Annu. Rev. Microbiol. 2001. 55:407–35
Copyright © 2001 by Annual Reviews. All rights reserved

INTERACTION OF BACTERIAL PATHOGENS WITH POLARIZED EPITHELIUM

B. I. Kazmierczak,[1] K. Mostov,[2] and J. N. Engel[3]

[1]*Department of Medicine, University of California, San Francisco, California 94143-0654; e-mail: barbkaz@itsa.ucsf.edu*

[2]*Departments of Anatomy and Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143-0502; e-mail: mostov@itsa.ucsf.edu*

[3]*Department of Medicine and Department of Microbiology and Immunology, University of California, San Francisco, California 94143-0654; e-mail: jengel@medicine.ucsf.edu*

Key Words bacterial pathogenesis, cell polarity, invasion, virulence, apical, basolateral

■ **Abstract** Many pathogens must surmount an epithelial cell barrier in order to establish an infection. While much has been learned about the interaction of bacterial pathogens with cultured epithelial cells, the influence of cell polarity on these events has only recently been appreciated. This review outlines bacterial-host epithelial cell interactions in the context of the distinct apical and basolateral surfaces of the polarized epithelium that lines the lumens of our organs.

CONTENTS

INTRODUCTION 408
POLARIZED EPITHELIUM 409
PEYER'S PATCHES AND M CELLS 411
IN VITRO TISSUE CULTURE MODELS 412
  MDCK Cells 412
  Other Cell Lines 412
SPECIFIC PATHOGEN-EPITHELIAL CELL INTERACTIONS 413
  The Pneumococcus: A Pathogen that Specifically Coopts an Apical Receptor for Transcytosis 413
  Enteropathogenic *Escherichia coli*: An Apical Pathogen that Supplies Both the Ligand and the Receptor 414
  Helicobacter pylori 415
  Salmonella: An Apical Pathogen that May Be Receptor Independent 416
  Mucosal Pathogens of the Genital Tract: *Neisseria gonorrhoeae* and *Chlamydia trachomatis* 417
  The Gonococcus 417
  Chlamydia spp. 418
  Shigella 419

0066-4227/01/1001-0407$14.00

Listeria 420
Yersinia Species 421
Pseudomonas aeruginosa: An Opportunistic Pathogen that Exploits Loss of Epithelial Cell Polarity for Invasion 423
Porphyromonas gingivalis: A Pathogen that Dissolves Tight Junctions to Reach BL Tissues by a Paracellular Route 424
SUMMARY AND FUTURE DIRECTIONS 425

INTRODUCTION

Most, if not all, microbial pathogens must surmount an epithelial cell barrier in order to successfully colonize humans and cause disease. Much has been learned about the host-pathogen interactions in cultured epithelial cells, but less is known about how pathogens interact with the polarized epithelial cells that line the mucosal surfaces of organs. These cells have developed distinct apical and basolateral (BL) surfaces that form selective permeability barriers between biological compartments. The apical and BL membrane domains are distinguished by unique assemblies of proteins, lipids, and other macromolecules and structures, such as the secreted mucous layer (104). M cells, which are specialized antigen-presenting cells, are intermixed with polarized epithelium at some surfaces, such as the gut and lung. For some pathogens, especially opportunistic pathogens, the distinct composition of the apical cell surface, combined with the physical barriers formed by both the secreted mucous layer and by intercellular junctions (including tight junctions and the zona adherens), prevents these microorganisms from establishing infection or from penetrating the epithelial cell barrier. Thus, infections develop only in patients with altered epithelial cell barriers, including direct trauma, indwelling catheters, or patients receiving cytotoxic chemotherapy. Other pathogens exploit the characteristics of the apical surface to establish infections at this site. In some cases, apical adhesion is followed by internalization and transcytosis of organisms to the BL surface. Several pathogens are able to induce transmigration of polymorphonuclear lymphocytes (PMNs) from the BL surface through the intercellular junctions, allowing the apically located microbes to travel the same intercellular route to reach the BL surface and tissues beneath. Others secrete proteases that disrupt intercellular junctions, allowing access to the BL surface and deeper tissues.

Although the use of in vitro cell culture systems utilizing transformed epithelial cells has taught us much about the interaction of microbial pathogens with host eukaryotic cells, only recently have we begun to study these interactions in more physiologic systems, such as monolayers of well-polarized epithelial cells. While these in vitro models are still far removed from the whole animal, they provide important insights into the interaction of pathogens with polarized epithelium. Studies of the interactions of viruses with polarized epithelial cells have received much attention. This review focuses on recent developments in the study of bacterial pathogens and their interactions with polarized epithelial surfaces. This review

primarily covers events that require direct host cell–bacterial contact rather than on secreted toxins or interactions with the innate immune system.

POLARIZED EPITHELIUM

Among the major portals of entry of pathogens into the human body are the skin, gastrointestinal, respiratory, and urogenital tracts. While the skin has a more complex structure, these lumenal surfaces are for the most part lined with a single layer of epithelial cells that are highly polarized, and their plasma membrane is divided into two domains (Figure 1). The apical surface faces the lumen of the cavity, while the BL surface faces adjoining cells and the underlying basement

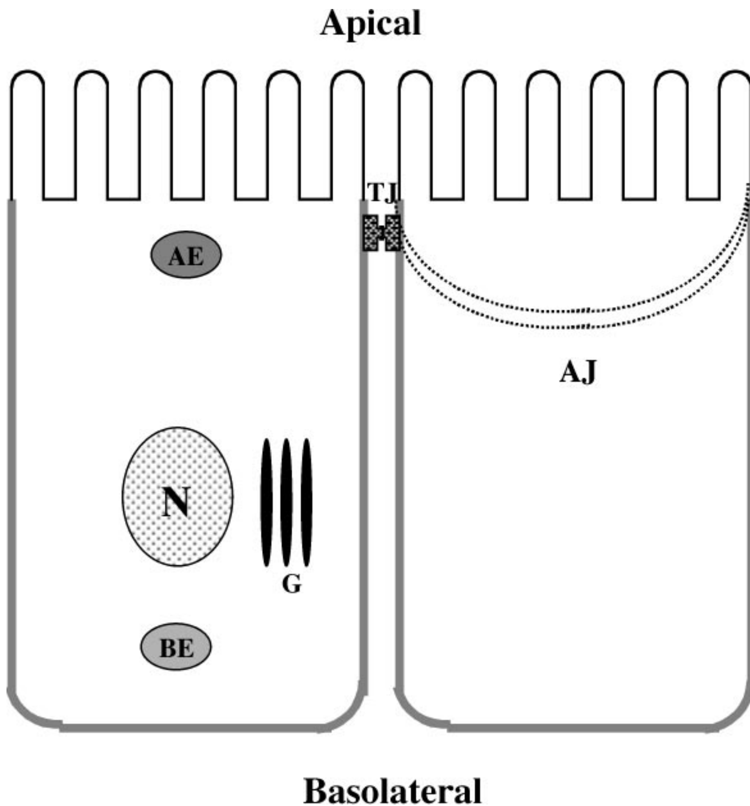


Figure 1 General features of polarized epithelial cells. Subcellular organelles are shown on the left cell, and specific features of the polarized cell are indicated on the cell on the right. AP, apical surface (thin line); BL, basolateral surface (thick line); TJ, tight junction; AJ, adherens junction; N, nucleus; G, Golgi; BE, basolateral endosome; AE, apical endosome.

membrane (104, 171). These two plasma membrane domains serve very different functions and therefore have almost completely different compositions. The apical surface contains transporters and enzymes that are specialized to interact with the external environment. The outer leaflet of the apical surface is highly enriched in glycosphingolipids and cholesterol, which may assemble into rafts (167). Proteins that are exposed on the outer surface of the apical plasma membrane are anchored to the membrane by a glycosylphosphatidyl-inositol (GPI) lipid anchor rather than a transmembrane segment.

The BL plasma membrane of the epithelial cell contains many transporters and receptors that are involved in taking up nutrients and hormones from the circulation (e.g., receptors for transferrin and low-density lipoproteins). In this regard, the BL plasma membrane seems to resemble the relatively nonspecialized plasma membrane of other cells, such as fibroblasts. The BL surface can be divided into lateral and basal domains, contacting other cells and the basement membrane, respectively. The lateral surface is highly specialized in that it contains several types of cell-cell junctions that are vital for epithelial functioning.

The tight junction (TJ) is located at the apical-most region of the lateral surface and defines the boundary between the apical and BL surfaces. The TJ serves two functions (100). First, it acts as a gate to prevent paracellular diffusion between the cells. This function enables the epithelial monolayer to be relatively impermeable to solutes (and larger particles). Second, the TJ acts as a fence to prevent diffusion of plasma membrane components between the apical and BL domains. The TJ contains major integral membrane proteins such as occludin and members of the claudin family. These proteins span the plasma membrane four times and have two large loops protruding from the outside of the cell. Homophillic interactions between these proteins on adjacent cells are thought to be the principal basis for the gate function of the TJ. A number of proteins, such as ZO-1, ZO-2, ZO-3, and cingulin, associate with the cytoplasmic surface of the TJ.

Adjacent to the TJ is the adherens junction, which also forms a circumferential belt around the cell. The adherens junction consists mainly of E-cadherin and other cadherins, which are integral membrane proteins whose large extracellular domains interact in a homophillic manner with cadherins on adjacent cells. The cytoplasmic domains of cadherins interact with members of the catenin family, which in turn interact with the actin cytoskeleton.

Proteins and lipids can reach the two plasma membrane domains by two major routes (101). Newly made proteins and lipids travel through the Golgi to the *trans*-Golgi network (TGN). Here they are packaged into vesicles that deliver them to either the apical or BL plasma membrane. Though this can be their final destination, they can be endocytosed from the BL surface and delivered to lysosomes. While most material endocytosed from the BL surface simply recycles to that surface, selected proteins and lipids are carried across the cell to the apical surface by transcytosis. Transcytosis can also occur from the apical to the BL surface, and although this process is exploited by certain pathogens such as the *Pneumococcus* (see below), it is less well understood.

The cytoskeleton of epithelial cells is also highly polarized. The apical surface generally contains microvilli, which can be tightly packed, as in the case of intestinal cells. Each microvillus has a central core of actin and other proteins. Underlying the microvilli is a dense network of actin and other cytoskeletal proteins, referred to as the terminal web. There is also a dense band of cortical actin underlying and anchored to the TJ and adherens junction. In contrast, cortical actin underlying the BL surface is relatively scarce, though some stress fibers are seen at the basal surface, at least in cultured cells.

Of particular relevance to interaction with bacteria is that endocytosis at the apical and BL surfaces seems to differ in several ways. The best-understood mechanism of endocytosis is through clathrin-coated pits. The concentration of clathrin-coated pits is equal at the two surfaces, but pits at the apical surface appear arrested at the shallow pit stage and are apparently impeded in progressing to deeply invaginated pits and then budding off into vesicles (107). The net result is that on a per-surface-area basis, clathrin-mediated endocytosis is approximately five- to tenfold less than at the BL surface. Unlike clathrin-mediated endocytosis at the BL surface, clathrin-mediated apical endocytosis is sensitive to the actin depolymerizing agent, cytochalasin D. This suggests that the specialized actin cytoskeleton underlying the apical surface is uniquely involved in apical endocytosis. Apical clathrin-mediated endocytosis is regulated by the small GTPase, ADP ribosylating factor 6 (ARF6), which is found only at the apical surface (2). ARF6 can act on enzymes that modify the lipid composition of the plasma membrane, as well as more directly on the actin cytoskeleton, and so there may be multiple mechanisms by which ARF6 controls apical clathrin-mediated endocytosis.

