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Towards a physiology of epithelial pathogens

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It has long been known that pathogens such as *Vibrio cholerae*, *Bordetella pertussis*, *Clostridium difficile* and *Escherichia coli* secrete toxins that produce specific changes in cell signalling in epithelial and other cells [7, 38]. These toxins have tended to be viewed as anomalies, useful as examples to stir the interest of medical students, and helpful for studies on signalling systems, but without great significance for our overall understanding of the interactions of pathogens with epithelia or the development of the signs and symptoms of the induced disease. Recent advances in the understanding of intracellular signalling systems, the sequencing of an increasing number of bacterial and viral genomes, and the availability of genetically modified mice, however, have resulted in an explosive growth in the knowledge of the molecular mechanisms by which pathogens affect epithelial function. Studies on the responses of epithelia to a wide variety of pathogens, including influenza viruses, rotaviruses, *Pseudomonas aeruginosa* and *Bordetella pertussis*, among others, have revealed a plethora of unsuspected acute and long-term effects of pathogens on epithelial function. Not only have they provided important new information on the mechanisms by which pathogens lead to disease, but they have also offered unexpected insights and tantalizing glimpses into the mechanisms controlling normal epithelial function.

In this overview, we will focus on the acute effects that pathogens have on epithelia, i.e. the effects occurring within minutes of the epithelium being exposed to a pathogen or its toxin. In particular, we will focus on recent progress in the elucidation of the roles of toxins and of the attachment of pathogens to the cell surface, as triggers for the pathological effects of bacteria and viruses on epithelia. The examples chosen are only illustrative and are intended merely to give some feeling about current progress in the field.

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The effects of toxins

As mentioned above, the concept that bacteria produce diarrhoea by secreting enterotoxins is well established. For example, *Vibrio cholerae* [38], enterotoxic *Escherichia coli* [4, 38] and *Clostridium difficile* [35] produce their effects by secreting protein toxins into the extracellular medium: cholera toxin irreversibly activates the G_s protein [38], *E. coli* heat-stable enterotoxin activates guanylate cyclase [4], and clostridial toxins A and B irreversibly inactivate the small G proteins rhoA, rac and cdc42 [35]. The role of the toxins secreted by *Bordetella pertussis* [2] in producing whooping cough is also becoming increasingly well defined.

Enteropathogenic *E.coli* [45], *Salmonella* spp. [9, 11], *Shigella* spp. [36], and *Pseudomonas aeruginosa* [47], however, like many other Gram-negative bacteria, have the capacity to adhere to the surface of the epithelium and to inject effector proteins directly into the cytosol [47]. Although the molecular targets for these injected proteins have been studied extensively, the mechanisms by which the proteins lead to altered epithelial transport are obscure in most cases [45]. *Salmonella* spp., for example, inject a variety of enterotoxins (Fig. 1), among which SopE2 and SopB stimulate the small G protein cdc42 [10, 49], and SopE stimulates cdc42 as well as the closely related rac1 [10]. In polarized epithelial cells, rac1 acts synergistically with the bacterial proteins SipA and SipC to produce the cytoskeletal rearrangements [11], evident as membrane ruffling, which mediate bacterial entry into the cell across the apical membrane [5]. In contrast, active cdc42 is the major mediator of bacterial entry across the basolateral membrane, as it is across the plasma membranes of nonpolarized cells, with rac1 playing a lesser role [5, 11]. Following bacterial entry, a third

