Microbiology Spectrum
American Society for Microbiology Press

Shigella Pathogenesis: New Insights through Advanced Methodologies

PAMELA SCHNUPF[1] and PHILIPPE J. SANSONETTI[2]

[1]Institut Imagine, Laboratory of Intestinal Immunity, INSERM UMR1163; Institut Necker Enfants Malades, Laboratory of Host-Microbiota Interaction, INSERM U1151; and Université Paris Descartes-Sorbonne, 75006 Paris, France; [2]Institut Pasteur, Unité de Pathogénie Microbienne Moléculaire, INSERM U1202, and College de France, Paris, France

ABSTRACT *Shigella* is a genus of Gram-negative enteropathogens that have long been, and continue to be, an important public health concern worldwide. Over the past several decades, *Shigella* spp. have also served as model pathogens in the study of bacterial pathogenesis, and *Shigella flexneri* has become one of the best-studied pathogens on a molecular, cellular, and tissue level. In the arms race between *Shigella* and the host immune system, *Shigella* has developed highly sophisticated mechanisms to subvert host cell processes in order to promote infection, escape immune detection, and prevent bacterial clearance. Here, we give an overview of *Shigella* pathogenesis while highlighting innovative techniques and methods whose application has significantly advanced our understanding of *Shigella* pathogenesis in recent years.

SHIGELLA AND SHIGELLOSIS

Shigella spp. are diarrheal pathogens closely related to *Escherichia coli*. They are named after Kiyoshi Shiga, who in 1898 identified its most virulent member, *Shigella dysenteriae*, as the causative agent of bacillary dysentery, also known as shigellosis (1). *Shigella* spp. are Gram-negative, non-spore-forming, facultative anaerobic bacilli that in humans and other primates cause diarrheal disease by invading the colonic epithelium. Spreading of the infection is generally limited to the intestinal lining, where it leads to colonic inflammation, mucosal ulceration, and a loss in intestinal barrier function. Shigellae are transmitted through the fecal-oral route or through ingestion of contaminated food and water. In most cases, *Shigella* spp. cause a self-limiting disease that can be effectively treated by oral rehydration or antibiotics, although a steady increase in the number of shigellosis cases caused by antibiotic-resistant *Shigella* strains has become a growing concern (2, 3). Shigellosis can be fatal in the very young and in infected individuals who are immunocompromised or do not have access to adequate medical treatment.

Clinical symptoms of shigellosis range from mild watery diarrhea to a bloody mucoid diarrhea accompanied by painful abdominal cramps and fever. The range of clinical symptoms is related to both the immune status of the host and the causative *Shigella* species, which differ in the presence of some critical virulence factors, including Shiga toxin. A major complication in infants and children is toxic megalocolon, while after clearance of the infection, other possible complications include hemolytic-uremic syndrome, characterized by renal failure, low platelet and red blood cell levels, and a 35% fatality rate, as well as postreactive arthritis, where patients may suffer from chronic arthritis of the joints for years after the shigellosis episode (4).

Shigella has a very low infectious dose, estimated to be 10 to 100 bacteria, and *Shigella* remains a major public health concern with an estimated 165 million cases

Received: 17 January 2019, **Accepted:** 15 February 2019, **Published:** 12 April 2019

Editors: Pascale Cossart, Institut Pasteur, Paris, France; Craig R. Roy, Yale University School of Medicine, New Haven, Connecticut; and Philippe Sansonetti, Institut Pasteur, Paris, France

Citation: Schnupf P, Sansonetti PJ. 2019. *Shigella* pathogenesis: new insights through advanced methodologies. *Microbiol Spectrum* 7(2): BAI-0023-2019. doi:10.1128/microbiolspec.BAI-0023-2019.

Correspondence: Pamela Schnupf, pamela.schnupf@inserm.fr; Philippe J. Sansonetti, philippe.sansonetti@pasteur.fr

occurring worldwide every year, including up to 100,000 deaths, particularly in children under 5 years of age (5, 6). The occurrence of shigellosis is largely restricted to developing countries where limited availability of clean drinking water and poor hygiene foster disease transmission and malnutrition contributes to disease severity. A vaccine for *Shigella* has not been licensed, partly due to the large repertoire of *Shigella* serotypes that need to be targeted in order for the vaccine to be globally effective (6).

