CHAPTER 2

Adherence, Anti-Adherence, and Oligosaccharides: Preventing Pathogens from Sticking to the Host

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Contents

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Abstract

For many pathogenic bacteria, infections are initiated only after the organism has first adhered to the host cell surface. If adherence can be inhibited, then the subsequent infection can also be inhibited. This approach forms the basis of anti-adherence strategies, which have been devised to prevent a variety of bacterial infections. In this chapter, the molecular basis by which respiratory, urinary, and gastrointestinal tract pathogens adhere to host cells will be described. The five general types of anti-adherence agents will also be reviewed. The most well-studied are the receptor analogs, which include oligosaccharides produced synthetically or derived from natural sources, including milk, berries, and other plants. Their ability to inhibit pathogen adherence may lead to development of novel, food-grade anti-infective agents that are inexpensive and safe.

I. INTRODUCTION

For most pathogenic bacteria, infection or colonization of host tissues depends, in large part, on the ability of the organism to somehow withstand the normal flux or flow within the particular tissue and to ''stick'' to the surfaces of the intended target. In fact, adherence of pathogens to host cell surfaces can be considered an essential first step in the infection process (Savage, 1977, 1984). Adherence, however, is not random or indiscriminant, but rather is cell- and tissue-dependent. That is, a particular organism recognizes specific receptors located on the host cell surface and then attaches itself to those receptors using specific adhesin molecules. Thus, an enteric pathogen, such as *Salmonella enterica*, expresses adhesins that bind to targets found in the gastrointestinal tract (GIT), but not in the urinary tract. Conversely, uropathogenic *Escherichia coli* (UPEC) can adhere to cells that line the urinary tract, but is poorly equipped to stick to other tissues. Importantly, adhesins are rightfully considered virulence factors; their absence renders the organism incapable of causing an infection. Likewise, if the receptor is absent or adherence is otherwise blocked, infection is similarly impeded.

For the majority of cases, carbohydrate- or oligosaccharide-bearing moieties are the most common host cell receptors recognized by pathogenic bacteria, although protein-type receptors also exist that are associated with adherence to connective tissues (collagens) or injured tissues that are on the mend (fibronectin, laminin). The identification and characterization of these specific bacteria–host interactions have not only revealed important insights into the molecular mechanisms of pathogenesis, but have also provided a basis for development of anti-infection agents. If the receptor targets are known, then it is possible to identify molecules that mimic those receptors and to use those molecules as decoys. Averting adherence by physically blocking bacterial contact with intended receptors has provided the rationale for development of several anti-adhesion strategies (Kahane and Ofek, 1996; Karlsson, 1998; Kelly and Younson, 2000; Ofek *et al.*, 2003b; Sharon and Ofek, 2000; Zorf and Roth, 1996). Such approaches are attractive for several reasons. First, anti-adherence approaches could provide an effective alternative to antibiotics, whose use has led to the emergence of antibiotic-resistant pathogens. Second, some of the proposed anti-adherence substrates occur naturally in milk, foods, and plants. Recently, for example, food-grade oligosaccharides that are used as prebiotics were also found to have anti-adhesive activity. Finally, the efficacy of many novel anti-adhesive agents has already been reported. These include adhesin-based vaccines, host-derived anti-adhesives, probiotics, adhesin analogs, and receptor analogs.

This chapter will provide an overview of the research on anti-adhesion agents. Particular attention will be devoted to the anti-adherence agents derived from or found naturally in foods. In addition, the pathogen infection process, the architecture of host epithelial cell surfaces, and the chemistry and mechanisms involved in bacterial interactions with host cell surfaces will also be reviewed.

II. ROUTE OF INFECTION

Enteric bacterial pathogens must maneuver through a lengthy stretch of hazardous terrain before they reach their intended target or infection site within a host. Initially, they must tolerate salivary enzymes having various hydrolytic activities in the mouth, followed by exposure to shedded epithelial cells in the esophagus that may prevent local bacterial adherence (Pearson and Brownlee, 2005). In the stomach, bacteria must endure another severe environment created by the secretion of digestive enzymes and hydrochloric acid (up to 0.1 M concentration and a pH as low as 1.0). Once bacteria reach the intestines, they then encounter mechanical,

chemical, and physical barriers in the form of luminal flow, a mucus layer, commensal bacteria, secreted anti-microbial proteins and peptides, and a host immune response (Pearson and Brownlee, 2005).

The severity of these barriers against pathogens within the intestines is dependent upon the physiological dynamics and the prepared mucosal defenses of the target tissues. In the small intestine, the rapid luminal flow, the low pH, and the presence of digestive enzymes ward off many invaders. The apical surface of the small intestine contains an abundance of intestinal villi ranging from 10 to 40 per mm^2 of mucosal surface area (Laux *et al.*, 2005), that, through ciliary action, also help avert bacterial adherence. These villi contain varying types of epithelial cells, including columnar absorptive enterocytes, goblet cells that secret mucus, and cells that secrete anti-microbial proteins (Falk *et al.*, 1998; Ouellette and Selsted, 1996). Additionally, there is a high rate of epithelial cell turnover that assists in preventing adherence (Laux *et al.*, 2005; Pearson and Brownlee, 2005).

The conditions within the large intestine are much less strenuous on invading pathogens. The luminal flow is less vigorous, there is a neutral pH, and the epithelial cell turnover is around tenfold slower than that of the small intestine (Falk *et al.*, 1998; Xu and Gordon, 2003). Moreover, the surface of the large intestine does not have villi and is relatively smooth, but still contains absorptive enterocytes, goblet cells, and anti-microbial secreting cells (Falk *et al.*, 1998).

Goblet cells are present in many of the gastrointestinal tissues of humans. They secrete a protective gel-like mucus layer that covers the stomach, small intestine, and large intestine (Allen *et al.*, 1984; Forstner *et al.*, 1995). This mucus is chiefly composed of large filamentous gel-forming glycoproteins called mucins (Forstner *et al.*, 1995), that provide both a loosely adherent surface layer and a firmly adherent underlying layer (Atuma *et al.*, 2001). Mucus is thought to act as a medium for protection, lubrication, and transport between the lumen and the epithelial cell surface (Forstner *et al.*, 1995). With respect to bacterial adherence in the GIT, however, the most important role of mucus is as a protective barrier against unwanted bacterial invaders. The mucus layer not only acts as a physical barrier by blocking binding sties at the epithelial cell surface, but it also contains proteins, lipids, and nucleic acids that may deter pathogen adherence, including defensins, lysozyme, anti-adherence molecules, secreted IgA and IgM, and other resident microorganisms (Pearson and Brownlee, 2005).

Although the mucus layer acts as a barrier to some invading pathogens, it also supports the growth and maintenance of a number of commensal bacteria in the GIT. It acts as an initial binding site, a source of nutrients for growth, and is a niche where these bacteria can replicate and potentially compete with other newly introduced bacteria (Laux *et al.*, 2005). Therefore, tissues that produce mucus have the potential to provide the host

with a well established microflora that is thought to be essential for GIT balance (Tannock, 1999). The thickness of the mucus layer differs in varying portions of the GIT. It is thinner and discontinuous in the small intestine and significantly thicker and continuous in the stomach and large intestine (Atuma *et al.*, 2001). The mucus layer in the stomach acts as a protectant against the digestive enzymes and high acid content that would otherwise destroy the tissue (Allen *et al.*, 1984), rather than for the growth and maintenance of microflora. The mucus layer in the large intestine fosters immensely diverse, highly competitive bacterial populations (Tannock, 1997, 1999). Consequently, pathogenic microorganisms that attempt to infect tissues that support large populations of indigenous microflora must vigorously compete with these bacteria to become established.

III. ADHERENCE BASICS

Many of the general molecular mechanisms involved in pathogen binding, including specificity, overall binding kinetics, and affinity, are now established (Ofek and Doyle, 2000). First, the ability of pathogens to survive and initiate infection within a host is reliant on their ability to adhere to host cell tissues. Secondly, adherence involves the interaction of complementary molecules on the surface of the bacteria and the surface of the host epithelium, respectively. These principles emphasize the importance of understanding the molecular mechanisms behind adherence. This is especially true when designing new approaches to prevent pathogen infection by interrupting the adhesion process.

A. Adherence kinetics

Adherence is very intricate and requires a strict series of events that eventually leads to stable bacterial–host interactions. As a bacterium approaches a host cell, it must overcome repulsive forces generated by the negative charges found on both the host tissues and the bacterial surface (Ofek *et al.*, 2003a). Varying attractions and interactions account for the ability of bacteria to prevail over these forces, including van der Waals' attractions, Coulombic forces, hydrophobic interactions, and eventually complementary interactions. The van der Waals' attractions and Coulombic forces allow a bacterium to move to within 5 nm of the host cell surface (Busscher and Weerkamp, 1987). Once this occurs, bacterial binding follows a two-step kinetic model (Hasty *et al.*, 1992). The first step occurs when the bacteria overcomes repulsive forces and becomes loosely and reversibly bound to the host cell surface through hydrophobic interactions. These interactions are mediated by

hydrophobins on the bacterial surface that interact with hydrophobic moieties (e.g., fatty acids) on the host cell surface (Rosenberg and Doyle, 1990; Rosenberg and Kjelleberg, 1986; Rosenberg *et al.*, 1996). The second kinetic step occurs when a strong irreversible complex is established between a specific bacterial adherence molecule and a complementary host receptor (Fig. 2.1) (Ofek and Doyle, 2000).

The precise chemical interactions between an adhesin and its receptor are also important. For example, direct- and water-mediated hydrogen bonds are the most important interactions within the carbohydrate-recognition domain in carbohydrate-binding adhesins on the host cell surface (Weis and Drickamer, 1996). Nonpolar van der Waals' interactions and hydrophobic ''stacking'' of the receptor oligosaccharide rings with aromatic amino acid side chains of the bacterial adhesin protein also contribute to oligosaccharide–protein interactions. X-ray structural

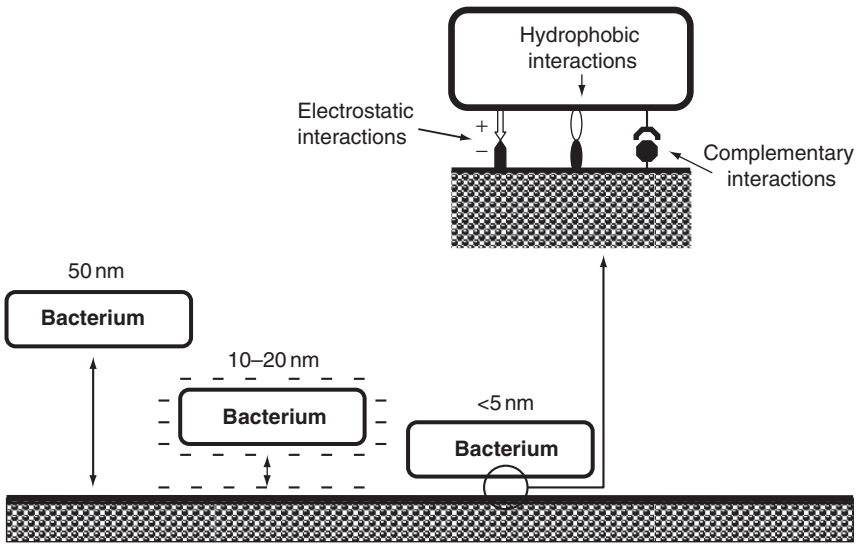

FIGURE 2.1 Description of bacterial adherence. Three distinct interaction regions are illustrated. When the bacterium is greater than 50 nm from the host cell, van der Waal's interactions occur. Upon moving to within 10–20 nm, both van der Waal's interactions and Coulombic forces attempt to overcome an intense repulsion generated by the net negative charges on opposing surfaces. Fimbriae and other polymers that have a small diameter can effectively overcome this repulsion, as repulsive forces decrease in proportion to the diameter of the particles approaching each other. When the bacterium reaches to within 5 nm, complementary binding (lectin–carbohydrate interactions) is required, which may also involve stabilizing hydrophobin–hydrophobin and charge–charge (electrostatic) interactions. Adapted from Busscher and Weerkamp (1987) and Ofek *et al.* (2003a).

analysis indicates that the hexose units of some oligosaccharide receptors interact with adhesins through hydrogen bonding, while nonpolar interactions form between other portions of the oligosaccharide and protein (Weis and Drickamer, 1996). Additionally, hydrophobic interactions and hydrogen bonds surrounding the adhesin–oligosaccharide complex promote more stable adherence (Duncan-Hewitt, 1990).