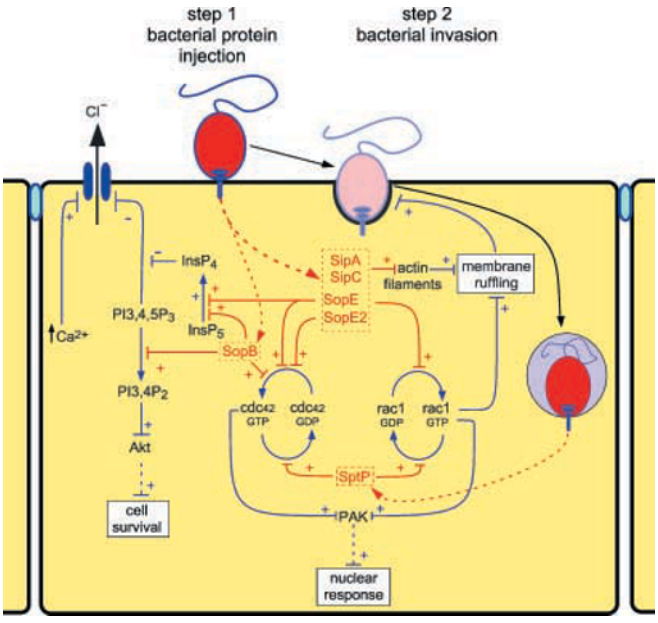

Fig. 1 Effects of the effector proteins injected by *Salmonella* species on signalling pathways in epithelial cells [9, 11]. The role of rac1 in regulating invasion of the bacterium across the apical membrane, in contrast to the more widely recognized role of both cdc42 and rac1 in regulating invasion across the basolateral membrane, is reported in Criss et al. [5]. The role of cdc42, rac and PAK kinases in triggering the responses of the nucleus to *Salmonella* infection is described in Galán and Zhou [11]. The roles of SopB, $PI3,4P_2$ and the Akt kinase in mediating the effects of *Salmonella* on cell survival are described in Marcus et al. [26] and in Steele-Mortimer et al. [39]. In step 1, a bacterium has engaged the apical membrane of the cell and is injecting proteins SipA, SipC, SopE, SopE2 and SopB (*red lettering* and *broken red arrows*) which then catalyse the transformations indicated. One outcome of the activation process is the initiation of membrane ruffling which, in turn, facilitates bacterial invasion. Once within the cell, the bacterium releases another protein (SptP) which acts on cdc42 and rac1 and reverses membrane ruffling. [$InsP_5$ Inositol 1,3,4,5,6 pentakisphosphate, $InsP_4$ inositol 1,4,5,6 tetrakisphosphate, $PI3,4,5P_3$ phosphatidylinositol 3,4,5 trisphosphate, $PI3,4P_2$ phosphatidylinositol 3,4 bisphosphate]

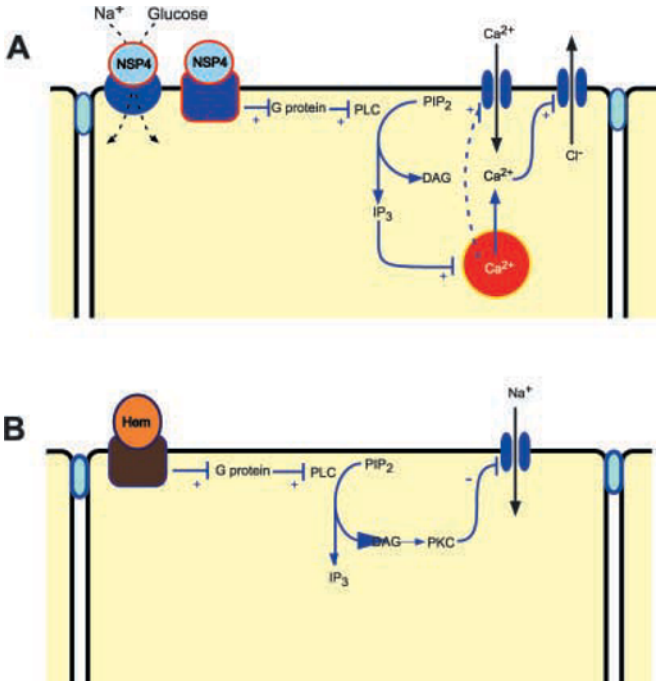

Fig. 2 **A** The mechanism by which the NSP4 enterotoxin of rotaviruses activates Cl^- channels [29] and inhibits SGLT1 [16] in intestinal epithelial cells. Although Ca^{2+} release from intracellular stores is shown as triggering Ca^{2+} influx across the apical membrane, in fact it is not yet known whether this influx takes place across the apical or the basolateral membrane, or both. **B** Mechanism by which influenza hemagglutinin inhibits epithelial Na^+ channels in respiratory and other epithelia [21]. [*DAG* Diacylglycerol, IP_3 inositol 1,4,5 trisphosphate, PIP_2 phosphatidylinositol 4,5-bisphosphate, *PKC* protein kinase C, *PLC* phospholipase Cb, *SGLT1* Na^+-glucose cotransporter 1]