Another type of endocytosis is via caveolae, which are found predominantly on the basal membrane of epithelial cells (79). Most bacteria probably do not enter epithelial cells through either clathrin-mediated endocytosis or caveolae, with the exception of uropathogenic *Escherichia coli*, which enter through caveolar structures. Rather they enter through less-well-understood mechanisms that resemble macropinocytosis or phagocytosis. There appears to be multiple types of phagocytosis, at least in professional phagocytic cells (32). This may also be true for epithelial cells, though it has not been well studied. These processes usually require gross rearrangements of the actin cytoskeleton. Nonclathrin, noncaveolar endocytosis can be induced at the apical surface of Madin-Darby canine kidney (MDCK) cells by a number of signaling molecules, including protein kinase C, protein kinase A, heterotrimeric G proteins, and the cyclooxygenase pathway (81). These pathways may be exploited by bacterial pathogens.

PEYER'S PATCHES AND M CELLS

Although this review focuses primarily on the effect of epithelial cell polarity on bacterial–host cell interactions at mucosal surfaces, the role of M cells that overlay Peyer's patches (PP) cannot be ignored and are considered where appropriate.

PP are major sites of antigen sampling from the intestinal lumen and induce efficient immune responses of the gut-associated lymphoid tissue or tolerance. As reviewed in detail elsewhere (109, 146), M cells have a distinct architecture that promotes adherence and transport of antigens and microorganisms. Like other polarized cells, M cells have distinct apical and BL surfaces, but they have fewer microvilli and lack the glycocalyx, which coats the microvilli of enterocytes. An additional BL membrane subdomain accounts for the short height of M cells, allowing transcytosed material to be efficiently presented to underlying lymphocytes and thereby initiating immune response of the gut-associated lymphoid tissue.

IN VITRO TISSUE CULTURE MODELS

MDCK Cells

The most widely used cell line for studying polarized epithelial cells in culture is the MDCK line. Although of renal origin, in many ways this cell line has proven useful as a model system for all simple epithelial lines. Its resemblance to gastrointestinal, airway, and corneal epithelia has been close enough to serve for studies of interactions with pathogens such as *Pseudomonas* and *Salmonella* (4, 36). When grown on permeable filter supports, MDCK cells can obtain their nutrients via their BL surface, thus allowing the cells to become fully polarized, similar to the in vivo setting in which epithelial cells obtain nutrients from the underlying blood vessels (42). When grown on impermeable surfaces, such as tissue culture dishes, the cells cannot do this and are forced to grow in a partially nonpolarized state. An additional feature is that the degree of polarization of MDCK and other epithelial cells can be experimentally manipulated by several techniques. These include varying the length of time a confluent monolayer is grown in culture (6, 82, 83), growing the cells in calcium-depleted medium (6, 170), or inducing depolarization of the cells by treatment with hepatocyte growth factor (HGF) (39). Finally, there are variant MDCK cell clones that differ in their degree of polarization.

Other Cell Lines

Although derived from a colon adenocarcinoma, Caco-2 cells are similar to enterocytes from the small intestine (129). When grown under standard culture conditions, they undergo dramatic changes over time that mimic the maturation process of intestinal epithelial cells during crypt-to-villus migration (119). Subconfluent Caco-2 cells form circular islets that spread progressively. The peripheral cells are undifferentiated and undergo cell division, whereas central cells differentiate and become polarized. After 5–6 days in culture, the entire monolayer is polarized, although the process of differentiation, which includes formation of the apical brush border, requires about 10 more days to complete.

The T84 cell line is a polarized human intestinal epithelial cell line that displays phenotypic and biophysical properties of natural intestinal crypt epithelia (29, 70,

113). This system has been extensively used to study the bacterial induction of PMN transmigration. T84 cells are plated on the underside of collagen-coated porous filter supports, such as Transwells™ (Corning costar). After formation of a polarized monolayer, the filters are placed right-side up (so that the apical surface of the cells is in the basal chamber) and the top of the filter is the equivalent of the matrix underlying the BL surface. PMNs can be added to the top of the filter (i.e., the BL side), and their ability to transmigrate through the filter across paracellular junctions can be measured by the azurophil granule marker, myeloperoxidase (113). PMN transmigration from the basal to apical chamber is observed on addition of the PMN chemoattractant fMLP (92). During active transepithelial migration of PMNs, TJ integrity, as assessed by biophysical and permeability characteristics, is reversibly lost. Subsequent PMN transepithelial migration leads to foci in which epithelial cells are separated from their neighbors, resulting in focal availability of BL ligands such as β1-integrins. This system has been used to study the effects of various bacterial pathogens [including *Salmonella*, *Shigella*, *Helicobacter pylori*, enteropathogenic *E. coli* (EPEC), and *Yersinia*] on basal to apical PMN transmigration.

Until recently, it has been possible to study only M cells in vivo because they could not be cultured in vitro. However, culture of Caco-2 cells on filters in the presence of PP lymphocytes in the BL compartment resulted in the appearance of cells with characteristics of M cells (76). These included disorganization of the brush border, the loss of apical sucrase-isomaltase, and a gain in transcytotic activity for both inert particles and noninvasive bacteria. The interactions of *Yersinia* spp. have now been studied in this in vitro M cell model, which may prove useful to the study of other enteric pathogens that interact with M cells in vivo.

SPECIFIC PATHOGEN-EPITHELIAL CELL INTERACTIONS

Polarized cells provide many barriers that guard against microbial invasion. Many microbes have adapted to this hostile environment by targeting specific epithelial cell structures. These include colonizing or directly entering epithelial cells from the apical surface, transcytosing from the apical membrane to the BL region, and creating direct access to BL domains. The latter can be achieved by direct cytotoxic injury, intercellular migration, disruption of the epithelial TJs, or indirectly by inducing PMN transmigration. Examples of each of these strategies are presented below in the context of the interaction of specific bacterial pathogens with polarized epithelium.

The Pneumococcus: A Pathogen that Specifically Coopts an Apical Receptor for Transcytosis

Streptococcus pneumoniae (the Pneumococcus) is a leading cause of pneumonia, sepsis, and meningitis in all age groups. The usual portal of entry is through the nasopharyngeal epithelium. The molecular mechanisms of pneumococcal

adherence and invasion are poorly understood. In addition to binding to the platelet-activating factor receptor (PAF) present on the surface of lung epithelial and vascular endothelial cells (23), bacterial cell surface phosphorylcholine also anchors a family of proteins, the choline-binding proteins, to the bacterial surface. These included pneumococcal surface protein A (172) and choline-binding protein A (CbpA) (128). CbpA is found in all pneumococcal serotypes tested (174), and genetic disruption of *cbpA* leads to decreased adherence to human cells and loss of nasopharyngeal colonization in rats (128). Affinity chromatography using CbpA as a ligand has identified the polymeric immunoglobulin receptor (pIgR) as a receptor for CbpA. This is the first example of a pathogen coopting the polymeric immunoglobulin (pIg) transcytosis machinery to promote translocation across a mucosal epithelial barrier. pIgR is required for transcytosis of pIg, including IgA and IgM, across mucosal epithelial cells (102). After synthesis by plasma cells, located in the lamina propria of mucosal surfaces, polymeric IgA and IgM bind covalently to pIgR expressed on the BL surface of the epithelial membrane. The pIg-pIgR complex is subsequently transcytosed across the epithelial cell to be released into mucosal secretions. The extracellular, ligand-binding portion of pIgR, known as the secretory component (SC), is proteolytically cleaved off from the apical surface of the epithelial cells and released complexed with the pIg, protecting pIg from proteolytic degradation by bacterial IgA proteases (77, 78). Of note, bacterial transcytosis occurs in the opposite direction to pIgA transport. It is interesting that human pIgR expression is high in upper respiratory epithelial cells but only trace amounts are detectable in the lower respiratory tract. Thus, pneumococcal CbpA-pIgR interaction may contribute to early steps in the pathogenesis of pneumococcal disease, such as colonization. PAF receptors present in the lung and vascular epithelium may be important in subsequent steps in the development of pneumonia, bacteremia, and meningitis. The observation that cytokines increase pIgR expression may explain the propensity of humans to develop pneumococcal pneumonia following viral and other bacterial upper and lower respiratory infections (102).

Enteropathogenic *Escherichia coli*: An Apical Pathogen that Supplies Both the Ligand and the Receptor

A number of intestinal pathogens, including EPEC, enterohemorrhagic *E. coli*, *Citrobacter* spp., and *Hafnia alvei*, provoke the formation of a so-called attaching and effacing lesion (A/E) in the absence of extracellular toxin production or bacterial internalization. The best studied of these A/E-inducing pathogens is EPEC (50). This intestinal bacterium is a major cause of diarrheal disease in young children in underdeveloped countries. The first step in the formation of the A/E lesion is loose bacterial attachment to epithelial cells, mediated by the plasmid-encoded bundle-forming pilus (3, 58, 152). Intimin, a surface-exposed, 94-kDa outer-membrane protein related in structure to *Yersinia invasin*, is the bacterial ligand required for intimate attachment to the host cell (65). Its receptor, TIR, is bacterially encoded and secreted into the host cell membrane by the bacterial type III secretion system

(75). Notably, purified intimin can bind to eukaryotic cells via β1-integrins in the absence of EPEC, but the biological significance of this finding is unclear (40).

The disruption of polarized epithelium integrity may contribute to the pathogenesis of diarrhea. Pedestal formation and loss of microvilli could lead to a loss of absorptive surfaces in intestinal epithelial cells. As suggested by studies using T84 cells, EPEC activation of NF-κB and subsequent interleukin (IL)-8 secretion and PMN transmigration (138, 139) may cause fluid and electrolyte leakage. Through a phospholipase C–mediated pathway, myosin light chain kinase (MLCK) is activated, resulting in phosphorylation of MLC_{20} (88, 173). The end result may be contraction of the perijunctional actinomysin ring and increased paracellular permeability. EPEC can decrease transepithelial resistance (TER) and cause relocalization of occludin from the TJ to an intracellular compartment, an event that is accompanied by dephosphorylation of occludin and can be prevented by the serine/threonine phosphatase inhibitor, calyculin A (147). These changes required an intact type III secretion system. EPEC can affect epithelium permeability and ion transport (22), possibly by protein kinase C–mediated phosphorylation of the cystic fibrosis transmembrane regulator (CFTR) and alterations in chloride transport (7). Activation of NF-κB leads to upregulation of galanin-1 receptors and increased chloride secretion (57). Finally, altered bicarbonate transport may contribute to the diarrheal disease associated with EPEC (56). Additional studies are required to further dissect this complex interplay between a mucosal pathogen and intestinal epithelium.

Helicobacter pylori

Infection with *H. pylori* is associated with chronic gastritis, peptic ulcer disease, and gastric adenocarcinoma and lymphoma (87). The bacteria adhere specifically to the apical surface of gastric epithelium, and adhesion is influenced by the lewis b antigen (10). A 40-kb pathogenicity island, the cag pathogenicity island (PAI), is associated with ulcer- and gastritis-producing strains (14). It encodes a type IV secretion system, which exports CagA, an immunodominant antigen (5, 111, 150). On secretion, this bacterial protein is inserted into the host cell membrane and becomes tyrosine phosphorylated, a scenario somewhat similar to the bacterially directed translocation and insertion into the host cell plasma membrane of the Tir protein of EPEC. Subsequent host cell phosphorylation and dephosphorylation events are observed (5). The presence of the cag PAI correlates with changes in the host cell cytoskeleton, including effacement of the microvilli at the site of attachment, cytoskeletal rearrangement directly beneath the bacteria, and pedestal formation at the site of attachment (143). Actin, α-actinin, and talin are involved in this process. It is interesting that the CagA protein is often found at the site of active actin reorganization (142). The cag PAI is also associated with the ability to activate NF-κB and to stimulate IL-8 production (20, 21, 144). In T84 monolayers, *H. pylori* induced PMN transmigation in an IL-8–, cag PAI–, and CagE-dependent manner (60). It will be interesting to determine whether cell polarity can affect the ability of *H. pylori* to colonize gastric epithelium and cause cytoskeletal changes.

