Acute diarrhoeal diseases

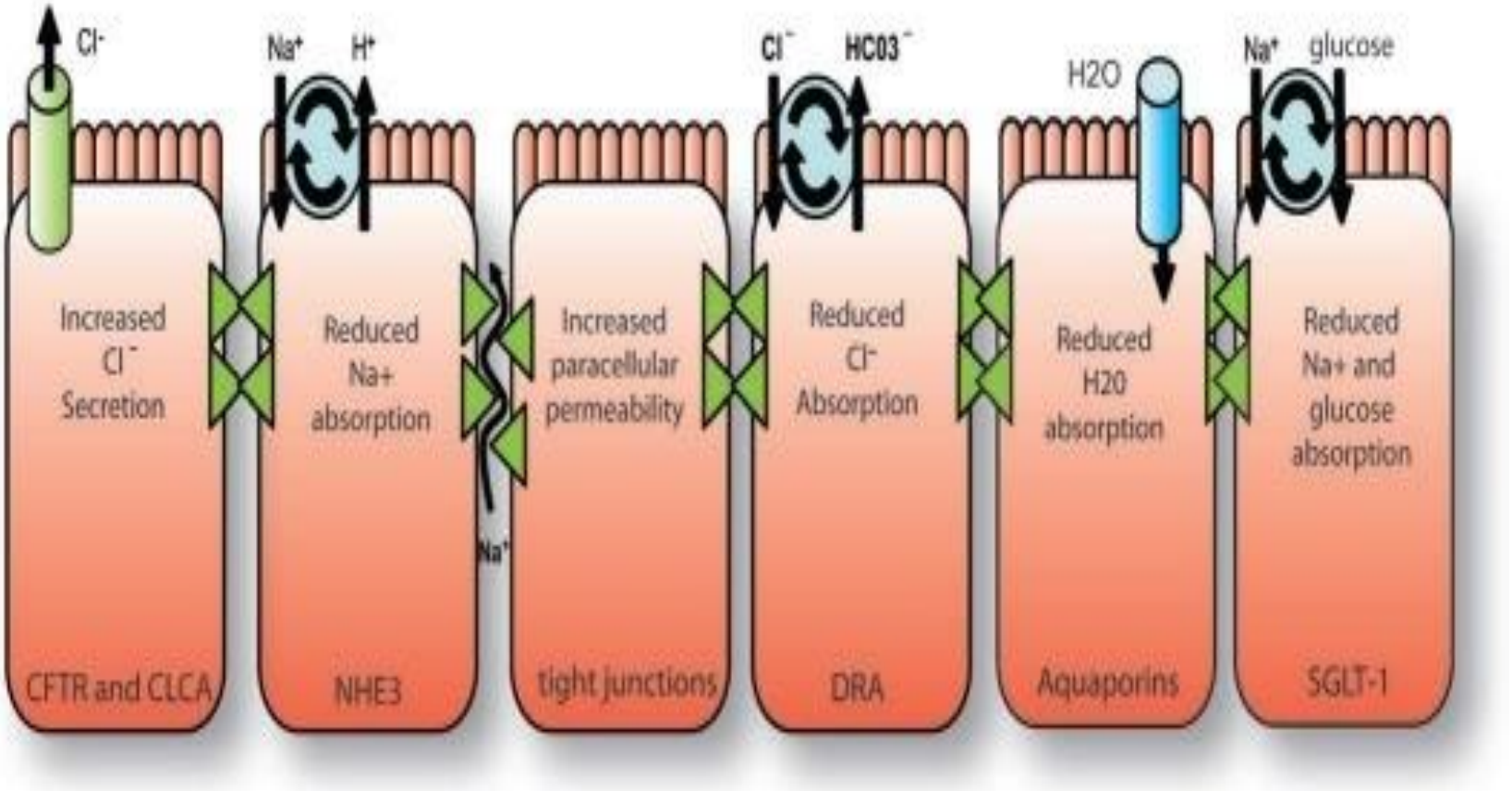
Causative Microorganism

Vibrio cholerae, E.coli, Shigella Species, *Clostridium difficile*

Diarrhea

- Diarrhea is simply an altered movement of ions and water that follows an osmotic gradient. Under normal conditions, the gastro-intestinal tract has tremendous capacity to absorb fluid and electrolytes, where 8–9 liters of fluid are presented to the intestine daily and only 100–200 ml are excreted in the stool. Enteric pathogens, however, can alter this balance towards net secretion, leading to diarrheal disease. The altered movement of ions can occur either through transporters or the lateral spaces between cells, which are regulated by tight junctions

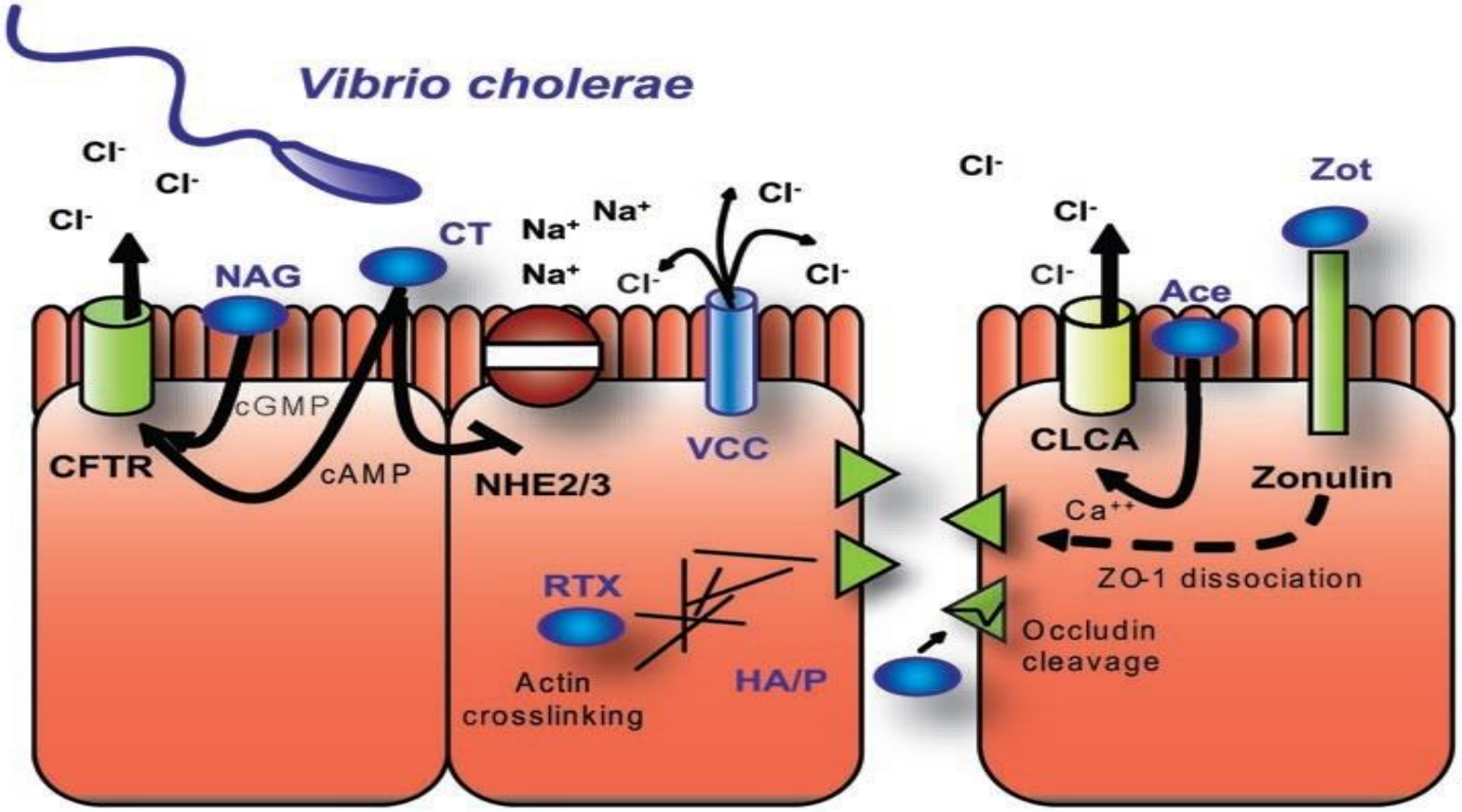
In this regard, some transporters seem to be tightly coupled with water movement, including sodium-dependent glucose transporter (SGLT1), Na^+/H^+ exchanger isoform 3 (NHE3) and the apicalCl^-/HCO_3^- exchanger, downregulated in adenoma (DRA). An overview of general mechanisms causing diarrhea. At the most basic level, diarrhea is caused by increased secretion or decreased absorption of fluids and electrolytes. Certain ion transport processes are particularly associated with diarrhea. These include CFTR and CLCA, which are chloride channels and the Na^+/H^+ exchange isoform, NHE3, which is involved in Na^+ absorption. Alterations in tight junctions create an important pathway for the movement of both ions and water. DRA is responsible for chloride absorption and is associated with congenital chloride diarrhea.