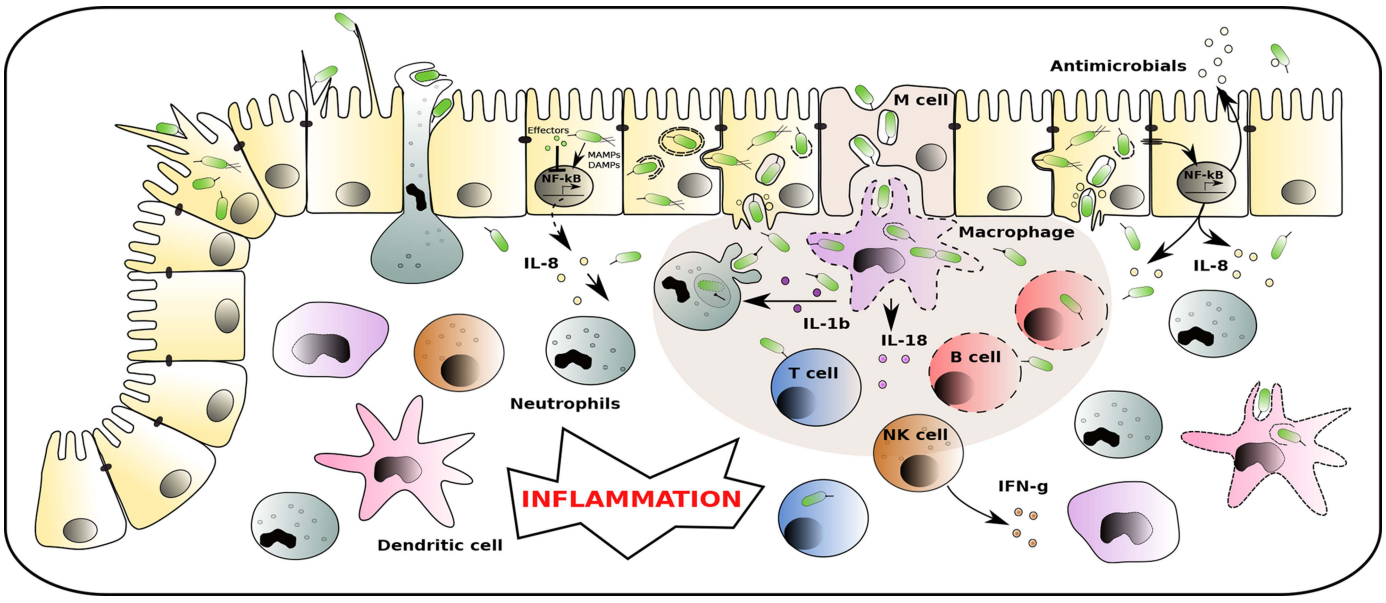
The genus *Shigella* is composed of four species: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. Each species represents a different serogroup (A through D, respectively) and is composed of multiple (15 to 20) serotypes, except for *S. sonnei*, which has only one (4). The large number of serotypes reflects the extensive variability in the composition, modification, and number of repeats of its lipopolysaccharide (LPS) O antigen, a major *Shigella* antigenic target of the humoral response of the host. *Shigella* spp. are endemic in a number of tropical and subtropical regions. Globally, *S. boydii* and *S. dysenteriae* are the least common agents of shigellosis, but they remain endemic in South Asia and sub-Saharan Africa, while *S. sonnei* is the most prevalent *Shigella* species linked to diarrheal disease in industrialized countries and an important cause of traveler's diarrhea (7). At 60% of all cases, *S. flexneri* is the most common cause of shigellosis worldwide. It is also the most widely studied *Shigella* species. *In vitro* and *in vivo* studies, in combination with clinical observations, have led to a detailed description of *S. flexneri* pathogenesis (3).

THE *SHIGELLA* INFECTIOUS CYCLE

Virulence of *Shigella* requires its type III secretion system (T3SS), a needle-like molecular syringe anchored in the bacterial cell wall. Activation of the T3SS occurs through contact of the needle tip with the host plasma membrane, resulting in the formation of a direct channel between the bacterial and host cytoplasm. *Shigella* injects a large number of bacterial effectors through this syringe to subvert many different host cell processes and to facilitate infection and dissemination.

