Microbes and Microbial Toxins: Paradigms for Microbial-Mucosal Interactions III. Shigellosis: from symptoms to molecular pathogenesis

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Sansonetti, Philippe J. Microbes and Microbial Toxins: Paradigms for Microbial-Mucosal Interactions. III. Shigellosis: from symptoms to molecular pathogenesis. Am J Physiol Gastrointest Liver Physiol 280: G319–G323, 2001.—Interaction of Shigella flexneri with epithelial cells includes contact of bacteria with the cell surface and release of Ipa proteins through a specialized type III secretory system. A complex signaling process involving activation of small GTPases of the Rho family and c-src causes major rearrangements of the subcellular cytoskeleton, thereby allowing bacterial entry by macropinocytosis. After entry, shigellae escape to the cell cytoplasm and initiate intracytoplasmic movement through polar nucleation and assembly of actin filaments caused by bacterial surface protein IcsA, which binds and activates neuronal Wiskoff-Aldrich syndrome protein (N-WASP), thus inducing actin nucleation in an Arp2/3-dependent mechanism. Actin-driven motility promotes efficient colonization of the host cell cytoplasm and rapid cell-to-cell spread via protrusions that are engulfed by adjacent cells in a cadherin-dependent process. Bacterial invasion turns infected cells to strongly proinflammatory cells through sustained activation of nuclear factor-kB. A major consequence is interleukin (IL)-8 production, which attracts polymorphonuclear leukocytes (PMNs). On transmigration, PMNs disrupt the permeability of this epithelium allow bacterial translocation. Subsequent apoptotic killing of macrophages in a caspase 1-dependent process causes the release of IL-1β and IL-18, which accounts for the initial steps of inflammation.

dysentery; epithelium; colon; inflammation

SHIGELLAe are gram-negative, nonsporulating, facultative anaerobic bacilli that belong to the family Enterobacteriaceae. They cause shigellosis, or bacillary dysentery, an invasive infection of the human colon that affects a spectrum of clinical presentations, from short-lasting watery diarrhea to acute inflammatory bowel disease, the classic expression of bacillary dysentery characterized by the triad of fever, intestinal cramps, and bloody diarrhea with mucopurulent feces (10). The etiological agents belong to the genus Shigella, which comprises four different species. S. flexneri (6 serotypes) and S. sonnei (1 serotype) account for the endemic disease, the former being prevalent in the developing world, the latter in the industrialized world. S. dysenteriae (16 serotypes) includes serotype 1, the “Shiga bacillus,” which accounts for deadly epidemics in the poorest countries, largely due to its capacity to produce shigatoxin, a potent cytoxin. S. boydii (8 serotypes) remains restricted to the Indian subcontinent. In terms of public health (13), shigellosis shows three major characteristics: 1) it is mostly a pediatric disease, >60% of the cases occurring in children between the ages of 1 and 5 yr; 2) it is a third-world disease, with ~150 million cases occurring every year, compared with 1.5 million cases in industrialized countries; and 3) it is also a deadly disease, with ~1 million deaths every year, again mostly infants and young children. Lack of hygiene is the major, if not exclusive, contributing factor, the disease being transmitted by person-to-person contact or contaminated food.

In addition to poverty being the primary factor favoring occurrence of shigellosis, other disease-specific parameters aggravate the public health burden of shigellosis. They are essentially four, all of which deserve increased research attention: 1) extension of antibiotic (multi-)resistance in both endemic and epidemic areas; 2) very low infectivity, 10–100 microorganisms administered orally being able to cause the disease in adult volunteers; 3) severity of acute complications, particularly in infants and malnourished children, with complex and yet often unexplained pathogeneses, some of them being lethal, as is the case for acute hypoglycemia. .
mia, seizures, toxic megacolon, pseudoleukemoid reaction and hemolytic-uremic syndrome, intestinal perforations, peritonitis, and Gram-negative septicemia; and 4) the recently recognized importance of delayed complications, characterized by a prolonged state of malnutrition whose pathogenesis is still unclear and indeed poses another challenge.

This situation makes vaccination a cost-effective approach, and the World Health Organization has put the development of a *Shigella* vaccine at the top of its priority list of awaited vaccines against enteric infections (22). Understanding the pathogenesis of shigellosis, the basis of the innate immune response that causes excessive inflammation leading to intestinal tissue destruction, as well as the basis of the protective adaptive immune response has become a priority topic in several laboratories worldwide, with the aim of developing a vaccine against the disease.