B. Adherence specificity

Bacterial adherence molecules are referred to as adhesins (Finlay and Falkow, 1989). The most well-studied and abundant group of adhesins (at least among bacterial pathogens) are the proteinaceous bacterial lectins that recognize complementary oligosaccharides on the host cell surface. Structurally, these lectin adhesins have a small globular carbohydrate-recognition domain that represents a relatively shallow indention on the surface of the protein (Weis and Drickamer, 1996). Subtle chemical diversity within this indentation allows for the selectivity of each adhesin to its target or cognate oligosaccharide receptor. Consequently, this results in the exquisite ability of bacteria to differentiate between slightly dissimilar oligosaccharide structures on the host cell surface. Thus, the overall specificity of a bacterium for a particular host is contingent on the presence of definitive oligosaccharide receptors (Ofek *et al.*, 1978; Sharon and Ofek, 1986). For example, *E. coli* strains that express the K99 adhesin bind specifically to *N*-glycolylneuraminyllactosyl ceramide. Animals, such as newborn piglets, that have these particular oligosaccharide sequences on their intestinal cell surface, are susceptible to infection by this pathogen. Humans do not possess these structures and are accordingly resistant to infection by K99-expressing *E. coli* (Ono *et al.*, 1989).

This binding specificity also explains the attraction a pathogen has for a particular host tissue. *Streptococcus pneumoniae* targets oligosaccharide structures present on human respiratory tract tissues (Andersson *et al.*, 1983; Smit *et al.*, 1984), while most diarrheagenic *E. coli* pursue the oligosaccharides on intestinal tissues (Ofek *et al.*, 1977). However, specificity is not entirely a function of the presence or absence of particular oligosaccharide structures. For example, *E. coli* that possess mannose-specific adhesins do not colonize all mannose-containing tissues (Ofek and Doyle, 2000). Therefore, adhesion is apparently due to a combination of factors including oligosaccharide presentation and orientation. Additionally, specificity may be context-dependent in that the structures surrounding the bacterial adhesin and complementary host cell receptor must be in suitable positions and have agreeable charges for any host–pathogen interactions to occur (Ofek and Doyle, 2000; Weis and Drickamer, 1996).

C. Adherence affinity

The affinity of an adhesin for an oligosaccharide is a significant feature of bacterial adherence. Generally, a single oligosaccharide molecule would have a low affinity for its corresponding protein, within, for example, a range of micro- to millimolar (Weis and Drickamer, 1996). However, increasing the valency of the protein–oligosaccharide interaction significantly enhances the affinity of the protein for its intended target. Bacteria create these multivalent oligosaccharide-binding proteins by assembling individual protein subunits that contain numerous individual oligosaccharide-binding sites into a filamentous structure (Vijayan and Chandra, 1999; Weis, 1997; Weis and Drickamer, 1996). These multi-unit structures can concurrently bind to numerous individual oligosaccharides on the host cell via a ''Velcro-like'' mechanism, increasing avidity for the target (Mulvey *et al.*, 2001).

IV. SPECIFIC PATHOGEN–HOST INTERACTIONS

Identifying and characterizing the direct molecular contact points between bacterial adhesins and host receptors are central to developing novel strategies to prevent infection via adhesin–receptor interference. Three main types of adhesin–receptor interactions have been described, lectin–carbohydrate, protein–protein, and hydrophobin–protein (Courtney *et al.*, 1990; Cywes *et al.*, 2000; Hanski *et al.*, 1992; Hasty *et al.*, 1992; Sylvester *et al.*, 1996; Szymanski and Armstrong, 1996; Wu *et al.*, 1996). Lectin–carbohydrate interactions are often found along the surface of the host cell, and involve the glycolipids, glycoproteins, and proteoglycans found in the glycocalyx layer. Protein–protein interactions usually involve the extracellular matrix (ECM) components of the host cell (discussed in Section IV.B.). Hydrophobin–protein interactions are thought to take place during the early stages of bacteria–host contact before specific lectin–carbohydrate or protein–protein interactions occur. Each of these will be discussed below in more detail.

A. Lectin–carbohydrate interactions

The lectins involved in lectin–carbohydrate interactions are either located on the bacterial cell surface or in the host epithelial cell surface. An example of the latter is the host cell lectin CD44 that binds to hyaluronic acid moieties in the capsule of the Gram-positive pathogen *Streptococcus pyogenes* (Cywes *et al.*, 2000). Host lectins have only recently been found to be complementary to Gram-negative pathogen oligosaccharide structures. Lipopolysaccharides (LPS) or lipooligosaccharides anchored to the outer membranes of *Vibrio mimicus* (Alam *et al.*, 1996) and *Pseudomonas aeruginosa* (Zaidi *et al.*, 1996) have been shown to serve as adhesins to mucosal cells and mucus components, most probably by recognizing lectins on the host cell surface (Jacques, 1996).

The most well-studied bacteria–host interactions are those involving bacterial protein lectins and complementary oligosaccharide ligands on the host cell surface. In Gram-negative bacteria, lectins usually exist in the form of polymorphic fimbriae or pili. They are often made up of hundreds of protein subunits that bind host oligosaccharides (Nuccio and Baumler, 2007; Ofek and Doyle, 1994; Sharon and Ofek, 1986). Lectins in Gram-positive bacteria are often positioned within the peptidoglycan layer or are anchored in the cytoplasmic membrane so that they traverse the peptidoglycan layer and extend beyond the cell wall (Ofek *et al.*, 2003a). Determining the specificity of bacterial lectins to their cognate oligosaccharide ligands has been the subject of considerable interest, but has also proven to be experimentally challenging. Several approaches have been described, including direct-binding assays, measurement of *in vitro* cell adherence to tissue culture cells in the presence of oligosaccharides, and determining virulence *in vivo* when exogenous oligosaccharide receptors are present (Ascencio *et al.*, 1993; Barthelson *et al.*, 1998; Brennan *et al.*, 1991; Firon *et al.*, 1987; Giannasca *et al.*, 1996; Hanisch *et al.*, 1993; Jagannatha *et al.*, 1991; Krivan *et al.*, 1988; Ofek *et al.*, 1977; Rajan *et al.*, 1999; Stromberg *et al.*, 1990; Teneberg *et al.*, 2004). The interactions between UPEC FimH adhesin and human host glycoproteins are among the best described, and are discussed, in detail, later in this chapter. Table 2.1 lists some of the oligosaccharide structures found on host cell surfaces that are complementary to pathogen lectins.

B. Protein–protein interactions

Protein–protein interactions are usually associated with the exposed ECM proteins and proteoglycans that are normally found at the basolateral surface of the mucosa. They become available for binding when the host cell surface has been compromised. There are a number of bacterial proteins that have been found to bind one or more of the ECM components including fibronectin, laminin, collagen, and elastin. These proteins are commonly referred to as MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) (Patti *et al.*, 1994). The best-illustrated protein–protein interactions are those involving fibronectin-binding bacterial proteins that, accordingly, adhere to fibronectin in the ECM of host enterocytes. Several bacteria have been found to express fibronectin-binding proteins (FnBPs) (Hasty *et al.*, 1994). However, only a few studies have shown a definitive interaction between an adhesin and fibronectin as it pertains to adherence. As a result, the frequency of this adherence mechanism in nature remains to be determined (Ofek *et al.*, 2003a). Over six different FnBPs have been identified in *S. pyogenes* (Finlay and Caparon, 2000); some of these will be discussed in more detail below (Section VI.B.).

TABLE 2.1 Examples of pathogen oligosaccharide adherence sites on host mucosal surfaces[a]

Organism	Target molecule	Target tissue
Escherichia coli		
Type 1 pili	Man(α1–3)[Man(α1–6)]Man	Urinary
P-fimbriae	Gal(α1–4)Gal	Urinary
S-fimbriae	NeuAc(α2–3)Gal(β1–3)GalNAc	Neural
CFA/1	NeuAc(α2–8)-	Intestinal
K1	GlcNAc(β1–4)GlcNAc	Endothelial
F5 (K99)	NeuGc(α2–3)Gal(β1–4)Glc	Intestinal
Bordetella pertussis	Gal(β1–3)GalNAc(β1–4)Gal(β1–4)-Glc	Respiratory
Haemophilus influenza	$[NeuAc(\alpha2–3)]_{0,1}$ Gal(β1–4)GlcNAc-(β1–3)Gal(β1–4)GlcNAc	Respiratory
Helicobacter pylori	NeuGc(α2–3)Gal(β1–4)Glc(NAc)	Stomach
	Fuc(α1–2)Gal(β1–3)[Fuc(α1–4)]Gal	Stomach
Klebsiella pneumoniae	Man	Respiratory
Mycococcus pneumoniae	NeuGc(α2–3)Gal(β1–4)Glc(NAc)	Respiratory
Neisseria gonorrhoea	Gal(β1–4)Glc(NAc)	Genital
Neisseria meningitidis	$[NeuAc(\alpha2–3)]_{0,1}$ Gal(β1–4)GlcNAc-(β1–3)Gal(β1–4)GlcNAc	Respiratory
Pseudomonas aeruginosa	Gal(β1–3)Glc(NAc)(β1–3)Gal(β1–4)-Glc	Respiratory
Salmonella typhimurium	Man	Intestinal
	Gal(β1–4)GalNAc	Intestinal
Streptococcus pneumoniae	$[NeuAc(\alpha2–3)]_{0,1}$ Gal(β1–4)GlcNAc-(β1–3)Gal(β1–4)GlcNAc	Respiratory
Streptococcus suis	Gal(α1–4)Gal(β1–4)Glc	Respiratory

[a] Adapted from Karlsson (1989) and Ofek and Doyle (1994).

C. Hydrophobin–protein interactions

Hydrophobin–protein interactions include those bacterial surface components that promote adhesion to host cell surfaces via hydrophobic moieties that are often thought to be nonspecific (Rosenberg and Doyle, 1990; Rosenberg and Kjelleberg, 1986; Rosenberg *et al.*, 1996).

Hydrophobin–protein interactions enable the bacterial cell to overcome any repulsive forces at the host cell surface. However, there may also be some degree of specificity involved, as in the case of lipoteichoic acid (LTA) found on the surface of *S. pyogenes* that exhibits reversible adherence (Courtney *et al.*, 1990; Hasty *et al.*, 1992).

V. INTESTINAL TARGET TISSUES

Epithelial cells of the mucosal surface are the first to come into contact with invading pathogens, and consequently are the first to be colonized during infection (Ofek *et al.*, 2003a). However, when epithelial cells are disrupted by injury or by tissue or organ insults, the exposed underlying structural components or ECM become prime targets for bacterial adherence. Therefore, understanding the biology of host cells and tissues is critical in identifying mechanisms of bacterial adherence, especially when developing novel methods to prevent selective pathogen adherence.

The intestinal epithelia is generally characterized as structurally simple, in that it is made up of a single layer where the apical side of each cell borders the lumen and each basal side sits on the basal lamina or basement membrane. The basal lamina is primarily composed by two-dimensional sheets of type IV collagen, but also includes laminins, heparan sulfate proteoglycans, fibronectin, and other ECM components (Erickson and Couchman, 2000). The epithelial cell layer itself, is made up of absorptive enterocytes (Trier and Madara, 1981), mucus secreting goblet cells (Moe, 1955), undifferentiated crypt cells (Trier and Madara, 1981), Paneth cells with large secretary granules (Elms, 1976; Erlandsen and Chase, 1972), enteroendocrine cells, and gut-associated lymphoid tissue (GALT) (Roy *et al.*, 1987) containing Peyer's patches (Trier and Madara, 1981), which are predominately composed of antigen-sampling M cells (Owen and Jones, 1974). Individual cells are bound to the ECM by transmembrane integrins, and are bound to each other via several multi-protein complexes including tight junctions (occludins and claudins), adherens junctions (cadherins), and gap junctions (Ofek *et al.*, 2003a). Beneath the basement membrane is a network of connective tissues including fibril-forming collagen (type I, II, and III), fibronectin, elastin, various cell types like mast cells and macrophages, and neural and vascular elements (Trier and Madara, 1981). Depending on the location within the GIT, the apical surface of the epithelium may be organized into microvilli, and is covered by a carbohydrate-rich glycocalyx layer and a mucus layer composed of high-molecular weight glycoproteins that collectively protect the epithelium from pathogens when structurally intact (Neutra and Forstner, 1987). However, when the integrity of the apical cell surface is compromised, or when the host is confronted by pathogens that

are capable of invading enterocytes from the basal side, the structural components associated with the basal membrane and ECM become key receptors in bacterial adherence.

