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Review

Bacterial Adhesins in Host-Microbe Interactions

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Most commensal and pathogenic bacteria interacting with eukaryotic hosts express adhesive molecules on their surfaces that promote interaction with host cell receptors or with soluble macromolecules. Even though bacterial attachment to epithelial cells may be beneficial for bacterial colonization, adhesion may come at a cost because bacterial attachment to immune cells can facilitate phagocytosis and clearing. Many pathogenic bacteria have solved this dilemma by producing an antiphagocytic surface layer usually consisting of polysaccharide and by expressing their adhesins on polymeric structures that extend out from the cell surface. In this review, we will focus on the interaction between bacterial adhesins and the host, with an emphasis on pilus-like structures.

Introduction

The concept of bacterial adhesion to host cells was first appreciated in 1908, when it was reported that *Escherichia coli* could hemagglutinate animal cells by appendages later determined to be multimeric pili. The continued characterization of pilus structure and function, as well as the discovery of monomeric adhesins and their roles in tissue tropism, set the stage for a vast field of study surrounding how bacteria adhere to their host and the host response to this interaction. It is now known that most commensal and pathogenic bacteria interacting with eukaryotic hosts express adhesive molecules on their surfaces that promote interaction with host cell receptors or with soluble macromolecules. Even though bacterial attachment to epithelial cells may be beneficial for bacterial colonization, adhesion may come at a cost because bacterial attachment can also stimulate immune cell infiltration, activation, and phagocytosis, which facilitate bacterial clearing. Many pathogenic bacteria have solved this dilemma by producing a surface layer that prevents immune recognition or phagocytosis and by expressing their adhesins on polymeric structures that extend out from the cell surface, allowing for initial host interactions at a "safe" distance. In this review, we focus on the interaction between extended polymeric bacterial adhesins and the host. We describe the best-characterized pilus structures, such as type 1 pili, P-pili, type IV pili, curli in Gram-negative bacteria, and several recently recognized pili in Gram-positive organisms, as well as their interaction with host cell receptors, such as toll-like receptors, thereby evoking proinflammatory responses. We delineate the role of non-pilus structures in adhesion and the importance of attachment to host cells for the delivery of effector proteins to host cells by type III and type IV secretion systems. Moreover, we address how adhesion to immune cells may lead to evasion of immune responses and increased survival inside phagocytes. Finally, we discuss novel approaches to inhibit bacterial adhesion as potential drug targets, as well as adhesins as potential candidates in protein-based vaccines.

Major Bacterial Adhesin Families and Their Ligands

Bacterial adhesive surface structures, especially bacterial pili or fimbrial adhesins in Gram-negative organisms, have historically been the predominating adhesins studied. However, over the years, a large number of monomeric surface-bound adhesive proteins have been identified. Some, but not all, of these adhesins are filamentous and can reach beyond a capsular layer. It was initially believed that bacterial adhesion to host cells only provided a means for bacteria to colonize a site and a way to avoid clearance by mucosal secretions and peristalsis. However, it has become clear that bacterial adhesins depend on ligand interactions to mediate a series of signaling events that may affect bacterial uptake or invasion and/or promote pro- or anti-inflammatory events by affecting innate immune receptors. A large number of bacterial surface-bound proteins interact with soluble ligands present in serum. Although these binding events are not adhesin-ligand interactions in a strict sense, such contacts do play a significant role during bacterial invasion, for example, by evading complement deposition (Friberg et al., 2008) or by binding of serum components belonging to the contact system, resulting in coagulation, release of proinflammatory molecules, and generation of cleavage products acting as antimicrobial peptides (Frick et al., 2006).

Chaperone-Usher Pili

The biogenesis and regulation of bacterial adhesins have been studied in detail for several pathogens. Early studies from Swedish groups revealed that *E. coli* isolates from children with acute kidney infection agglutinated human erythrocytes in a P-blood group-specific manner and identified the receptor as galα(1–4)βgal present in the globoseries of glycolipids (Kallenius et al., 1981; Leffler and Svanborg-Eden, 1981). It was subsequently determined that two adhesive properties were encoded by two different pilus operons in uropathogenic *E. coli* (UPEC), in which the *fim* operon encoded type 1 pili-expressing mannose-sensitive hemagglutination and the *pap* operon encoded P- or Pap-pili responsible for the interaction with the digalactoside unit in the P-blood group antigen. Whereas the *fim* operon was present in all *E. coli* isolates, *pap* was part of a pathogenicity island also encoding other putative virulence determinants, such as hemolysin. Both types of pili were found to be heteropolymeric structures composed of one major pilus subunit protein building up the pilus stalk and several minor subunit proteins at

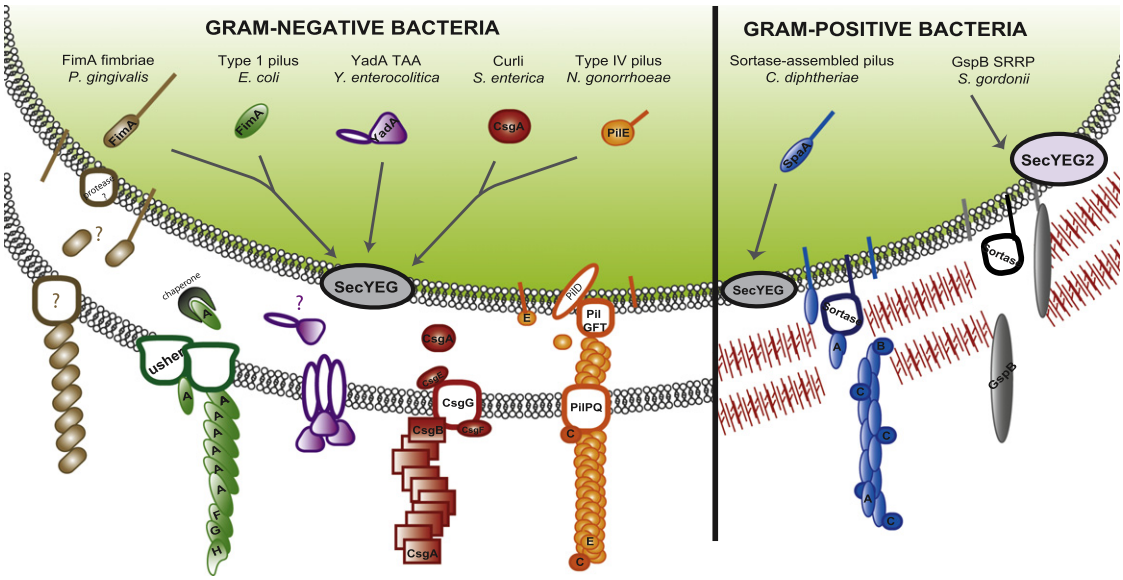

Figure 1. Schematic Representation of Bacterial Pili or Pilus-Like Adhesive Structures, Their Secretion, and Assembly