Infection by vibreo choleri

- *V. cholerae* have several toxins, which are used to compromise the host, with the most important of these being cholera toxin (CT) itself. CT consists of an A subunit bound to a pentameric ring of B subunits, where the B subunits are responsible for delivery of the A subunit into the cell.[3] The A subunit ADP-ribosylates a GTPase, which regulates adenylate cyclase resulting in elevated cAMP production[4] (**Fig. 2**). The production of cAMP activates PKA, which then phosphorylates the regulatory domain of CFTR.
- In addition to increased Clsecretion, the absorption of Na^+ is decreased through a cAMP-dependent mechanism where the activity of both apical sodium transporters, NHE2 and NHE3, is decreased[8] (**Fig. 2**). Together, this leads to an increase in NaCl levels in the intestinal lumen by enhancing secretion or decreasing absorption.
- In addition to CT, *V. cholerae* encodes several other toxins, which modulate ion secretion and perturb barrier function to cause massive diarrhea. The toxins that affect ion secretion directly include accessory cholera toxin (ACE), which stimulates Ca^{2+} dependent Cl^- secretion; NAG-stable toxin, which activates guanylyl cyclase, thus stimulating cGMP production, which leads to PKG-mediated activation of CFTR; and, finally *V. cholerae* cytolysin (VCC), which creates anion permeable pores[9–11] (**Fig. 2**). One of the phenotypes associated with VCC toxicity has been linked with the newly described phenomenon autophagy.[12] The VCC toxin causes large vacuoles to form in host cells in addition to its cytolytic effect on red blood cells.
- The *V. cholerae* toxins which alter intestinal barrier function include hemagglutinin/protease or HA/P, RTX and Zot. HA/P is an extracellular protease, which cleaves a tight junction structural protein, occludin, that is known to regulate paracellular permeability