A. Cell surface structures

Under normal circumstances, bacteria first encounter the carbohydrate-rich mucus and glycocalyx layers on the apical surface of the gastrointestinal epithelium. The mucus layer is mainly composed of large filamentous gel-forming glycoproteins called mucins that may or may not be membrane-bound, are naturally highly sialylated, and are continuously in motion down the GIT (Forstner *et al.*, 1995). Pathogens must either penetrate through or bind to the mucus layer to initiate infection (Ofek *et al.*, 2003a). Consequently, bacterial motility and chemotaxis through the mucus layer may play very important roles in adherence for many pathogens (Eaton *et al.*, 1992; Freter *et al.*, 1981; Takata *et al.*, 1992; Young *et al.*, 2000). On the other hand, some nonmotile pathogenic bacteria are still capable of complete colonization and full virulence. For example, nonmotile, flagellated *S. enterica* serovar Typhimurium and *E. coli* F-18 remain virulent in animal models (McCormick *et al.*, 1988, 1990). However, it should be noted that the involvement of motility and chemotaxis in mucus penetration is not well understood (Laux *et al.*, 2005). Additionally, many human and animal enteric pathogens have been shown to bind to mucus components *in vitro*, including enteropathogenic *E. coli* (EPEC) (Mack and Sherman, 1991; Smith *et al.*, 1995), pathogenic *E. coli* strains with K88 (Cohen *et al.*, 1983), K99 (Laux *et al.*, 1984; Lindahl and Carlstedt, 1990), RDEC-1 (Mack and Sherman, 1991), and 987P adhesins (Dean, 1990), *Clostridium difficile* (Karjalainen *et al.*, 1994, 2001; Tasteyre *et al.*, 2001; Waligora *et al.*, 2001), *Campylobacter* spp. (Sylvester *et al.*, 1996), *Salmonella* serovars (Vimal *et al.*, 2000), *Yersinia* spp. (Mantle and Husar, 1994), *Shigella dysenteriae 1* (Sudha *et al.*, 2001), and *Helicobacter pylori* (Van de Bovenkamp *et al.*, 2003). It is unclear how binding of mucus components facilitates *in vivo* adhesion to the epithelial cell surface. However, it is likely that adherence to mucus components *in vitro* reflects events associated with the early stages of infection rather than later stages of colonization (Laux *et al.*, 2005), and that in some cases, binding of mucus components appears to be positively correlated to enhanced colonization (Cohen *et al.*, 1985; Vimal *et al.*, 2000).

The glycocalyx is a general term that refers to the dense mat of variable, highly glycosylated integral membrane glycoproteins, glycolipids, and proteoglycans that are presented on the epithelial cell surface (Ito, 1969). They are thought to play a recognition role in cell growth, differentiation, and cell–cell interactions (Brandley and Schnaar, 1986; Roseman, 1985), as well as in malignancy (Hakomori, 1984; Prokazova

et al., 1988; Yogeeswaran, 1983) and modulation of receptor-mediated membrane processes (Hanai *et al.*, 1988; Usuki *et al.*, 1988). The diversity and density of saccharides in the glycocalyx layer make it an exceptionally appealing surface for lectin-bearing bacteria (Mirelman and Ofek, 1986). Several studies have shown that bacteria are capable of binding internal or terminal saccharide sequences of glycolipids (Bock *et al.*, 1985; Karlsson, 1989; Krivan *et al.*, 1988). For example, a number of bacteria have been shown to bind specifically to lactosylceramide, including *Bacteroides* spp., *Clostridium* spp., *Shigella* spp., *S. enterica* serovar Typhimurium, and *E. coli* spp. (Karlsson, 1989). Interestingly, lactosylceramide is not present in the human small intestinal epithelium (Bjork *et al.*, 1987), but is found in abundance in the colonic epithelium (Holgersson *et al.*, 1988), which appears to coincide with the target tissue for most of the aforementioned pathogens. Thus, receptor specificity to particular saccharide structures in the glycocalyx is of utmost importance to many enteric pathogens.

B. ECM components

The components of the ECM are also important bacterial receptors when they become accessible to invading pathogens. Studies have shown that several pathogenic bacteria bind to ECM components, including collagens (Holderbaum *et al.*, 1986; Visai *et al.*, 1990; Wagner *et al.*, 2007), laminins (Moran *et al.*, 2005; Plotkowski *et al.*, 1996; Speziale *et al.*, 1982; Switalski *et al.*, 1987; Valkonen *et al.*, 1994), fibronectin (Dorsey *et al.*, 2005; Dramsi *et al.*, 2004; Froman *et al.*, 1984, 1987; Monteville and Konkel, 2002), vitronectin (Liang *et al.*, 1997; Valentin-Weigand *et al.*, 1988), hyaluronan (Cywes *et al.*, 2000), elastin (Downer *et al.*, 2002), and proteoglycans (Alvarez-Dominguez *et al.*, 1997; Ascencio *et al.*, 1993; Bergey and Stinson, 1988; Fleckenstein *et al.*, 2002; Guo *et al.*, 1998; Isaacs, 1994). In general, these proteins and proteoglycans function together, when necessary, to facilitate wound healing and inflammation, resulting in a vulnerable provisional ECM that is susceptible to bacterial colonization and host cell entry (Preissner and Singh Chhatwal, 2005). Besides the recuperative aspect of ECM components, they are essential contributors to the cellular shape, orientation, differentiation, and metabolism of a variety of cellular systems (Ruoslahti and Obrink, 1996; Timpl and Brown, 1996).

C. Host cell adhesive components

Other cellular components that mediate cell–cell or cell–ECM interactions have also been shown to have bacterial specificity, including integrins (Coburn *et al.*, 1998; Leong *et al.*, 1990; Rezcallah *et al.*, 2005; Wang *et al.*, 2006; Watarai *et al.*, 1996), cadherins (Mengaud *et al.*, 1996), and selectins

(Ho *et al.*, 1998; Sandros *et al.*, 1994). Integrins are expressed ubiquitously and have multiple functions depending on their location in the body. In the small intestine, integrins have been shown to not only mediate cell interactions with the ECM and basement membrane, but also activate various signaling pathways that leads to the modulation of gene expression (Lussier *et al.*, 2000). For example, *Shigella flexneri* (Watarai *et al.*, 1996), *Staphylococcus aureus* (Fowler *et al.*, 2000), and *Yersinia* spp. (Isberg *et al.*, 2000) use integrin, cadherin, and selectin receptors, either directly or indirectly, to make cellular contact and then initiate invasion pathways into epithelial cells.

With every new bacterial receptor identified, an exciting opportunity emerges for receptor decoy discovery that could prevent infection by these pathogens. Table 2.2 lists some of the specific interactions of bacterial species with host ECM and adhesive components.

VI. BACTERIAL ADHESINS

The initial adherence of pathogens to host cell surfaces is considered an essential step in colonization and infection (Savage, 1977, 1984). Therefore, identifying the bacterial molecules that mediate adherence has been a major area of research, especially since these molecules may serve as targets for anti-adherence strategies. As discussed previously (Section VI), the detailed interactions between a pathogen and a host cell are often mediated by proteinaceous surface structures on the bacterial surface. These bacterial proteins are referred to as adhesins (Finlay and Falkow, 1989), and are most often found on the tips of bacterial fimbriae or pili (fimbrial adhesins), but may also be anchored in the bacterial membrane so that it can be presented on the bacterial outer membrane (afimbrial adhesins) (Sharon and Ofek, 1986). Models of fimbrial and afimbrial adhesins of some human pathogens are discussed here.

A. Fimbrial adhesins

Often UPEC strains carry many different adhesins, two of these are type 1 fimbriae (*fim*) and pyelonephritis-associated pili (*pap*) (Berglund and Knight, 2003; Mulvey, 2002; Schilling *et al.*, 2001). These adhesins allow the organism to take advantage of its local environment by regulating cross-talk between the *fim* and *pap* operons (Xia *et al.*, 2000), ultimately resulting in a genetic on/off switch. In the lower urinary tract, type 1 fimbriae binds a high-mannose glycoprotein, uroplakin Ia (Firon *et al.*, 1987; Wu *et al.*, 1996; Zhou *et al.*, 2001), resulting in cystitis (Ronald *et al.*, 2001). The P-pili are used by UPEC in escalating urinary tract infections (UTI) to bind galabiose-containing glycolipid receptors in the kidney that

TABLE 2.2 Important extracellular matrix and adhesive components use as receptors by pathogens[a]

ECM/adhesive components	Microorganism	Adhesin
Fibronectin	*Staphylococcus aureus*	FnbA, FnbB
	Group A streptococci	PrtF1/Sfb1, PrtF2, LTA
		SOF/SfbII, M3 protein
	Group C streptococci	FnBA, FNnBB
	Group G streptococci	FnB, GfbA
	Listeria monocytogenes	FbpA
	Campylobacter jejuni	CadF
	Salmonella enterica serotype *Typhimurium*	ShdA, MisL
Collagen	*Staphylococcus aureus*	Can
	Group A streptococci	M proteins, FNB54
	Streptococcuc parasanguis	FimA
	Legionella pneumophila	Mip
Vitronectin	*Staphylococcus aureus*	60-kDa protein
Laminin	*Neisseria meningitidis*	NhhA
	Staphylococcus aureus	
	Group A streptococci	
Integrins	*Shigella*	IpaB, IpaC
	Yersinia	Invasin
Heparan sulfate	*Neisseria gonorrhoeae*	Opa proteins
E-cadherin	*Listeria monocytogenes*	Internalin A
Uroplakin	*E. coli*	Type 1 fimbriae
CD48	*E. coli*	Type 1 fimbriae

[a] Adapted from Finlay and Caparon (2005) and Ofek *et al.* (2003a).

initiates pyelonephritis (Dodson *et al.*, 2001; Lund *et al.*, 1987). The role of type 1 fimbriae in the GIT has not been elucidated (Bloch *et al.*, 1992), and the receptors are undefined in this milieu (Bouckaert *et al.*, 2005). However, the type 1 fimbriae are by far the most prevalent adhesin in UPEC strains (Brinton, 1959; Buchanan *et al.*, 1985). Many proteins are necessary to construct these fimbriae including FimA that forms a rigid helical rod, FimF and FimG subunits that makes up the short tip fibrillum, and FimH presented at the end of the tip fibrillum that is responsible for adherence through a carbohydrate-recognition domain for mannose (Jones *et al.*, 1995; Krogfelt *et al.*, 1995). Although the primary FimH receptor is

uroplakin Ia, FimH also recognizes glycoproteins with one or more *N*-linked high-mannose structures and is able to agglutinate yeast cells (Firon *et al.*, 1984; Krogfelt *et al.*, 1995; Ofek *et al.*, 1977). More recently, the FimH lectin has been shown to bind with a high affinity to butyl α-*D*-mannosides particularly those with longer alkyl tails, aryl mannosides, and fructose (Bouckaert *et al.*, 2005). Accordingly, inhibiting FimH–receptor interactions have been shown to prevent bacterial adherence to the bladder epithelium and as a result may prevent infection (Langermann and Ballou, 2003; Langermann *et al.*, 1997, 2000; Thankavel *et al.*, 1997). Both natural and synthetic mannose terminal structures have been shown to interrupt FimH-mediated UPEC adherence (Aronson *et al.*, 1979; Firon *et al.*, 1987; Nagahori *et al.*, 2002; Old, 1972), and with the recent discovery of high affinity mannoside and fructose receptors, more anti-adherence candidates are sure to follow, increasing the likelihood of developing potential vaccines against these pathogens.

B. Afimbrial adhesins

Many afimbrial adhesins have been identified in pathogenic bacteria including *E. coli, H. pylori, Bordetella pertussis, Neisseria* species, *Yersinia* species, *Haemephilus influenzae, Campylobacter jejuni, S. aureus,* and *Streptococcus* species (Finlay and Caparon, 2005). These adhesins have been shown to bind ECM components in an attempt to initiate infection. Some of the most well-studied afimbrial adhesins are FnBPs belonging to the MSCRAMM family of adhesins that are expressed by *S. aureus* and *S. pyogenes* (Patti *et al.*, 1994). Fibronectin is a large dimeric glycoprotein found in plasma, the ECM, and on eukaryotic cell surfaces. It is responsible for host cellular processes like adhesion, migration, and differentiation (Hynes, 1990), but is also a common substrate for bacterial attachment that ultimately results in host cell internalization (Fowler *et al.*, 2000). The FnBPs of *S. aureus* and *S. pyogenes* have similar structural organization and Fn-recognition mechanisms (Joh *et al.*, 1999). In general, FnBPs are surface proteins anchored in the cell wall that contain an LPXTG motif found in most surface-associated proteins of Gram-positive bacteria (Fischetti *et al.*, 1990), and have a short positively charged C-terminal tail where Fn recognition occurs within sequence repeats of 35–40 amino acid residues (called FnBRs) (Patti *et al.*, 1994). In some instances, two Fn-binding domains have been identified, as in the case of UR, which is upstream of *sfbI* in *S. pyogenes*.