Bacterial cell depicted at the top in green with representative adhesive structural subunits shown in the cytoplasm. Each structure and associated assembly machinery is color coordinated. Subunits with extended lines indicate sequences, in addition to Sec secretion signal sequences, that are cleaved prior to assembly on the bacterial surface (i.e., for *P. gingivalis* FimA and *N. gonorrhoeae* PilE). The subunit for each structure is indicated in the cytoplasm, and arrows indicate the Sec translocon through which the subunits pass. Additional dedicated membrane translocation/processing machinery are indicated by an open circle at the membrane, and additional subunits (if present) are labeled on the assembled structure. Question marks next to cytoplasmic subunits indicate that the exact form of the protein in the periplasm is not known. Details of each pilus secretion and assembly pathway are in the text.

the distal end. One of these minor proteins, PapG and FimH, respectively, represented the actual adhesin (Figure 1). Each adhesin has two domains, a pilin domain allowing copolymerization and a second carbohydrate-binding lectin domain (Hultgren et al., 1989; Schilling et al., 2001).

The ordered assembly of type 1 and P-pili has been described in molecular detail and forms the paradigm for the chaperone-usher pathway of pilus formation. This pathway involves the secretion of structurally incomplete pilin subunits across the cytoplasmic membrane via the SecA/Y pathway, the completion of the pilin fold by interaction with a periplasmic chaperone, and the polymerization of pilin subunits across an outer-membrane usher or translocator via a process in which the incoming pilin replaces the chaperone to complete the fold by donor strand complementation. The outer-membrane usher exists as a twin pore structure in which one pore is used for subunit secretion and the other remains plugged for secretion (Remaut et al., 2008). It is proposed that two ushers facilitate reiterative binding of chaperone/subunit complexes at one usher and subsequent interaction with the previously assembled subunit at the second usher. The actual adhesins FimH and PapG, respectively, end up at the distal end because the chaperone-adhesin complex has the highest affinity for the outer-membrane usher (Remaut et al., 2006, 2008). A large number of pilus adhesins of enteric organisms are assembled via the chaperone-usher pathway, including the colonization factor antigens expressed by enterotoxigenic *E. coli* (ETEC) (Poole et al., 2007) and the Dr fimbrial adhesin of UPEC (Piatek et al., 2005).

Type IV Pili

In contrast to the chaperone-usher assembled pili, type IV pili expressed by Gram-negative pathogens such as *Neisseria spp.*, *Pseudomonas aeruginosa*, and enteropathogenic *E. coli*, as well as by Gram-positive pathogens such as *Clostridium perfringens* and *Streptococcus sanguis*, are assembled via a completely different process (Figure 1). Type IV pilin proteins are cotranslationally translocated across the inner membrane as pre-pilins, whereupon the inner-membrane pre-pilin peptidase recognizes and cleaves a conserved N-terminal leader sequence, releasing a mature pilin peptide. Studies of the prototypic neisserial type IV pili indicate that, after pilin monomers are released from the inner membrane, the pilus fiber is assembled in the periplasm by an unknown mechanism that requires an ATPase and four additional proteins of unknown function (Carbonnelle et al., 2005, 2006; Wolfgang et al., 2000). An outer-membrane secretin pore is required for the translocation of the pilus to the cell surface. Pilin of *N. gonorrhoeae* has two posttranslational modifications in the surface exposed and highly antigenically variable regions of the pilin structure: an O-linked phosphocholine or phosphoethanolamine and an O-linked glycosylation (Hansen and Forest, 2006). The presence and characteristics of glycosylated bacterial adhesins are becoming increasingly appreciated (Chamot-Rooke et al., 2007; Vik et al., 2009), and it has been proposed that these modifications may contribute to antigenic variation of the pilus or function in pilus biogenesis; however, their true physiological function is unknown.

Despite considerable efforts over more than two decades, it is fair to say that we still do not know how type IV pili mediate attachment. In addition to the major type IV pilin subunit, *N. gonorrhoeae* encodes a set of five proteins that are structurally similar to the pilus subunit and, when inactivated, result in a dramatically reduced type IV pilus expression. It is not known whether one or more of these minor putative pilins are also acting

as adhesins (Winther-Larsen et al., 2005). PilC has been reported to be the neisserial type IV pilus tip adhesin, as well as to be associated with the outer membrane of the bacterium. PilC can mediate pilus-dependent adherence to epithelial cells (Rudel et al., 1995). CD46 was the first cellular receptor identified for the neisserial pilus, but this observation has been controversial (Gill and Atkinson, 2004), in part because no direct interaction between the adhesin and putative ligand has been demonstrated. Further, whether the major pilin subunit executes host ligand binding or whether minor pilus-associated proteins such as PilC carry out this function is still unclear. Recent studies demonstrate CD46-independent interaction of PilC with a proteinaceous ligand expressed on immortalized epithelial cells (Kirchner et al., 2005; Kirchner and Meyer, 2005), the identity of which may shed light onto a bona fide neisserial pilus receptor. Finally, studies in primary cervical epithelial cells show that the I domain of integrins interacts with *N. gonorrhoeae* pili, and this interaction is required for adherence and invasion (Edwards and Apicella, 2005).

Curli

A subset of enteric commensals and pathogens, such as *E. coli* and *Salmonella spp*., as well as a number of environmental Gram-negative organisms express adhesive amyloids. These structures, called curli or thin aggregative fimbriae, are formed in a nucleation-dependent process in which the major subunit protein, CsgA, is secreted across the inner membrane via Sec and across the outer membrane via a multimeric outer-membrane CsgG pore in a soluble form but undergoes a conformational change when interacting with a related surface-bound CsgB subunit to form insoluble amyloid fibers (Figure 1). Curli amyloids are notoriously sticky without exhibiting a clear ligand-binding specificity. Most commensal isolates of *E. coli* and *Salmonella* only express curli at room temperature. However, a number of clinical *E. coli* urosepsis isolates also express curli at 37°C, suggesting a role in pathogenicity (Bian et al., 2000). Curli have been shown to be one component in the matrix formed by enterics during their sessile mode of growth and may interact with cellulose, which is the other main matrix component (Zogaj et al., 2001). Interestingly, Alteri et al. recently described the presence of pili in *Mycobacterium tuberculosis*, which had morphological, biochemical, and functional properties similar to curli amyloid fibers found in Gram-negative bacteria (Alteri et al., 2007).

