependent targeting of human cellular integrins by *N. meningitidis*. Opc-expressing *N. meningitidis* (Nm) interacts with RGD-bearing serum proteins such as vitronectin and fibronectin present in the blood and subsequently via the formation of a trimolecular complex with integrin receptors on the apical surface of endothelial cells. This leads to cytoskeletal mobilisation and cellular invasion. *Inset*: Scanning electron micrograph of a fractured endothelial cell showing efficient invasion of human endothelial cells by capsule-deficient Opc-expressing bacteria (B) [46, 48]. **b** The Tir-Intimin system of *E. coli*. Enteropathogenic *E. coli* use their own protein Tir, which is injected into eukaryotic cells via the bacterial type three secretion system (TTSS). Tir is then inserted into the host plasma membrane and, in its dimeric form, binds to the intimin adhesin of the pathogens and leads to pedestal (P) formation on target cells at which bacteria become located [21, 23]. For detailed mechanisms see reviews [7, 9].

that can cause opportunistic infections in patients with AIDS. The bacteria contain β1 integrin subunit-like structures that enable them to bind to the integrin ligands including laminin, collagen and fibronectin, a process that is inhibited by anti-β1 integrin antibodies [31].

Other strategies of interest relate to mechanisms that lead to increased avidity of binding which can ultimately result in cellular invasion. Such a goal achieved via interactions involving multiple receptors on host cells have been illustrated in Figs. 1 and 3f for *N. meningitidis*. In other cases, distinct sites on a single receptor may be targeted. For example, some RGD-binding integrins have been shown to accommodate microbially coded RGD-bearing ligands and glycolipids at distinct sites. Such dual engagement appears to be required for cellular invasion [39]. Increased avidity may also be attained by upregulation of receptor expression on target cells. Numerous examples of such a strategy are available including *B. pertussis* upregulation of macrophage CR3 integrin via several mechanisms. Subsequent binding of the adhesin FHA to the integrin leads to macrophage entry that bypasses the phagocyte oxidative burst [35]. In other cases, target cell receptors upregulated via inflammatory cytokines are believed to form a potential basis of increased host susceptibility to infection (discussed below) or severity of infection. An example of the latter is the special pathology of acute cerebral malaria which appears to result from excessive adherence of infected erythrocytes to vascular endothelial CD36, ICAM-1, ELAM-1, and other receptors which are upregulated by circulating TNF-α during malarial infections [28].

Lectin-like interactions: As this subject has been addressed in the other chapters, only a few aspects relating to bacterial lectins are reiterated. In contrast to protein–protein interactions that can achieve a high degree of specificity and avidity, bacterial protein/host carbohydrate interactions generally allow greater promiscuity in receptor targeting via the common glycan substitutions of eukaryotic glycoproteins. In relation to the affinity of binding, some bacterial lectins can alter their conformational state in different environments to acquire high affinity for their targets. For example, it is proposed that high shear stress created by urine flows induces structural changes in the FimH lectin of uropathogenic *Escherichia coli* (UPEC) giving rise to a conformation with high affinity for its mannosyl receptor. This strategy may allow bacteria to attach to the colonising surface under high shear stress whilst allowing the bacterium to move by transient detachment under low shear stress [40].

Bacteria that generate their own receptors: Rather than using host molecules, enteropathogenic *E. coli* (EPEC) generate their own adhesion receptors. In a remarkable strategy, these pathogens utilize an injection system (the type three secretion system, TTSS) comprising a needle complex that inserts into the target cell membrane to deliver effector proteins such as Tir (translocated intimin receptor). Tir subsequently inserts into the host cell plasma membrane and acts as a receptor for the bacterial adhesin, intimin [21] (Figs. 2b).

The above examples were chosen to illustrate the diversity in microbial mode of niche colonisation. To stress the multifactorial and dynamic nature of colonisation and infection processes, the example of *N. meningitidis* is illustrated below.