enterotoxin, SptP, is injected which reverses both the activation of the G proteins and the cytoskeletal changes [11]. Tantalizingly, SopB also acts as an inositol phosphatase that increases the cytosolic concentration of the activator of Ca^{2+}-activated Cl^- channels, inositol 1,4,5,6-tetrakisphosphate (IP_4) [9], while decreasing the concentration of the inhibitor of these channels, phosphatidylinositol 3,4,5-trisphosphate (PI 3,4,5P_3) [26]. In keeping with the functional redundancy among the toxins injected by *Salmonella*, SopE also appears to be able indirectly to stimulate the breakdown of IP_4 [49]. These changes in phosphoinositide levels may act synergistically with the increase in intracellular Ca^{2+} that accompanies cellular invasion by *Salmonella* [11, 31] to activate Ca^{2+}-activated Cl^- channels. This attractive model for the stimulation of fluid and electrolyte secretion by *Salmonella*, however, has not been tested extensively and the precise links between the injected bacterial toxins and the derangements in epithelial transport they produce remain unclear. In particular, none of these toxins has yet been linked to the increase in expression of epithelial Galanin-1 receptors which, on the basis of studies in mutant mice, has been postulated as an essential precursor to the onset of diarrhoea [27].

Among the known targets of toxin action, rho, rac and cdc42 are notable for being targeted by many secreted [35] and injected toxins [9, 11, 36, 45]. These toxins are usually thought to be concerned principally with triggering cytoskeletal re-organization leading to alterations in paracellular permeability [11, 37], although recent reports that these small G proteins regulate transporters, including Na^+-H^+ exchangers (NHEs) [40, 43] and aquaporins [20], suggest that they may play an important role in producing the transport defects that accompany the early phase of epithelial infections.

Additional targets for bacterial toxins continue to be identified. The α-hemolysin of uropathic *E. coli*, for example, has recently been shown to induce Ca^{2+} oscillations in renal epithelial cells by a mechanism that appears to involve both L-type Ca^{2+} channels and IP_3-gated Ca^{2+} stores [44]. Similarly, recent studies have shown that *Pseudomonas aeruginosa* induces apoptosis in epithelial cells by stimulating Jun N-terminal kinases and up-regulating CD95 [19], which in turn inhibits Na^+-H^+ exchange in T-lymphocytes [24].

Bacteria are not the only pathogens that disturb epithelial function by means of toxins. Rotaviruses, an important cause of diarrhoeal disease in children, have been shown to produce an enterotoxin, NSP4. This viral glycoprotein activates phospholipase C in colonic crypt cells leading to the production of inositol 1,4,5-trisphosphate, increased intracellular Ca^{2+} [6, 30, 48] and Cl^- secretion from crypt cells (Fig. 2A; [30]): it also alters paracellular permeability [41] and noncompetitively inhibits the intestinal Na^+-glucose cotransporter, SGLT1 [16].

The effects of attachment

A striking finding of recent studies with both bacteria and viruses is that attachment of the pathogen to the epithelium, the first stage in the process of infection, is itself sufficient to trigger pathological changes in epithelial function. Thus, binding of *Pseudomonas aeruginosa* to airway epithelium has been reported to inhibit transepithelial Na^+ transport [8] and to trigger mucin overproduction via the Ras-MAPK-pp90rsk pathway [25]. Which receptor is responsible for these effects is unclear. At least in the case of increased mucin production [25], the Toll-like receptor 4 (TLR4) [42] and CD14 [1], which are believed to mediate epithelial responses to lipopolysaccharide from *Pseudomonas aeruginosa* and other Gram-negative bacteria, appear not to be involved. Conversely, binding of lipopolysaccharide to the cystic fibrosis transmembrane conductance regulator (CFTR) has been proposed as a critical step in the endocytosis by epithelia of Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Salmonella* spp. [33].

Viral attachment also influences epithelial transport. Thus, we have recently demonstrated that influenza virus induces a marked down-regulation of epithelial Na^+ channel activity in respiratory, gastrointestinal and renal epithelia [21]. This down-regulation is the result of the hemagglutinin in the viral coat binding to an apical membrane receptor which then activates phospholipase C and protein kinase C (Fig. 2B). This effect of influenza hemagglutinin can be reproduced by other hemagglutinins, including concanavalin A [21] and the B-oligomer of pertussis toxin (Kunzelmann and Cook, unpublished data) and, unlike the rotavirus NSP4 enterotoxin [16], does not affect SGLT1 activity [21]. Furthermore, epithelia have been reported to show increased activation of the Raf/MEK/ERK signalling cascade within 5 min of exposure to influenza virus [34]. It thus appears likely that other infectious agents with hemagglutinating activity, for example rhinoviruses and parainfluenza viruses, may similarly lead to decreased Na^+ transport and fluid accumulation in respiratory epithelia. Recent reports that respiratory syncytial virus activates protein kinase C and MAP kinase [28], that rhinoviruses activate p38 MAP kinase [15], and that the B-oligomer of pertussis toxin activates p42/p44 MAP kinase [12] further suggest that receptor binding by viruses and other pathogens may be an important trigger in epithelia for the changes in the rate of transport of electrolytes as well as the secretion of proteins such as cytokines and mucins which accompany infection. Interestingly, the surface receptors for respiratory syncytial virus appear to include both TLR4 and CD14 [23], the same two proteins that mediate responses to lipopolysaccharide.