Trimeric Autotransporter Adhesins

An increasing number of adhesins of Gram-negative proteobacteria, such as *Yersinia enterocolitica*, *Neisseria meningitidis*, and *Haemophilus influenzae*, belong to the trimeric autotransporter adhesins (TAA) family (Hoiczyk et al., 2000; Serruto et al., 2009). Secretion of each autotransporter begins with Sec-dependent translocation across the inner membrane. Subsequently, the transporter domains of each of the three subunits insert into the outer membrane to form a translocator, which allows for the autotransport of linked passenger domains across the outer membrane (Figure 1). These adhesins are characterized by the ability to form highly stable trimers on the bacterial surface and by a common mechanism of secretion, which is linked to their trimerization (Cotter et al., 2005; Linke et al., 2006). The TAAs have a head-stalk-anchor architecture, in which the heads are the primary mediators of attachment. The TAAs mediate bacterial interaction with either host cells or extracellular matrix proteins and, in some cases, induce invasion of target cells (Girard and Mourez, 2006; Scarselli et al., 2006).

Sortase Assembled Pili

Pili in Gram-positive bacteria were first described in 1968 in *Corynebacterium renale* (Yanagawa et al., 1968) but have recently been observed in many potentially pathogenic Gram-positive organisms, such as *Corynebacterium diphtheriae*, *Streptococcus agalactiae* (also called Group B streptococci or GBS), *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Enterococcus faecalis*, and *Actinomyces naeslundii*, among others. In contrast to pili in Gram-negative bacteria, pili in Gram-positive organisms are composed of covalently linked subunits. After Sec-mediated secretion of the pilins, transpeptidases called sortases link the pilin monomers to each other and eventually transfer the pilus to the peptidoglycan cell wall (Figure 1) (Mandlik et al., 2008a; Proft and Baker, 2009; Scott and Zahner, 2006). An interesting paradigm is emerging among some Gram-positive cocci, whereby pilus secretion and assembly occur in a coordinated and focal pattern at the cell surface (Falker et al., 2008; Kline et al., 2009). In *E. faecalis*, sortase enzyme focal localization is mediated by a positively charged cytoplasmic domain within the enzyme itself. Focal assembly of pili may be energetically favorable given the multiple biological processes, including pilin secretion, subunit polymerization, cell wall synthesis, and pilus fiber attachment to the cell wall that must be spatially and temporally coordinated for efficient fiber biogenesis.

Adhesin-Receptor Interactions at Mucosal Surfaces

Together with secreted proteins, adhesins are among the first bacterial molecules to make physical contact with the host. Interactions between microbial adhesins and host cell receptors can lead to signaling events that trigger host inflammatory responses. Hence, both pilus-associated adhesins as well as non-pilus structures and their specific receptors in this interaction will influence the mucosal responses obtained. Attachment to host cells is also important, enabling secretion of effector proteins through bacterial secretion systems.

Pilus-Associated Adhesins

Most studies on bacterial adhesins and associated inflammatory mucosal responses emanate from studies on UPEC-mediated urinary tract infections (UTI), in which it was demonstrated that bacterial adherence to the uroepithelium was associated with inflammation, including a mucosal cytokine response. The underlying mechanisms for adhesin-mediated proinflammatory responses have been harder to pinpoint. In human challenge studies, *E. coli* expressing P-pili mediating adherence to galα(1–4)βgal-containing glycosphingolipids have been shown to elicit an inflammatory response in bladder epithelium, whereas type 1-piliated *E. coli*, usually associated with acute cystitis and binding to mannosylated glycoproteins (Figure 2) in the uroepithelium, did not evoke an inflammatory response in volunteers (Bergsten et al., 2007; Wullt et al., 2001). However, when mucosal biopsies from various parts of the urinary tract were exposed to either type 1 or P-piliated *E. coli*, a rapid cytokine response was observed that exceeded that obtained by non-piliated controls (Bergsten et al., 2007). In most studies, the adhesin-mediated proinflammatory response in the murine urinary tract has been linked to toll-like receptor 4 (TLR4)