Currently, SfbI is the model FnBP for Fn recognition. It contains two high-affinity and several lower-affinity binding sites for Fn (Schwarz-Linek *et al.*, 2003, 2004, 2006). Each FnBR in the C-terminus can potentially bind to one dimer of Fn, which in turn, contains two binding domains for integrins (Schwarz-Linek *et al.*, 2006). Therefore, SfbI is thought to serve as a molecular bridge between the bacteria and host cell integrins in a

FnBP-mediated internalization of *S. pyogenes* (Ozeri *et al.*, 1998). Additionally, SfbI has been shown to recruit other ECM components like collagen I and IV that aid in escaping the host immune system (Dinkla *et al.*, 2003). Around 50% of the *S. pyogenes* clinical isolates express the *sfbI* gene (Natanson *et al.*, 1995). However, only five different FnBPs contain FnBRs that can potentially assist bacteria in adherence and invasion (Schwarz-Linek *et al.*, 2006).

The presence of multiple FnBPs could possibly explain how *S. pyogenes* is able to colonize different host tissue and confer various tissue tropisms. The identification of the SfbI adhesin has contributed to the recent development of vaccines composed of SfbI-derived peptides conjugated to either the diphtheria toxoid or used with the Lipid Core Peptide (LCP) delivery system. These vaccines have been shown to confer protective immunity to BALC/c mice when challenged intranasally with lethal doses of *S. pyogenes* (Olive *et al.*, 2007; Schulze *et al.*, 2006).

VII. COMMON BACTERIAL ADHERENCE MECHANISMS

Human microbial pathogens that possess the ability to adhere to host tissues have a distinct advantage over those that do not, in that they are better equipped to evade and resist the defense systems of their host. There are numerous adherence mechanisms that have been described to date. However, those that are most commonly studied originate from pathogens that colonize the GIT and genital-urinary tract, including *Salmonella, H. pylori*, and pathogenic serotypes of *E. coli*.

A. *Salmonella*

The adherence mechanisms involved in *Salmonella* infection have been studied in great deal. Disease associated with *S. enterica* serovars is initiated by attachment to and invasion of host cells, followed by subsequent inflammation of the lamina propria and lymph nodes (Darwin and Miller, 1999). Several genetically defined fimbrial or piliar adhesins contribute to the initial attachment and the overall infection process of *Salmonella*. Some of these include type 1 fimbriae (Fim), plasmid-encoded (PE) fimbriae, long polar (LP) fimbriae, and thin aggregative fimbriae (curli). However, many other putative fimbrial operons have been identified within various *S. enterica* serovar genomes, but the expression of these proteins is currently undefined.

The type 1 fimbriae in *S. enterica* serovars are encoded by the *fimAICDHF* operon (Collinson *et al.*, 1996b) and are morphologically similar to, but antigenicly different from the type 1 fimbriae of *E. coli* (Korhonen *et al.*, 1980). These fimbriae are composed primarily of FimA

protein subunits, but binding specificity is determined by the FimH subunit on the tip of the fimbrial shaft, which has an affinity to mannose residues, as described previously (Clegg and Swenson, 1994; Klemm and Krogfelt, 1994). Recent studies have shown that the *S. enterica* serovar Typhimurium FimH adhesin mediates adherence to HeLa, HEp-2, and mouse intestinal epithelial cells (Boddicker *et al.*, 2002; Hancox *et al.*, 1997; Thankavel *et al.*, 1997). Additionally, both *S. enterica* serovar Tyhimurium and *S. enterica* serovar Enteritidis have been shown to bind human colon carcinoma cell line HT-29 and human bladder cancer cell line Hu1703He via their type 1 fimbriae FimH adhesin (Kisiela *et al.*, 2006). However, there appears to be significant heterogeneity in receptor specificities for particular mannosylated compounds among type 1 fimbriae, not only from different bacterial genera, but also from within the same species. These differences have been attributed to allelic variants in the FimH adhesin, where a disparity in one or two amino acid residues leads to differing receptor affinities and specificities (Boddicker *et al.*, 2002; Kisiela *et al.*, 2006; Sokurenko *et al.*, 1994, 1995). These allelic differences likely mediate host specificity and target tissue specificity via the type I fimbriae adhesion.

The *Salmonella* LP fimbriae, encoded by the *lpfABCDE* fimbrial operon, were first identified in *S. enterica* serovar Typhimurium and thought to have been acquired by horizontal transfer during evolution, as the flanking sequences of this operon are homologous to those in *E. coli* K-12 (Baumler, 1997). Although the expression of the *lpf* operon in a nonfimbriated *E. coli* strain results in the appearance of polar filaments, there is no conclusive evidence that LP fimbriae are polar on *Salmonella*. The *lfpABCDE* operon has been implicated in the colonization of murine Peyer's patches and reported to be important in the early stages of oral infection (Baumler *et al.*, 1996c; Norris *et al.*, 1998). Using the mouse small intestine model of infection, a mutation in an outer membrane protein (OMP) thought to be an usher for fimbrial assembly (*lpfC*), resulted in reduced colonization of the Peyer's patches, but not villous enterocytes (Baumler *et al.*, 1996c). *In vivo*, this mutation alone had a minimal effect on virulence in BALC/c mice, as did a single mutation in *invA*, a type III secretion system (TTSS) gene required for invasion. However, these mutations in conjunction, led to a 150-fold increase in oral LD_{50} compared to the wild type or either single mutant, leading to the conjecture that *Salmonella* must be intimately adhered to target cells to invade a murine host via its TTSS and that LP fimbriae may be the means by which *Salmonella* accomplishes this feat (Jones *et al.*, 1994; Norris *et al.*, 1998).

The PE fimbriae, encoded by the *pefBACD* operon contained on the virulence plasmid pSLT, has been found in only four *Salmonella* serotypes, *S. typhimurium*, *S. choleraesuis*, *S. paratyphi*, and *S. enteritidis* (Baumler *et al.*, 1997). The PE fimbria has been demonstrated to mediate adherence to the

mouse small intestine and also appears to be involved in the initiation of fluid accumulation (Baumler *et al.*, 1996a,b). Nicholson and Low (2000) reported that the expression of the *pefBACD* operon is under methylation-dependent transcriptional regulation, similar to the *pap* operon in *E. coli*, and that expression only occurs in conditions of low pH and O_2 rich medium. Additionally, the expression of SrgA, a disulfide oxidoreductase, is also required for the production of PE fimbriae (Bouwman *et al.*, 2003). Specifically, the disulfide bond within the major structural subunit of the PE fimbriae, PefA, must be oxidized by SrgA in order for the fimbriae to be assembled and for the maintenance of PefA stability.

Another fimbrial adhesin that mediates the adherence of *Salmonella* to host cells is the thin aggregative fimbriae, often referred to as Tafi. Collinson *et al.* (1991) identified this adhesin in *Salmonella enteritidis*, and the operon was termed *agf*. However, because the Tafi homolog in *E. coli* was first termed curli and the operon termed *csg* (Arnqvist *et al.*, 1992; Collinson *et al.*, 1992), Tafi was renamed with the *csg* nomenclature (Romling *et al.*, 1998). The curli fimbriae have been found to be essential for numerous *Salmonella* virulence mechanisms including accelerating amyloidosis in mice, binding fibronectin, and enhancing adherence and invasion of eukaryotic cells (Arnqvist *et al.*, 1992; Dibb-Fuller *et al.*, 1999; Kim and Kim, 2004; La Ragione *et al.*, 2000; Lundmark *et al.*, 2005; Sukupolvi *et al.*, 1997). Curli-producing bacteria tend to auto-aggregate, which has been suggested to enhance the survival of *Salmonella* spp., in hostile environments like stomach acid or other detrimental milieus (Collinson *et al.*, 1993). Interestingly, the genes involved in curli production are organized into two adjacent divergently transcribed operons, *agfBAC* and *agfDEFG* (Collinson *et al.*, 1996a), which are both required for curli biosynthesis and assembly (Collinson *et al.*, 1993). Additionally, curli are the only fimbriae dependent on the extracellular nucleation–precipitation assembly pathway, which deviates from other assembly pathways as fiber growth occurs extracellularly (Hammar *et al.*, 1996).

Recently, a non-fimbrial adhesin, SiiE, has been identified in *S. enterica* serovar Typhimurim. Although little is known about SiiE, it has been found to mediate contact-dependent adhesion to HeLa cell surfaces (Gerlach *et al.*, 2007). SiiE is a type 1 secretion system (T1SS) secreted protein encoded in the *Salmonella* pathogenicity island 4 and might functionally resemble the type 1 fimbrial adhesins. More work is needed to elucidate the true role of SiiE in adherence *in vivo*.

B. *Helicobacter pylori*

H. pylori is one of the main causes of human chronic gastritis, resulting in various diseases including peptic ulcers, gastric adenocarcinomas, and mucosa-associated lymphoid tissue (MALT) lymphomas (Williams

and Pounder, 1999). At least 50% of the world population is infected with *H. pylori* (Hocker and Hohenberger, 2003), but less than 30% of those are symptomatic (Das and Paul, 2007). The variability in disease frequency and severity of clinical outcome has been attributed, in part, to variable expression of at least two virulence genes, the cytotoxin associated gene (*cagA*), which is encoded within the *cag* pathogenicity island (PAI) and the vacuolating toxin A (*vacA*). Importantly, virulence is also associated with expression of an outer membrane-bound adhesin encoded by *babA2* (Atherton *et al.*, 1995; Blaser, 1996; Gerhard *et al.*, 1999).

Due to the very low pH in the human stomach (pH 1–2), adherence and colonization are a significant challenge. Thus, rather than colonizing the intensely acidic stomach lumen, *H. pylori* colonizes the mucin layer that covers the gastric mucosa. This latter microenvironment has a near neutral pH and is much more suitable for survival (Salyers and Whitt, 2002). Therefore, *H. pylori* must not only be highly motile in an effort to penetrate the mucus barrier, but must be able to maintain their presence there. Not surprisingly, adhesins associated with the carbohydrate-containing moieties within the mucus layer have been identified, including *H. pylori*–neutrophil-activating protein (HP–NAP). In addition, adhesins have been described that allow *H. pylori* to adhere directly to the epithelial cell surface. These include BabA, AlpA and AlpB, SabA, HopH, HopZ, and HorB, all of which belong to the *H. pylori* OMP family 1 (Alm *et al.*, 2000).

Originally, HP–NAP was found to induce neutrophil adhesion to endothelial cells *in vitro* and *in vivo* (Kurose *et al.*, 1994; Yoshida *et al.*, 1993). This is supported by the observation that HP–NAP has a high affinity for glycosphingolipids ending in a linear NeuAcα3Galβ4GlcNAc β3Galβ4GlcNAcβ sequence (Teneberg *et al.*, 1997), which is found in the glycosphingolipid fraction of human neutrophils (Karlsson *et al.*, 2001). However, more recently, HP–NAP has been isolated from the OMP fraction of *H. pylori* and shown to have an entirely different function in the bacterial cell membrane (Namavar *et al.*, 1998). Namavar *et al.* (1998) found that HP–NAP may be responsible for the adhesion of *H. pylori* to sulfated mucins. Consistent with these findings, HP–NAP has a high affinity for sulfatide (SO_3Gal1β-Cer) and gangliotetraosyl ceramide (SO_3Galβ3GalNAcβ4Galβ4Glcβ1-Cer), which are not found on human neutrophils (Teneberg *et al.*, 1997). Additionally, *H. pylori* and purified HP–NAP bind to SO_3–3-Gl, SO_3–NAcGlc, the blood group antigen SO_3–3-Lewis a, and sulfo-Lewis x and Lewis x antigens (Namavar *et al.*, 1998). Moreover, bovine milk and fucoidan, components of dietary seaweed that have been shown to have an anti-ulcer effect (Shibata *et al.*, 1998), block sulfatide-mediated and Lewis^b-mediated adherence of *H. pylori* to gastric cells (Hata *et al.*, 1999; Shibata *et al.*, 1999, 2003).

As noted above, BabA is the best-characterized *H. pylori* adhesin. BabA binds Lewis^b moieties on gastric epithelial cell surfaces. However, not all

H. pylori isolates express the BabA protein. In fact, only about half of the isolates studied by Hennig *et al.* (2004) expressed a detectible level of BabA protein and of those, there was considerable variation with regard to binding Lewis^b *in vitro*. Additionally, Ilver *et al.* (1998) reported that 63 (66%) out of 95 *H. pylori* isolates were able to bind Lewis^b moieties. On the other hand, Gerhard *et al.* (1999) reported that the presence of *babA2* genotype is a good indicator for the ability of strains to express the Lewis^b-binding adhesin, and that there is a strong correlation among the expression of *babA2*, Lewis^b adherence, and gastric cancer.