Salmonella: An Apical Pathogen that May Be Receptor Independent

Salmonella enteriditis (including *S. enteriditis v. typhimurium*) is one of the most common causes of food-borne gastrointestinal infections in humans. In vivo, as the bacterium approaches the epithelial surface, the microvilli in the immediate vicinity degenerate. Long, fibrous structures can be observed that link the organism with the apical surface. The apical cytoplasm close to the organism begins to bleb and swell, distorting outward, eventually internalizing the bacterium in a membrane-bound vesicle that may be surrounded by cytoplasmic extrusion. Epithelial cell invasion involves the SPI1 pathogenicity island that encodes a type III secretion system (reviewed in 34). Infection with *Salmonella typhimurium* triggers rearrangement of polymerized actin and other cytoskeleton-associated proteins, including α-actinin, tropomyosin, talin, and ezrin (37). Recently, two effectors secreted by the SPI-1–encoded type III system, SopE and SptP, have been implicated in the regulation of membrane ruffles and bacterial uptake (41, 176). Both modulate the activity of Rac and CDC42: SopE by acting as a GTPase exchange factor (GEF), and SptP as a GTPase activating protein (GAP). These findings suggest that *Salmonella* entry could be receptor independent, as the delivery of SopE via the type III secretion system might be sufficient to induce membrane ruffling and bacterial uptake. However, whether specific bacterial adhesins bind to apical receptors to allow engagement of the type III secretion system remains unknown.

In contrast to typhoid fever caused by *Salmonella typhi* in humans and *Salmonella typhimurium* in mice, *Salmonella* diarrheal infections are characterized by disruption of the normal movement of electrolytes and water across the gut wall (130) and a large influx of PMNs into the intestinal mucosa and lumen from the underlying vasculature (155, 160). Apical addition of *Salmonella* to T84 monolayers cultured with PMNs leads to transepithelial PMN migration; notably, under the conditions of the experiment, transcytosis or BL appearance of bacteria is not observed. IL-8 synthesis and BL secretion occur, which is thought to recruit PMNs through the lamina propria to the subepithelial space but not to induce PMN transmigration. Evidence suggests that a novel soluble factor, pathogen-elicited epithelial chemoattractant (PEEC), is necessary for the final step of PMN transmigration (93). The ability of *Salmonella* serotypes to elicit PMN transmigration in vitro correlates with their ability to cause diffuse enteritis (defined histologically as transepithelial migration of neutrophils) but not typhoid fever in humans (91). This in vitro PMN transmigration system has been used to identify specific bacterial factors necessary for the process, including a putative PEEC. Several putative type III secreted effectors of *Salmonella dublin* that affect fluid secretion and PMN influx have been identified including SopA (168), SopB, an inositol phosphate phosphatase (110), and SopD (46, 67, 161).

An interesting twist to the interaction of *Salmonella* with T84 cells is the finding that nonpathogenic *Salmonella* can prevent the induction of IL-8 secretion by pathogenic *Salmonella* (108). The nonpathogenic *Salmonella* block IκB-α

degradation, which prevents subsequent nuclear translocation of the active NF-κB dimer. Although IκB-α is phosphorylated, its subsequent polyubiquination is specifically inhibited. The implication of these findings is that commensal bacteria of the gut could themselves be responsible for the unique tolerance of the gastrointestinal mucosa to the proinflammatory stimuli presented by the enormous numbers of bacteria inhabiting the normal gut. The identification of the bacterial and/or host molecules responsible for the downregulation of the specific enzyme involved in ubiquination and the determination whether nonpathogenic *E. coli* can also prevent the induction of IL-8 will be exciting areas of future research.

Mucosal Pathogens of the Genital Tract: *Neisseria gonorrhoeae* and *Chlamydia trachomatis*

Sexually transmitted pathogens, such as *N. gonorrhoeae* and *C. trachomatis*, adhere to and enter the polarized epithelium lining the genital tract. No M cells or follicle-associated epithelium have been identified in genital tract epithelia. Almost all studies of gonococcus (GC) have been carried out in tissue culture or organ culture, as no animal models exist for this human-specific pathogen (and humans are not usually eager volunteers). In the case of *C. trachomatis*, both animal and tissue culture models are available but often lead to contradictory results. Moreover, the absence of genetic transfer methods for *Chlamydia* has been severely limiting. Nevertheless, understanding the complex interplay between these important human pathogens and their host epithelial cells is essential to developing new therapeutic approaches and vaccines.

The Gonococcus

Interactions of GC with the epithelial cell surface have been studied extensively using organ culture (human fallopian tubes) and immortalized human epithelial cells grown in vitro, although the role of epithelial cell polarity per se has not been explicitly tested. On contact with the apical surface, the bacteria form a loose association with the epithelial cell (27, 162), adhering as microcolonies at the tips of clusters of elongated microvilli and on the plasma membrane (54). Subsequently, the bacterial aggregates disappears and GC tightly adhere to the host cell surface (94). The phase variable Opa family members play roles in adhesion, invasion, and tissue tropism of the gonococcus; receptors for this gene family include cell surface heparan sulfate proteoglycans, glycosaminoglycans, vitronectin, and members of the CD66 family of transmembrane receptors (11, 16, 49, 52, 158, 159). CD66 molecules are found on the apical membranes of polarized T84 monolayers. Type IV pili are another critical adhesin (153), which, via binding of the tip protein PilC1 to CD46, promote initial attachment of the GC to epithelial cells (69). This pilus-mediated adhesion induces the release of intracellular calcium by epithelial cells and causes novel GC receptors to appear on the plasma membrane (68). Adhesion via the type IV pilus induces formation of cortical plaques beneath adherent bacteria, which are enriched in two Opa receptors (CD66 and HSPG) in

receptor tyrosine kinases (intercellular adhesion molecule 1 and epidermal growth factor receptor) and in cortical actin (97–99).

Both P+Opa- and P-Opa+ strains of GC transcytose across polarized epithelium and exit the basal surface (95) without damaging the monolayer (98). IgA protease and pili mediate the speed of transepithelial trafficking, the latter in a manner that is independent of its role in attachment (61, 98). The exact role of pili in virulence remains undetermined, however, because pilin-defective strains can colonize human volunteers (66) and behave identically to wild-type strains in human fallopian tube organ cultures (18). A genetic screen to identify mutants of GC exhibiting accelerated transcytosis in polarized T84 monolayers identified four mutants defining three genetic loci. These mutants adhere to and invade cells at wild-type levels and do not affect the monolayer TER, which suggests that the integrity of the cellular barrier is not compromised. Although these loci may affect trafficking per se, it is more likely that increased intracellular quantities of bacteria lead to the increased numbers that are transcytosed. The isolation of such mutants implies that GC negatively regulate their intracellular growth and suggests that the GC may respond to a stimulus encountered when cultured with epithelial cells (62).

Chlamydia spp.

Chlamydiae are obligate intracellular parasites that are important causes of sexually transmitted disease as well as trachoma (87). *Chlamydia psittaci* is primarily an animal pathogen, but accidental acquisition by humans leads to psittacosis, a rare cause of atypical pneumonia and endocarditis in humans. *Chlamydia pneumoniae* is a significant cause of upper and lower respiratory tract infections in humans and has been implicated in the pathogenesis of atherosclerosis.

Although much is known about the intracellular life of *Chlamydia* within host cells, the initial interactions at the cell surface remain controversial (reviewed in 55). No bacterial adhesin has been conclusively identified, although a novel trimolecular mechanism of attachment has been proposed in which a chlamydia-specific glycosaminoglycan bridges receptors on the infectious particle (the elementary body, or EB) and the host cell (15, 123, 151, 175). Likewise, no host cell receptor has been identified and the mechanism of internalization is unclear (reviewed in 55). This may be due in part to multiple modes of entry that are exhibited in both a strain-dependent and host cell–dependent manner (105, 124). Although chlamydial internalization via actin-dependent clathrin-mediated endocytosis has been reported (59, 121, 125, 169), others have failed to observe coated pits (13, 121, 163). Entry is not prevented in cells expressing a dominant negative form of dynamin, indicating that clathrin-independent uptake can occur (9). The effects of actin cytoskeleton inhibitors in infection have been variable, depending on the serovar, cell type, and conditions of infection (121, 140, 163). These inhibitors have little effect if added just after infection, which suggests that they inhibit entry and not subsequent steps in the intracellular life cycle (9). Actin rearrangements in the vicinity of the vacuole have been observed (85, 86, 157).

Few studies of chlamydial internalization have been carried out using polarized epithelial cells. When human endometrial gland epithelial cells were grown on collagen-coated filters, endocytosed EBs were more frequently observed in clathrin-coated vesicles than when the same cells were cultured on plastic surfaces. Similar results were observed with HeLa cells, again suggesting that there are multiple routes of entry that are influenced by culture conditions and perhaps by the polarization state of the host cell (169). The explicit effect of cell polarity on *C. trachomatis* infection in vitro is under investigation. Understanding the basis for such differences remains a key problem in the area of chlamydial cellular microbiology.

Shigella

Shigella is a gram-negative bacterium that causes bacillary dysentery, a bloody diarrhea that is endemic worldwide but particularly important in young children in developing nations. Animal studies suggest that Shigellae penetrate the intestinal epithelium primarily by M cells (164), but entry into epithelial cells in vitro has been most extensively studied. Products of the type III secretion system, encoded on the 220-kb virulence plasmid and including IpaA, -B, -C, and -D, allow the organism to penetrate M cells as well as nonpolarized or nonconfluent epithelial cells (134). This involves a complex set of carefully orchestrated changes in the actin cytoskeleton that result in the formation of ruffles and cellular extensions, which eventually merge and engulf the bacteria, a process lasting 5–10 min (for a comprehensive review, see 134). Underlying events include activation of Rho family GTPases (1, 166), activation of vinculin (156) and src kinase (28, 31), and recruitment of focal adhesion proteins including ezrin, paxillin, and $p125^{FAK}$ (165) in order to form a pseudo-focal adhesion complex (148). The bacterium is taken up into a vacuole, lyses the phagosome membrane using IpaB (IpaC may also be involved), escapes into the cytosol, and spreads from cell to cell by actin-based motility (8, 48). E-cadherin, a BL protein, is required for efficient intercellular spread (135). Besides being involved in entry and vacuole lysis, the IpaB gene product also activates caspase-1/interleukin-converting enzyme 1B and induces secretion of the proinflammatory cytokine IL1-β (177–179).

In confluent, polarized epithelial cell layers such as Caco-2 or T84 cell lines, very little adherence of *Shigella* to the apical surface is observed (136). Only direct contact with the BL surface, induced by disrupting the monolayer with a calcium chelator such as EDTA, allows efficient entry into polarized cells (106). Therefore, the issue of how *Shigella* accesses the BL surface in intact colonic mucosa remains to be solved. One possible mechanism is suggested by the finding that apically located *Shigella* stimulate transmigration of basal PMNs (114, 115). The subsequent disruption of the intercellular junctions may be sufficient to allow the bacteria to traverse the same route and access the BL surfaces, allowing more efficient epithelial cell invasion as well as stimulation of the host immune response. The mechanism of this apical signaling is unknown.