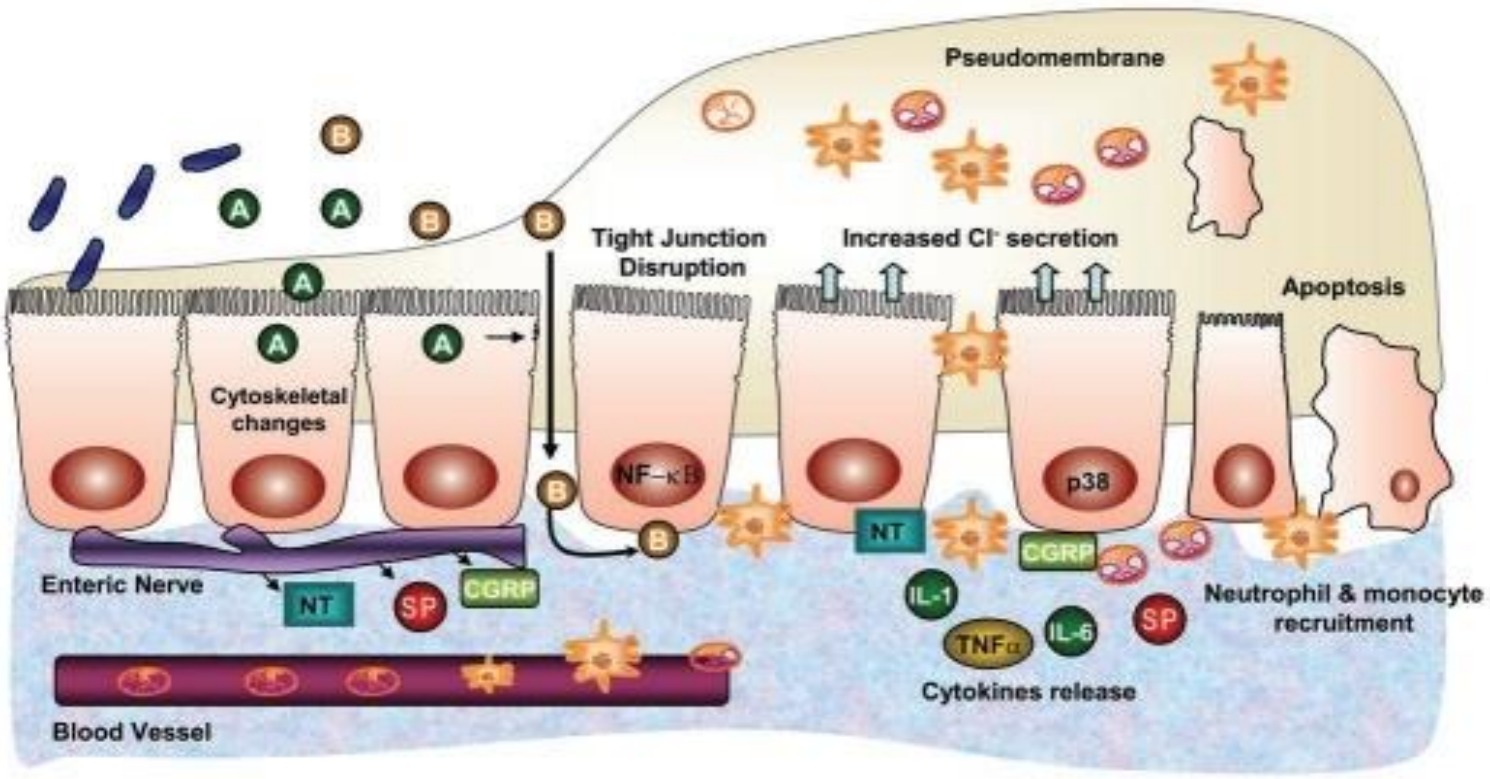
Mechanisms underlying *V. cholerae*-induced diarrhea: Cholera is characterized by severe watery diarrhea due to changes in ion secretion and absorption. Both CLCA and CFTR-dependent Cl^- secretion are activated, the first by Ace and the second by cholera toxin and NAG heat-stabile toxin. Increased cAMP levels also block sodium absorption through NHE2 and NHE3. *V. cholerae* also creates anion permeable pores through insertion of VCC. In concert with changes in ion transport, paracellular permeability is and rTX interferes with the contractile actin also decreased. Zot interaction with zo