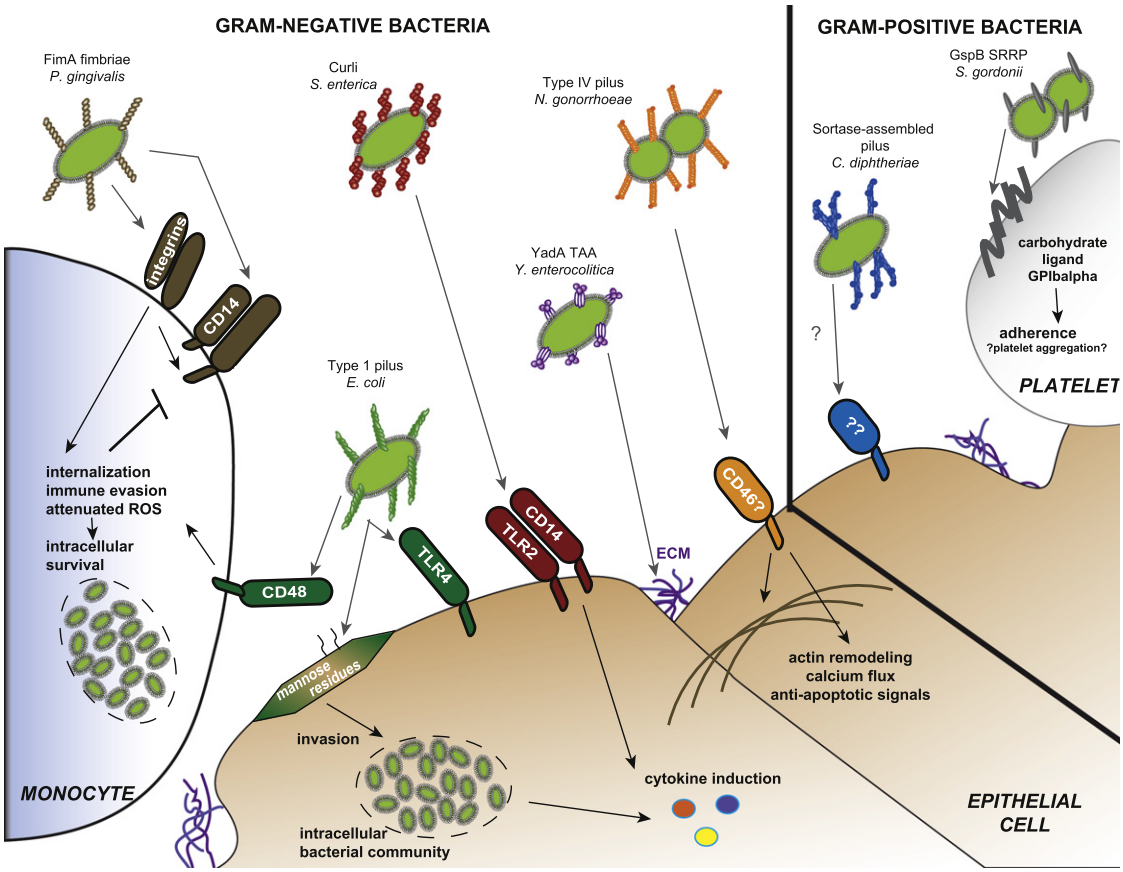

Figure 2. Bacterial Pili or Pilus-Like Adhesive Structures and Host Targets

Host cell receptors are color coded to correspond to the interactive microbial adhesive structure. Arrows indicate known host ligand and/or receptor interactions, downstream signals, and functional outcomes associated with the adhesin-ligand interaction. Question marks indicate controversial or unknown points of interaction. The details of each adhesin-host interaction are described in the text. ROS, reactive oxygen species; ECM, extracellular matrix proteins; GPIbalpha, glycoprotein Ibalpha.

activation on both the bladder epithelial cells and immune cells (Schilling et al., 2003). It has been suggested that the PapG adhesin of *E. coli* P-pili triggers TLR4 activation independent of lipopolysaccharide (LPS) by binding to glycosphingolipid receptors. A proposed mechanism is that glycosphingolipid receptors for P-fimbriae can recruit TLR4 as coreceptors (Frendeus et al., 2001). Murine models have been extensively used to demonstrate a role for the FimH adhesin of type 1 pili in activating lipid raft-dependent internalization into bladder epithelial cells (Figure 2). Interestingly, this internalization process is considerably higher in the absence of TLR4 (Song et al., 2007). TLR4 signaling through increased production of the secondary messenger, cAMP, negatively regulates lipid raft-mediated bacterial invasion (Bishop et al., 2007). Each internalized UPEC bacterium can rapidly replicate to as many as 10^5 bacteria per cell, resulting in the maturation of intracellular bacterial communities (IBCs), a niche with biofilm-like properties protected from innate defenses and antibiotics (Anderson et al., 2003; Justice et al., 2006). Bacteria within each IBC of the infected bladder epithelium have the potential to undergo morphological changes, flux out of the infected cell, and go on to infect neighboring cells. Within IBCs, bacteria produce type 1 pili, and in vivo repression of piliation abrogates formation of IBCs, arguing for a role of type 1 fimbriae in intracellular biofilm formation (Wright et al., 2007). The multitude of biological effects mediated by type 1 fimbriae could depend on the fact that the FimH adhesin binds to mannose residues on glycoproteins. Hence, a multitude of host cell glycoproteins are potential receptors for the FimH lectin. The mechanisms for FimH-mediated direct activation of TLR4 (Mossman et al., 2008) might also be due to the lectin-binding nature of FimH.

Curli elicit an inflammatory response in the host (Figure 2). *E. coli* and *Salmonella* curli can bind and assemble the contact-phase system on the bacterial surface, leading to the release of bradykinin, which is a potent inducer of fever, pain, and hypotension (Herwald et al., 1998). In a murine sepsis model, curliated *E. coli* evoked a more dramatic drop in blood pressure as compared to noncurliated mutants (Bian et al., 2001). The drop in blood pressure was dependent on inducible nitric oxide synthase 2 expression. It was subsequently shown that the major curli subunit in *Salmonella*, CsgA, behaves as a microbial-associated molecular pattern (MAMP) for TLR2 and that purified CsgA protein elicited interleukin-8 (IL-8) production in epithelial cells transfected with TLR2/CD14 (Tukel et al., 2005). It will be

interesting to identify precisely which sequence in CsgA promotes TLR2 activation and how this sequence correlates to the proinflammatory effects of human amyloids such as amyloid-β peptide, which plays a central role in the pathogenesis of Alzheimer's disease. It should be noted, though, that direct TLR4 and TLR2 stimulation by pili and curli, respectively, has been controversial in the literature because it is hard to purify these components and there is a risk for contamination by LPS and lipopeptides.

As obligate human pathogens, *N. gonorrhoeae* and *N. meningitidis* must adhere to host mucosal surfaces to persist within their hosts. The pathogenic *Neisseriae* utilize type IV pili for initial attachment, often adhering as microcolonies whose formation is also dependent on type IV pili (Lappann et al., 2006). Adherence to host epithelial cells leads to Ca^{2+} flux and intracellular kinase activity, resulting in downregulation of proapoptotic proteins (Figure 2) (Howie et al., 2008; Merz and So, 2000). In addition to cytoprotective effects, these signaling events also result in local remodelling of host cell membranes and the actin cytoskeleton and in cortical plaque formation, proposed to facilitate further signaling to modulate the host inflammatory response. Interestingly, *N. meningitidis* appear to have made use of host cytoskeletal rearrangements such that the bacteria specifically induce host cell filipodium formation to create a protective "nest" around bacterial microcolonies, rendering them resistant to shear stress likely to be encountered in the bloodstream (Mikaty et al., 2009). A common property of type IV pili is their ability to retract. The retractile force generated by bundled pili is as high as 1 nN (Biais et al., 2008). These strikingly high mechanical forces are critical to many interactions between *N. gonorrhoeae* and epithelial cells, including microcolony and cortical plaque formation leading to cytoprotection (Higashi et al., 2007). Hence, the initial attachment of the microbe to host cells by pilus-associated adhesins influences host inflammatory responses and leads to cytoprotection and remodelling of the host cell membrane and cytoskeleton.

Bacterial Adhesins Associated with or Presented on Non-Pilus Surface Structures

Adhesins belonging to the TAA family, such as NhhA from *N. meningitidis*, UspA1 from *Moraxella catarrhalis*, or YadA from enteropathogenic *Yersinia*, share a common binding affinity to components of the extracellular matrix (Figure 2) (Conners et al., 2008; Hoiczyk et al., 2000; Scarselli et al., 2006). Some of these interactions can result in invasion into host cells. In the case of HadA from certain strains of *H. influenzae*, the invasion process occurs independently of clathrin, indicating the induction of signaling events distinct from those of zippering bacteria (Serruto et al., 2009). Notably, most TAAs also seem to confer serum resistance to bacteria by binding components of the complement system (Conners et al., 2008; Kirjavainen et al., 2008; Sjolinder et al., 2008).