Although *Shigella* do not enter cells through classical receptor-mediated endocytosis pathways, it is presumed that the Ipa proteins must nonetheless interact with specific surface molecules in order to trigger changes in the actin cytoskeleton. The Ipa complex binds the fibronectin receptor, the $\alpha 5\beta 1$integrin (165), but integrins do not appear to be exclusive receptors for the IpaB-C complex. Indeed, evidence that another cell surface molecule, CD44, may bind IpaB has been recently published (136). Whether the putative receptors for the Ipa complexes are basolaterally segregated in polarized epithelium remains to be investigated.

M cell infection by *Shigella flexneri* is associated with membrane remodelling that is similar to that seen following invasion of cultured epithelial cells. In contrast to *Salmonella*, no cytotoxicity is observed with *Shigella*, and the bacteria are translocated to intraepithelial and subepithelial tissues. No invasion of adjacent enterocytes is observed (133).

Listeria

Listeria monocytogenes is a facultative intracellular bacterium that causes life-threatening infections, including bacteremia and meningitis. These infections occur primarily in the context of pregnancy, aging, and immunosuppression (87). The interaction of *L. monocytogenes* with cultured macrophages and epithelial cells has been extensively studied (for a review, see 19). Internalization occurs in an actin-dependent manner and is inhibited by cytochalasin D. Bacteria are taken up into a membrane-bound compartment that is lysed by the action of Listeriolysin *O*, allowing bacteria to escape into the cytoplasm. The organism recruits cellular actin to the polarly localized ActA surface protein and moves within the cytoplasm as well as from cell to cell by actin-based motility.

The *L. monocytogenes* invasin internalin (InlA) mediates entry into Caco-2 cells (19, 44) by a process requiring the extracytoplasmic domain of E-cadherin (96). The binding of the C terminus of E-cadherin to β-catenin and subsequent interactions with α-catenin and actin likely explains why bacterial uptake is sensitive to cytochalasin D. A hybrid consisting of the ectodomain of E-cadherin and the actin-binding domain of α-catenin was sufficient to support *Listeria* entry into fibroblasts. α- and β-catenins, as well as E-cadherin, clustered and colocalized at the entry site, where F-actin then accumulated. How *Listeria* accesses BL E-cadherin to gain entry across the intestinal epithelium is unknown. The induction of signaling events may lead to a transient depolarization of the intestinal epithelium, allowing InlA-E–cadherin interactions. E-cadherin is also expressed in the choroid plexus and may play an important role in the spread of *Listeria* across the blood-brain barrier.

The closely related protein InlB mediates *Listeria* invasion of a wide variety of cultured cell lines (30, 63, 80, 112). Two host cell receptors have been identified for this molecule. gC1q-R, the receptor for the globular head of the complement component C1q, binds InlB in a calcium-dependent manner (12). This ubiquitously expressed protein does not possess a transmembrane domain or a consensus site

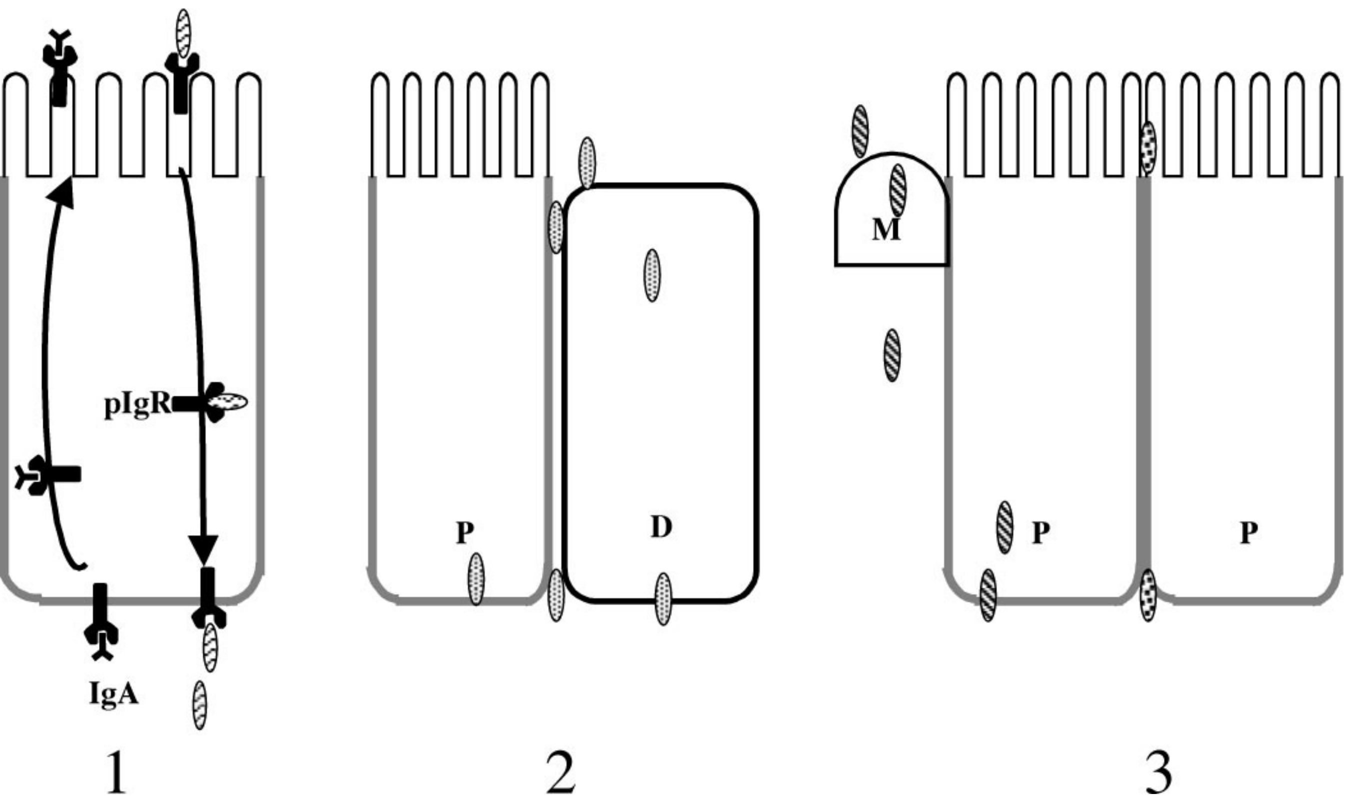
for glycosylphosphatylinositol membrane anchoring, raising the possibility that a coreceptor is required for bacterial internalization. The second receptor identified for InlB, the Met tyrosine kinase (Met), may play such a role (145). Met, which is a high-affinity receptor for HGF, binds InlB in a calcium-independent manner. It is tyrosine phosphorylated following treatment of cells with purified InlB and is required for downstream-signaling events that accompany *Listeria* internalization, such as tyrosine phosphorylation of Gab1 and Cbl and interaction of Gab1 with p85. InlB-mediated internalization of *Listeria* may play an important role in bacterial traversal of the blood-brain barrier and/or in fetal infection, as *L. monocytogenes* invasion of endothelial cells is InlB dependent but InlA independent (53, 112).

The entry of *L. monocytogenes* into Caco-2 cells as a function of cell polarization and differentiation has been studied (45). *Listeria* entered through the entire surface of nonpolarized cells but predominantly through the BL surface of polarized cells. However, electron micrographs show attachment and entry of *L. monocytogenes* into the apical surface of Caco-2 cells (71), though the frequency of this mode of internalization is unknown (45). Calcium chelators reduce the uptake of *Listeria* despite their ability to disrupt intercellular junctions and allow bacteria access to the BL surface (71). This may reflect the inability of either InlA or InlB to bind their cognate receptors in the absence of divalent cations. Of note, these studies did not determine whether the increased entry into nonpolarized cells reflects an increased density of receptors or of other components of the internalization pathway.

In vivo, the primary sites of entry of *L. monocytogenes* have not been clearly identified. In mice, the organism multiplies in ileal Peyers's patches (84, 90). In orally infected rats, dividing *Listeria* were observed in absorptive intestinal epithelial cells within 3 h after ingestion. At later times, the bacteria were found in phagocytic cells present in the lamina propria (122). Other studies failed to reveal invasion of *Listeria* into M cells (89). It is not clear whether enterocytes are infected from their apical pole and represent a site of translocation across the intestinal barrier or if they are infected from their BL surface after translocation of the bacteria through M cells or between cells. Alternatively, infection of enterocytes could result from direct cell-to-cell spread of bacteria from infected M cells or macrophages.

Yersinia Species

The interaction of *Yersinia* spp. with epithelial cells and macrophages, especially *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*, have been extensively studied and have provided paradigms for bacterial internalization (reviewed in 33, 35, 64). In vitro internalization assays identified three surface molecules involved in invasion: Inv, Ail, and YadA. The identification of β1-integrins as the receptor for Inv and YadA poses an interesting paradox of how this luminal pathogen binds to a receptor found on the BL surface. One explanation may be the identification of β1-integrins on the apical surface of M cells cultured in vitro (141). Alternatively, at least in polarized MDCK cells, *Yersinia* can attach to β1-integrins at TJs, disrupt


pIgR
IgA
1
P
D
2
M
P
P
3

the structures via delivery of YopE, a type III–secreted GTPase activating protein (GAP), and access BL surfaces by paracellular diffusion (154). Finally, neutrophil transmigration across intercellular junctions may allow access to BL ligands and thus provide a portal of entry for *Yersinia* (92). The relative contribution of each of these processes to in vivo *Yersinia* infections remains to be determined. Quite possibly, the redundancy of invasion and entry pathways contributes to this organism's virulence.

Pseudomonas aeruginosa: An Opportunistic Pathogen that Exploits Loss of Epithelial Cell Polarity for Invasion

P. aeruginosa provides an example of a bacterium whose virulence at mucosal surfaces is augmented by the disruption of a highly polarized epithelial monolayer. This opportunistic gram-negative pathogen is responsible for a large number of nosocomial infections in the setting of preexisting epithelial tissue damage, including superinfection of burns, corneal ulcer formation following contact lens–associated trauma, and tracheobronchitis and pneumonia in mechanically ventilated patients (131). Highly polarized cells are relatively resistant to infection by cytotoxic or invasive strains (38). Manipulations that disrupt polarity and/or monolayer integrity, including disruption of the monolayer by mechanical wounding (120), treatment of cells with HGF (39), disruption of TJs by exposure to calcium chelators (38, 116, 120), and growth under tissue culture conditions that do not promote the formation of polarized monolayers (120), enhance internalization by invasive strains and epithelial cell damage by cytotoxic strains. These results are taken to show that *P. aeruginosa* internalization or cytotoxicity preferentially occurs following bacterial interaction with the BL domain of polarized epithelial cells. However, the identity of the BL determinant responsible for *P. aeruginosa* internalization has remained elusive. The finding that bacterial internalization is decreased in cell lines lacking the cystic fibrosis transmembrane receptor (CFTR) and that internalization can be restored on transfection with CFTR has suggested that CFTR itself may be the receptor for invasion (117, 118). This hypothesis is

Figure 2 *Panel 1*: Transcytosis of Pneumococcus by pIgR. The pIgR first carries polymeric immunoglobulin from the basolateral (BL) to the apical surface. At the apical surface, the pIgR binds the Pneumococcus (wavy-lined bacteria) and transports it to the BL surface, where it is released. *Panel 2*: *Pseudomonas aeruginosa* (stippled bacteria) enters into depolarized cells at the edge of a wound (cell labeled 'D') or at BL surfaces between cells. *Panel 3*: Pathogens such as *Yersinia* and *Shigella* (striped bacteria) may access the BL surface by entry through apical receptors on M cells (labeled 'M'). *P. gingivalis* (dotted bacteria) secretes proteases that disrupt the TJ to allow intercellular access. The apical surface is depicted by thin lines, and the BL surface is depicted by a thick line.

attractive in that it explains the unique susceptibility of CF patients to chronic *P. aeruginosa* infections. However, the apical location of this receptor, the fact that cells lacking CFTR can still internalize *P. aeruginosa*, and the finding that overexpression of the CFTR in MDCK cells, which produce no or very little CFTR, does not increase *P. aeruginosa* internalization suggest that other receptors may also play important roles for *P. aeruginosa* invasion (47). In recent studies, CD95 (the fas receptor) mediated apoptosis of lung epithelial cells in some clinical isolates of *P. aeruginosa* (51).