3 Adhesion Mechanisms and Host Susceptibility: The Paradigm of *Neisseria*

The colonisation spectrum within respiratory neisserial species: commensalism to pathogenesis. Within the genus *Neisseria,* there are several species for which the only known host is man. Survival of the host must thus be a critical factor in influencing the evolution of their interaction mechanisms. Many neisserial species are harmless commensal organisms of the nasopharynx and are rarely associated with disease and include *N. lactamica* that shares numerous features with *N. meningitidis*. The latter, on the other hand, may colonise asymptomatically in up to 30% of a healthy population but has the capacity to become pathogenic and can cause fatal illness unless intervention measures are taken promptly. In this case, the disease is rare when compared to the occurrence of the bacterium in a healthy population and disease may be regarded as an accidental event, often arising as a result of immunedeficiency of the patient or other pre-disposing factors such as prior viral infections discussed below [4, 26].

Receptor modulation and transition from commensalism to pathogenesis. Susceptibility to infection by several respiratory colonisers including *N. meningitidis, H. influenzae* and *M. catarrhalis* increases markedly following influenza and/or respiratory syncytial virus (RSV) infections [1, 2, 4, 10, 12]. Further, epidemiological studies have shown spatial and temporal association between particular respiratory bacterial and viral infections. Since circulating inflammatory cytokines increase significantly following viral infections and since certain cellular receptors are upregulated in response to inflammatory cytokines, one potential mechanism may involve the upregulation of host receptors targeted by bacteria. Below, in vitro studies that examined this hypothesis have been briefly described.

Modulation of receptors by IFN-γ and consequences on bacterial adhesion and invasion was examined in a recent investigation. Besides epidemiological links between inflammation and infection, further impetus for this study was derived from the fact that the three mucosal opportunistic bacteria mentioned above all target CEACAMs (CEA-related cell adhesion molecules belonging to human carcinoembryonic antigen family (Fig. 3a), and that CEACAMs are expressed normally at low levels and are subject to upregulation by cytokines such as IFN-γ. In addition, studies on integrin upregulation have previously suggested that receptor density, by increasing the affinity of bacteria–host interactions, may encourage bacterial infiltration [19]. Indeed, transfected cell lines or IFN-γ treated epithelial cells expressing high levels of CEACAMs resulted in enhanced cellular invasion by *N. meningitidis* [10, 33]. Notably, whilst capsulate piliated bacterial interactions with cells expressing low levels of receptors do not lead to significant cellular invasion, a different scenario is observed in the setting of high surface density of CEACAMs. In this case, the meningococcal adhesins pili and Opa act synergistically in host cell binding which leads to greater invasion by capsulate bacteria (Fig. 3f). Since capsulate bacteria are serum resistant and meningococci can grow rapidly in blood, traversal of such a phenotype