Summary

The study of the mechanisms by which viral and bacterial toxins and attachment factors alter epithelial function is progressing rapidly. The recent molecular identification of key epithelial transporters such as rSK4 [46], the increasing sophistication of our understanding of the mechanisms regulating epithelial transporters [3, 17, 18, 22], and the ongoing development of novel techniques for measuring the properties of epithelia [13, 14] all suggest that this process will accelerate. The substantial commonality of these mechanisms among viruses and bacteria suggests that the new knowledge gained in this area will be of general significance to the understanding of epithelial infection, rather than of just limited applicability to a single organism or strain. Furthermore, studies on the signalling systems used by these pathogens to modify epithelial behaviour will shed new light on the control of normal epithelial function.

Given recent discussion about the future of physiology [32], it is reassuring that physiologists can look forward not only to playing a key role in linking genes to function, but also in making a major contribution to the understanding of how infectious diseases produce their characteristic pathologies.

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References

1. Becker MN, Diamond G, Verghese MW, Randell SH (2000) CD14-dependent lipopolysaccharide-induced beta-defensin-2 expression in human tracheobronchial epithelium. J Biol Chem 275:29731–29736
2. Belcher CE, Drenkow J, Kehoe B, Gingeras TR, McNamara N, Lemjabbar M, Basbaum C, Relman DA (2000) The transcriptional responses of respiratory epithelial cells to *Bordetella pertussis* reveal host defensive and pathogen counter-defensive strategies. Proc Natl Acad Sci USA 97:13847–13852
3. Bouritius H, Oprins JC, Bindels RJM, Hartog A, Groot JA (1998) Neuropeptide Y inhibits ion secretion in intestinal epithelium by reducing chloride and potassium conductance. Pflügers Arch 435:219–226
4. Charney AN, Egnor RW, Alexander-Chacko JT, Zaharia V, Mann EA, Giannella RA (2001) Effect of *E. coli* heat-stable enterotoxin on colonic transport in guanyl cyclase C receptor deficient mice. Am J Physiol 280:G216–G221
5. Criss AK, Ahlgren DM, Jou TS, McCormick BA, Casanova JE (2001) The GTPase rac1 selectively regulates *Salmonella* invasion at the apical plasma membrane of polarized epithelial cells. J Cell Sci 114:1331–1341