Further dissection of these events has been made possible by the use of confluent MDCK monolayers assayed before complete polarization is achieved. *P. aeruginosa* enters nonpolarized confluent monolayers through a toxin-B–sensitive pathway that involves activation of RhoA (73; B.I. Kazmierczak, K. Mostov, J.N. Engel, manuscript submitted). In polarized MDCK cells, internalization of *P. aeruginosa* is downregulated 10 to 100-fold, which correlates with a loss of the RhoA-dependent pathway (74). Whether the downregulation occurs at the level of the receptor or at subsequent steps prior to the activation of Rho remains to be determined. RhoA activation and *P. aeruginosa* internalization are not dependent on type III–secreted bacterial effectors, but they do require type IV pili (17; B.I. Kazmierczak, K. Mostov, J.N. Engel, manuscript submitted). These results suggest a mechanism for the increased susceptibility of wounded epithelia to *P. aeruginosa* internalization. Previously, this phenomenon has been ascribed to increased levels of a putative pilin receptor, asialoGM$_1$ (24–26) or to the upregulation of fibronectin and the integrin $\alpha_5\beta_1$ during wound repair (126). However, RhoA activation also occurs following epithelial injury (137); this activation may promote *P. aeruginosa* internalization.

Porphyromonas gingivalis: A Pathogen that Dissolves Tight Junctions to Reach BL Tissues by a Paracellular Route

P. gingivalis is a gram-negative bacterium that is one of the primary causes of adult periodontal disease. A key to its pathogenic potential is its ability to reach subepithelial connective tissue structures. *P. gingivalis* adheres to and invades oral epithelial cells in vitro in a process that may involve fimbriae (132, 149). Using MDCK cells as a model for polarized epithelial cell layers, BL-applied *P. gingivalis* can degrade occludin, E-cadherin, and β1-integrin found in TJs, adherens junctions, and cell-cell matrix junctions that serve to limit paracellular diffusion in intact epithelial cell layers (72). These effects were observed even when the cells were grown on 0.4-μm filters, which suggests that a diffusible molecule was responsible for the degradation. Similar effects were observed with apically applied bacteria, although the time course was slower. *P. gingivalis* culture supernatants were observed to degrade immunoprecipitated E-cadherin and occludin molecules. *P. gingivalis* was not directly cytotoxic to MDCK cells and could cross from the apical to the BL compartment, but not vice versa. Together, these results suggest that *P. gingivalis* can reach subepithelial tissues by specifically degrading the

cell-cell junction complexes. The identity and specificity of the proteases and why this effect occurs preferentially from the BL surface will be interesting to investigate.

SUMMARY AND FUTURE DIRECTIONS

We are now beginning to appreciate that most pathogens must surmount an epithelial cell barrier in order to cause disease. Some bacteria exploit unique features of the apical or BL surface to facilitate colonization, infection, or penetration of the epithelial barrier (Figure 2). For example, gonococci require one or more receptors found primarily on the apical surface for invasion, apoptosis, or transcytosis. In the case of Pneumococcus, the host cell receptor, pIgR, is found both basolaterally and apically, but first encounters *S. pneumoniae* at the apical surface after the release of polymeric immunoglobulin. Other pathogens must reach the BL surface to establish an infection, either by entering and crossing M cells or by directly disrupting the epithelial cell monolayer. Epithelial disruption may result indirectly when pathogens stimulate PMN transmigration (*Shigella*, *Yersinia*, *Listeria*, and possibly *H. pylori*), or directly following pathogen-mediated degradation of TJ components (*P. gingivalis*). Conversely, pathogens that induce their own uptake or supply their own surface receptor (for example, *S. typhimurium* or EPEC) may be relatively insensitive to cell polarity. Still other pathogens (such as *P. aeruginosa*) appear to interact preferentially with epithelial cells that are no longer polarized, as may occur following epithelial cell injury. It will be interesting to determine whether some microorganisms are able to reverse or alter cell polarity to allow colonization and damage.

In addition to requiring unique apical or BL receptors, pathogens may also exploit the distinct properties of the apical or BL actin cytoskeleton to facilitate adherence and/or internalization. Specialized membrane subdomains, such as lipid rafts, may also be required for interaction of certain pathogens with epithelial cells, as suggested by the recent reports that caveolae mediate uptake of some pathogens into macrophages, including mycobacteria, uropathogenic *E. coli*, and *Campylobacter jejeuni* (127). The in vitro study of pathogen–host cell interactions has revealed a wealth of information regarding mechanisms of disease and host cell biology. However, we anticipate that examining how pathogens interact with the polarized epithelial tissues they encounter in vivo will further expand our knowledge of pathogenesis, provide vital insights into host cell biology, and illuminate new targets for disease prevention and treatment.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health (AI42806, HL55980, AI24436, AI44148, AI01636), the American Lung Association, and the Howard Hughes Medical Institute Physician Postdoctoral Fellowship.

Visit the Annual Reviews home page at www.AnnualReviews.org

LITERATURE CITED

1. Adam T, Giry M, Boquet P, Sansonetti P. 1996. Rho-dependent membrane folding causes *Shigella* entry into epithelial cells. *EMBO J.* 15:3315–21
2. Altschuler Y, Liu S-H, Katz L, Tang K, Hardy S, et al. 1999. ADP-ribosylation factor 6 and endocytosis at the apical surface of Madin-Darby canine kidney cells. *J. Cell Biol.* 147:7–12
3. Anantha RP, Stone KD, Donnenberg MS. 1998. Role of BfpF, a member of the PilT family of putative nucleotide-binding proteins, in type IV pilus biogenesis and in interactions between enteropathogenic *Escherichia coli* and host cells. *Infect.Immun.* 66:122–31
4. Apodaca G, Bomsel M, Lindstedt R, Engel J, Frank D, et al. 1995. Characterization of *Pseudomonas aeruginosa*-induced MDCK cell injury: glycosylation defective host cells are resistant to bacterial killing. *Infect. Immun.* 63:1541–51
5. Backert S, Ziska E, Brinkmann V, Zimny-Arnt U, Fauconnier A, et al. 2000. Translocation of the *Helicobacter pylori* CagA protein in gastric epithelial cells by a type IV secretion apparatus. *Cell Microbiol.* 2:155–64
6. Balcarova-Stander J, Pfeiffer SE, Fuller SD, Simons K. 1984. Development of cell surface polarity in the epithelial Madin-Darby canine kidney (MDCK) cell line. *EMBO J.* 3:2687–94
7. Baldwin T, Brooks S, Knutton S, Manjarrez Hernandez H, Aitken A, Williams P. 1990. Protein phosphorylation by protein kinase C in HEp-2 cells infected with enteropathogenic *Escherichia coli*. *Infect. Immun.* 58:761–65
8. Bernardini ML, Mounier J, d'Hauteville H, Coquis-Rondon M, Sansonetti PJ. 1989. Identification of icsA, a plasmid locus of *Shigella flexneri* that governs bacterial intra- and intercellular spread through interaction with F-actin. *Proc. Natl. Acad. Sci. USA* 86:3867–71
9. Boleti H, Benmerah A, Ojcius DM, Cerf-Bensussan N, Dautry-Varsat A. 1999. Chlamydia infection of epithelial cells expressing dynamin and Eps15 mutants: clathrin-independent entry into cells and dynamin-dependent productive growth. *J. Cell Sci.* 112:1487–96
10. Borén T, Falk P, Roth K, Larson G, Normark S. 1993. Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. *Science* 262:1892–95
11. Bos MP, Grunert F, Belland RJ. 1997. Differential recognition of members of the carcinoembryonic antigen family by Opa variants of *Neisseria gonorrhoeae*. *Infect. Immun.* 65:2353–61
12. Braun L, Ghebrehiwet B, Cossart P. 2000. gC1q-R/p32, a C1q-binding protein, is a receptor for the InlB invasion protein of *Listeria monocytogenes*. *EMBO J.* 19:1458–66
13. Byrne GI, Moulder JW. 1978. Parasite-specified phagocytosis of *Chlamydia psittaci* and *Chlamydia trachomatis* by L and HeLa cells. *Infect. Immun.* 19:598–606
14. Censini S, Lange C, Xiang Z, Crabtree JE, Ghiara P, et al. 1996. cag, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. *Proc. Natl. Acad. Sci. USA* 93:14648–53
15. Chen JC-R, Stephens RS. 1994. Trachoma and LGV biovars of *Chlamydia trachomatis* share the same glycosaminoglycan-dependent mechanism for infection of eukaryotic cells. *Mol. Microbiol.* 11:501–7
16. Chen T, Belland RJ, Wilson J, Swanson J.

1995. Adherence of pilus- Opa+ gonococci to epithelial cells in vitro involves heparan sulfate. *J. Exp. Med.* 182:511–17
17. Comolli J, Waite L, Mostov K, Engel JN. 1999. The interaction of *Pseudomonas aeruginosa* pili and asialo-GM1 stimulates epithelial cell cytotoxicity and bacterial internalization. *Infect. Immun.* 67:3207–14
18. Cooper M, McGee Z, Mulks M, Kooney J, Hindman T. 1984. Attachment to and invasion of human fallopian tube mucosa by an IgA1 protease deficient mutant of *Neisseria gonorrhoeae* and its wild type parent. *J. Infect. Dis.* 150:737–44
19. Cossart P, Lecuit M. 1998. Interactions of *Listeria monocytogenes* with mammalian cells during entry and actin-based movement: bacterial factors, cellular ligands and signaling. *EMBO J.* 17:3797–806
20. Crabtree JE, Covacci A, Farmery SM, Xiang Z, Tompkins DS, et al. 1995. *Helicobacter pylori* induced interleukin-8 expression in gastric epithelial cells is associated with CagA positive phenotype. *J. Clin. Pathol.* 48:41–45
21. Crabtree JE, Xiang Z, Lindley IJ, Tompkins DS, Rappuoli R, Covacci A. 1995. Induction of interleukin-8 secretion from gastric epithelial cells by a cagA negative isogenic mutant of *Helicobacter pylori*. *J. Clin. Pathol.* 48:967–69
22. Crane JK, Oh JS. 1997. Activation of host cell protein kinase C by enteropathogenic *Escherichia coli*. *Infect. Immun.* 65:3277–85
23. Cundell DR, Gerard NP, Gerard C, Idanpaan-Heikkila I, Tuomanen EI. 1995. *Streptococcus pneumoniae* anchor to activated human cells by the receptor for platelet-activating factor. *Nature* 377:435–38
24. de Bentzmann S, Plotkowski C, Puchelle E. 1996. Receptors in the *Pseudomonas aeruginosa* adherence to injured and repairing airway epithelium. *Am. J. Respir. Crit. Care Med.* 154:S155–62
25. de Bentzmann S, Roger P, Dupuit F, Bajolet-Laudinat O, Fuchey C, et al. 1996. Asialo GM1 is a receptor for *Pseudomonas aeruginosa* adherence to regenerating respiratory epithelial cells. *Infect. Immun.* 64:1582–88
26. de Bentzmann S, Roger P, Puchelle E. 1996. *Pseudomonas aeruginosa* adherence to remodelling respiratory epithelium. *Eur. Respir. J.* 10:2145–50
27. Dehio C, Gray-Owen SD, Meyer TF. 1998. The role of neisserial Opa proteins in interactions with host cells. *Trends Microbiol.* 6:489–95
28. Dehio C, Prévost MC, Sansonetti PJ. 1995. Invasion of epithelial cells by *Shigella flexneri* induces tyrosine phosphorylation of cortactin by a pp60c-src-mediated signalling pathway. *EMBO J.* 14:2471–82
29. Dharmsathaphorn K, Madara J. 1990. Established intestinal cell lines as model systems for electrolyte transport sudies. *Methods Enzymol.* 192:354–89
30. Dramsi S, Biswas I, Maguin E, Braun L, Mastroeni P, Cossart P. 1995. Entry of *Listeria monocytogenes* into hepatocytes requires expression of InlB, a surface protein of the internalin multigene family. *Mol. Microbiol.* 16:251–61
31. Dumenil G, Sansonetti P, Van Nhieu GT. 2000. Src tyrosine kinase activity down-regulates Rho-dependent responses during *Shigella* entry into epithelial cells and stress fibre formation. *J. Cell Sci.* 113:71–80
32. Edgar BA. 1999. From small flies come big discoveries about size control. *Nat. Cell Biol.* 1:E191–93
33. Falkow S, Isberg RR, Portnoy DA. 1992. The interaction of bacteria with mammalian cells. *Annu. Rev. Cell Biol.* 8:333–63
34. Finlay BB. 1994. Molecular and cellular mechanisms of *Salmonella* pathogenesis. *Curr. Top. Microbiol. Immunol.* 192:163–85
35. Finlay BB, Falkow S. 1997. Common