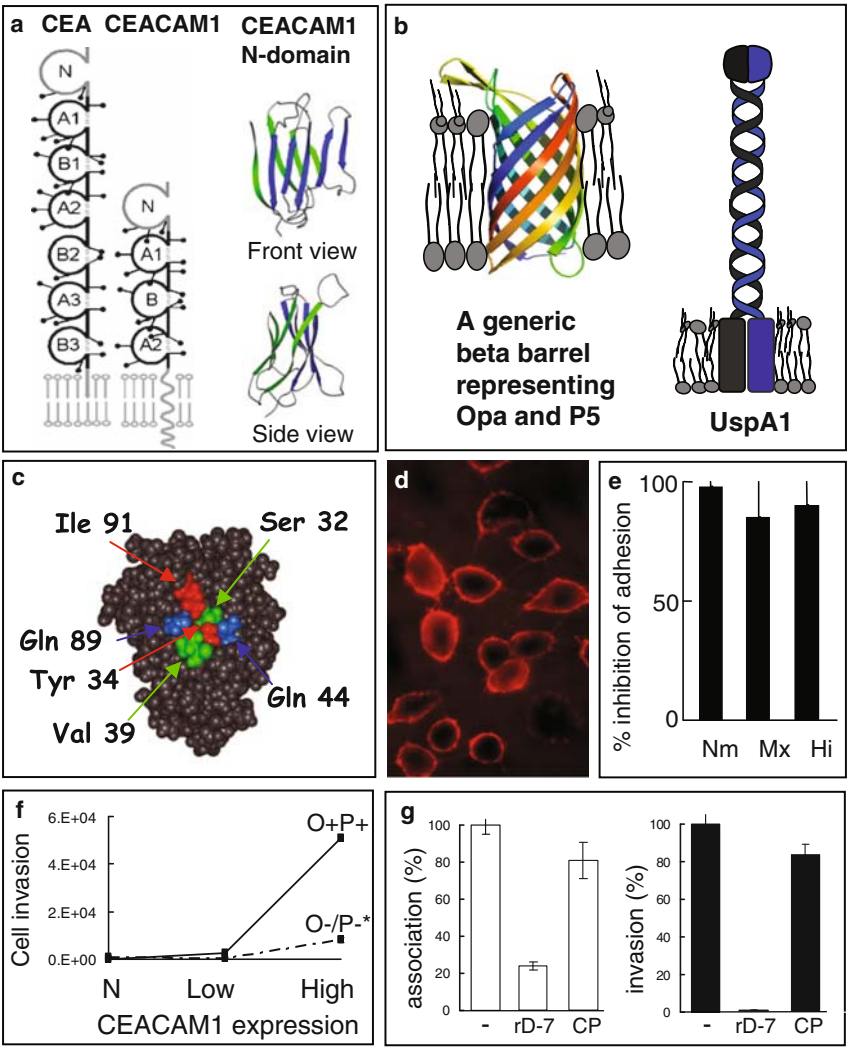

Fig. 3 CEACAM targeting of mucosal opportunistic bacteria. **a** Structure of the CEA-related cell adhesion molecules (CEACAMs). The molecular architectures of the GPI-anchored CEA and the transmembrane CEACAM1, two of the most widely expressed receptors, are presented. Glycans are shown as lollipop structures (http://cea.klinikum.uni-muenchen.de/) [17]. The family of molecules are characterised by their immunoglobulin variable (IgV)-like N-terminal domains and may contain none or several of Ig constant (Ig-C2)-like domains designated A1, B, etc. Ribbon diagrams of two views of the N-terminal domain (N-domain) of CEACAM1 (kindly provided by Dr. Andrea Hadfield, Department of Biochemistry, University of Bristol), required for the binding of several mucosal bacteria are shown. The immunoglobulin fold of the N-terminal domain consists of nine anti-parallel beta strands joined by loop regions arranged in two sheets (faces), ABED (*shown in green*) and C"C'CFG (CFG, for brevity; *shown in blue*). The ABED face is glycosylated whereas the CFG face is not; thus this protein face is fully exposed. **b** The molecular architectures of three of the bacterial ligands that bind to overlapping binding regions in the non-glycosylated CFG

across respiratory epithelium carries a high risk of widespread disease especially in a host with inadequate immunity against the pathogen (Fig. 4). In summary, adhesion appears not only to be essential for mucosal colonisation, but certain adhesin-receptor interactions could be key to microbial pathogenic potential.