To invade the host, *Shigella* must first withstand the physiochemical conditions encountered through its transit of the digestive system and cross the mucus layer of the colon. *Shigella* then traverses the colonic epithelial barrier through M (microfold) cells of the specialized epithelium overlying lymphoid follicles (Fig. 1). M cells

FIGURE 1 *Shigella* pathogenesis. *Shigella* infects the colonic epithelium at the follicle-associated epithelium and near the opening of colonic crypts. Invasion of M cells leads to *Shigella* transcytosis and release of *Shigella* at the basolateral side of the epithelium. *Shigella* can be taken up by macrophages and dendritic cells, which subsequently undergo pyroptosis, stimulating inflammation through the release of IL-1β and IL-18, which recruit neutrophils and activate innate defenses. *Shigella* also efficiently invades the basolateral side of the colonic epithelium from the lamina propria to reach its major replicative niche and the epithelial cell cytosol and propagate infection through cell-to-cell spread.



deliver *Shigella* to the basolateral side of the epithelium, where the bacteria are phagocytosed by antigen-presenting cells such as macrophages and dendritic cells residing within or near the M cell pocket. *Shigella* rapidly lyses the phagosomal compartment of the macrophage in a T3SS-dependent manner, and after limited replication in the cytosol, escapes the macrophage through activation of a lytic inflammatory cell death called pyroptosis. Macrophage pyroptosis, a process actively promoted by *Shigella*, is accompanied by caspase-l-mediated proteolytic activation and subsequent secretion of the proinflammatory cytokines interleukin 1β (IL-1β) and IL-18. Lytic cell death of infected macrophages releases *Shigella* at the basolateral side of colonic epithelial cells. *Shigella* interacts with host cell proteins found at the basolateral side of epithelial cells and forces its uptake into epithelial cells by inducing epithelial cell macropinocytosis. Invasion depends on the T3SS and a first wave of effectors to subvert the host cell cytoskeleton and mediate vacuolar escape.

Once in the cytosol, *Shigella* replicates and uses actin-based motility to escape host cell innate defenses such as autophagy and to spread to neighboring cells. Lysis of the double membrane of secondary phagosomes formed by *Shigella* physically pushing into neighboring epithelial cells again releases *Shigella* into its replicative niche, the host cell cytosol. Cytosolic replication of *Shigella* is also facilitated through the injection of a second wave of T3SS effectors that function to damp the host inflammatory response, promote host cell survival, and counteract antimicrobial processes. This ensures the integrity of its cytosolic niche and favorable conditions for replication and continued dissemination.

Although the inflammatory response is antagonized by *Shigella* effectors in infected epithelial cells, neighboring bystander cells actively participate in the inflammatory process by secreting the neutrophil chemoattractant IL-8 (8). Release of IL-8 from epithelial cells, in addition to release of the proinflammatory cytokines IL-1β and IL-18 by pyroptotic macrophages, fosters inflammation, the recruitment and activation of immune cells, including natural killer (NK) cells and particularly neutrophils, and antimicrobial defenses. Transmigration of neutrophils to the luminal side participates in the destabilization of the epithelial barrier and provides a means for luminal *Shigella* to translocate to the basolateral side. The host inflammatory response thereby can facilitate *Shigella* dissemination.

However, *Shigella* has devised numerous strategies to inhibit the host inflammatory response, subvert host innate immune responses, and maintain its privileged cytosolic replicative niche in order to prolong survival in the host. Ultimately, however, inflammation and neutrophil-mediated killing of *Shigella* lead to clearance of the infection. Neutrophils are particularly important in *Shigella* containment and resolution of infection, as they are more resistant than macrophages to *Shigella*-mediated cell death and can kill *Shigella* through phagocytosis, production of reactive oxygen species, and release of microbicidal molecules from their granules (degranulation) (9). In addition, *Shigella* induces neutrophil extracellular traps, whereby neutrophil death is accompanied by the release of nuclear chromatin and bactericidal proteins to immobilize and kill *Shigella* (10). Notably, *Shigella* also uses its T3SS and effectors to block the host from mounting an efficient adaptive immune response not only by suppressing innate immune response but also by directly targeting and subverting host processes of B and T cells.