themes in microbial pathogenicity revisited. *Microbiol. Mol. Biol. Rev.* 61:136–69

36. Finlay BB, Gumbiner B, Falkow S. 1988. Penetration of *Salmonella* through a polarized Madin-Darby canine kidney epithelial cell monolayer. *J. Cell Biol.* 107:221–30
37. Finlay BB, Ruschkowski S, Dedhar S. 1991. Cytoskeletal rearrangements accompanying *Salmonella* entry into epithelial cells. *J. Cell Sci.* 99:283–96
38. Fleiszig SM, Evans DJ, Do N, Vallas V, Shin S, Mostov K. 1997. Epithelial cell polarity affects susceptibility to *Pseudomonas aeruginosa* invasion and cytotoxicity. *Infect. Immun.* 65:2861–67
39. Fleiszig SM, Vallas V, Jun C, Mok L, Balkovetz D, et al. 1998. Susceptibility of epithelial cells to *Pseudomonas aeruginosa* invasion and cytotoxicity is upregulated by hepatocyte growth factor. *Infect. Immun.* 66:3443–46
40. Frankel G, Lider O, Hershkoviz R, Mould AP, Kachalsky SG, et al. 1996. The cell-binding domain of intimin from enteropathogenic *Escherichia coli* binds to beta1 integrins. *J. Biol. Chem.* 271:20359–64
41. Fu Y, Galan JE. 1999. A *Salmonella* protein antagonizes Rac-1 and Cdc42 to mediate host-cell recovery after bacterial invasion. *Nature* 401:293–97
42. Fuller SD, Simons K. 1986. Transferrin receptor polarity and recycling accuracy in "tight" and "leaky" strains of Madin-Darby canine kidney cells. *J. Cell Biol.* 103:1767–79
43. Deleted in proof
44. Gaillard J-L, Berche P, Frehel C, Gouin E, Cossart P. 1991. Entry of *L. monocytogenes* into cells is mediated by internalin, a repeat protein reminiscent of surface antigens from gram-positive cocci. *Cell* 65:1127–41
45. Gaillard J-L, Finlay BB. 1996. Effect of cell polarization and differentiation on entry of *Listeria monocytogenes* into the enterocyte-like Caco-2 cell line. *Infect. Immun.* 64:1299–308
46. Galyov EE, Wood MW, Rosqvist R, Mullan PB, Watson PR, et al. 1997. A secreted effector protein of *Salmonella dublin* is translocated into eukaryotic cells and mediates inflammation and fluid secretion in infected ileal mucosa. *Mol. Microbiol.* 25:903–12
47. Gerçeker AA, Zaidi T, Marks P, Golan DE, Pier GB. 2000. Impact of heterogeneity within cultured cells on bacterial invasion: analysis of *Pseudomonas aeruginosa* and *Salmonella enterica* serovar typhi entry into MDCK cells by using a green fluorescent protein-labelled cystic fibrosis transmembrane conductance regulator receptor. *Infect. Immun.* 68:861–70
48. Goldberg MB, Theriot JA. 1995. *Shigella flexneri* surface protein IcsA is sufficient to direct actin-based motility. *Proc. Natl. Acad. Sci. USA* 92:6572–76
49. Gómez-Duarte OG, Dehio M, Guzmán CA, Chhatwal GS, Dehio C, Meyer TF. 1997. Binding of vitronectin to opa-expressing *Neisseria gonorrhoeae* mediates invasion of HeLa cells. *Infect. Immun.* 65:3857–66
50. Goosney DL, de Grado M, Finlay BB. 1999. Putting *E. coli* on a pedestal: a unique system to study signal transduction and the actin cytoskeleton. *Trends Cell Biol.* 9:11–14
51. Grassmé H, Kirschnek S, Riethmueller J, Riehle A, von Kürthy G, et al. 2000. CD95/CD95 ligand interactions on epithelial cells in host defense to *Pseudomonas aeruginosa*. *Science* 290:527–30
52. Gray-Owen SD, Lorenzen DR, Haude A, Meyer TF, Dehio C. 1997. Differential Opa specificities for CD66 receptors influence tissue interactions and cellular response to *Neisseria gonorrhoeae*. *Mol. Microbiol.* 26:971–80
53. Greiffenberg L, Goebel W, Kim KS, Weiglein I, Bubert A, et al. 1998. Interaction of *Listeria monocytogenes* with

human brain microvascular endothelial cells: InlB-dependent invasion, long-term intracellular growth, and spread from macrophages to endothelial cells. *Infect. Immun.* 66:5260–67

54. Griffiss JM, Lammel CJ, Wang J, Dekker NP, Brooks GF. 1999. *Neisseria gonorrhoeae* coordinately uses Pili and Opa to activate HEC-1–B cell microvilli, which causes engulfment of the gonococci. *Infect. Immun.* 67:3469–80
55. Hackstadt T. 1999. Cell biology. In *Chlamydia: Intracellular Biology, Pathogenesis, and Immunity*, ed. RS Stephens, pp. 101–38. Washington, DC: ASM
56. Hecht G, Koutsouris A. 1999. Enteropathogenic *E. coli* attenuates secretagogue-induced net intestinal ion transport but not Cl-secretion. *Am. J. Physiol. Gastrointest. Liver Physiol.* 276:G781–G88
57. Hecht G, Marrero JA, Danilkovich A, Matkowskyj KA, Savkovic SD, et al. 1999. Pathogenic *Escherichia coli* increase Cl-secretion from intestinal epithelia by upregulating galanin-1 receptor expression. *J. Clin. Invest.* 104:253–62
58. Hicks S, Frankel G, Kaper JB, Dougan G, Phillips AD. 1998. Role of intimin and bundle-forming pili in enteropathogenic *Escherichia coli* adhesion to pediatric intestinal tissue in vitro. *Infect. Immun.* 66:1570–78
59. Hodinka RL, Davis CH, Choong J, Wyrick PB. 1988. Ultrastructural study of endocytosis of *Chlamydia trachomatis* by McCoy cells. *Infect. Immun.* 56:1456–63
60. Hofman V, Ricci V, Galmiche A, Brest P, Auberger P, et al. 2000. Effect of *Helicobacter pylori* on polymorphonuclear leukocyte migration across polarized T84 epithelial cell monolayers: role of vacuolating toxin VacA and cag pathogenicity island. *Infect. Immun.* 68:5225–33
61. Hopper S, Vasquez B, Merz A, Clary S, Wilbur JS, So M. 2000. Effects of the immunoglobulin A1 protease on *Neisseria gonorrhoeae* trafficking across polarized T84 epithelial monolayers. *Infect. Immun.* 68:906–11
62. Hopper S, Wilbur JS, Vasquez BL, Larson J, Clary S, et al. 2000. Isolation of *Neisseria gonorrhoeae* mutants that show enhanced trafficking across polarized T84 epithelial monolayers. *Infect. Immun.* 68:896–905
63. Ireton K, Payrastre B, Chap H, Ogawa W, Sakaue H, et al. 1996. A role for phosphoinositide 3-kinase in bacterial invasion. *Science* 274:780–82. Erratum. 1997. *Science* 275(5299):464
64. Isberg RR. 1996. Uptake of enteropathogenic *Yersinia* by mammalian cells. *Curr. Top. Microbiol. Immunol.* 209:1–24
65. Jerse A, Kaper J. 1991. The eae gene of enteropathogenic *Escherichia coli* encodes a 94–kilodalton membrane protein, the expression of which is influenced by the EAF plasmid. *Infect. Immun.* 59:4302–9
66. Johannsen C, Johnston D, Koymen H, Cohen M, Cannon J. 1999. A *Neisseria gonorrhoeae* immunoglobulin A1 protease mutant is infectious in the human challenge model of urethral infection. *Infect. Immun.* 67:3009–13
67. Jones MA, Wood MW, Mullan PB, Watson PR, Wallis TS, Galyov EE. 1998. Secreted effector proteins of *Salmonella dublin* act in concert to induce enteritis. *Infect. Immun.* 66:5799–804
68. Källström H, Islam MS, Berggren PO, Jonsson AB. 1998. Cell signaling by the type IV pili of pathogenic *Neisseria*. *J. Biol. Chem.* 273:21777–82
69. Källström H, Liszewski MK, Atkinson JP, Jonsson AB. 1997. Membrane cofactor protein (MCP or CD46) is a cellular pilus receptor for pathogenic *Neisseria*. *Mol. Microbiol.* 25:639–47
70. Kaoutzani P, Colgan S, Cepek K, Burkard P, Carlson S, et al. 1994. Reconstitution of cultured intestinal epithelial monolayers with a mucosal-derived