C. difficile associated diarrhea (CDAD)

- The pathogenic process of *C. difficile* infection starts with initial colonization followed by the production of two distinct exotoxins, Toxin A and B (TcdA and TcdB), as well as an additional toxin called binary toxin (CDT) which is found in some hypervirulent strains of *C. difficile*.[23] TcdA binds effectively to the apical side of the host cell to glycoprotein gp96, which forms part of the receptor in humans.[24] In contrast, TcdB gains access to the basolateral side of the cell after tight junction disruption and binds preferentially to an unidentified receptor[25,26] (**Fig. 3**). TcdA and TcdB are potent cytotoxic enzymes that specifically glucosylate the small GTPase protein Rho, which leads to disruption of cytoskeletal integrity and cytotoxic effects.[27] CDT, an actin-specific ADP ribosyltransferase, potentiates the toxicity of TcdA and B and may increase the severity of CDAD
- Besides the direct effects of the toxins, other mechanisms underlying *C. difficile* associated diarrhea include inflammation and activation of neuropeptides. The *C. difficile* toxins initiate an extensive inflammatory cascade that causes increased damage to host tissues resulting in fluid exudation. TcdA causes release of several proinflammatory cytokines such as leukotriene, PGE_2, and tumor necrosis factor (TNFα in vivo).[33] It also directly activates monocytes to release IL-1 and IL-6,[34] and increase neutrophil migration in vitro.[35] Other toxin-mediated inflammatory effects include release of reactive oxygen species, activation of mitogen-activated protein kinases and NFκB activation.[33] A number of studies suggest that important cellular responses to *C. difficile* toxins such as p38 MAP kinase activation, mitochondrial damage and IL-8 release occur prior to and independently of Rho glucosylation

Pathogenesis of *C. diffcile*-associated diarrhea. *C. difficile* produces toxin A and toxin B (TcdA and TcdB). TcdA binds to the apical side of the cell and, after internalization, causes cytoskeletal modification and disruption of tight junctions. The resulting loss of epithelial barrier function facilitates TcdA and TcdB to cross the epithelium with preferential binding of TcdB to the basolateral cell membrane. Both toxins are cytotoxic and lead to production of proinflammatory cytokines, increase in vascular permeability, recruitment of neutrophils and monocytes, epithelial cell apoptosis and connective tissue degradation, resulting in pseudomembrane formation and diarrhea. Further, the activation and release of various neuropeptides by the toxins stimulates ENS to elicit fluid secretion, causing diarrhea.



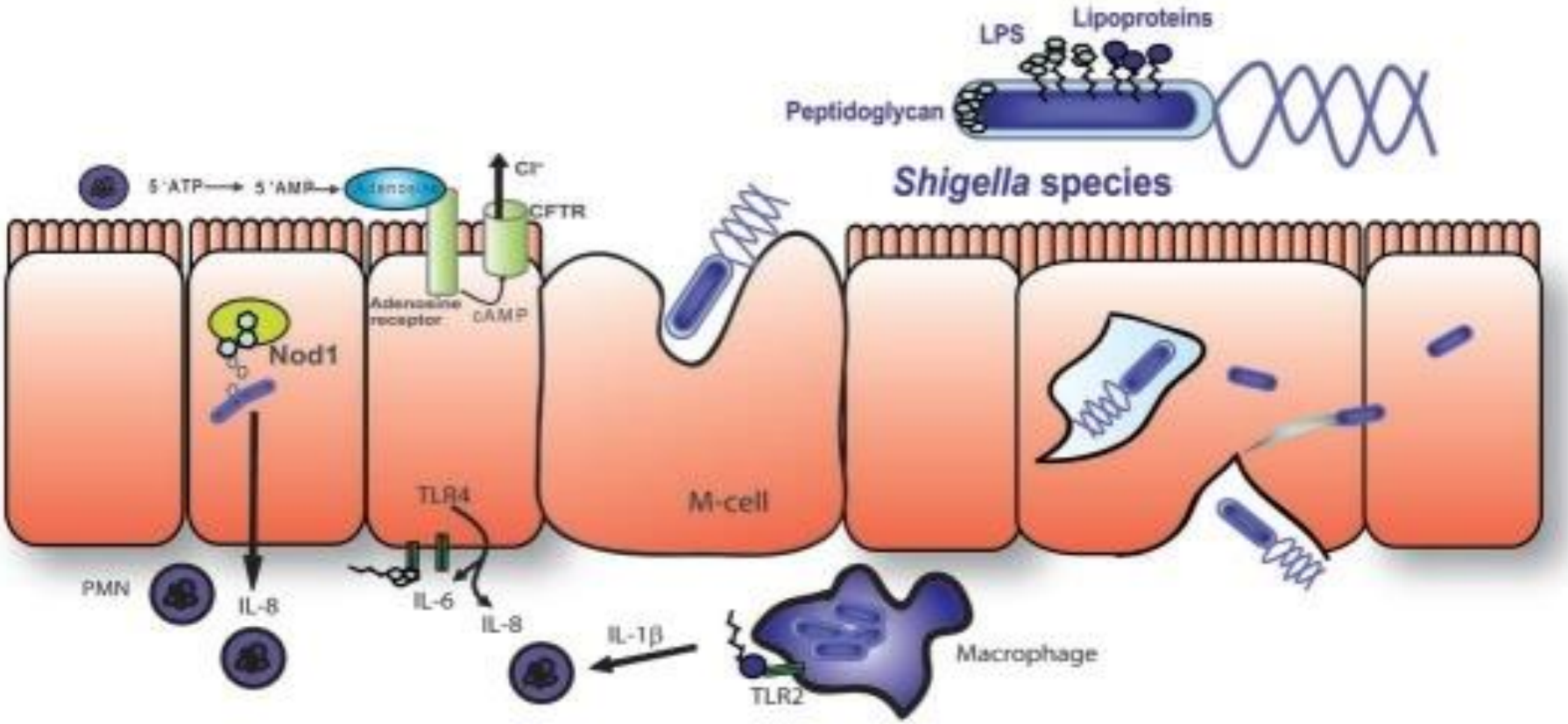
Shigella species.