Fig. 3 (continued) face of the receptor are shown [14, 16, 47]. The CEACAM-binding Opa proteins of *N. meningitidis* and P5 protein of *H. influenzae* are eight-stranded beta barrel molecules represented here by a generic structure based on that of NspA. However, at the amino acid level, the two proteins have little homology (11% similarity between P5 of *H. influenzae* strain Rd and OpaB of *N. meningitidis* strain C751) [16]. The genome of *N. meningitidis* encodes up to four different Opa proteins with variable surface-exposed loops. These variant Opa proteins nonetheless are able to bind to CEACAMs. The *M. catarrhalis* adhesin UspA1 that binds to CEACAMs is an extended 'lollipop'-like structure that exists as a trimer in the outer membrane. Notably, all three adhesins share little sequence similarities that could be assigned to their shared CEACAM-binding property. **c** A space filler model of the human CEACAM1 N-terminal domain showing the residues (in the CFG face) that are important in binding of the Opa proteins, with Ile-91 playing a central role. This residue is also central to the recognition of P5 and UspA1 [45]. **d–g** Complexities of ligand/receptor interplay and attempts at blocking invasion. A recombinant polypeptide (termed rD-7) based on UspA1, was produced that bound to CEACAM1 [15]. The efficient binding of rD-7 to surface expressed CEACAM1 is demonstrated by the red fluorescence of the indirectly labelled rD-7 in **d**. This immunofluorescence micrograph shows transfected HeLa cells expressing CEACAM1, which were incubated with rD-7 and the bound polypeptide was detected using rabbit antiserum against rD-7 followed by rhodamine conjugated anti-rabbit antibodies. The recombinant molecule rD-7 inhibits binding of multiple strains of the three species to HeLa-CEACAM1 cells (Nm: *N. meningitidis*, Mx: *M. catarrhalis* and Hi: *H. influenzae*) (**e**). In each case, mean % inhibition (and the range) of binding of ten strains in the presence of rD-7 compared to a control peptide is shown. The cellular invasion of capsulate *N. meningitidis* expressing either Opa or pili or both the adhesins was assessed using cell lines with distinct levels of surface CEACAM1 (**f**). For these experiments, untransfected HeLa cells were used as negative controls (N) in conjunction with HeLa transfectants expressing low or high receptor densities. Significant invasion of cells was only observed in cell lines expressing high levels of CEACAMs and both pili and Opa-expression was required for such invasion demonstrating the synergism between the adhesins in facilitating invasion by capsulate serum resistant phenotype. *Since the expression of Opa alone or pili alone in capsulate bacteria did not result in significant invasion, a single line is shown on the graph which represents their approximate invasion levels (O-/P-). The results illustrate one possible manner in which host cell susceptibility to invasion might increase during inflammation when circulating cytokines lead to receptor augmentation [10, 33]. The efficacy of rD-7 in blocking cellular adhesion or invasion was assessed in the setting of high receptor density. Capsulate (Opa+, pili+) meningococcal adhesion was reduced but not abrogated in the presence of the peptide, whereas invasion was completely abolished (**g**). Control peptide (CP) had no significant effect on cellular adhesion or invasion. The data indicate the key role of Opa-CEACAM interactions in driving invasion, and the potential of specific intervention measures in preventing this step