H. pylori also expresses a sialic acid-binding adhesin (SabA), which binds inflamed gastric mucosa (Mahdavi *et al.*, 2002). The expression of SabA receptors, sialyl-Lewis x (sLex) and sialyl-Lewis a (aLea) glycans, is activated by an inflammatory response, in which they help recruit white blood cells to tissue in peril (Alper, 2001). SabA has also been shown to be a prerequisite for the nonopsonic activation of human neutrophils, an inducer of oxidative metabolism, and to be essential for phagocytosis induction via binding sialylated neutrophil receptors (Petersson *et al.*, 2006; Unemo *et al.*, 2005). The minimal binding epitope described for SabA is a NeuAcα2–3Gal disaccharide, but longer gangliosides and glycoconjugates allow better binding, as do sialylated structures lacking fucose constituents (Aspholm *et al.*, 2006).

Both AlpA and AlpB have been shown to be involved in *H. pylori* adherence to gastric epithelial cells and gastric tissue sections, *in vitro* (Obenbreit *et al.*, 1999, 2000). Additionally, recent studies have shown that AlpB and, to a lesser extent, AlpA are required for colonization of the guinea pig stomach (de Jonge *et al.*, 2004). Additionally, the *alp* genes may play an important role in the early stages of infection, as the transcription of *alpA* is tenfold higher 1 h after infection versus 1 week after infection. However, *alpA* is also transcribed throughout the first 3 months of infection *in vivo*, suggesting an active role in maintaining infection (Rokbi *et al.*, 2001). More work is needed to elucidate the complete function of *alpA* and *alpB* in *H. pylori* infection in humans.

The role of other putative OMP adhesins, including HopZ, HopH, and HorB is still relatively poorly understood. Peck *et al.* (1999) showed that a *hopZ* isogenic mutant greatly reduced adherence to AGS cells (human gastric adenocarcinoma epithelial cells). The HopH protein once designated the ''outer inflammatory protein'' (OipA), because it was associated with an increase in interleukin-8 secretion from epithelial cells *in vitro* and heightened gastric inflammation *in vivo*, has also been implicated in bacterial adherence. However, de Jonge *et al.* (2004) showed that both *hopZ* and *hopH* isogenic mutants were able to colonize guinea pigs and the wild-type strain, confirming previous observations in a mouse model (Yamaoka *et al.*, 2002). The HorB protein has also been found to be involved in the adherence of *H. pylori* to AGS cells and in the production

of LPS *O*-polysaccharide chains (Snelling *et al.*, 2007), which has been suggested to have a role in adherence to gastric sections (Edwards *et al.*, 2000), albeit later studies have discredited *H. pylori* LPS as an adhesin (Mahdavi *et al.*, 2003). The loss of HorB production reduces the ability of *H. pylori* to colonize *Helicobacter*-free BALB/c mice and the expression of *horB* has been detected in gastric biopsies of two culture-positive human subjects (Snelling *et al.*, 2007). More research is needed to completely elucidate the role of these putative adhesins in *H. pylori* infection. Undoubtedly, more OMP adhesins will be identified. However, these adhesins mentioned above and other more recently discovered OMPs must be confirmed as true adhesins and not simply auxiliary proteins that are necessary for the presentation of a functional adhesin.

C. Enteropathogenic *Escherichia coli* (EPEC)

Infections by EPEC strains are one of the major causes of infant diarrhea in developing countries (Cravioto *et al.*, 1991; Levine *et al.*, 1988), and recently have been recognized as a contributing factor in childhood diarrhea in the United States as well (Cohen *et al.*, 2005). Generally, infection causes acute and chronic diarrhea, vomiting, and low-grade fever with a higher mortality rate in developing countries where treatment may be inadequate (Clausen and Christie, 1982; Levine and Edelman, 1984). EPEC is characterized according to the unique genetic attributes that encode its distinctive multi-step infection process, and, in particular, the formation of ''attaching and effacing'' (A/E) lesions on the brush border surface of the small intestine (Moon *et al.*, 1983). The organism is also known for its ability to form three-dimensional microcolonies on the surface of host gastrointestinal epithelium cells (Kaper, 1996). Scaletsky *et al.* (1984) described this microcolony formation as ''localized adherence'' (LA). A/E lesion formation is manifested by a degeneration of the intestinal brush border surface at the site of attachment, followed by microvilli effacement, and the assemblage of highly organized pedestal structures mediated by individual bacteria (Frankel *et al.*, 1998). The genes necessary for A/E lesion formation are found in the locus of enterocyte effacement (LEE) PAI and include structural components of the TTSS apparatus and secreted translocator and effector proteins (Frankel *et al.*, 1998).

Before LA and A/E lesion formation can occur, however, EPEC must first attach to the host cell surface via adhesins. Several adhesins have been implicated in the initial adherence of EPEC to small intestine enterocytes including type IV bundle forming pili (BFP), TTSS EspA filaments, intimin, and flagella. However, in a recent study, Cleary *et al.* (2004) found no evidence that adhesive factors other than BFP and EspA are able to support initial EPEC adherence. Recently, LifA, whose gene sequence has significant homology to the *efa-1* and *toxB* genes in enterohemorrhagic

Escherichia coli, EHEC O111 and EHEC O157:H7, respectively, has also been suggested to play a role in adherence in the absence of BFP (Badea *et al.*, 2003).

The BFP of EPEC has been shown to be very important in the pathogenicity of this organism (Bieber *et al.*, 1998). It serves as a contact for bacteria–bacteria interactions and microcolony formation (Giron *et al.*, 1991), auto-aggregation (Vuopio-Varkila and Schoolnik, 1991), dispersal of bacteria from microcolonies (Bieber *et al.*, 1998; Knutton *et al.*, 1999), and, as noted above, has been implicated in the initial adherence of EPEC to host epithelial cells (Donnenberg *et al.*, 1992; Giron *et al.*, 1991; Tobe and Saskawa, 2002). Although strains that lack BFP are still capable of causing ''diffuse'' binding and promoting pedestal formation *in vitro* (Cleary *et al.*, 2004; Rosenshine *et al.*, 1996), they are about 200-fold less virulent than the wild-type parent strain *in vivo* (Bieber *et al.*, 1998). Thus, BFP appears to be a convincing candidate as the primary adherence factor.

Among the BFP receptor molecules that have been proposed, most are oligosaccharides. Exogenous molecules that resemble BFP receptors interfere with LA, thus competitive-binding inhibition assays are very useful when investigating these candidate receptors. For example, *N*-acetylgalactosamine completely inhibits LA to HeLa cells, thus, it may act as a receptor for BFP on the host cell surface (Scaletsky *et al.*, 1988). Another group found that locally adhering EPEC bound to asialo-GM1, asialo-GM2, globoside, and lacto-*N*-neotetraose, which all share the sequence GalNAc(β1–4)Gal (Jagannatha *et al.*, 1991). In addition, LA was inhibited by fucosylated tetra- and pentasaccharides in several strains of BFP-expressing EPEC (Cravioto *et al.*, 1991), suggesting a role of these sugars as binding sites. Additionally, phosphatidylethanolamine (PE) has also been implicated as a potential receptor for BFP (Foster *et al.*, 1999), and recently, Hyland *et al.* (2008) reported that *N*-acetyllactosamine (LacNAc) was a possible receptor for EPEC with BFP composed of α-bundlin.

Because EPEC are still able to bind to host cells, albeit less efficiently, without BFP, other factors may also be involved in adherence. Recent studies have demonstrated that EspA filaments promote an attenuated adherence of BFP deficient EPEC strains to the brush borders of Caco-2 cells (Knutton *et al.*, 1998). EspA is the major component of the long hollow filamentous needle complex of the TTSS that directly contacts the host cell and that facilitates injection of bacterial effector proteins, EspB and EspD, into the host cytoplasm for A/E lesion formation (Daniell *et al.*, 2001). EspA-mediated adherence is less efficient than BFP binding, presumably due to the small number of filaments (∼12 EspA filaments) produced per bacterium (Daniell *et al.*, 2001). The nature of the interaction is currently unknown (Cleary *et al.*, 2004), and host cell receptors have not been thoroughly investigated. However, recent data suggests that cholesterol may play an important role in adherence and type III secretion

in the absence of BFP. Allen-Vercoe *et al.* (2006) found that bacterial adherence and delivery of effector proteins were abolished after treatment of HeLa cells with the cholesterol-depleting agent, methyl-β-cyclodextrin. Additionally, lipid rafts, where cholesterol is localized, were necessary for pedestal formation by EPEC. Therefore, cholesterol may serve as an adherence site for EPEC when BFP is absent.

Flagella are also thought to play a role in the initial attachment of EPEC to host cells (Giron *et al.*, 2002). Giron *et al.* (2002) discovered that flagella extended outward from microcolonies while BFP were tightly associated with the microcolony, suggesting that flagella may tether the bacteria to the host and BFP may simply mediate microcolony formation. However, other workers suggested that flagella were produced by adherent EPEC, but found no evidence that implicated flagella in adherence (Cleary *et al.*, 2004). Consequently, more work is needed in this area.

Several other proteins have been suggested to act as putative adhesins, but additional work is needed to determine their precise role in adherence. Intimin, for example, is an OMP that directly binds Tir, a translocated intimin receptor inserted in the host cell membrane by the bacteria through the TTSS after initial contact has occurred. This interaction ultimately prompts A/E lesion formation (Frankel *et al.*, 1998; Kenny *et al.*, 1997). Cleary *et al.* (2004) suggested that intimin cannot support initial bacterial adhesion as EPEC strains that lacked BFP and EspA could not adhere to two types of epithelial cells in the absence of Tir. However, intimin has been shown to bind other host cell receptors, such as β1 integrins (Frankel *et al.*, 1996) and nucleolin (Sinclair and O'Brien, 2002, 2004). These receptors have been shown to be necessary for EPEC to modulate host cell tight junctions (Dean and Kenny, 2004), an event that may precede the onset of diarrhea (Guttman *et al.*, 2006). In a recent study, Hernandes *et al.* (2008) found that atypical EPEC strain 1551–2 that lacks BFP was able to form loose microcolonies after 6 h of infection via intimin omicron, suggesting that intimin omicron is responsible for the LA phenotype observed and that this strain may express an additional novel adhesive structure.

The protein LifA, which has been characterized as the EPEC toxin lymphostatin (Klapproth *et al.*, 1996, 2000), has also been implicated as a potential EPEC adherence factor. The *lifA* gene has high homology to the *efa-1* and *toxB* genes in EHEC strains (Badea *et al.*, 2003), which have been proposed to influence adherence to epithelial cells (Stevens *et al.*, 2002; Tatsuno *et al.*, 2001). However, the true role of these genes in EHEC adherence is still unclear (Torres *et al.*, 2005). An initial EPEC adherence study using a *lifA* mutant found no difference in adherence to cultured epithelial cells (Klapproth *et al.*, 2000). However, a subsequent study found that LifA may play a role in adherence in the absence of BFP (Badea *et al.*, 2003). Therefore, the function of *lifA* in adherence remains to be established.

D. Enterohemorrhagic *Escherichia coli* (EHEC)

EPEC and EHEC strains share many of the same virulence and adherence factors. In particular, EHEC strains also contain the LEE, PAI and they form characteristic A/E lesions on cultured mammalian cells and in animals (Knutton *et al.*, 1989; Tzipori *et al.*, 1986). In fact, intimin, which is essential in EPEC pathogenesis as discussed above, is also found in EHEC O157 and other EHEC serotypes (Huppertz *et al.*, 1996; Yu and Kaper, 1992). Although intimin is one of the only adhesins in EHEC demonstrated to play a role in colonization *in vivo* (Donnenberg *et al.*, 1993), many other putative fimbrial and afimbrial adhesins have been identified in EHEC strains including Efa1 (Nicholls *et al.*, 2000), LP fimbriae (Torres *et al.*, 2002), curli (Kim and Kim, 2004), F9 (type I pilus homolog) (Low *et al.*, 2006), *E. coli* common pilus (ECP) (Rendon *et al.*, 2007), and type IV pilus (TFP) (Srimanote *et al.*, 2002). However, their role in *in vivo* adherence and host colonization remains unclear. The most recent and well-studied EHEC adhesins are discussed below.

The Efa1 adhesin was identified in 2000 by Nicholls *et al.* (2000) through transposon mutagenesis of a clinical non-O157:H7 EHEC isolate of serotype O111:H-. They found that Efa1 promotes adherence to Chinese hamster ovary (CHO) cells, human red blood cells agglutination, and autoaggregation. Additionally, the *efa1* gene was present in 116 strains of attaching–effacing EPEC and EHEC, but not in 91 nonattaching–effacing *E. coli* strains. *E. coli* O157:H7 strains lack *efa1*, but do encode *toxB*, a truncated version of the *efa1* gene. A *toxB* mutant exhibits reduced adherence to cultured epithelial cells (Stevens *et al.*, 2004). However, *toxB* had an indirect effect on adherence to epithelial cells by modulating the production and secretion of proteins that play a role for A/E formation in EHEC. The receptors for Efa1 and ToxB have not been identified and their true role *in vivo* is unknown.