Relatively recently, the adhesive properties of flagella have been recognized. The adherence of ETEC to intestinal cells was previously thought to depend only on the production of different pilus-like colonization factors. However, ETEC flagella have now also been implicated in adhesion. The two-partner secretion (TPS) adhesin EtpA was shown to specifically bind to conserved residues of the flagellin positioned at the tips of mature flagella. These flagellin domains become accessible to the EtpA adhesin by the loss of the FliD cap protein complex (Roy et al., 2009). The attachment of enterohemorrhagic *E. coli* (EHEC) O157 to bovine rectal epithelial cells is also dependent on the flagellum (Roy et al., 2009). Whether this binding is executed by the flagellum itself or via its H7 binding adhesin, as for ETEC, is currently unknown. Given the wide variety of bacterial species that express flagella, as well as TPS proteins, this interactive mode of adhesion may represent a common but overlooked paradigm for bacterial-host interactions. In this context, it is interesting to analyze whether bound adhesins interfere with the established role of flagellin as a proinflammatory TLR5-ligand. Further, because flagella not only provide motility, but also represent a type III secretion apparatus, flagellar-mediated adhesion and host effector secretion might be linked.

The adhesive nature of different bacterial secretion structures has been largely unstudied. For the type III secretion apparatus to deliver effector proteins into target cells, bacterial adhesion is necessary. In the case of different enteropathogenic *Yersinia* species, the three adhesins, invasin, YadA, and Ail, have been shown to promote T3SS-dependent delivery of *Yersinia* effector proteins (Rosqvist et al., 1990). However, these adhesins are not part of the type III secretion apparatus itself but are expressed as outer-membrane proteins. The attaching and effacing lesions (A/E) seen upon intestinal infections with EHEC and the closely related enteropathogenic *E. coli* (EPEC) depend on bacterial type III secretion of TIR that act as a translocation receptor for the adhesin intimin (Kenny et al., 1997). The forces created by this interaction, however, are not sufficient for firm EPEC attachment but require other adhesin-ligand interactions, such as the bundle-forming pilus (BFP) that also mediates microcolony formation, curli, and the recently described *E. coli* common pilus (ECP) (Saldana et al., 2009a, 2009b). Like type 1 pili and curli, ECP pili are present in most isolates of *E. coli*, including intestinal and extraintestinal *E. coli* pathogens (Rendon et al., 2007).

The type IV Cag secretion apparatus from *Helicobacter pylori* may also carry specialized adhesins. The CagL protein of the type IV secretion pilus was shown to mediate $\alpha_5\beta_1$ integrin binding, promoting delivery of the CagA effector protein as well as activation of cellular tyrosine kinases (Kwok et al., 2007). It is likely that the typical adhesion of *H. pylori* to the rims of gastric epithelial cells (Su et al., 1998) is due to integrin binding, which is in contrast to the binding phenotype seen in fixed gastric tissue sections. This is dominated by the outer membrane BabA and SabA lectins recognizing nonsialylated and sialylated Lewis antigens, respectively (Ilver et al., 1998). The *H. pylori* BabA and SabA adhesins may act sequentially in infection as nonsialylated glycans dominate at the onset of *H. pylori* infection, and sialylated glycans are induced during chronic infection (Mahdavi et al., 2002). There also seems to be a functional connection between the Cag type IV secretion apparatus and adhesion to Lewis antigens, as *cag*-carrying *H. pylori* strains upregulate glycosyl transferases required for synthesis of Lewis antigens (Marcos et al., 2008). In conclusion, this indicates the importance of non-pilus adhesins for host-pathogen interactions, and it has become more evident that structures not primarily implicated in attachment, such as flagella or secretion systems, can mediate adhesion to host cells. Therefore, signaling events triggered by adhesion of these structures could contribute to the overall host response.

Bacterial Adhesins Interacting with Phagocytes and Subsequent Evasion of Innate Immune Responses

Phagocytes, such as neutrophils, macrophages, and dendritic cells, are important effector cells of the innate immune response that rapidly can phagocytose bacteria and alert the immune system to danger. However, bacteria have evolved ways to avoid getting killed by phagocytes and may instead use these cells in order to hide from the immune system or the action of antibiotics, to constitute a reservoir for reinfection, and to spread within the body. Uptake by complement receptor 3 (CR3) does not generate toxic oxygen products and can therefore be utilized as a port of entry for bacteria, including *Bordetella pertussis.* The filamentous hemagglutinin (FHA) of *B. pertussis* binds to an integrin signal transduction complex on phagocytes consisting of the leukocyte response integrin (LRI) and the integrin-associated protein (IAP) through RGD motifs in FHA (Ishibashi et al., 1994). This leads to upregulation of CR3, to which FHA binds, and subsequently to internalization and persistence of the bacteria inside the macrophages (Ishibashi et al., 1994). FimH, the adhesin of *E. coli* type 1 pili, can bind to macrophages via the receptor CD48 (Figure 1). Under nonopsonic conditions, FimH-mediated internalization leads to attenuated release of intracellular free radicals and reduced acidification of the vesicles containing FimH-positive bacteria, promoting survival of the bacteria inside the phagocytes (Baorto et al., 1997). Another *E. coli* adhesin interacting with phagocytes is the autotransporter protein antigen 43 (Ag43). Bacteria expressing Ag43 are taken up to a higher extent by neutrophils and survive longer in these cells (Fexby et al., 2007).