SHIGELLA T3SS AND T3SS EFFECTORS

The T3SS apparatus and the majority of the ~30 T3SS effectors secreted by the T3SS are encoded on a large (~200-kb) virulence plasmid (Fig. 2) (11). Genes encoding the structural components of the T3SS apparatus, effectors involved in cellular invasion, and the main transcriptional regulators VirB and MxiE are located in a 31-kb pathogenicity island termed the entry region (12). Conversely, effectors whose genes are scattered throughout the plasmid are generally involved in post-invasion processes. Expression of the T3SS and its effectors is under tight control of a regulatory network that responds to environmental cues encountered during transit through the host. The major trigger for the expression of the T3SS apparatus and the first wave of effectors involved in the host cell invasion is a shift to 37°C. Other factors that affect virulence gene expression are pH, osmolarity, and iron availability (3). A shift to 37°C induces the transcription of the transcriptional activator VirF, leading to the expression of IcsA, an adhesin and the actin nucleator required for cytosolic actin-based motility, as well as the transcription factor VirB. VirB, in turn, induces transcription of structural components of the T3SS apparatus (*mxi-spa* genes), effectors that mediate bacterial entry, and some additional effectors (3). A second wave of effectors, under the control of MxiE, is transcriptionally induced upon activation of the T3SS (Fig. 2A).

The T3SS is composed of a basal body spanning both the inner and outer bacterial membrane, a long needle-like structure that protrudes from the bacterium and a tip complex composed of the translocators IpaB and IpaD (Fig. 2B). The needle is composed of repeating MxiH