The *lpfABCC'DE* chromosomal fimbrial operon identified in EHEC O157:H7 has high similarity to the LP fimbriae (*lfp*) operon of *S. enterica* serovar Typhimurium (Perna *et al.*, 2001). In one recent study (Torres *et al.*, 2002), LP fimbriae were proposed to participate in the interactions of EHEC with eukaryotic cells by assisting in microcolony formation, as isogenic *E. coli* O157:H7 *lpf* mutants showed slight reductions in adherence to tissue culture cells and formed fewer microcolonies compared to the wild-type strain. A second locus within the EHEC O157:H7 genome has homology to LP fimbriae in *Salmonella* and shares an overall 31% identity to proteins in the previously mentioned *lpf* operon in EHEC O157:H7. Additionally, a similar region has been identified in Shiga toxin-producing *E. coli* O113:H21 (Doughty *et al.*, 2002). A mutation in the O113:H21 LP fimbriae results in decreased adherence to epithelial cells. Together, these data suggest that LP fimbriae may play a role in

EHEC adherence *in vitro*. However, the role of these LP fimbriae *in vivo* remains undefined.

The TFP in EPEC, termed BFP, have been shown to play a very important role in infection in animal models as discussed above. However, until recently, no TFP has been identified in EHEC strains. Xicohtencatl-Cortes *et al*. (2007) identified hemorrhagic coli pilus (HCP), a TFP, in EHEC O157:H7. It is composed of a 19-kDa pilin subunit (HcpA) and is encoded by the *hcpA* gene. The HCP was found to be composed of bundles of fibers that formed physical bridges between bacteria adhering to human and bovine host cells. Although the expression of HCP was only induced under strict growth and environmental conditions *in vitro*, the authors showed that *hcpA* was expressed *in vivo* by testing the sera of HUS patients and healthy individuals for antibodies against HcpA. Only HUS patient sera had antibodies that recognized HcpA. Additionally, the disruption of *hcpA* gene reduces EHEC adherence to cultured epithelial cells and bovine and porcine explants. Consequently, these data suggests that EHEC O157:H7 possesses TFP that are important intestinal colonization factors that contribute to the pathogenesis of this microorganism.

Recently, other putative EHEC fimbrial adhesins have been identified, including the ECP and the F9 fimbriae. The ECP is composed of a 21-kDa pilin subunit whose amino acid sequence corresponds to the *ecpA* gene present in all *E. coli* genomes. Isogenic *ecpA* mutants of EHEC O157:H7 or fecal commensal *E. coli* demonstrated significant reduction in adherence to cultured epithelial cells (Rendon *et al.*, 2007). The F9 fimbriae in EHEC O157:H7 were identified by Low *et al.* (2006) and found to promote colonization in 1–2-week-old calves. Mutation of the major F9 subunit gene in EHEC O157:H7 resulted in reduced levels of shedding in weaned calves, but did not reduce the level of colonization at the terminal rectum, indicating that the adhesin is not responsible for rectal colonization, but may contribute to colonization at other intestinal sites.

E. Uropathogenic *Escherichia coli* (UPEC)

UPEC is the primary cause of UTI in the developed world. These infections are ascending infections in that they usually start in the bladder and move up the urinary tract toward the kidneys, and then possibly entering the bloodstream. If the bacteria do enter the bloodstream, any organ is susceptible to infection and other more life threatening conditions may develop, including pneumonia and meningitis. As with other pathogens, the ability of UPEC to colonize the bladder and kidney in animal models is dependent on its ability to adhere to uroepithelial cells (Hagberg *et al.*, 1983). The most common UPEC adhesins are type I, P, F1C, S, and Auf fimbriae and the Afa/Dr afimbrial adhesins (Oelschlaegar *et al.*, 2002). Recently, the sequencing of three UPEC genomes have revealed the

presence of several additional gene clusters with homology to some of the existing UPEC fimbrial adhesins already identified (Brzuszkiewicz *et al.*, 2006; Chen *et al.*, 2006; Welch *et al.*, 2002). The type I fimbriae will not be discussed here as it has been described above for *E. coli* and *Salmonella*.

The pyelonephritis-associated pili (P fimbriae), like the type I fimbriae, is a chaperone-usher class of fimbriae and is the most extensively studied adhesin. These fimbriae are used by UPEC to bind galabiose-containing glycolipid receptors in the kidney that initiates pyelonephritis (Dodson *et al.*, 2001; Lund *et al.*, 1987). Acute pyelonephritis is the most serious UTI in that it may lead to scarring of the kidneys, resulting in kidney damage, kidney failure, and even sepsis. The P fimbriae are encoded by the *pap* genes. They are structurally comprised of around 1000 copies of PapA, the major subunit protein, which polymerize to form a rigid structure that connects to PapE and PapF (minor subunit structures), and PapG, the receptor-binding adhesin, at the distal end (Kuehn *et al.*, 1992; Lindberg *et al.*, 1987). There are three different PapG isoreceptor-binding variants (PapGI, -II, and -III) (Stromberg *et al.*, 1990). Each PapG variant binds different isoreceptors that contain a common Gal(α1–4)Gal moiety linked to a ceramide group that anchors the receptor in the lipid bilayer of the host cell (Hakomori, 1990). Differences in the PapG receptor type and distribution on different host cell surfaces have been shown to dictate variations in host tropism of P-fimbriated *E. coli* (Stromberg *et al.*, 1990). For example, the class II *papG* allele has been shown to be primarily associated with pyelonephritis and bacterimia, while the class III *papG* allele is associated with human cystitis and with genitourinary infections in dogs and cats (Johnson, 1998; Johnson *et al.*, 2000; Otto *et al.*, 1993). However, little is known about the clinical association of the class I *papG* allele, as this allele is found less frequently. Recently, an extensive review has been published by Lane and Mobley (2007) detailing the role of P fimbriae in adherence and persistence in UPEC.

Several other fimbrial adhesins have been identified in UPEC strains; however, much less is known about these adhesins. The F1C fimbriae (Foc) resembles type I fimbriae in genetic organization and organelle structure (Klemm *et al.*, 1994; van Die *et al.*, 1991). Backhed *et al.* (2002) identified two F1C receptors, galactosylceramide and globotriaosylceramide, both with phytosphingosine and hydroxy fatty acids. However, the ceramide portion of the glycosphingolipid receptor was found to confer binding specificity. The authors also reported that human renal epithelial cells produce proinflammatory chemokine interleukin-8 in response to F1C-mediated attachment, suggesting a role in F1C-mediated attachment in mucosal defense against bacterial infection. The S-fimbriae, which resembles the F1C fimbriae at the amino acid level (van Die *et al.*, 1991), mediates adherence to sialic acid-containing glycolipids or glycoproteins, and are associated with sepsis and meningitis in newborn infants

(Korhonen *et al.*, 1985; Parkkinen *et al.*, 1986). The Auf fimbriae, encoded by the *aufABCDEFG* gene cluster, were found to be significantly associated with UPEC (Buckles *et al.*, 2004). Although the deletion of the entire *auf* gene cluster had no effect on the ability of UPEC to colonize the kidney, bladder, or urine in a murine model, *aufA* was detected in the urine from infected mice by RT-PCR. Therefore, the true role of the Auf fimbriae in UPEC pathogenesis is still unclear.

The Afa/Dr family of UPEC afimbrial adhesins has also been shown to promote initial attachment to host cells. Interestingly, the Afa/Dr family consists of both fimbrial and afimbrial members as determined by electron microscopy (Bilge *et al.*, 1989; Garcia *et al.*, 1996). However, recent high-resolution structural studies have shown that the previously characterized afimbrial adhesin, Afa-3, may comprise fine filaments that are not detectible after preparation for electron microscopy (Anderson *et al.*, 2004). The Afa/Dr adhesins are encoded by genes designated A through E, with E typically encoding the structural adhesin (Le Bouguenec *et al.*, 2001). Most of the Afa/Dr adhesins bind to the Dr^a blood group antigen present on CD55 (also known as decay-accelerating factor, DAF). DAF is an eukaryotic cell membrane protein that regulates complement cascade and protects cells for autologous complement-mediated damage (Lublin and Atkinson, 1989). In fact, studies have shown that the level of host cell colonization by Afa/Dr-expressing *E. coli* is directly proportional to the extent of host cell CD55 expression (Selvarangan *et al.*, 2000). Other Afa/Dr adhesins, including F1845, Dr, and Afa-3 have also been shown to bind to three members of the carcinoembryonic antigen-related adhesin molecules (CEACAM) family: CEA, CEACAM1, and CEACAM6 in diffusely adhering *E. coli* (Berger *et al.*, 2004). Additionally, many studies have indicated that both Dr and Afa-3 adhesins recognize $\alpha5\beta1$ integrin (Guignot *et al.*, 2001; Plancon *et al.*, 2003). While other fimbrial and afimbrial adhesins such as F9 (Ulett *et al.*, 2007a) and Antigen 43 (Ulett *et al.*, 2007b) have been shown to promote persistence in the UTI, their proposed function relates to aggregation and biofilm formation, rather than initial attachment and thus are not discussed here.

VIII. ANTI-ADHESIVES

The ability to adhere to host tissues is an essential step for infection by many pathogenic microorganisms (Finlay and Falkow, 1989). Adherence not only provides the pathogen with the means to initiate colonization of the host cell surface, but it also enhances resistance against host cleansing or clearing mechanisms, such as flow of lumen contents in the intestinal tract, airflow through the lungs, and urine flow through the urinary tract. In addition, once bacteria have adhered, they are privy to nutrients that

support colonization and survival (Ofek and Doyle, 1994). Adhered bacteria are also positioned to facilitate toxin delivery or to invade host tissues. It is now well accepted that the initial phase of most bacterial infections involves attachment of the pathogen to host cell receptors via bacterial adhesins, as discussed above in Section VI (Fig. 2.2A). Therefore, by disrupting adhesin–receptor interactions, it may be possible to prevent initial adherence and subsequent infection.

The disruption or inhibition of pathogen attachment to host cells via anti-adherence agents has attracted considerable attention for several reasons. First, this approach is considered more gentle and ecologically sound compared to alternative approaches, such as using chemotherapy or antibiotic treatments (Karlsson, 1998). Some of the candidate anti-adhesive agents are even found naturally in foods. In addition, although some resistance to anti-adhesive agents may possibly occur, dissemination of bacterial strains that are resistant to anti-adhesives will likely occur at a significantly lower rate compared to antibiotic-resistant strains (Ofek *et al.*, 2003a).

There are some potential limitations of anti-adhesive strategies that must also be recognized. Pathogenic bacteria often encode genes for more than one type of adhesin, and, via a process known as phase variation (Henderson *et al.*, 1999), express adhesins on either a random or perhaps ''as-needed'' basis. Therefore, a cocktail of different anti-adhesive agents

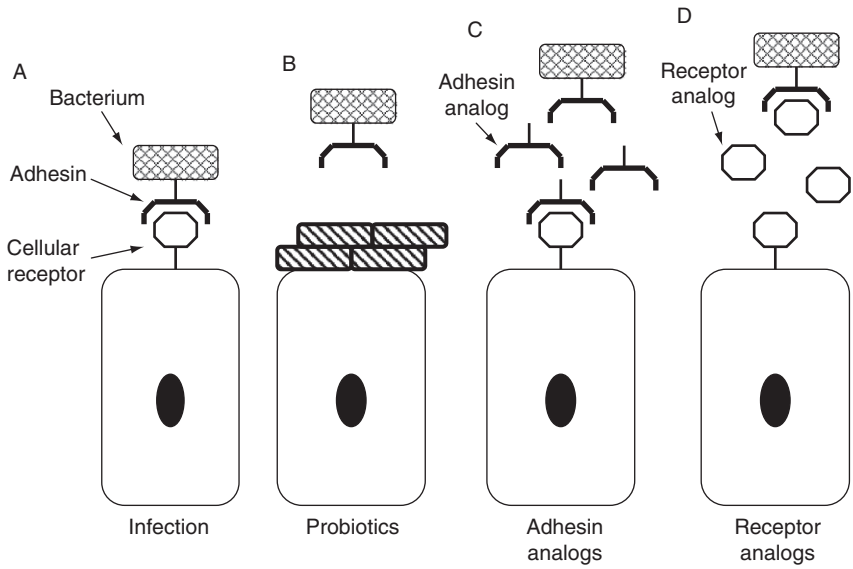

FIGURE 2.2 Schematic illustration of adherence (A) and anti-adhesive agents: probiotics (B), adhesin analogs (C), and receptor analogs (D).

that target several adhesins or a single agent that has a broad spectrum of anti-adhesion activity may be necessary (Ofek *et al.*, 2003a).