lymphocyte cell line. *J. Clin. Invest.* 94:788–96

71. Karunasagar I, Senghaas B, Krohne G, Goebel W. 1994. Ultrastructural study of *Listeria monocytogenes* entry into cultured human colonic epithelial cells. *Infect. Immun.* 62:3554–58
72. Katz J, Sambandam V, Wu JH, Michalek SM, Balkovetz DF. 2000. Characterization of *Porphyromonas gingivalis*-induced degradation of epithelial cell junctional complexes. *Infect. Immun.* 68: 1441–49
73. Kazmierczak BI, Jou T-S, Mostov K, Engel J. 2001. Rho-GTPase activity modulates *Pseudomonas aeruginosa* internalization by epithelial cells. *Cell Microbiol.* 3:85–98
74. Deleted in proof
75. Kenny B, DeVinney R, Stein M, Reinsheid J, Frey EA, Finlay BB. 1997. Enteropathogenic *E. coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells. *Cell* 91:511–20
76. Kernéis S, Bogdanova A, Kraehenbuhl JP, Pringault E. 1997. Conversion by Peyer's patch lymphocytes of human enterocytes into M cells that transport bacteria. *Science* 277:949–52
77. Kilian M, Mestecky J, Russel M. 1988. Defense mechanisms involving Fc-dependent functions of immunoglogulin A and their subversion by bacterial immunoglobulin A proteases. *Microbiol. Rev.* 52:296–303
78. Kobayashi K. 1971. Studies on human secretory IgA. Comparative studies of the IgA-bound secretory piece and the free secretory piece protein. *Immunohistochemistry* 8:785–800
79. Kurzchalia TV, Parton RG. 1999. Membrane microdomains and caveolae. *Curr. Opin. Cell Biol.* 11:424–31
80. Lingnau A, Domann E, Hudel M, Bock M, Nichterlein T, et al. 1995. Expression of the *Listeria monocytogenes* EGD inlA and inlB genes, whose products mediate bacterial entry into tissue culture cell lines, by PrfA-dependent and -independent mechanisms. *Infect. Immun.* 63:3896–903
81. Llorente A, van Deurs B, Garred O, Eker P, Sandvig K. 2000. Apical endocytosis of ricin in MDCK cells is regulated by the cyclooxygenase pathway. *J. Cell Sci.* 113:1213–21
82. Low S-H, Miura M, Roche PA, Valdez AC, Mostov KE, Weimbs T. 2000. Intracellular redirection of plasma membrane trafficking after loss of epithelial cell polarity. *Mol. Biol. Cell* 11:3045–60
83. Low S-H, Roche PA, Anderson HA, van Ijzendoorn SCD, Zhang M, et al. 1998. Targeting of SNAP-23 and SNAP-25 in polarized epithelial cells. *J. Biol. Chem.* 273:3422–30
84. MacDonald TT, Carter PB. 1980. Cell-mediated immunity to intestinal infection. *Infect. Immun.* 28:516–23
85. Majeed M, Gustafsson M, Kihlström E, Stendahl O. 1993. Roles of Ca^{2+} and F-actin in intracellular aggregation of *Chlamydia trachomatis* in eucaryotic cells. *Infect. Immun.* 61:1406–14
86. Majeed M, Kihlström E. 1991. Mobilization of F-actin and clathrin during redistribution of *Chlamydia trachomatis* to an intracellular site in eucaryotic cells. *Infect. Immun.* 59:4465–72
87. Mandell GL, Bennett JE, Dolin R. 2000. *Principles and Practice of Infectious Diseases*. New York: Churchill Livingstone. 3264 pp. 4th ed.
88. Manjarrez-Hernandez HA, Baldwin TJ, Williams PH, Haigh R, Knutton S, Aitken A. 1996. Phosphorylation of myosin light chain at distinct sites and its association with the cytoskeleton during enteropathogenic *Escherichia coli* infection. *Infect. Immun.* 64:2368–70
89. Marco AJ, Altimira J, Prats N, López S, Dominguez L, et al. 1997. Penetration of *Listeria monocytogenes* in mice infected by the oral route. *Microb. Pathog.* 23:255–63
90. Marco AJ, Prats N, Ramos JA, Briones V,

Blanco M, et al. 1992. A microbiological, histopathological, and immunohistochemical study of the intragastric inoculation of *L. monocytogenes* in mice. *J. Comp. Pathol.* 107:1–9
91. McCormick BA, Miller SI, Carnes D, Madara JL. 1995. Transepithelial signaling to neutrophils by *Salmonellae*: a novel virulence mechanism for gastroenteritis. *Infect. Immun.* 63:2302–9
92. McCormick BA, Nusrat A, Parkos CA, D'Andrea L, Hofman PM, et al. 1997. Unmasking of intestinal epithelial lateral membrane $\beta 1$ integrin consequent to transepithelial neutrophil migration in vitro facilitates inv-mediated invasion by *Yersinia pseudotuberculosis*. *Infect. Immun.* 65:1414–21
93. McCormick BA, Parkos CA, Colgan SP, Carnes DK, Madara JL. 1998. Apical secretion of a pathogen-elicited epithelial chemoattractant activity in response to surface colonization of intestinal epithelia by *Salmonella typhimurium*. *J. Immunol.* 160:455–66
94. McGee A, Johnson A, Taylor-Robinson D. 1981. Pathogenic mechanisms of *Neisseria gonorrhoeae*: observations on damage to human Fallopian tubes in organ culture by gonococci of colony type 1 or type 4. *J. Infect. Dis.* 143:413–22
95. McGee Z, Horn R. 1988. Phagocytosis of gonococci by nonprofessional phagocytic cells. *Rev. Infect. Dis.* 10:158–61
96. Mengaud J, Ohayon H, Gounon P, Mege RM, Cossart P. 1996. E-cadherin is the receptor for internalin, a surface protein required for entry of *L. monocytogenes* into epithelial cells. *Cell* 84:923–32
97. Merz AJ, Enns CA, So M. 1999. Type IV pili of pathogenic *Neisseriae* elicit cortical plaque formation in epithelial cells. *Mol. Microbiol.* 32:1316–32
98. Merz AJ, Rifenbery DB, Arvidson CG, So M. 1996. Traversal of a polarized epithelium by pathogenic *Neisseriae*: facilitation by type IV pili and maintenance of epithelial barrier function. *Mol. Med.* 2:745–54
99. Merz AJ, So M. 1997. Attachment of piliated, Opa- and Opc-gonococci and meningococci to epithelial cells elicits cortical actin rearrangements and clustering of tyrosine-phosphorylated proteins. *Infect. Immun.* 65:4341–49
100. Mitic LL, Van Itallie CM, Anderson JM. 2000. Molecular physiology and pathophysiology of tight junctions I. Tight junction structure and function: lessons from mutant animals and proteins. *Am. J. Physiol. Gastrointest. Liver Physiol.* 279:G250–54
101. Mostov K, Apodaca G, Aroeti B, Okamoto C. 1992. Plasma membrane protein sorting in polarized epithelial cells. *J. Cell Biol.* 116:577–83
102. Mostov K, Kaetze C. 1999. Immunoglobulin transport and the polymeric immunoglobulin receptor. In *Mucosal Immunology*, ed. P Orgra, J Mestecky, N Lamm, W Strober, J Bienenstock, J McGhee, pp. 181–211. San Diego: Academic
103. Deleted in proof
104. Mostov KE, Verges M, Altschuler Y. 2000. Membrane traffic in polarized epithelial cells. *Curr. Opin. Cell Biol.* 12:483–90
105. Moulder JW. 1991. Interaction of chlamydiae and host cells *in vitro*. *Microbiol. Rev.* 55:143–90
106. Mounier J, Vasselon T, Hellio R, Lesourd M, Sansonetti PJ. 1992. *Shigella flexneri* enters human colonic Caco-2 epithelial cells through the basolateral pole. *Infect. Immun.* 60:237–48
107. Naim HY, Dodds DT, Brewer CB, Roth MG. 1995. Apical and basolateral coated pits of MDCK cells differ in their rates of maturation into coated vesicles, but not in the ability to distinguish between mutant hemagglutinin proteins with different internalization signals. *J. Cell Biol.* 129:1241–50
108. Neish A, Gewirtz A, Zeng H, Young A,

Hobert M, et al. 2000. Prokaryotic regulation of epithelial responses by inhibition of IκB-α ubiquination. *Science* 289: 1560–63

109. Neutra M, Mantis N, Frey A, Giannasca P. 1999. The composition and function of M cell apical membranes: implications for microbial pathogenesis. *Semin. Immunol.* 11:171–81
110. Norris FA, Wilson MP, Wallis TS, Galyov EE, Majerus PW. 1998. SopB, a protein required for virulence of *Salmonella dublin*, is an inositol phosphate phosphatase. *Proc. Natl. Acad. Sci. USA* 95:14057–59
111. Odenbreit S, Püls J, Sedlmaier B, Gerland E, Fischer W, Haas R. 2000. Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion. *Science* 287:1497–500
112. Parida S, Domann E, Rohde M, Muller S, Darji A, et al. 1998. Internalin B is essential for adhesion and mediates the invasion of *Listeria monocytogenes* into human endothelial cells. *Mol. Microbiol.* 28:81–93
113. Parkos C, Delp C, Arnaout M, Madara J. 1991. Neutrophil migration across a cultured intestinal epithelium: dependence on a CD11b/CD18–mediated event and enhanced efficiency in the physiologic direction. *J. Clin. Invest.* 88:1605–12
114. Perdomo J, Cavaillon J, Huerre M, Ohayon H, Gounon P, Sansonetti P. 1994. Acute inflammation causes epithelial invasion and mucosal destruction in experimental shigellosis. *J. Exp. Med.* 180: 1307–19
115. Perdomo J, Gounon P, Sansonetti P. 1994. Polymorphonuclear leukocyte transmigration promotes invasion of colonic eptihelial monolayer by *Shigella flexneri*. *J. Clin. Invest.* 93:633–43
116. Pereira SHM, Cervante MP, deBentzmann S, Plotkowski MC. 1997. *Pseudomonas aeruginosa* entry into Caco-2 cells is enhanced in repairing wounded monolayers. *Microb. Pathog.* 23:249–55
117. Pier GB, Grout M, Zaidi TS. 1997. Cystic fibrosis transmembrane conductance regulator is an epithelial cell receptor for clearance of *Pseudomonas aeruginosa* from the lung. *Proc. Natl. Acad. Sci. USA* 94:12088–93
118. Pier GB, Grout M, Zaidi TS, Olsen JC, Johnson LG, et al. 1996. Role of mutant CFTR in hypersusceptibility of cystic fibrosis patients to lung infections. *Science* 271:64–67
119. Pinto M, Robine-Leon S, APpay M, Kedinger M, Triadou N, et al. 1983. Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture. *Biol. Cell* 47:323–30
120. Plotkowski MC, de Bentzmann S, Pereira SH, Zahm JM, Bajolet-Laudinat O, et al. 1999. *Pseudomonas aeruginosa* internalization by human epithelial respiratory cells depends on cell differentiation, polarity, and junctional complex integrity. *Am. J. Respir. Cell Mol. Biol.* 20:880–90
121. Prain CJ, Pearce JH. 1989. Ultrastructural studies on the intracellular fate of *Chlamydia psittaci* (strain guinea pig inclusion conjunctivitis) and *Chlamydia trachomatis* (strain lymphogranuloma venereum 434): modulation of intracellular events and relationship with endocytic mechanism. *J. Gen. Microbiol.* 135:2107–23
122. Racz P, Tenner K, Mero E. 1972. Experimental *Listeria* enteritis. I. An electron microscopic study of the epithelial phase in experimental *Listeria* infection. *Lab. Invest.* 26:694–700
123. Rasmussen-Lathrop S, Koshiyama K, Phillips N, Stephens R. 2000. Chlamydia-dependent biosynthesis of a heparan sulphate-like compound in eukaryotic cells. *Cell Microbiol.* 2:137–44
124. Raulston JE. 1995. Chlamydial envelope components and pathogen-host cell interactions. *Mol. Microbiol.* 15:607–16
125. Reynolds DJ, Pearce JH. 1990. Characterization of the cytochalasin D-resistant (pinocytic) mechanisms of endocytosis