Porphyromonas gingivalis is a Gram-negative anaerobic oral pathogen associated with chronic peridontitis and atherosclerosis. *P. gingivalis* pili are unique in that they contain an extra-long signal peptide and require additional proteases for fimbrial maturation (Figure 1). These pili can activate a proadhesive pathway in human monocytes that is dependent on CR3 and also involves TLR2 and CD14, as well as Ras-related C3 botulinum toxin substrate 1 (Rho family, small GTP-binding protein Rac1) and phosphoinositide 3-kinase (PI3K) (Hajishengallis et al., 2006b; Harokopakis et al., 2006) (Figure 2). The major fimbrillin FimA, together with LPS, has the ability to activate TLR2. However, although *P. gingivalis* fimbriae can employ either TLR1 or TLR6 for cooperative TLR2-dependent signaling, LPS display a preference for TLR1 (Hajishengallis et al., 2006a). *P. gingivalis* fimbriae may also limit TLR2 activation in human monocytes or mouse macrophages by inhibiting CXCR4-mediated activation of cAMP-dependent protein kinase A (PKA), which, in turn, inhibits TLR2-induced NF-κB (nuclear factor-κB) activation in response to *P. gingivalis* (Hajishengallis et al., 2008). By interacting with CR3 on immune cells, *P. gingivalis* fimbriae may also mediate subversion of innate immune responses. In contrast to TLR2 activation, this interaction depends upon the minor fimbrial components FimCDE. Mutants lacking FimCDE but expressing FimA were less persistent and virulent than the wild-type organism both in vitro and in vivo (Wang et al., 2007).

Bacterial polysaccharides interact with phagocytes and can be recognized by several different receptors, such as lectins, TLRs, and scavenger receptors (SRs), leading to uptake and clearance of the bacteria. Certain TLRs recognize bacterial polysaccharides, with the most well-characterized interactions being those of TLR4 and LPS of Gram-negative bacteria and TLR2 and lipopeptides. Although TLRs may not be phagocytic receptors per se, their activation can induce upregulation of scavenger receptors, such as MARCO and SR-A (Amiel et al., 2009; Plüddemann et al., 2009). Scavenger receptors can also directly bind to bacterial polysaccharides, such as lipoteichoic acid, which promotes phagocytosis of the bacteria (Areschoug and Gordon, 2008). However, the polysaccharide capsule of *S. agalactiae* has been shown to protect the bacterium from phagocytosis mediated by scavenger receptors, thereby evading recognition and clearance (Areschoug et al., 2008).

Bacteria have been shown to interact with the dendritic cell-specific intracellular adhesion molecule-grabbing nonintegrin (DC-SIGN, CD209) and its murine homolog SIGN- R1 via their LPS or capsular polysaccharide. SIGN-R1 has been shown to protect mice against lethal pneumococcal challenge. The receptor is expressed on marginal zone macrophages, where it binds to the capsular polysaccharide of *S. pneumoniae*, which leads to activation of the complement cascade and clearance of the bacteria (Kang et al., 2006). The functions of DC-SIGN can also be modulated by bacteria. For example, the LPS of *Y. pestis* binds to DC-SIGN, promoting uptake and possibly also intracellular survival of the bacteria (Zhang et al., 2008). Taken together, the interaction between bacterial adhesins and phagocytic cells leads to uptake and invasion of host cells and, subsequently, killing of the bacteria or immune escape where bacterial survivors may spread in the body. Thus, phagocytosis may not always be regarded as beneficial to the host but can also act as a virulence mechanism of bacteria.

Adhesins in Gram-Positive Bacteria and Their Interactions with the Host

Head-Stalk-Type Adhesins

Gram-positive firmicutes produce putative head-stalk-type adhesins. The best-studied group is a family of serine-rich repeat proteins (SRRP). These proteins are large glycosylated cell wall-anchored proteins that require a specialized *secY2A2* secretion system (Figure 1). The amino-terminal region of SRRPs is highly basic and acts as an adhesin by binding to sialylated glycoconjugates. The SSRPs of *Streptococcus parasanguis*, *Streptococcus gordonii*, and *Staphylococcus aureus* play a role in biofilm formation, in the colonization of the dental surface, in platelet aggregation, and in the development of endocarditis (Figure 2) (Bensing et al., 2004; Chen et al., 2004; Siboo et al., 2005). Clones of *Streptococcus pneumoniae* associated with high invasiveness have been found to carry *psrP-secY2A2*, a 37 kb pathogenicity island (Blomberg et al., 2009). The pneumococcal PsrP is an SSRP adhesin that was recently shown to facilitate bacterial persistence in the lungs of mice (Rose et al., 2008). The identification of SRRPs in a growing number of important pathogens, as well as the presence of specialized secretion systems, indicates important roles in the pathogenesis of Gram-positive organisms. However, we are only beginning to understand the functions of these highly unusual proteins.

Sortase-Assembled Pili

Sortase-assembled pili of Gram-positive bacteria were only recently identified, so our knowledge of their role in pathogenesis and interaction with host cells is limited. As is the case for many

pili of Gram-negative bacteria, sortase-assembled pili play roles in adhesion to host cells and tissue tropism (Mandlik et al., 2008b; Proft and Baker, 2009). Pili in *C. diphtheriae* influence adhesion to pharyngeal cells; in *S. pneumoniae*, to lung epithelial cells; in *S. pyogenes*, to tonsil epithelium; and in *S. agalactiae*, to lung and cervical epithelial cells. Interestingly, in all of these pathogens, adhesion is mediated by their minor pilin subunits and not by the major pilus stalk protein. Streptococcal pili were first described in *S. parasanguis*, colonizing the oral cavity of humans and animals (Wu and Fives-Taylor, 2001). The structure and biogenesis of pili in *C. diphtheriae*, the cause of the respiratory tract infection diphtheria, is best understood among sortase-assembled pili (Figure 1). So far, data suggest a similar organization for the other pathogenic sortase-assembled pili in which the pilus shaft is made of covalently linked major pilin subunits with minor pilins inserted. In *C. diphtheriae*, three different pilus gene clusters have been described, named *spaA*, *spaD*, and *spaH*. The major pilin subunit SpaA builds up the pilus shaft, whereas the minor pilin subunit SpaC is located at the tip, and SpaB is located along the structure. Minor pilin subunits SpaB and SpaC are involved in adhesion to and colonization of human pharyngeal cells. Further, a strain that expresses SpaA-type pili only adheres well to pharyngeal cells, whereas SpaD- and SpaH-type pili are involved in adhesion to laryngeal and lung epithelial cells, suggesting a role for pilus type in tissue tropism.