Many bacterial adhesins and host receptors have been identified and studied in great depth, as described in Section VII. Therefore, with every new adhesin or receptor discovered, a new opportunity arises to develop an anti-adherence mechanism that may inhibit or block the adhesin–receptor interaction, which is the ultimate aim of anti-adhesion therapy (Kahane and Ofek, 1996; Moricout *et al.*, 1990; Ofek and Doyle, 1994). In actual practice, there are several types of anti-adhesive mechanisms including adhesin-based vaccines, innate host-derived anti-adhesives, probiotics, adhesin analogs, and receptor analogs.

A. Adhesin-based vaccines

Averting infection by blocking adhesion with adhesin vaccines can be conferred both passively and actively (Ofek *et al.*, 2003a). Passive immunity was shown in a study that demonstrated that suckling piglets acquired immunity from their mother who were previously vaccinated with K88 fimbriae and other related adhesins from enterotoxigenic *E. coli* (ETEC) while pregnant (Moon and Bunn, 1993). It was assumed that milk-secreted antibodies prevented infection by blocking bacterial adherence (Ofek *et al.*, 2003a). In another study, anti-*Streptococcus mutans* monoclonal antibodies against SA I/II adhesins were applied to the tooth surfaces of human volunteers that had been chemically cleared of oral *S. mutans* microflora (Ma *et al.*, 1998). The control subjects were positive for *S. mutans* within 2–3 months, while the passively immunized group was free of *S. mutans* for at least a year. However, this passive immunity may have been the result of competitive exclusion, as SA I/II antibodies were not detectable one day after infection (Kelly and Younson, 2000). Nonetheless, adhesin-based vaccines appear to be a promising area of study, and may some day, greatly contribute to reducing infections in some populations.

Active immunity appears to be functional in animal models through the stimulation of both IgG and secretory IgA antibodies (Ofek and Doyle, 1994). It has been suggested that mucosal IgG-mediated immunity, as opposed to systemic immunity, can improve the protective effect of adhesin-based vaccines (Mestecky *et al.*, 1997; Wisemann *et al.*, 1999). Active immunity against UTI has been extensively investigated. The type 1 fimbriae, FimH adhesin complex and its periplasmic chaperone, FimC, provided immunity against *E. coli* in the urinary tract of both mice and nonhuman primates. The potential effectiveness of adhesin vaccines in animals has gained considerable attention, and development of multiple adhesin vaccines in humans is likely to follow.

B. Host-derived anti-adhesins

The innate immunity of a host plays a significant role in preventing infection. Body fluids have an abundance of potential anti-adhesive molecules. However, only a few of them have been found to be effective against pathogens (Ofek *et al.*, 2003b). The anti-adhesive agents that have the most potential to inhibit bacterial attachment are those associated with the host mucosal surface including secreted mucins (Ofek *et al.*, 2003a). Mucins, as discussed previously in Section II, protect the mucosal surface from invading pathogens by a permissive protective barrier. However, other components of the mucus layer may provide some innate immunity, as recent studies have shown that some mucins and other mucosal surface components can inhibit bacterial adherence *in vitro* (Mack and Sherman, 1991; Wu *et al.*, 1996). This is promising data, but may only be effective in luminal areas where the mucus flow is relatively fast so that the bacteria can be cleared with the mucus. In luminal areas where the mucus layer is thick and moving slowly, bacterial binding to these potential anti-adhesins may accomplish the opposite effect by facilitating bacterial penetration of the mucus so infection can continue. Much more research is needed in this area to determine efficacy of host-derived anti-adhesins.

C. Probiotics as anti-adhesives

Probiotic bacteria are by definition, ''nonpathogenic, live microbial, mono- or mixed-culture preparations, which, when administered to humans or animals in adequate amounts, confer a health benefit on a host by improving intestinal microbial balance'' (Fuller, 1989; Havenaar and Huis in't Veld, 1992; Havenaar *et al.*, 1992; Salminen *et al.*, 1998). The most widely used probiotics are lactobacilli and bifidobacteria, but other microorganisms, including *E. coli*, enterococci, bacilli, and yeasts have also been used (Holzapfel and Schillinger, 2002). Both lactobacilli and bifidobacteria have been shown to have an inhibitory effect on many enteric pathogens (Saavedra, 1995). A number of mechanisms have been attributed to these antagonist effects, such as decreasing the luminal pH by the production of short chain fatty acids, competition with pathogens for nutrients, production of inhibitory compounds like bacteriocins, and competing for adhesin receptors on the host cell surface thereby inhibiting pathogen adherence (Bernet *et al.*, 1994; Sanders, 1993). All of these mechanisms are important and potentially play a role in probiotic functionality. However, the competitive binding of probiotics to host tissues at the expense of pathogens is clearly an anti-adhesive effect (Fig. 2.2B).

Several probiotic organisms have been shown to adhere to the intestinal mucosa (Jacobsen *et al.*, 1999). For example, *Lactobacillus acidophilus*

(Bernet *et al.*, 1994; Coconnier *et al.*, 1992) and *L. casei* (Hudault *et al.*, 1997) were shown to bind to Caco-2 cells at relatively high numbers, and this adherence may reduce or displace adherence of enteric pathogens, including *Samonella enterica* serovar Typhimurium, EPEC, and *Yersinia enterolitica*. In some cases, the probiotics do not have to be viable or even bind directly to the pathogen target receptor to affect adherence of pathogens. In one study, heat-killed *L. acidophilus* LB was shown to inhibit ETEC attachment to polarized Caco-2 cells (Chauviere *et al.*, 1992). However, elevated concentrations of heat-killed *L. acidophilus* were required. Because ETEC utilize CFA/I, CFA/II, and CFA/III receptors to bind host cells and *L. acidophilus* does not express adhesins for these receptors, the authors suggested steric hindrance as the explanation for adherence inhibition.

Recently, surface-layer proteins (Slps) extracted from *Lactobacillus helveticus* were shown to act as anti-adhesives against *E. coli* O157:H7 (Johnson-Henry *et al.*, 2007). The Slps in many *Lactobacillus* species have been found to, among other things, assist in bacterial adherence to host tissues (Frese *et al.*, 2005). Extracted Slps from *L. helveticus* reduced *E. coli* O157:H7 adherence and A/E lesion formation on both HEp-2 and T84 cells, suggesting that probiotic binding may interrupt the infectious process of some intestinal pathogens (Johnson-Henry *et al.*, 2007). The role of probiotics as anti-adhesives is somewhat unclear in that, although *in vitro* studies are promising, there have been no carefully controlled clinical human studies to test the effect of probiotics as anti-adhesives (Ofek *et al.*, 2003a).

D. Adhesin analogs

The rationale for adhesin analogs is based on the assumption that soluble, exogenous bacterial adhesins will bind to their intended receptor, thereby competitively blocking pathogen adherence to those same receptors (Fig. 2.2C). This type of anti-adhesive has been mostly impractical to use because they are almost always macromolecules, are not readily available, must be used in high concentrations, and due to their innate nature may be toxic and/or immunogenic (Ofek *et al.*, 2003a). Despite these drawbacks, however, new technologies have allowed the development of some potential adhesin analogs.

Both proteinaceous and non-proteinaceous analogs have been studied. Examples include a synthetic 20 amino acid adhesin peptide sequence copied from *S. mutans* and LTA of groups A and B streptococci. The synthetic peptide mimics a *S. mutans* adhesin that binds a salivary protein on dental surfaces and was shown to inhibit bacterial adherence to immobilized salivary receptors *in vitro*. *In vivo*, this peptide hindered the recolonization by *S. mutans* on teeth that had been cleared of the

normal microflora as compared to the control groups that had been treated with saline or placebo peptides (Kelly *et al.*, 1999). Although these results would appear promising, *S. mutans* is able to utilize other adhesins that bind to cell surfaces, especially in the presence of sucrose. Thus, the application of multiple analogs may be required for this approach to be effective (Ofek *et al.*, 2003a).

In another study, non-proteinaceous LTA was used as an anti-adhesive against group A streptococci (Dale *et al.*, 1994). The nasal cavities of mice were treated with the adhesin analog and a group A streptococcal suspension was then administered. Colonization and death were significantly reduced in the LTA-treated mice compared to the control mice. Although the potential of adhesin analogs was demonstrated in this study, LTA may not be a practical anti-adhesive because of its potential toxicity. Group A streptococci are also thought to bind the CD44 receptor on epithelial cells via the presence of a hyaluronic acid capsule (Cywes *et al.*, 2000). Thus, the anti-adhesive properties of hyaluronic acid have also been examined. Mice that had been orally treated with hyaluronic acid and then challenged by group A streptococci have fewer adhered cells and resisted colonization (Cywes *et al.*, 2000). However, the exact mechanisms accounting for this inhibition are not known.

E. Receptor analogs

Inhibition of bacterial adherence via receptor analogs is the most well-studied of the anti-adhesive mechanisms. This strategy is based on the observation that bacterial adherence is often mediated by interactions between bacterial surface proteins and complimentary oligosaccharide receptors located at the surface of host cells. Soluble oligosaccharides that resemble or mimic host oligosaccharide receptors interrupt the adherence process by acting as receptor analogs or decoys. More precisely, rather than binding to host cells, pathogens bind to the soluble oligosaccharide decoys and are displaced from the intestinal tract preventing infection initiation and subsequent host tropism (Fig. 2.2D). Although many of the receptor analogs that have been studied are derived synthetically, there are numerous reports describing anti-adherence activities from natural sources, such as milk, berries, and other foods (Table 2.3). Moreover, there is now considerable evidence demonstrating that soluble oligosaccharides specific for an adhesin can competitively inhibit binding to target cells not only in the GI tract, but also in a variety of other tissues (Aronson *et al.*, 1979; Barthelson *et al.*, 1998; Bouckaert *et al.*, 2005; Firon *et al.*, 1987; Hyland *et al.*, 2006; Nagahori *et al.*, 2002; Zorf and Roth, 1996).

TABLE 2.3 Synthetic and naturally occurring anti-adhesive receptor analogs

Receptor analogs	Pathogen	Tissue
Synthetic		
N-acetyl-galactosamine	Enteropathogenic *E. coli*	Intestinal
Methyl α-mannoside	Uropathogenic *E. coli*	Urinary
Sialylated NeuAcα2–3(or 6) Galβ1	*Streptococcus pneumoniae*	Respiratory
Globotriose	*E. coli* shiga toxins	Intestinal
Commercial galactooligosaccharides	Enteropathogenic *E. coli*	Intestinal
Natural		
Human milk oligosaccharides	*Shigella*	Intestinal
	Campylobacter jejuni	Intestinal
	Pathogenic *E. coli* varotypes	Intestinal
Egg-yolk-derived sialyloligosaccharides	*Salmonella enteritidis*	Intestinal
Cranberry extracts	*Helicobacter pylori*	Intestinal
	Uropathogenic *E. coli*	Urinary
	Streptococcus mutans	Oral
Green tea extracts	*Helicobacter pylori*	Intestinal
	Proprionibacterium acnes	Epithelial
	Staphylococcus aureus	Epithelial
Carrot extracts	Enteropathogenic *E. coli*	Intestinal
Mannooligosaccharides	*Salmonella*	Intestinal
	Klebsiella	Respiratory/ Urinary
	Uropathogenic *E. coli*	Urinary

1. Studies with synthetically derived analogs

As described earlier in Section IV.A., several of the carbohydrate sequences that serve as receptors for enteric pathogens have been identified. For EPEC, these receptors are located on the surface of host epithelial cells and are often comprised of galactose, *N*-acetyl-galactosamine, lactosyl

glycans, and fucosylated and sialylated oligosaccharides (Vanmaele *et al.*, 1999). These residues, if derived synthetically and added exogenously, would, therefore, have the potential to serve as effective anti-adhesives. Several studies support this hypothesis (Alvarez-Dominguez *et al.*, 1997; Ascencio *et al.*, 1993; Hyland *et al.*, 2006; Klapproth *et al.*, 2000). In several of these studies, carbohydrate receptors were conjugated to bovine serum albumin (BSA), forming lactosyl-BSA, *N*-acetyl-galactosamine-BSA, or other BSA-glycoconjugates, and then used in adherence assays (Hyland *et al.*, 2006). Adherence inhibition of nearly 99% was achieved for *N*-acetyl-galactosamine-BSA. Galactosyl-, fucosyl-, and other mixed glycoconjugates gave intermediate levels of inhibition (about 50%).