utilized by chlamydaie. *Infect. Immun.* 58:3208–16
126. Roger P, Puchelle E, Bajolet-Laudinat O, Tournier JM, Debordeaux C, et al. 1999. Fibronectin and $\alpha 5\beta 1$ integrin mediate binding of *Pseudomonas aeruginosa* to repairing airway epithelium. *Eur. Respir. J.* 13:1301–9
127. Rosenburger C, Brummel J, Finlay B. 2000. Lipid rafts as pathogen portals. *Curr. Biol.* 10:R823–25
128. Rosenow C, Ryan P, Weiser JN, Johnson S, Fontan P, et al. 1997. Contribution of novel choline-binding proteins to adherence, colonization and immunogenicity of *Streptococcus pneumoniae*. *Mol. Microbiol.* 25:819–29
129. Rousset M. 1986. The human colon carcinoma cell lines HT-29 and Caco-2: two in vitro models for the study of intestinal differentiation. *Biochemie* 68:1035–40
130. Rout W, Formal S, Dammin G, Giannella R. 1974. Pathophysiology of *Salmonella* diarrhea in the Rhesus monkey: intestinal transport, morphological and bacteriological studies. *Gastroenterology* 67:59–70
131. Salyers AA, Whitt DD, eds. 1994. *Bacterial Pathogenesis: A Molecular Approach*. Washington, DC: ASM
132. Sandros J, Papapanou P, Namnark U, Dahlen G. 1994. *Porphyromonas gingivalis* invades pocket epithelium in vitro. *J. Perodontal. Res.* 29:62–69
133. Sansonetti PJ, Arondel J, Cantey JR, Prévost MC, Huerre M. 1996. Infection of rabbit Peyer's patches by *Shigella flexneri*: effect of adhesive or invasive bacterial phenotypes on follicle-associated epithelium. *Infect. Immun.* 64:2752–64
134. Sansonetti PJ, Arondel J, Huerre M, Harada A, Matsushima K. 1999. Interleukin-8 controls bacterial transepithelial translocation at the cost of epithelial destruction in experimental shigellosis. *Infect. Immun.* 67:1471–80
135. Sansonetti PJ, Mounier J, Prevost MC, Mege RM. 1994. Cadherin expression is required for the spread of *Shigella flexneri* between epithelial cells. *Cell* 76:829–39
136. Sansonetti PJ, Van Nhieu GT, Egile C. 1999. Rupture of the intestinal epithelial barrier and mucosal invasion by *Shigella flexneri*. *Clin. Infect. Dis.* 28:466–75
137. Santos MF, McCormack SA, Guo Z, Okolicany J, Zheng Y, et al. 1997. Rho proteins play a critical role in cell migration during the early phase of mucosal restitution. *J. Clin. Invest.* 100:216–25
138. Savkovic SD, Koutsouris A, Hecht G. 1997. Activation of NF-kappaB in intestinal epithelial cells by enteropathogenic *Escherichia coli*. *Am. J. Physiol. Cell Physiol.* 273:C1160–67
139. Savkovic SD, Koutsouris A, Hecht G. 1996. Attachment of a noninvasive enteric pathogen, enteropathogenic *Escherichia coli*, to cultured human intestinal epithelial monolayers induces transmigration of neutrophils. *Infect. Immun.* 64:4480–87
140. Schramm N, Wyrick PB. 1995. Cytoskeletal requirements in *Chlamydia trachomatis* infection of host cells. *Infect. Immun.* 63:324–32
141. Schulte R, Kerneis S, Klinke S, Bartels H, Preger S, et al. 2000. Translocation of *Yersinia enterocolitica* across reconstituted intestinal epithelial monolayers is triggered by *Yersinia* invasin binding to $\beta 1$–integrins apically expressed in M-like cells. *Cell Microbiol.* 2:173–85
142. Segal ED, Cha J, Lo J, Falkow S, Tompkins LS. 1999. Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by *Helicobacter pylori*. *Proc. Natl. Acad. Sci. USA* 96:14559–64
143. Segal ED, Falkow S, Tompkins LS. 1996. *Helicobacter pylori* attachment to gastric cells induces cytoskeletal rearrangements and tyrosine phosphorylation of host cell proteins. *Proc. Natl. Acad. Sci. USA* 93:1259–64
144. Segal ED, Lange C, Covacci A, Tompkins LS, Falkow S. 1997. Induction of

host signal transduction pathways by *Helicobacter pylori*. *Proc. Natl. Acad. Sci. USA* 94:7595–99

145. Shen Y, Naujokas M, Park M, Ireton K. 2000. InlB-dependent internalization of *Listeria* is mediated by the met receptor tyrosine kinase. *Cell* 103:501–10
146. Siebers A, Finlay BB. 1996. M cells and the pathogenesis of mucosal and systemic infections. *Trends Microbiol.* 4:22–29
147. Simonovic I, Rosenberg J, Koutsouris A, Hecht G. 2000. Enteropathogenic *Escherichia coli* dephosphorylates and dissociates occludin from intestinal epithelial tight junctions. *Cell Microbiol.* 2: 305–15
148. Skoudy A, Van Nhieu GT, Mantis N, Arpin M, Mounier J, et al. 1999. A functional role for ezrin during *Shigella flexneri* entry into epithelial cells. *J. Cell Sci.* 112:2059–68
149. Sojar HT, Han Y, Hamada N, Sharma A, Genco RJ. 1999. Role of the amino-terminal region of *Porphyromonas gingivalis* fimbriae in adherence to epithelial cells. *Infect. Immun.* 67:6173–76
150. Stein M, Rappuoli R, Covacci A. 2000. Tyrosine phosphorylation of the *Helicobacter pylori* CagA antigen after cag-driven host cell translocation. *Proc. Natl. Acad. Sci. USA* 97:1263–68
151. Stephens RS, Fawaz FS, Kennedy KA, Nichols B, Koshiyama K, et al. 2000. Eukaryotic cell uptake of heparin-coated microspheres: a model of host cell invasion by *Chlamydia*. *Infect. Immun.* 68:1080–85
152. Stone KD, Zhang HZ, Carlson LK, Donnenberg MS. 1996. A cluster of fourteen genes from enteropathogenic *Escherichia coli* is sufficient for the biogenesis of a type IV pilus. *Mol. Microbiol.* 20:325–37
153. Swanson J. 1973. Studies on the gonococcus infection. IV. Pili: their role in attachment of gonococci to tissue culture cells. *J. Exp. Med.* 137:571–89
154. Tafazoli F, Homstrom A, Forsberg A, Magnusson K-E. 2000. Apically exposed, tight junction-associated β1-integrins allow binding and YopE-mediated perturbation of epithelial barriers by wild-type *Yersinia* bacteria. *Infect. Immun.* 68:5335–43
155. Turnbull P, Richmond J. 1978. A model of *Salmonella* enteritis: the behaviour of *Salmonella enteriditidis* in the chick intestine studied by light and electron microscopy. *Br. J. Exp. Pathol.* 59:64–75
156. Van Nhieu GT, Ben-Ze'ev A, Sansonetti PJ. 1997. Modulation of bacterial entry into epithelial cells by association between vinculin and the *Shigella* IpaA invasin. *EMBO J.* 16:2717–29
157. van Ooij C, Apodaca J, Mostov K, Engel J. 1997. Characterization of the *Chlamydia trachomatis* vacuole and its interaction with the host endocytic pathway in HeLa cells. *Infect. Immun.* 65:758–66
158. van Putten JP, Hayes SF, Duensing TD. 1997. Natural proteoglycan receptor analogs determine the dynamics of Opa adhesin-mediated gonococcal infection of Chang epithelial cells. *Infect. Immun.* 65:5028–34
159. Virji M, Evans D, Hadfield A, Grunert F, Teixeira AM, Watt SM. 1999. Critical determinants of host receptor targeting by *Neisseria meningitidis* and *Neisseria gonorrhoeae*: identification of Opa adhesiotopes on the N-domain of CD66 molecules. *Mol. Microbiol.* 34:538–51
160. Wallis TS, Hawker RJH, Candy DCA, Qi GM, Clarke GJ, et al. 1989. Quantification of the leukocyte influx into rabbit ileal loops induced by strains of *Salmonella typhimurium* of different virulence. *J. Med. Microbiol.* 30:149–56
161. Wallis TS, Wood M, Watson P, Paulin S, Jones M, Galyov E. 1999. Sips, Sops, and SPIs but not stn influence *Salmonella* enteropathogenesis. *Adv. Exp. Med. Biol.* 473:275–80
162. Wang J, Gray-Owen SD, Knorre A, Meyer TF, Dehio C. 1998. Opa binding to cellular CD66 receptors mediates the

transcellular traversal of *Neisseria gonorrhoeae* across polarized T84 epithelial cell monolayers. *Mol. Microbiol.* 30:657–71

163. Ward ME, Murray A. 1984. Control mechanisms governing the infectivity of *Chlamydia trachomatis* for HeLa cells: mechanisms of endocytosis. *J. Gen. Microbiol.* 130:1765–80
164. Wassef JS, Keren DF, Mailloux JL. 1989. Role of M cells in initial bacterial uptake and ulcer formation in the rabbit intestinal loop model in Shigellosis. *Infect. Immun.* 57:858–63
165. Watarai M, Funato S, Sasakawa C. 1996. Interaction of Ipa proteins of *Shigella flexneri* with alpha5beta1 integrin promotes entry of the bacteria into mammalian cells. *J. Exp. Med.* 183:991–99
166. Watarai M, Kamata Y, Kozaki S, Sasakawa C. 1997. Rho, a small GTP-binding protein, is essential for Shigella invasion of epithelial cells. *J. Exp. Med.* 185:281–92
167. Weimbs T, Low S-H, Chapin SJ, Mostov KE. 1997. Apical targeting in polarized epithelial cells: there's more afloat than rafts. *Trends Cell Biol.* 7:393–99
168. Wood M, Jones M, Watson P, Siber A, McCormick B, et al. 2000. The secreted effector protein of *Salmonella dublin*, SopA, is translocated into eukaryotic cells and influences the induction of enteritis. *Cell Microbiol.* 2:293–303
169. Wyrick P. 1989. Entry of genital *Chlamydia trachomatis* into polarized human epithelial cells. *Infect. Immun.* 57:2378–89
170. Yeaman C, Grindstaff KK, Nelson WJ. 1999. New perspectives on mechanisms involved in generating epithelial cell polarity. *Physiol. Rev.* 79:73–98
171. Yeaman C, Le Gall AH, Baldwin AN, Monlauzeur L, Le Bivic A, Rodriguez-Boulan E. 1997. The O-glycosylated stalk domain is required for apical sorting of neurotrophin receptors in polarized MDCK cells. *J. Cell Biol.* 139:929–40
172. Yother J, White J. 1994. Novel surface attachment mechanism of the *Streptococcus pneumoniae* protein PspA. *J. Bacteriol.* 176:2976–85
173. Yuhan R, Koutsouris A, Savkovic SD, Hecht G. 1997. Enteropathogenic *Escherichia coli*-induced myosin light chain phosphorylation alters intestinal epithelial permeability. *Gastroenterology* 113:1873–82
174. Zhang J-R, Mostov K, Lamm M, Nanno M, Ohwaki M, Tuomanen E. 2000. The polymeric immunoglobulin receptor translocates Pneumococci across human nasopharyngeal epithelial cells. *Cell.* 102: 827–37
175. Zhang JP, Stephens RS. 1992. Mechanism of *Chlamydia trachomatis* attachment to eukaryotic host cells. *Cell* 69: 861–69
176. Zhou D, Mooseker MS, Galan JE. 1999. An invasion-associated *Salmonella* protein modulates the actin-bundling activity of plastin. *Proc. Natl. Acad. Sci. USA* 96:10176–81
177. Zychlinsky A, Fitting C, Cavaillon JM, Sansonetti PJ. 1994. Interleukin 1 is released by murine macrophages during apoptosis induced by *Shigella flexneri*. *J. Clin. Invest.* 94:1328–32
178. Zychlinsky A, Kenny B, Menard R, Prevost M-C, Holland IB, Sansonetti PJ. 1994. IpaB mediates macrophage apoptosis induced by *Shigella flexneri*. *Mol. Microbiol.* 11:619–27
179. Zychlinsky A, Prevost MC, Sansonetti PJ. 1991. *Shigella flexneri* induces apoptosis in infected macrophages. *Nature* 358:167–69