6. Dong Y, Zeng CQ, Ball JM, Estes MK, Morris AP (1997) The rotavirus enterotoxin NSP4 mobilizes intracellular calcium in human intestinal cells by stimulating phospholipase C-mediated 1,4,5-trisphosphate production. Proc Natl Acad Sci USA 94: 3960–3965
7. Epple HJ, Kreusel KM, Hanski C, Schilzke JD, Riecken EO, Fromm M (1997) Differential stimulation of intestinal mucin secretion by cholera toxin and carbachol. Pflügers Arch 433: 638–647
8. Evans DJ, Matsumoto PS, Widdicombe JH, Li-Yun C, Maminishkis AA, Miller SS (1998) *Pseudomonas aeruginosa* induces changes in fluid transport across airway surface epithelia. Am J Physiol 275:C1284–C1290
9. Feng Y, Wente SR, Majerus PW (2001) Overexpression of the inositol phosphatase SopB in human 293 cells stimulates cellular chloride influx and inhibits nuclear mRNA export. Proc Natl Acad Sci USA 98:875–879
10. Friebel A, Ilchmann H, Aepfelbacher M, Ehrbar K, Machleidt W, Hardt WD (2001) SopE and SopE2 from *S. typhimurum* activate different sets of rhoGTPases of the host cell. J Biol Chem 276: 34035–34040
11. Galán JE, Zhou D (2000) Striking a balance: modulation of the actin cytoskeleton by *Salmonella*. Proc Natl Acad Sci USA 97:8754–8761
12. Garcia JGN, Wang P, Liu F, Hershenson MB, Borbiev T, Verin AD (2001) Pertussis toxin directly activates endothelial cell p42/p44 MAP kinases via a novel signalling pathway. Am J Physiol 280:C1233–C1241
13. Gitter AH, Bertog M, Schulzke J-D, Fromm M (1997) Measurement of paracellular epithelial conductivity by conductance scanning. Pflügers Arch 434:830–840
14. Gitter AH, Bendfeldt K, Schulzke J-D, Fromm M (2000) Trans/paracellular, surface/crypt, and epithelial/subepithelial resistances of mammalian colonic epithelia. Pflügers Arch 439:477–482
15. Griego SD, Weston CB, Adams JL, Tal-Singer R, Dillon SB (2000) Role of p38 mitogen-activated protein kinase in rhinovirus-induced cytokine production by bronchial epithelial cells. J Immunol 165:5211–5220
16. Halaihel N, Lievin V, Ball JM, Estes MK, Alvarado F, Vasseur M (2000) Direct inhibitory effect of rotavirus NSP4(114–135) peptide on the Na^+-D-glucose symporter of rabbit intestinal brush border membrane. J Virol 74:9464–9470
17. Heitzmann D, Warth R, Bleich M, Henger A, Nitschke R, Greger R (2000) Regulation of the Na^+-$2Cl^-$-K^+ cotransporter in isolated rat colon crypts. Pflügers Arch 439:378–384
18. Inglis SK, Collett A, McAlroy HL, Wilson SM, Olver RE (1999) Effect of luminal nucleotides on Cl^- secretion and Na^+ absorption in distal bronchi. Pflügers Arch 438: 621–627
19. Jendrossek V, Grassme H, Mueller I, Lang F, Gulbins E (2001) *Pseudomonas aeruginosa*-induced apoptosis involves mitochondria and stress-activated protein kinases. Infect Immun 69:2675–2683
20. Klussmann E, Tamma G, Lorenz D, Wiesner B, Maric K, Hofmann F, Aktories K, Valentini G, Rosenthal W (2001) An inhibitory role of rho in the vasopressin-mediated translocation of aquaporin-2 into cell membranes of renal principal cells. J Biol Chem 276: 20451–20457
21. Kunzelmann K, Beesley AH, King NJ, Karupiah G, Young JA, Cook DI (2000) Influenza virus inhibits amiloride-sensitive Na^+ channels in respiratory epithelia. Proc Natl Acad Sci USA 97:10282–10287
22. Kunzelmann K, Schreiber R, Nitschke R, Mall M (2000) Control of epithelial Na^+ conductance by the cystic fibrosis transmembrane conductance regulator. Pflügers Arch 440: 193–201
23. Kurt-Jones EA, Popova L, Kwinn L, Haynes LM, Jones LP, Tripp RA, Walsh EE, Freeman MW, Golenbock DT, Anderson LJ, Finberg RW (2000) Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. Nat Immunol 1:398–401
24. Lang F, Madlung J, Bock J, Lükewille U, Kaltenbach S, Lang KS, Belka C, Wagner CA, Lang HJ, Gulbins E, Lepple-Wienhues A (2000) Inhibition of Jurkat-T-lymphocyte Na^+/H^+-exchanger by CD95 (fas/Apo-1)-receptor stimulation. Pflügers Arch 440:902–907
25. Li JD, Feng W, Gallup M, Kim JH, Gum J, Kim Y, Basbaum C (1998) Activation of NF-KappaB via Src-dependent Ras-MAPK-pp90rsk pathway required for *Pseudomonas aeruginosa* induced mucin overproduction in epithelial cells. Proc Natl Acad Sci USA 95:5718–5723
26. Marcus SL, Wenk MR, Steele-Mortimer O, Finlay BB (2001) A synaptojanin-homologous region of *Salmonella typhimurium* SigD is essential for inositol phosphatase activity and AKt activation. FEBS Lett 494:201–207
27. Matkowskyj KA, Danilkovich A, Marrero J, Savkovic SD, Hecht G, Benya RV (2000) Galanin-1 receptor up-regulation mediates the excess colonic fluid production caused by infection with enteric pathogens. Nature Med 6:1048–1051
28. Monick M, Staber J, Thomas K, Hunninghake G (2001) Respiratory syncytial virus infection results in activation of multiple protein kinase C isoforms leading to activation of mitogen-activated protein kinase. J Immunol 166:2681–2687
29. Morris AP, Estes MK (2001) Microbes and microbial toxins: paradigms for microbial-mucosal interactions. VIII. Pathological consequences of rotavirus infection and its enterotoxin. Am J Physiol 281:G303–G310
30. Morris AP, Scott JK, Ball JM, Zeng CQ, O'Neal WK, Estes MK (1999) NSP4 elicits age-dependent diarrhea and Ca^{2+}-mediated I^- influx into intestinal crypts of CF mice. Am J Physiol 277:G431–G444
31. Pace J, Hayman MJ, Galán JE (1993) Signal transduction and invasion of epithelial cells by *S. typhimurium*. Cell 72: 505–514
32. Petersen OH (2001) Physiologists, physiology departments and universities: discipline and interdisciplinarity. Pflügers Arch 441:725–727
33. Pier GB (2000) Role of cystic fibrosis transmembrane conductance regulator in innate immunity to *Pseudomonas aeruginosa* infections. Proc Natl Acad Sci USA 97:8822–8828
34. Pleschka S, Wolff T, Ehrhardt C, Hobom G, Planz O, Rapp UR, Ludwig S (2001) Influenza virus propagation is impaired by inhibition of the Raf/MEK/ERK signalling cascade. Nature Cell Biol 3:301–305
35. Pothoulakis C, Lamont JT (2001) Microbes and microbial toxins: paradigms for microbial-mucosal interactions II. The integrated response to *Clostridium difficile* toxins. Am J Physiol 280:G178–G183
36. Sansonetti PJ (2001) Microbes and microbial toxins: paradigms for microbial-mucosal interactions. III. Shigellosis: from symptoms to molecular pathogenesis. Am J Physiol 280: G319–G323
37. Sears CL (2000) Molecular physiology and pathophysiology of tight junctions. V. Assault of the tight junction by enteric pathogens. Am J Physiol 279:G1129–G1134
38. Sears CL, Kaper JB (1996) Enteric bacterial toxins: mechanisms of action and linkage to intestinal secretion. Microbiol Rev 60:167–215
39. Steele-Mortimer O, Knodler LA, Marcus SL, Scheid MP, Goh B, Pfeifer CG, Duronio V, Finlay BB (2000) Activation of Akt/protein kinase B in epithelial cells by *Salmonella typhimurum* effector sigD. J Biol Chem 275:37718–37724
40. Szaszi K, Kurashima K, Kapus A, Paulsen A, Kaibuchi K, Grinstein S, Orlowski J (2000) RhoA and rho kinase regulate the epithelial Na^+/H^+ exchanger NHE3. Role of myosin light chain phosphorylation. J Biol Chem 275:28599–28606
41. Tafazoli F, Zeng CQ, Estes MK, Magnusson KE, Svensson L (2001) NSP4 enterotoxin of rotavirus induces paracellular leakage in polarized epithelial cells. J Virol 75:1540–1546
42. Tapping RI, Akashi S, Miyake K, Godowski PJ, Tobias PS (2000) Toll-like receptor 4, but not toll-like receptor 2, is a signaling receptor for *Escherichia* and *Salmonella* lipopolysaccharides. J Immunol 165:5780–5787