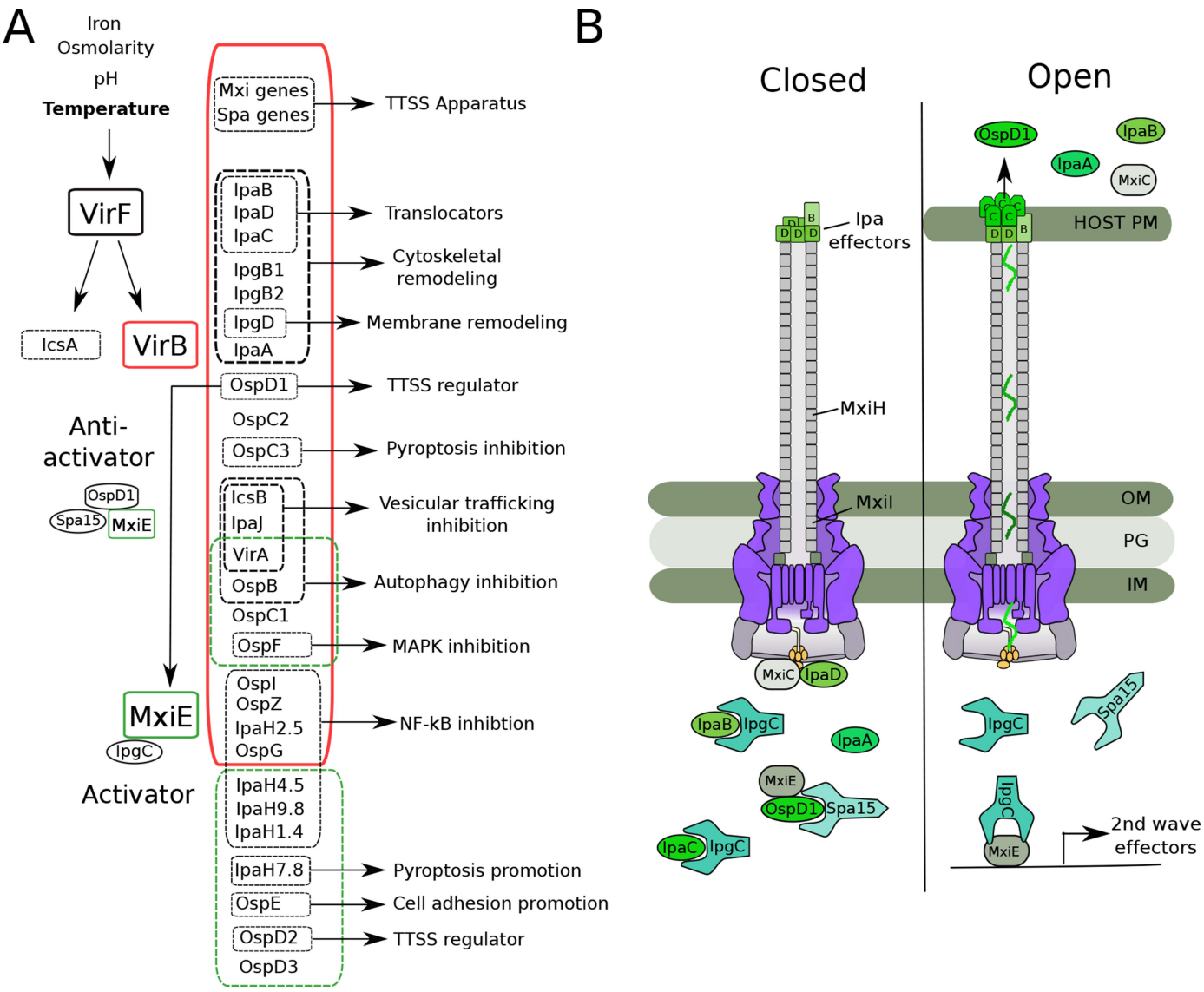


FIGURE 2 *Shigella* T3SS and effectors. (**A**) Expression of the T3SS apparatus and its effectors is regulated by a number of environmental factors that, through the transcription factor VirF, control the expression of the transcription factor VirB, which controls the expression of the T3SS apparatus and the first wave of effectors. Upon activation of the T3SS apparatus, MxiE is released from its inhibition and stimulates the transcription of the second wave of effectors. (**B**) When the T3SS is closed, first-wave effectors are stored in the bacterial cytoplasm with or without chaperones, while the gatekeeper MxiC and the translocator proteins IpaB and IpaD at the T3SS tip prevent effector secretion. Upon activation of the T3SS, effectors are secreted into the host cell cytosol, and expression of second-wave effectors is mediated by MxiE in complex with IpgC. IM, inner membrane; OM, outer membrane; PM, plasma membrane.

subunits and has been resolved to a striking precision of 0.4 Å using a combination of cryo-electron microscopy and solid-state nuclear magnetic resonance (13). The high resolution obtained through this hybrid approach, together with alanine scanning mutagenesis of MxiH and effector translocation studies (14), supports a model whereby the translocation of unfolded effector proteins through the T3SS needle lumen is mediated by effector interactions with side chains of several luminal MxiH amino acids (K79 and K72) while interaction with the D73 side chain regulates substrate release from the needle together with the translocators at the needle tip.

Three-dimensional reconstruction of transmission electron microcopy images of the T3SS needle tip revealed IpaD to be a homopentamer at the needle tip with the distal domain in an elongated conformation that controls the stepwise assembly of the tip complex (Fig. 2B) (15). Bile salt recognition leads to conformational changes in IpaD and promotes the recruitment of the hydrophobic IpaB effector distal to IpaD (16). IpaD and IpaB keep the needle complex in an "off" state so that deletion of either IpaD or IpaB renders the T3SS constitutively active or in a perpetual "on" state. Activation of the T3SS is achieved through the interaction of the needle tip complex with cholesterol and lipid rafts of the host plasma membrane (17, 18); although activation can be artificially induced through the addition of the dye Congo red. T3SS activation involves structural changes in IpaB, recruitment of the hydrophobic effector IpaC, their secretion, and the insertion of an IpaB-IpaC pore into the host membrane. Successful pore formation and opening of the T3SS lead to the secretion of MxiC, an effector that serves as a gatekeeper protein through direct interaction with IpaD to ensure that translocon proteins (IpaD, IpaB, and IpaC) are secreted before effectors (19). Removal of MxiC allows injection of the first wave of VirB-dependent *Shigella* effectors that are prestored in the bacterial cytoplasm and many of which are associated with their chaperone until the T3SS is activated (11).