The ability of synthetically derived carbohydrate analogs to protect against infection *in vivo* (in animals) was first reported by Aronson *et al.* (1979). They found that methyl α-mannoside inhibited UTI in mice that were administered *E. coli* expressing type 1 fimbriae. Subsequently, polyvalent sialylated oligosaccharides that terminate in NeuAcα2–3(or 6) Galβ1 were shown to prevent nine strains of *S. pneumoniae* from binding to human cells derived from the upper respiratory tract (Barthelson *et al.*, 1998). Finally, it also appears that oligosaccharide receptor analogs may not only prevent the adhesion of bacterial cells to host tissues, they also neutralize toxins produced by some bacteria. Shiga toxins (Stx), Stx1 and Stx2c, produced by Stx-producing *E. coli* (STEC) human strains were neutralized by globotriose-expressing recombinant *E. coli*, revealing a potential treatment for these infections (Paton *et al.*, 2001).

2. Studies with naturally occurring analogs

The possibility that pathogens can be inhibited by naturally occurring anti-adhesive substances is especially attractive and has captured significant attention. The initial evidence that such substances might exist was based on the long-standing observation that breast-fed infants appeared to suffer from fewer diarrheal diseases than formula-fed infants (Dewey *et al.*, 1995; Grulee *et al.*, 1934; Hagberg *et al.*, 1983; Huffman and Combest, 1990; Kramer *et al.*, 2001; Kunz and Rudloff, 1993; Newburg *et al.*, 2005). This apparent reduction in infection by diarrheal pathogens has been attributed to several components in human breast milk, including lactoferrin, casein peptides, and human milk oligosaccharides (HMOs) (Coppa *et al.*, 2006; de Araujo and Giugliano, 1999; Rhoades *et al.*, 2005). The concentration of free oligosaccharides, in particular, may reach levels as high as 10 g/L in mature milk (Chaturvedi *et al.*, 2001; Newburg *et al.*, 2004), which would make these oligosaccharides the third largest solid constituent in human milk (Newburg, 2000). These oligosaccharides can be found in nonconjugated (free) or conjugated form (glycolipids, glycoproteins). Some of the oligosaccharides appear to function as prebiotics (Coppa *et al.*, 2004), which stimulate the growth of lactobacilli and

bifidobacteria in the infant gut (Gibson and Roberfroid, 1995), and, by virtue of short chain, volatile acid production, inhibit enteric pathogens. While the prebiotic effect of milk oligosaccharides likely accounts for a substantial positive effect on gut and overall health of nursed infants, it is now evident that HMOs also act as adhesin analogs against invading pathogens (Brand-Miller *et al.*, 1994; Crane *et al.*, 1994; Kobata, 2003; Morrow *et al.*, 2004; Newburg *et al.*, 2004; Ruiz-Palacios *et al.*, 2003).

In recent years, human milk-derived oligosaccharides have been reported to have anti-adhesive activity against several pathogenic bacteria. For example, HMOs were reported to inhibit *Shigella* and *Campylobacter* and various pathotypes of *E. coli* via an anti-adherence mechanism (Kunz and Rudloff, 1993; Kunz *et al.*, 2000; Newburg, 1997; Sharon, 2006). The oligosaccharide fraction of human colostrums also inhibited adherence of EPEC to HEp-2 cells (Cravioto *et al.*, 1991). In addition, the fucosylated fraction of HMOs inhibited binding of *C. jejuni* to HEp-2 tissue culture cells (Ruiz-Palacios *et al.*, 2003) and protected infants from diarrheal diseases (de Araujo and Giugliano, 1999; Morrow *et al.*, 2005). Similarly, the fucosylated fraction of HMOs that contain H-2 blood group epitope was found to inhibit the binding of *C. jejuni* to monolayers of HEp-2 cells *in vitro* (Ruiz-Palacios *et al.*, 2003). This fraction also inhibited *Campylobacter* colonization of mice *in vivo* and inhibited binding of invasive pathogenic campylobacter to human intestinal mucosa *ex vivo*. Another study showed that the sialylated oligosaccharide fraction prevented enterotoxigenic and uropathogenic strains of *E. coli* from binding and agglutinating calf and human erythrocytes (Martin-Sosa *et al.*, 2002). In fact, in one of the first reports demonstrating the protective effects of milk oligosaccharides, the authors suggested that sialic acid-linked oligosaccharides were involved (Gyorgy *et al.*, 1974). Conversely, others have suggested that neutral, rather than sialylated breast milk oligosacccharides were responsible for adherence inhibition (Asakuma *et al.*, 2007; Coppa *et al.*, 1990; Newburg, 1997). Collectively, the data strongly suggest that HMOs are exceptional anti-adhesives.

Several other dietary saccharides have also been found to inhibit bacterial adherence in both animals and humans. In one report, egg-yolk-derived sialyloligosaccharides and their derivatives were found to inhibit *S. enteritidis* infection and lethality in BALC/c mice when administered orally (Sugita-Konishi *et al.*, 2002). This study also established that an immune response was not stimulated in cultured macrophages by these oligosaccharides, ruling out an immunological response as the cause for reducing infection and death. Similar anti-adherence activity against *S. enteritidis, S. enterica* serovar Typhimurium, and *E. coli* O157:H7 was also reported using non-immunized egg yolk powder (Kassaify *et al.*, 2005). In this study, a high density lipoprotein fraction reduced adherence

of *Salmonella* and, to a lesser extent, *E. coli* O157:H7, although the exact mechanism responsible for this inhibition could not be established.

Numerous studies have shown that cranberry extract has extensive anti-infection, and, in particular, anti-adhesive properties (Burger *et al.*, 2002; Howell, 2007; Howell *et al.*, 2001; Puupponen-Pimia *et al.*, 2005; Sharon and Ofek, 2002). Initially, cranberry juice became an area of interest due to its well-known beneficial effects in UTI (Moen, 1962; Papas *et al.*, 1968; Sternlieb, 1963). Several clinical trials have substantiated these initial observations. In one study, elderly women drank 300 ml of cranberry cocktail or a placebo every day for 6 months (Avorn *et al.*, 1994). In another study, young women drank 50 ml of cranberry–lingonberry juice concentrate diluted in 200 ml of water every day for 6 months (Kontiokari *et al.*, 2001). Both studies showed a significant reduction in the incidence of bacteria in urine samples as compared to the placebo. In another recent study, cranberry juice was administered to patients with *H. pylori* infections and who were receiving antibiotic therapy (Shmuely *et al.*, 2007). Although there were no overall differences in eradication of *H. pylori* among the treatments and control groups, eradication in female subjects fed the cranberry juice was significantly higher.

Direct evidence showing that cranberry juice components have anti-adhesive activity have also been described (Howell, 2007; Shmuely *et al.*, 2007). The high concentration of fructose that is found in cranberry juice has an affect on adherence, in that it has been found to inhibit, *in vitro*, type 1 fimbriae-mediated *E. coli* adhesion (Zafriri *et al.*, 1989). However, fructose would not be expected nor as evidence been shown, to indicate that it has anti-adherence activity *in vivo* (Howell, 2007). Rather, at least two other components of cranberries are now thought to be responsible for the anti-adherence activity. In particular, several studies have shown that proanthocyanidins (a flavonoid, also referred to as a condensed tannin) and other high-molecular weight compounds inhibit adherence of UPEC (Howell *et al.*, 1998, 2001; Shmuely *et al.*, 2004). Despite these reports, however, the precise mechanism for the observed inhibition in adherence is not clear. Howell (2007) has suggested that the cranberry components act as receptor analogs and inhibit adherence of *E. coli* fimbriae to host cell surfaces. In addition, alteration in cell surface properties or electric potential, cell morphology, or fimbrial length may also contribute to reduced adherence (reviewed in Howell, 2007).

In addition to reducing urinary and stomach infections, cranberry juice has also been shown to have anti-adhesive activity against oral bacteria, such as *S. mutans* (Guo *et al.*, 1998; Weiss *et al.*, 2002). In the latter study, the ability of saliva-coated *S. mutans* to adhere to saliva- or glucan-coated hydroxyapatite in the presence of 25% cranberry juice was greatly reduced by 40–85% as compared to the control, indicating that cranberry

juice effectively blocked bacterial adherence to binding sites in salivary pellicle and in glucans (Koo *et al.*, 2006).

Pectic-type and other water soluble oligosaccharides have also been suggested to have anti-adherence activity (Guggenbichler *et al.*, 1997; Kastner *et al.*, 2002; Lee *et al.*, 2006). In one recent report (Lee *et al.*, 2006), a high-molecular weight (80,000 Da) extract from green tea reduced adherence of *H. pylori* by up to 40% to a human gastric epithelial cell line. In addition, similar levels of inhibition of *Propionibacterium acnes* (a skin pathogen) and *S. aureus* to a fibroblast epithelial cell line were also observed. In another study, an aqueous extract from carrots blocked EPEC to HEp-2 cells and to human mucosal cells (Kastner *et al.*, 2002). The active material was found to be an acidic oligosaccharide containing trigalacturonic acid.

Finally, another group of oligosaccharides that have attracted attention for their potential anti-adhesive activity are the mannooligosaccharides (MOS). The MOS can be extracted from natural sources, produced synthetically, or can also be derived inexpensively from food-grade yeast cell walls, which are rich in mannan. These MOS products are sometimes included in feed rations for beef cattle, swine, and poultry, although their use in humans has not yet been considered (Castillo *et al.*, 2008; Franklin *et al.*, 2005; Hooge, 2004). Importantly, mannan contains α-linked mannose residues that are known to inhibit the adhesion of many enterobacterial species including *Salmonella, Klebsiella,* and *E. coli* (Bouckaert *et al.*, 2006; Sharon, 2006). In the latter study, UPEC, and other *E. coli* pathotypes, were found to vary as much as 100-fold in their affinity to various oligomannosides, suggesting that receptor analog activity depends on structure and bond type, and that some analogs may be better than others as anti-adhesive agents.

3. Commercial prebiotics as anti-adhesives

More recently, commercial prebiotic oligosaccharides have been reported to also have adherence–inhibition activity. Ordinarily, prebiotics are thought to exert beneficial effects to the host primarily by selectively influencing the growth of desirable lactobacilli and bifidobacteria in the colon (Gibson and Roberfroid, 1995). However, the similarity of some of these prebiotics to those found in nature, particularly those found in human breast milk (discussed above in Section VIII.E.2.), would suggest that they may also function as anti-adherence agents. Although commercially available galactooligosaccharides (GOS) clearly have a different composition from that of the natural GOS present in human milk, they do share a general structural similarity. Therefore, commercial GOS

would be expected to have many of the same attributes that naturally occurring, milk-derived GOS possess (Boehm and Stahl, 2007). Indeed, commercial GOS, like human milk GOS, is bifidogenic, both *in vitro* and when fed to infants.

Recently, the Hutkins' lab evaluated several such prebiotics for anti-adherence activity (Fig. 2.1), and showed that GOS, obtained as a food-grade material, significantly inhibited adherence of EPEC to HEp-2 and Caco-2 cells (Shoaf *et al.*, 2006). In addition, GOS also reduced the number of adhered microcolonies by 50% and the microcolony size (number of cells per microcolony) by 70%. This suggests that GOS may specifically be targeted to an adherence factor that is also responsible for microcolony formation. Specifically, BFP have been shown to mediate both microcolony formation (Giron *et al.*, 1991) and LA. Thus, the ability of GOS to interfere with BFP formation is especially important, given the role of BFP as an initial adherence factor in EPEC pathogenesis.

The application of commercial oligosaccharides as anti-adhesive agents is not restricted to the GIT, but may also extend to the urogenital tract, where mannosides and yeast mannan have been shown to have anti-adherence activity (Aronson *et al.*, 1979; Ofek *et al.*, 1977). These and other findings provide convincing evidence that commercial, food-grade oligosaccharides may serve as anti-infective agents against pathogenic microorganisms. This approach offers tremendous advantages as these agents are food-grade, safe, inexpensive, and importantly, could reduce reliance on antibiotics. Over-administration of antibiotics, both clinically and in animal agriculture, has led to bans in the European Union and a search for alternative treatments to reduce bacterial infections (Mountzouris *et al.*, 2006).

IX. CONCLUSIONS AND FUTURE PROSPECTS

Research aimed at understanding bacterial pathogenesis has established the importance of bacterial adherence in disease. This research has led to the identification of a number of both bacterial adhesins and potential host cell receptors. By understanding the detailed interactions between a bacterial adhesin and host receptor, it is possible to develop new mechanisms to prevent bacterial adhesion, thereby averting disease. Many promising anti-adhesion mechanisms have been developed and studied, but much more work is needed, both *in vitro* and *in vivo*, to establish the feasibility of these mechanisms.

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