- There are four major Shigella species that cause diarrheal disease. The most common species in the U.S. and other developed countries is *S. sonnei* followed by *S. flexneri*.[40] Two other Shigella species, *S. dysenteriae* and *S. boydii*, are found very rarely in developed countries and have a generally low infection rate over all, however, *S. dysenteriae* causes the most life-threatening of all of these infections due to the production of Shiga toxin, which can lead to hemolytic uremic syndrome (HUS). While all four species of Shigella are invasive due to a large virulence plasmid, there is some variation in the plasmid and *S. flexneri* is the best studied in this regard. The invasion process is complicated and occurs through a trigger mechanism on the basolateral side of epithelial cells after the bacteria have already passed through M-cells and potentially, macrophages

- . Shigella causes an inflammatory diarrhea and the cellular response to various steps of the invasion process are the primary cause of inflammation (**Fig. 4**). The destruction of macrophages after emergence from M-cells causes an initial release of IL-1β, which attracts PMNs.[44,45] PMNs release a precursor to the secretagogue adenosine, which activates Cl^-secretion. This early step in inflammation is exacerbated by the presence of free bacteria on the basolateral side of cells, which allows access to toll-like receptors. Shigella LPS is capable of activating TLR4, although recent studies suggest that it is about 50% less active than LPS from *E. coli* in activating NFκB due to a reduced level of acetylation.[46] Therefore, to some extent, Shigella actively evade TLR4 recognition, while TLR2 is activated by Shigella through lipoproteins.[47] In addition, the interaction of Nod1 with shed peptidoglycan from intracellular bacteria also leads to NFκB activation and IL-8 production.[48] IL-8 is another pro-inflammatory cytokine, which attracts PMNs. In this case, PMNs cause many of the symptoms of the disease but also lead to its eventual clearance.

Figure 4

Invasion and inflammation caused by Shigella. Shigella species cross the epithelial barrier through M-cells where they encounter and eliminate macrophages. Binding of lipoprotein to TLR2 results in the production of the chemoattractant IL-1β. After translocation through M-cells LPS can bind to basolateral TLR4 which causes the production of IL-6 and IL-8. This effect is somewhat diminished due to the acetylation of LPS in Shigella. IL-8 is a potent chemoattractant for PMNs and is also produced due to activation of intracellular Nod1 by peptidoglycan. PMNs are the primary destructive force in Shigella infection. PMNs cause Cl^- secretion through generation of a precursor to the secretagogue adenosine and can also cause ulceration of the epithelium, which results in a decrease in the absorptive surface but also maximizes permeability and allows easy access of gut flora to the basolateral surface of cells further driving inflammation.