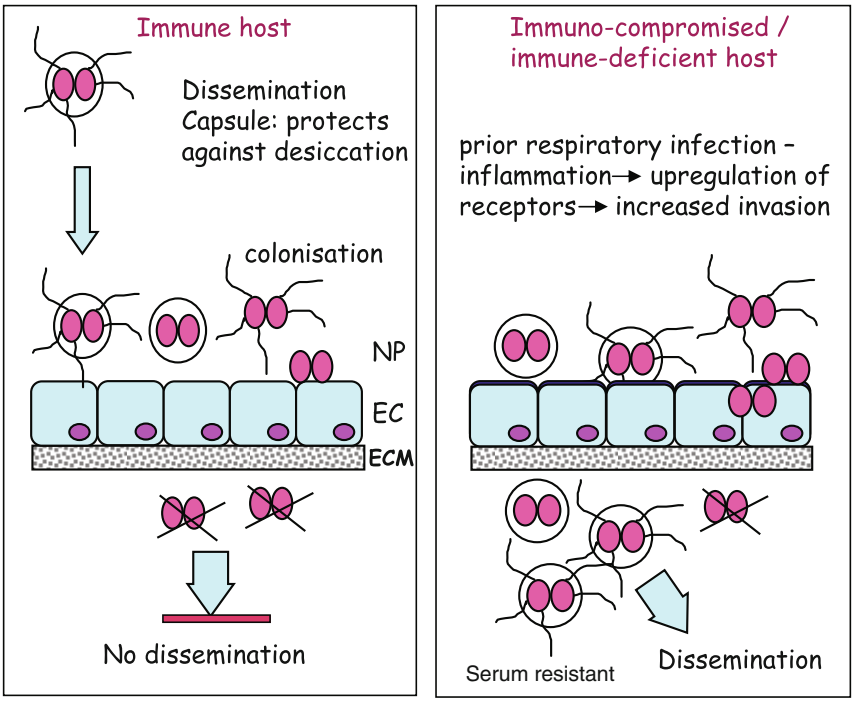

Fig. 4 Possible stages in the colonisation and pathogenic processes of *N. meningitidis.* A model based on epidemiological and in vitro experimental observations. Phase variation of surface structures is common in *N. meningitidis* and capsule-deficient phenotypes are found in the nasopharynx. Such a phenotype is invasive but is serum sensitive and does not survive in the blood. In the case illustrated on the right, where infiltration of capsule-expressing bacteria is possible, dissemination throughout the body may ensue unless the host has the ability to clear this phenotype efficiently from the blood (NP: Nasopharynx; EC: epithelial cell; ECM: extracellular matrix)

4 Potential Measures to Prevent Microbial Infection and Concluding Comments

The importance of invasion: Microbes require a foothold in a niche whilst avoiding elimination by the host. Within the array of mechanisms that have evolved to meet the latter requirement, epithelial cell invasion is regarded by many as a potential mechanism of hiding from professional phagocytes such as macrophages and neutrophils as well as other anti-microbials (antibodies, complement components, antimicrobial peptides, etc.). Whilst some microbes have evolved strategies to enter eukaryotic cells and multiply within, others may use this strategy merely to survive for periods and persist in the niche [6]. As noted above, meningococcal location within tonsillar epithelial cells has been reported. Bacteria then exit from these cells in either the apical direction to transmit to a new host or in the basolateral direction to enter the body. Armed with anti-phagocytic/anti-immune strategies such as

capsule, bacteria may survive in the blood and disseminate throughout the body (Fig. 4). Whilst this could be one scenario leading to disease, it is not a foregone conclusion that tissue invasion is required for infection. Indeed, mucosal damage, however small, may achieve the same effect by enabling bacteria to enter the body unhindered. However, the ability of a large number of mucosal pathogens to invade target cells or cross intact epithelial barriers does draw our attention to this potentially crucial step in the process of infection.

5 Natural and Artificial Anti-Adhesion/Anti-Invasion Measures

Receptor decoys in protein–glycan/protein–protein interactions. The intestinal pathogens *E. coli* and *Salmonella* use their mannose-binding lectins to target the abundant glycans of CEACAMs [22]. It has been suggested that in an evolutionary counter strategy, the GPI-anchored member, CEA (product of *ceacam5* gene), is shed daily in large amounts in the faeces, thus reducing pathogen load. CEA may thus be regarded as an arm of innate immunity that utilizes shed receptors as decoys [13]. In the case of the more recently recognised interactions of respiratory pathogens *N. meningitidis, H. influenzae* and *M. catarrhalis* with CEACAMs, it is becoming clear that the majority of the strains of these species bind preferentially to the transmembrane molecule CEACAM1, which may not be as readily shed. Interestingly, the pathogens utilise structurally divergent adhesins (Fig. 3b) to bind to a common protein site on the receptor (Fig. 3c) [3, 47, 49]. This focuses our attention on whether a common anti-adhesion strategy can be devised to control infections caused by them. Whether receptor decoys (perhaps in the form of small peptides) will prove effective in controlling bacterial infection in humans is at present unclear. However, since CEACAMs participate in several important physiological functions including homotypic interactions, any measures that may interfere with natural receptor functions need to be carefully evaluated. In a somewhat variant approach, infection preventing property of a CEACAM-binding recombinant peptide fashioned after a bacterial ligand, has been examined in vitro as outlined below.