43. Tominaga T, Ishizaki T, Narumiya S, Barber DL (1998) p160ROCK mediates RhoA activation of Na-H exchange. EMBO J 17:4712–4722
44. Uhlen P, Laestadius A, Jahnokainen T, Soderholm T, Backhed F, Celsi G, Brismar H, Normark S, Aperia A, Richter-Dahlfors A (2000) Alpha-haemolysin of uropathogenic *E. coli* induces Ca^{2+} oscillations in renal epithelial cells. Nature 405:694–697
45. Vallance BA, Finlay BB (2000) Exploitation of host cells by enteropathogenic *Escherichia coli*. Proc Natl Acad Sci USA 97:8799–8806
46. Warth R, Hamm K, Bleich M, Kunzelmann K, von Hahn T, Schreiber R, Ullrich E, Mengel M, Trautmann N, Kindle P, Schwab A, Greger R (1999) Molecular and functional characterization of the small Ca^{2+}-regulated K^+ channel (rSK4) of colonic crypts. Pflügers Arch 438:437–444
47. Winstanley C, Hart CA (2001) Type III secretion systems and pathogenicity islands. J Med Microbiol 50:116–126
48. Zhang M, Zeng CQ, Morris AP, Estes MK (2000) A functional NSP4 enterotoxin peptide secreted from rotavirus-infected cells. J Virol 74:11663–11670
49. Zhou D, Chen LM, Hernandez L, Shears SB, Galssn JE (2001) A Salmonella inositol polyphosphatase acts in conjunction with other bacterial effectors to promote host cell actin cytoskeleton rearrangements and bacterial internalization. Mol Microbiol 39:248–259