Upon activation, secretion of IpaB and IpaC frees up their chaperone, IpgC, while secretion of OspD1 dissociates OspD1 from its chaperone Spa15 and the transcriptional activator MxiE, alleviating inhibition of MxiE by OspD1 (20). MxiE is free to interact with its coactivator IpgC, newly liberated from its interaction of IpaB and IpaC, to form an active MxiE-IpgC complex. The MxiE-IpgC complex drives transcription of "second-wave" effectors involved in subverting host immune responses, host innate defenses, and host cell death pathways (Fig. 2A). In addition, MxiE further upregulates expression of some VirB-regulated effectors containing an MxiE recognition sequence (called the MxiE box) as part of their promoters.

The highly coordinated and hierarchical regulatory network that controls the synthesis of the T3SS and its effectors maximizes energy usage and ensures controlled release of effectors in time and space. This was recently investigated using a novel transcription-based secretion activity reporter in combination with fluorescence recovery after photobleaching (21). In this system, control of green fluorescent protein synthesis was placed downstream of the MxiE-regulated promoter of the second-wave effector IpaH7.8 and thereby served as a visual reporter for MxiE activity and a surrogate for T3SS activity during infection. The secretion activity reporter revealed tight control of T3SS activity during infection whereby the T3SS is active upon entry, shut off during cytosolic growth, and reactivated when bacteria form protrusions into neighboring cells for cell-to-cell spread.

Functional screening using a novel bottom-up, reductionist approach of heterologous expression of the *Shigella* T3SS and individual effectors in *E. coli* recently identified OspD2 as a T3SS regulator that limits effector secretion during cytosolic growth (22). By limiting VirA secretion, OspD2 functionally reduces epithelial cell death fostered by VirA-mediated calpain activation (23). In addition, T3SS shutdown during cytosolic growth allows the resynthesis and storage of effectors required for phagosomal lysis and facilitates escape from the secondary vacuole during cell-to-cell spread (21).

Shigella effectors and virulence factors exhibit various biochemical activities to subvert host cell processes. These include phosphorylation, dephosphorylation, deamination, demyristoylation, stimulation of GTP hydrolysis of small GTPases, promotion of GDP-GTP exchange of small GTPases, and ubiquitination. Polyubiquitination generally leads to degradation of the tagged protein by host proteasomes, although ubiquitination can also function in host cell signaling, transcriptional regulation, DNA damage control, and membrane trafficking. Ubiquitination is a recurring feature in the subversion of the host by *Shigella*, mainly because *Shigella* codes for a large IpaH effector family composed of five plasmid-encoded members and seven chromosome-encoded paralogs (24). IpaHs contain an N-terminal leucine-rich repeat domain involved in specific binding to target proteins and a C-terminal region with E3 ubiquitin ligase activity that mediates the ubiquitination of a bound target protein and subsequent ubiquitin-dependent degradation by host proteasomes (25, 26).

SHIGELLA OVERCOMES THE PHYSICOCHEMICAL BARRIERS OF THE HOST AND THE GUT MICROBIOTA TO REACH THE COLONIC EPITHELIUM

Shigella infects the colonic epithelium but first needs to overcome multiple barriers to reach the epithelial surface. For one, *Shigella* has to compete with the resident microbiota and overcome the microbiota-mediated colonization resistance. Streptomycin treatment, and thus reduction in the resident microbiota, facilitates *Shigella* infection (27), but how the resident microbiota restricts *Shigella* infection remains somewhat unclear.

Microbiota-mediated colonization resistance can be either indirect, by the stimulation of host defenses such as antimicrobial peptides, or direct, by, for example, the secretion of small-molecule inhibitors such as colicins to kill phylogenetically related strains, scavenging of essential nutrients, or contact-dependent injection of toxins through the T6SS. To be competitive in the interbacterial war of the microbiota, *Shigella* has been described to resist colicins, through modulation of the O-antigen chain length of its LPS, as well as to itself produce colicins to kill competing bacterial strains (2). In addition, some *Shigella* spp., such as *S. sonnei*, code for a T6SS that provides a competitive advantage over T6SS-deficient strains to infect the host (28).