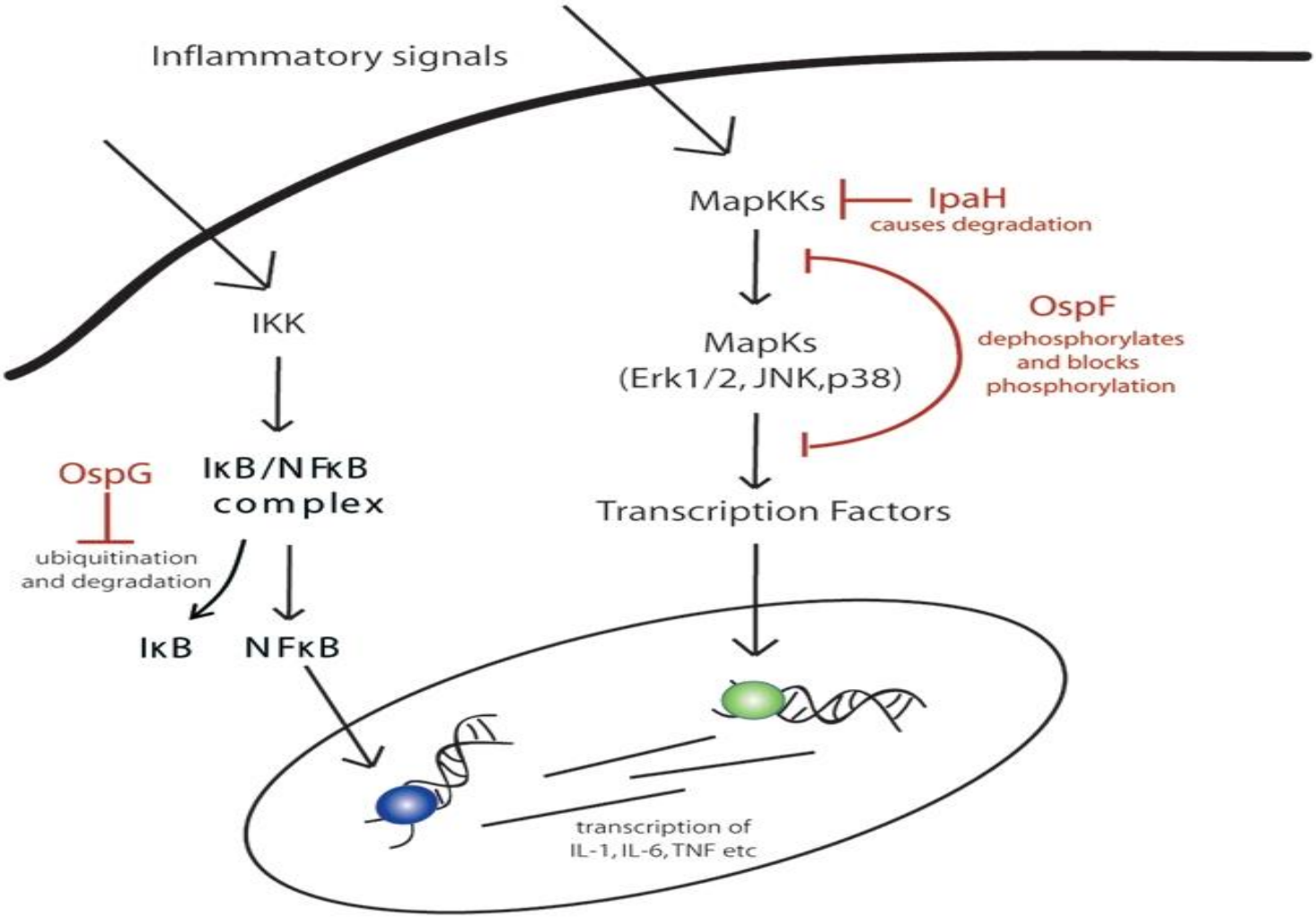


Type III secreted effector proteins modulate inflammation

- Shigella have a type III secretion system, which injects a number of effector proteins required for initial invasion, escape from the vacuole and movement within the cell. Because shigellosis is primarily due to inflammation, bacterial proteins that modulate the host immune response can differentially modulate the host diarrheal response as well. There are three effector proteins that meet this criteria: OspF, OspG and IpaH (**Fig. 5**). The first, OspF, has a unique way of inhibiting MAP kinase signaling. It is an entirely new type of enzyme which does not exist in mammalian cells called a phospho-threonine lyase.[49] OspF is capable of dephosphorylating threonine residues in a way that not only removes the phosphate group but also the oxygen atom, which is normally part of the amino acid, as well as a hydrogen atom from the adjacent carbon. In doing so it creates a carbon-carbon double bond within the backbone of the amino acid. In this form there is no hydroxyl group capable of being phosphorylated, thus the process is essentially irreversible. Several MAP kinases are affected by OspF, including Erk1/2, p38 and JNK.[49]

Figure 5

Modulation of pro-inflammatory signaling by Shigella T3SS effectors. Several type III secreted effector proteins have been shown to modulate the host immune response during Shigella infection. The first is OspG which interferes with the ubiquitination of phosphorylated IκB, preventing its degradation, thereby effectively blocking NFκB translocation into the nucleus. In contrast, IpaH actually induces ubiquitination and degradation of MapKKs, preventing further cascade activation. Finally, OspF has a unique way of cleaving the phosphate group and a few extra atoms from MapKs including Erk1/2, JNK and p38 which prevents further phosphorylation.



diffusely adherent *E. coli* (DAEC)

- Enterotoxigenic *E. coli*.