Similarly, in *S. agalactiae* (GBS), a major causative agent of neonatal pneumonia, sepsis, and meningitis, adhesion to lung and cervical epithelial cells involves minor pilins, whereas the major pilin is dispensable for adhesion. However, deletion of the GBS pilus backbone protein reduced the capacity of the pathogen to transcytose through human epithelial cells, whereas mutants in a minor pilin had no translocation defect (Pezzicoli et al., 2008). GBS pili have also been shown to contribute to adherence and invasion of endothelial cells of the human blood-brain barrier (Maisey et al., 2007). Three related pilus-encoding genomic islands have been found in GBS, and examination of clinical isolates showed that the pilus sequences in each island are conserved (Margarit et al., 2009). *S. pyogenes* (Group A streptococcus or GAS) lacking pili have been shown to have decreased adherence to pharyngeal cells. GAS pili show tissue tropism because pili are important for attachment to human tonsil epithelial cells and skin keratinocytes, two main sites of infection by this human-specific pathogen, whereas they do not affect adherence to liver or kidney cell lines. GAS colonizes the oropharynx, and saliva induces bacterial aggregation, which may lead to bacterial clearance. Recently it was shown that GAS pili contribute to cell aggregation in saliva, suggesting a role for pili in host defense. Two pilus islets have been recognized in *S. pneumoniae*. PI-1 consists of three structural proteins and three pilin sortases and is important for colonization, virulence, and the inflammatory response in mice (Barocchi et al., 2006). The minor pilin RrgA was found to be the major adhesin mediating attachment to lung epithelial cells (Nelson et al., 2007). The second functional pilus (PI-2) mediates adhesion to epithelial cells. Though PI-1 is present in some major antibiotic-resistant clones spreading around the world (Sjostrom et al., 2007), PI-2 is preferentially found among serotypes not harboring PI-1 (Bagnoli et al., 2008). The nosocomial opportunist *E. faecalis* most commonly causes UTIs but is also a common cause of endocarditis. A non-piliated *E. faecalis* mutant was found to be defective in biofilm formation as well as attenuated in a rat endocarditis model and in murine UTI (Nallapareddy et al., 2006; Singh et al., 2007). Genes encoding the *ebp* pilus have been found in all strains analyzed to date, and high titers of antibodies against the pilin proteins EbpA, EpbB, and EbpC have been found in endocarditis patient sera (Nallapareddy et al., 2006).

A. naeslundii, a dental pathogen causing caries and peridontitis, harbors two types of pili, type 1 and type 2 pili. Both of these pilus types are necessary for adherence and colonization of the oral cavity, and studies of their binding partners constitute the best-described host receptors for sortase-assembled pili (Yeung, 2000). Type 1 pili bind to salivary proline-rich proteins (PRPs) that coat the tooth enamel. The longer type 2 pili coaggregate with viridans streptococci and mediate adhesion to various host cells such as erythrocytes, epithelial cells, and leukocytes via carbohydrate receptors such as Gal-β1→3-Gal-NAc or Gal-β1→3-Gal, leading to phagocytosis and bacterial killing, as well as release of mediators such as superoxide that may contribute to the initiation of gingival inflammation. Biofilm formation in flowing saliva is also promoted by binding of receptor polysaccharide of oral streptococci such as *S. oralis* to *A. naeslundii*.

A growing number of cell wall-associated surface adhesins of Gram-positive bacteria also recognize adhesive matrix proteins and are therefore members of a group of bacterial adhesins termed MSCRAMMs. Prominent examples are the fibrinogen-binding clumping factor A (ClfA) of *S. aureus* and SdrG of *Staphylococcus epidermidis*, but MSCRAMMs may also bind $\alpha_{IIb}\beta_3$ integrin on platelets, causing platelet aggregation. Structural and functional studies of these MSCRAMMs suggest a dynamic "dock, lock, and latch" model for substrate binding, in which the adhesin adopts an open conformation that allows ligand access to a binding region between two protein domains (N2 and N3). A flexible C-terminal extension of the N3 domain is redirected to cover the ligand and to "lock" it in place. Next, the C-terminal part of this extension interacts with the N2 domain and forms a β strand complementing a β sheet in the N2 domain. This inserted β strand will function as a latch to form a stable adhesin-ligand complex (Deivanayagam et al., 2002; Ganesh et al., 2008).

Approaches to Inhibit Bacterial Adhesion

A number of different approaches have been taken over the years to inhibit bacterial adhesion (Table 1). One example is the design of competitive inhibitors based on detailed information on the adhesin-ligand interaction, such as the use of multivalent galabiose as an inhibitor for attachment mediated by P-pili (Salminen et al., 2007). Likewise, the detailed information on FimH-ligand interactions has allowed identification of α-D-mannose-based inhibitors that block *E. coli* adhesion on uroepithelial cells and also antagonize invasion and biofilm formation (Wellens et al., 2008). Small molecules that inhibit formation of the chaperone-usher class of pilus adhesins have also been identified. Bicyclic 2-pyridones, termed pilicides, bind to the surface of the chaperone, which interacts with the outer-membrane usher translocator (Pinkner et al., 2006). Substrate-derived inhibitors for the housekeeping sortase A of *S. aureus* have been

Table 1. Adhesins as Therapeutic Targets and/or Vaccine Candidates

Bacterial Adhesin Class	Pathogens	Composite Adhesive Structure	Adhesins	Ligand/ Receptor	Virulence in Animals[a]	In Vitro Inhibitors	Protection in Animals	References
Chaperon-Usher-Assembled Pili	UPEC	Pap pilus	PapG	digalactoside	yes	multivalent galabiose	vaccine protection in UTI	Hagberg et al., 1983; O'Hanley et al., 1985; Salminen et al., 2007
	UPEC	type 1 pilus	FimH	mannosylated glycoproteins	yes	α-D-mannose derivatives	vaccine protection in UTI	Langermann et al., 1997, 2000; Wellens et al., 2008
	ETEC	CFA fimbriae	CfaE	ND[c]	yes	ND	vaccine protection in diarrhoeal disease	Svennerholm and Tobias, 2008
	DAEC[b]/UPEC	Dr fimbriae	DraE	DAF/CEACAM	yes	pilicides against the whole class	vaccine protection in UTI	Goluszko et al., 2005; Korotkova et al., 2008
Curli	*E. coli*	curli fibers	CsgA	contact phase system	yes	QFGGGNPP	ND	Bian et al., 2001; Herwald et al., 1998
	S. enterica	curli fibers	CsgA	contact phase system	ND	ND	ND	Herwald et al., 1998
Flagella	ETEC	Flagellum	EtpA	ND	yes	ND	vaccine protection after challenge with Flagellin	Roy et al., 2009
Serine-Rich Repeat Protein (SRRP)	*S. pneumoniae*	NA[d]	PsrP	sialylated carbohydrates	yes	sortase inhibitors	vaccine protection in pneumonia	Blomberg et al., 2009; Rose et al., 2008
	S. gordonii	NA	GspB	ND	yes	sortase inhibitors	ND	Bensing et al., 2004
Trimeric Autotransporter Adhesins (TAAs)	*Y. enterocolitica*	NA	YadA	diverse	yes	ND	strain-specific vaccine protection	Hoiczyk et al., 2000; Vogel et al., 1993
	N. meningitidis	NA	NhhA	diverse	yes	ND	nonprotective antibodies induced	Bowe et al., 2004; Scarselli et al., 2006
Type IV Pili	*N. gonorrhoeae*	pilus	PilC	CD46[e], integrin, proteinacious ligand	yes	ND	pilus vaccine failed to protect against disease	Boslego et al., 1991
	V. cholerae	TCP	TcpA	ND for human receptor	yes	virstatin	vaccine protection	Shakhnovich et al., 2007; Sun et al., 1990
Sortase-Assembled Pili	GBS	Gbs pili	GBS1472	ND	yes	sortase inhibitors	vaccine protection	Dramsi et al., 2006; Margarit et al., 2009
	GAS	Spy pili	ND	ND	yes	sortase inhibitors	vaccine protection	Manetti et al., 2007; Mora et al., 2005
	S. pneumoniae	Rrg pili	RrgA	ND	yes	sortase inhibitors	vaccine protection	Barocchi et al., 2006; Gianfaldoni et al., 2007; Nelson et al., 2007
	E. faecalis	Ebp pili	ND	ND	yes	sortase inhibitors	ND	Nallapareddy et al., 2006; Singh et al., 2007