To prime itself for infection and activate its own defenses, *Shigella* also senses environmental changes specific to the host as it transits through the intestine. *Shigella* survives the acid environment of the stomach through an effective acid resistance system (3, 29). In the small intestine, *Shigella* becomes primed for adhesion through the presence of bile salts. First, the surface protein IcsA, whose transcription is temperature dependent and is induced when *Shigella* enters the host, undergoes bile salt-dependent structural changes that lead to enhanced polar adhesion of *Shigella* to epithelial cells (30). In addition, the transcription of the effectors OspE1 and -2 is induced by bile salts and leads to an OspE1/2-mediated increase in cellular adhesion (31). In the colon, *Shigella* secretes mucinases to facilitate their crossing of the mucus layer in order the reach the epithelial surface. There, oxygen sensing plays an integral part in priming *Shigella* for infection. While strict anaerobes are largely restricted to the colon, where luminal oxygen concentrations are very low, oxygen concentrations are elevated at the epithelial surface due to diffusion of oxygen from the oxygenated epithelium. Oxygen levels remain highest at the epithelial surface, as oxygen diffusion may be limited by the thick mucus layer. While the transcription of the T3SS is inhibited under anaerobic conditions (32), *Shigella* senses the increased oxygen concentration at the epithelial surface to activate their T3SS and enhance invasion, thereby avoiding unnecessary activation of its T3SS when physically far from its cellular target (33).

SHIGELLA INVADES COLONOCYTES AND ELICITS HELP FROM THE HOST TO REACH THE EPITHELIUM

Once *Shigella* crosses the mucus layer, it reaches the colonic epithelium. This single-layer intestinal epithelium provides a physical barrier between the contents of the intestinal lumen and the immune system of the host and is a critical part of the host defense system. Tight junctions located near the apical side link neighboring epithelial cells together to form a fluid-impermeable barrier. However, regulated sampling of luminal contents does occur through specialized M cells of the follicle-associated epithelium (FAE) overlying Peyer's patches and smaller mucosal lymphoid tissues, such as cryptopatches, isolated lymphoid follicles, and colonic patches. Experiments in monkeys and morphological analysis of rectal biopsy samples from shigellosis patients identified the FAE of the colon as the first site targeted by *Shigella* (34, 35). More targeted analysis of *Shigella* interactions with the FAE of small intestinal Peyer's patches using rabbit ileal loop models supported the early interaction of *Shigella* with the FAE and specifically implicated M cells as the entry portal (36–38). These observations, together with the observation that *Shigella* invades the basolateral side of polarized colonocytes *in vitro* much more efficiently than the apical side, established M cells as the main entry portal of *Shigella*. However, the early steps of invasion are still not clear. The relatively recently developed colonic guinea pig infection model (39) has highlighted *Shigella*'s ability to also interact and invade the apical side of colonocytes. At the same time, *Shigella* infection of epithelial cells *in vitro* has provided interesting insights into the interaction of *Shigella* with the apical side of epithelial cells.

Shigella is nonflagellated and therefore does not use flagellum-mediated motility to either reach the colonic mucosa or carry out close interaction with the host epithelium. Instead, *Shigella* elicits the help of the host to reach the epithelial surface. Time-lapse video microscopy of *Shigella* infection of epithelial cells *in vitro* has revealed that *Shigella* can be captured by nanometer-width micropodial extensions resembling filopodia that extend from the epithelial cell body (40). Capture of *Shigella* triggers retraction of the filopodia, effectively bringing *Shigella* into close proximity to the cell body, where invasion can then proceed. Filopodium-mediated motility can be considered a pathogenic feature of *Shigella*, as it is dependent on the T3SS and particularly on a functional IpaB and IpaD tip complex. Thus, *Shigella* T3SS mutants are captured less often, and capture does not stimulate filopodial retraction. During infection of polarized colonocytes, these filopodial-capture events mostly occur at cell-cell junctions, and in addition to enhancing invasion efficiency, filopodial capture of *Shigella* facilitates the entry of multiple bacteria within one entry focus.

Filopodium-mediated capture by *Shigella* is enhanced by host cell signaling in response to *Shigella* infection

through Ca^{2+}-mediated release of ATP via connexin hemichannels at the plasma membrane. ATP sensing then leads to Erk1/2 activation, which stimulates filopodium retraction through enhancement of actin retrograde flow (40). Measurements of stall forces for the retraction of single filopodia using optical tweezers suggests that interactions of only a limited number of *Shigella* T3SS with receptors on the filopodial tip is sufficient for a high retraction stall force of 10 pN (41). In comparison, a maximal stall force of 8 pN was reached with beads heavily coated with *Yersinia* invasin, a β1 integrin ligand.