- ETEC causes toxigenic secretory diarrhea characterized by massive intestinal fluid secretion. The key virulence attributes of ETEC include adherence to epithelial cell surfaces by colonization factors and elaboration of heat labile (LT) and heat stable (ST_a) enterotoxins. Some strains of ETEC may also express Entero-aggregative heat stable toxin 1 (EAST1). Similar to cholera toxin, heat labile toxins elicit increases in Cl^-secretion via activation of cAMP. ST_a, however, is known to evoke secretion and diarrhea by elevation of intracellular cGMP. After binding to its receptor, guanylyl cyclase C (GC-C), ST_a through cGMP dependent pathways is known to stimulate CFTR translocation to the surface of villus enterocytes causing its activation.[57] There is net Cl^-, HCO_3^-, and water secretion as well as inhibition of Na^+/H^+ exchange in jejunal enterocytes by ST_a

- Enteropathogenic *E. coli* (EPEC).
- EPEC is a major cause of persistent, watery diarrhea in infants, often accompanied by low-grade fever and vomiting. The pathogenic mechanisms of EPEC remained elusive for many years because, in contrast to prototypic enteric bacterial pathogens, EPEC does not produce classical enterotoxins such as LT in order to influence host cell pathways. In addition, EPEC is typically regarded as non-invasive in comparison to bacteria such as Shigella and Salmonella, although a limited number of the bacteria are internalized. However, EPEC does encode a T3SS and produces a characteristic attaching and effacing (A/E) lesion which is marked by effacement of microvilli on the epithelial surface at the site of bacterial attachment.[65] In addition, there is an accumulation of cytoskeletal proteins beneath adherent microcolonies leading to actin cup or pedestal formation, depending on cell type. This profound change in intestinal epithelial cells induced by A/E lesions contributes to the diarrheal phenotype due to loss of overall absorptive surface.[66] However, diarrhea occurs as quickly as 3–4 h after the ingestion of the pathogen, suggesting that mechanisms other than malabsorption are at work

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