**MOLECULAR AND CELLULAR PATHOGENESIS OF SHIGELLOSIS: AN UPDATE**

The ability of *Shigella* to invade and colonize the intestinal epithelium is a key determinant of the disease. However, the pathogenesis of shigellosis is a subtle combination, particularly at the early stage of the disease, of 1) the capacity for the bacteria to cross the epithelium in selected areas corresponding to M cells of the follicle-associated epithelium (FAE) that covers the mucosa-associated lymphoid follicles, the inductive sites of local immune responses (28); 2) the intrinsic invasive properties of the bacteria for epithelial cells (21); and 3) the inflammatory response achieved by the cellular components of the intestinal barrier that disrupt the coherence of this barrier and facilitate bacterial invasion (37). Expression of the *Shigella* invasive phenotype in the presence of the various cell populations that constitute the intestinal barrier (Fig. 1), particularly M cells, epithelial cells, resident macrophages, and polymorphonuclear leukocytes (PMNs), engages variable interactions whose result constitutes the overall process leading to rupture, invasion, and inflammatory destruction of the intestinal barrier.

**Genetic and Molecular Basis of the Shigella Invasive Phenotype**

In *S. flexneri* and other *Shigella* species, a 214-kb virulence plasmid contains most of the genes required to express the key steps of the invasive phenotype (5, 26). The coding sequences are scattered over the entire virulence plasmid, essentially separated by multiple, often incomplete, insertion sequences. One 30-kb block, however, shows a dense pattern of genes, the *ipa/mxi-spa* locus that can be considered the main *Shigella* pathogenicity island (PAI). This PAI is necessary and sufficient to cause entry into epithelial cells via macropinocytosis, macrophage apoptotic death, and activation of PMNs. It primarily encodes a type III secretory system, a flagella-like structure able to deliver *Shigella* effector proteins, particularly Ipa proteins, straight from the bacterial cytoplasm into the cytoplasmic membrane of the eukaryotic cell target or its cytoplasm.

**EPITHELIAL CELLS**
- Entry by macropinocytosis & escape into the cytoplasm.
- Intracellular & intercellular spread.
- NFkB activation, production of IL-8 and other.

**MONOCYTES / MACROPHAGES**
- Phagocytosis.
- Caspase 1 activation / Apoptosis.
- Maturation-Release of IL-1β & IL-18.

**POLYMORPHONUCLEAR LEUCOCYTES**
- Phagocytosis.
- Release of granular content.
- Increased adherence.
- Killing of bacteria.
Interaction of Shigella with M cells and epithelial cells. In vivo evidence indicates that expression of the invasive phenotype is required for Shigella to translocate at high frequency through M cells (23). It is therefore likely that the Shigella PAI mediates efficient invasion of both M cells and epithelial cells. However, whereas Shigella can enter via the apical pole of M cells, they are very inefficient, if not unable, in entering via the apical pole of epithelial cells whose basolateral pole, on the other hand, is very permissive for entry (16). M cell translocation, therefore, is likely to allow access of shigellae to the basolateral pole of these epithelial cells, where efficient internalization proceeds. Bacterial signals and cell responses mediating entry of Shigella into epithelial cells have mostly been studied in the epitheliod HeLa cell line. These data have been summarized in a recent review (33). The Shigella type III secreton allows the insertion of a pore into the cytoplasmic membrane of the eukaryotic cell target. This pore, which contains a complex of the IpaB and IpaC proteins, expresses a dual function: it induces early events of actin polymerization via the COOH terminal domain of IpaC (32), and it is also likely to introduce into the eukaryotic cell cytoplasm a series of plasmid-encoded proteins (~15) that are known, in vitro, to be secreted through the type III secreton (5). Among these, IpaA and IpgD are involved in the maturation of the entry focus (4, 17), whereas the possible function of the others is unknown.

While entering cells, Shigella causes the formation of filopods that are quickly remodeled in lamellipods, thus resulting in a structure that entraps the microorganism (1). Further remodeling causes the formation of a macropinocytic vacuole that eventually completes internalization. These events result from a cross-talk between the bacterium and the host cell signaling pathways that regulate the cytoskeleton, essentially causing actin nucleation and polymerization at the eukaryotic cell membrane (33). The major target of IpaC is the cascade of a complex between IcsA/VirG, N-WASP, and Arp 2/3 that is known, in vitro, to be secreted through the type III secreton (5). Among these, IpaA and IpgD are involved in the maturation of the entry focus (4, 17), whereas the possible function of the others is unknown.