[a] Virulence in animals is defined as attenuated bacterial adherence in vivo in the absence of the adhesin or composite adhesive structure, except for curli, wherein in vivo effect was scored as a drop in blood pressure.
[b] Diffusely adhering *Escherichia coli*.
[c] No data available.
[d] Not applicable.
[e] No direct interaction between CD46 and the neisserial pilus adhesin has been demonstrated.

described (Scott et al., 2002). The accumulating knowledge on structure-function relationships for housekeeping and pilus-specific sortases will likely lead to the development of additional sortase inhibitors.

The similarity between bacterial curli and human amyloid deposits makes curli inhibitors of potential interest to prevent other types of amyloid formation. The QFGGN amyloid motif from the curli subunit CsgA, when conjugated to proline residues, has been shown to act as a peptide inhibitor of bacterial curli formation (Cherny et al., 2005). Virstatin, 4-[N-(1,8-naphthalimide)]-n-butyric acid, was shown to block both cholera toxin production and the toxin-coregulated pilus by inhibiting dimerization of the ToxT transcriptional activator (Shakhnovich et al., 2007). It is likely that development of novel live detection screens, such as those used when discovering that salicylanilides were type III secretion inhibitors, will yield a multitude of other classes of potential virulence inhibitors, some of which will act at the level of bacterial adhesion (Kauppi et al., 2003).

Adhesive Proteins as Protective Antigens

Complete sequencing of multiple genomes from a given bacterial species has provided an astonishingly high number of different types of adhesin genes, suggesting that there is no single route to bacterial attachment (Brzuszkiewicz et al., 2006). This redundancy puts a constraint on the use of adhesins as vaccines. Several adhesins have been shown to give protection in animal models, suggesting them to be potential candidates in protein-based vaccines (Table 1). Among Gram-negative bacterial adhesins, FimH in a complex with the FimC chaperone has been shown to be protective against UTI in murine and primate models (Langermann et al., 2000). Further, *E. coli* Dr fimbrial antigen was protective against UTI in mice upon challenge with a Dr adhesin-expressing strain (Goluszko et al., 2005). Microparticles containing the SafD/F adhesin chaperone complex gave some protection against invasive *Salmonella enteritidis* infection after oral challenge (Strindelius et al., 2004). A synthetic-peptide consensus-sequence vaccine (Cs1) that targets the host receptor-binding domain of the type IV pilus of *P. aeruginosa* showed increased protection against challenge in mice by four piliated strains (Kao et al., 2007). A vaccine that protects against travelers diarrhea caused by ETEC is under development. This vaccine consists of killed bacteria carrying dominant fimbrial antigens, such as the CF antigen I and CS1–CS6, as well as the heat-labile LT toxoid. Unfortunately, no ETEC vaccine candidate has been shown to be effective in the most important target groups, which are infants and young children in endemic areas (Svennerholm and Tobias, 2008).

Bacterial surface proteins binding complement regulators such as factor H are promising vaccine candidates. In *N. meningitidis*, the factor H binding 27 kD lipoprotein GNA1870 or fHbp is present in all strains and elicits protective bactericidal antibodies. It is currently being evaluated as one component in a multiprotein-based broad vaccine against meningococcal disease (Madico et al., 2006). The detailed interaction between fHbp and factor H was recently worked out and provides an explanation as to why meningococcal fHbp binds human, but not murine, factor H, resulting in murine resistance to meningococcal infection (Schneider et al., 2009). The inability of murine factor H to bind to the surface of *N. meningitidis* might allow for complement-mediated killing and could potentially explain murine resistance to meningococcal infection.

Sortase-assembled pili seem to be highly immunogenic. Thus, GBS pilus components mediated protection in mice against all group B streptococcal challenge strains tested (Margarit et al., 2009). Similar levels of protection in mice have been achieved against GAS and pneumococci using recombinant pilus antigens. Also, in human infections, antibodies are generated against pili of Gram-positive bacteria. Hence, sera from patients affected by GAS-mediated pharyngitis recognized recombinant pili proteins, and pneumococcal pilin proteins were found to raise serum antibodies in high titers in patients (Mandlik et al., 2008b; Proft and Baker, 2009).

Conclusions

This review has focused on the role played by bacterial adhesins in host-microbe interactions, leaving out many aspects on adhesin biogenesis and regulation. Even though significant progress has been made on how individual bacterial adhesins recognize host ligands and thereby generate or modulate various host responses, many questions still remain. We are still largely ignorant of the possible co-operative effects that might be mediated by the multitude of adhesins expressed by many pathogens. Are we dealing with a highly redundant system in which one bacterial adhesin can easily be replaced by another one? Or is there a need for a multitude of co-operative interactive adhesin-ligand events, well orchestrated in time and space, for productive infection of a host?

In the future, structural-functional correlates of bacterial adhesion will be further elucidated, additional receptors characterized, and associated signaling pathways worked out. Even though bacterial pathogen interactions with epithelial cells have been the focus for many years, little is still known on how commensal bacterial binding in the gut and at other colonized mucosal surfaces might contribute to the normal homeostasis, development, and function of mucosal barriers. Likewise, we need to learn more about the role played by bacterial adhesins in the interaction with immune cells and how they might affect innate and adaptive immune responses.

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