The use of CEACAM-blocking peptides to inhibit invasion. Based on *M. catarrhalis* UspA1 adhesin that binds to CEACAMs, a recombinant peptide was generated with the capacity to bind specifically to CEACAMs (Fig. 3d) and block the binding of *N. meningitidis, H. influenzae and M. catarrhalis* to CEACAM expressing cell lines [15] (Fig. 3e). In vitro, the peptide significantly inhibits Opa-CEACAM-mediated cellular invasion of the Opa+, pili+ phenotype whilst not eliminating pilus-mediated adhesion which occurs via a distinct receptor. Importantly, this occurs in the post-inflammation models of infection in which CEACAM density of target cells is enhanced supporting high levels of cellular invasion [10, 33] (Fig. 3g). Thus, if the hierarchical importance of the receptors involved in mucosal barrier penetration can be identified, then it may be possible to inhibit selectively the route of cellular invasion without abrogating bacterial adhesion. This may have an added advantage in the case of the frequent mucosal

colonisers since the possibility remains that elimination of such bacteria could lead to colonisation by other unwanted agents and their presence on mucosa may serve to balance the local ecology and boost immunity. Whether such anti-invasive measures will be effective in vivo and whether they can be fashioned to prevent bacterial interactions without receptor interference and resultant side effects remains to be seen.

Vaccines. Perhaps the most effective strategy is to immunise with key adhesins/invasins to induce endogenous blocking antibodies. This approach, however, requires an extensive knowledge of the precise binding mechanisms of bacterial adhesins, which have proved extremely difficult in some cases. Adhesins with variant structures able to target a single receptor are not uncommon as exemplified by the Opa proteins of *N. meningitidis*. Such structural/antigenic variation is perhaps the greatest challenge to devising anti-adhesion vaccines based on bacterial ligands. Where the knowledge is available and the adhesin is largely invariant, successful use of adhesins has been made to prevent colonisation and disease in animal models, e.g., the FimH and the PapG adhesins of pathogenic *E. coli* [29, 32].

Anti-adhesion therapy for lectin-like interactions: the evidence for their efficacy. As is pertinent for this book, the final reflection should be on lectin adhesins of microbes. A number of enteric infections have been shown to be successfully prevented by administration of receptor mimics and have been reviewed recently [36]. One important example that illustrates the protective effects of glycans is the observation that infants fed on milk with high oligosaccharide levels are largely spared from high incidence of diarrhoea compared with those fed on low carbohydrate milk. This has been assigned to the presence of fucosylated oligosaccharides which are effective inhibitors of attachment of the enteric pathogen *Campylobacter jejuni* and of the stable toxin of enterotoxigenic *E. coli* [25]. Additionally, both carbohydrate mimics and antibodies to adhesins have been shown to be effective in a number of animal studies. However, human clinical trials using adhesion-inhibiting oligosaccharides have been disappointing for the respiratory pathogens *H. influenzae and S. pnemoniae*. Neither their incidence of colonisation nor otitis media could be prevented [41]. Similarly, for the enteric bacterium *Helicobacter pylori* (a causative agent of peptic ulcers and cancers), no reduction in colonisation was obtained [27], perhaps reflecting the multifactorial and dynamic nature of bacterial adhesion processes. It is entirely possible that high affinity blocking agents and cocktails of anti-adhesion drugs will finally succeed against some such pathogens! The only question is: Do we have the tools to study the complexities of in vivo dynamics at the host–microbe interface in order to devise effective anti-adhesion measures to combat problematic microbial diseases?

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