Once internalized, the phagocytic vacuole is quickly lysed by the invading bacterium, thereby allowing its escape into the host cell cytoplasm, where it nucleates and assembles an F-actin comet at one of its poles (3). This results in the bacterium moving inside epithelial cells and passing from cell to cell, thereby causing a very efficient process of intracellular colonization. Shigella actin-based motility is mediated by a single outer membrane protein, IcsA/VirG (14, 15). IcsA/VirG is unable to directly induce actin nucleation, indicating that recruitment of a cytosolic component is required. Glycine-rich repeats in the NH2-terminal end of IcsA/VirG bind neuronal Wiskoff-Aldrich syndrome protein (N-WASP) (30), a member of the WASP family of Cdc-42-dependent mediators of actin nucleation via the Arp 2/3 complex. For-
This causes massive decrease in the IL-1RA-IL-1 ratio, a characteristic of severe inflammatory processes. Conversely, perfusion of IL-1RA during the course of experimental shigellosis causes considerable attenuation of the severity of lesions, thus emphasizing the major role that IL-1 is playing in Shigella-mediated inflammation (24). Recent experiments have provided evidence on the respective roles of IL-1β and IL-18 release following caspase 1 activation by IpaB in infected macrophages (29). Release of IL-1β early after infection causes rupture of the epithelial barrier and destabilization of tissue homogeneity, which favors bacterial diffusion, massive tissue invasion, and enhancement of inflammation and tissue destruction. On the other hand, the parallel release of IL-18, which is a potent interferon (IFN)-γ inducer, allows the innate immune system to establish proper conditions for eradication of the Shigella inoculum, since IFN-γ is essential for the killing of Shigella (34). In consequence, it is likely that macrophage and, possibly, dendritic cell apoptosis occurring early after bacteria have crossed the FAE causes both IL-1β-mediated inflammation participating in the inflammatory rupture of the epithelial barrier and facilitation of bacterial dissemination as well as IL-18-programmed control of Shigella growth.

**Bacterial Invasion Causes Epithelial Cells to Produce Proinflammatory Molecules: Consequences of the Epithelium Becoming a Participant in Inflammation**

In response to bacterial invasion, it is now well established that colonic epithelial cells produce a large array of proinflammatory cytokines and chemokines, such as IL-8 (12). Invasion of epithelial cells by Shigella activates high and sustained nuclear translocation of nuclear factor-κB (20), which accounts for IL-8 production by infected cells. This process that may occur at a distance from the FAE as epithelial invasion proceeds, once IL-1β has started to disrupt integrity of the epithelial barrier, and accounts for attraction of PMNs in subepithelial tissues, followed by their transmigration through the epithelium, which extends the zone of invasion and causes major tissue destruction. Neutralization of PMN transmigration either in vitro (19) or in vivo (18), using an anti-CD18 monoclonal antibody that neutralizes binding of PMNs to epithelial cells, dramatically decreases both bacterial invasion and inflammatory destruction of the epithelium. In addition, neutralization of IL-8 during experimental infection by Shigella causes a dramatic decrease in PMN invasion of the epithelium and epithelial destruction. On the other hand, bacteria that have been able to cross the epithelial barrier grow freely in the lamina propria, unchecked by PMNs, and subsequently diseminate in the blood stream (25). In consequence, IL-8 production by invaded epithelial cells and their neighboring cells accounts for Shigella control at the epithelial level, but at the cost of massive epithelial destruction, particularly by PMNs. It is therefore likely that the spread of invasive bacteria, at a distance from the FAE, is constantly maintained by an influx of PMNs that subverts epithelial integrity and facilitates further bacterial invasion. These various elements of Shigella pathogenesis are summarized in Fig. 3.

**CONCLUSION**

Recent research has illustrated the power of combining microbial genetics and cell biology to decipher the cross-talks established between bacterial pathogens such as Shigella and their eukaryotic cell targets. We tried to summarize here some of the major features of the Shigella invasive process that leads to rupture, invasion, and inflammatory destruction of the intestinal barrier. We have now reached the stage at which these data must be integrated in a global scheme of signaling studied at the level of infected tissues, with...
particular interest in understanding engagement of the innate immune response and how this affects the adaptive immune response itself. This is essential to strengthening our future approaches aimed at developing an anti-Shigella vaccine.

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REFERENCES