In addition, it was recently shown that *Shigella* membrane proteins are targeted by human defensin 5 (HD5) and that this interaction promotes *Shigella* adhesion and invasion of colonocytes both *in vitro* and in several *in vivo* models (42). Enhanced attachment and invasion were specific to HD5 and did not occur with mouse cryptdins, leaving open the possibility that HD5-mediated infection participates in the narrow host range of *Shigella*. The investigation of the interaction and invasion of the apical side of colonocytes by *Shigella* has been facilitated by new *in vivo* and *ex vivo* models. Indeed, the analysis of *Shigella* invasion of the colon, the natural site of infection, was long hindered due to the lack of a suitable infection model. This limitation was recently overcome through the development of a guinea pig colonic infection model that largely recapitulates the disease phenotype in humans (39).

Although the guinea pig model generally suffers from a shortage of available reagents to interrogate the host immune response, infection with *Shigella* can be monitored using simple fluorescent markers of the actin cytoskeleton. Interrogation of *Shigella* infection of the guinea pig colon using advanced quantitative bioimaging and correlative light and electron microscopy revealed that *Shigella* preferentially associates with colonic crypts and invades colonocytes located at the crypt mouth while rarely reaching the base of the crypt, where stem cells are located in their protected niche (43). Similarly, *Shigella* is preferentially found at crypt openings during early interactions with human colonic explants (44). In the guinea pig model, *Shigella* infection of cryptoproximal colonocytes is followed by spreading of the bacteria within the epithelium and increasing levels of tissue destruction until the infection is controlled by the host immune system. As seen in human rectal biopsy samples, *Shigella* infection does not generally reach the level of the intestinal crypt, although shigellosis and the associated vascular lesions do lead to an increase in mitotic activity in the crypt (43). Under these circumstances, direct invasion of colonocytes near the crypt opening likely delays the interaction of *Shigella* with macrophages to a later time point in the infection process.

SHIGELLA LIKELY TRAVERSES THE COLONIC EPITHELIUM THROUGH M CELLS

M cells are part of the specialized epithelium overlying mucosa-associated lymphoid tissue, including cryptopatches, isolated lymphoid follicles, and Peyer's patches of the small intestine as well as colonic patches (45). These secondary lymphoid tissues are inductive sites for adaptive B and T cell immune responses against antigens found in the intestinal lumen. Compared to neighboring villus epithelial cells, the FAE is characterized by reduced levels of mucus, antimicrobial peptides, and fucosylation and a site more easily accessible to antigens and microbes. M cells are best characterized as part of the small intestinal Peyer's patches, as these are large and accessible lymphoid structures. Lymphoid tissues of the large intestine resemble Peyer's patches but are smaller and less discernible with fewer follicles and smaller germinal centers, the sites where B cells are activated, proliferate, switch immunoglobulin class, and increase immunoglobulin antigen affinity by somatic hypermutation. Small intestinal Peyer's patches instead bulge out of the intestinal wall, allowing easy identification, and therefore often serve as proxies for the interaction of microbes with lymphoid tissue.

M cells are involved in immunosurveillance by the host and are characterized by their lack of microvilli, an apical microfold structure, and a large pocket-like invagination on their basolateral side (45). These cells are thereby structured to mediate the controlled sampling of antigens and bacteria from the intestinal lumen and transcytose these to the basolateral side, where antigen-presenting cells such as macrophages and dendritic cells are strategically located in or near the M cell pocket to kill commensals and process antigens. Like other enteropathogenic bacteria, *Shigella* can hijack this specialized M cell portal to breach the protective intestinal barrier and gain access to the basolateral side of the epithelium.

In both the mouse and rabbit ileal loop models, *Shigella* invades M cells overlying Peyer's patches early during infection (36, 37, 46). Notably, little is still known about the interaction of *Shigella* with M cells, as this interaction remains to be investigated in detail on a cellular level. However, time course analysis coupled with transmission electron microscopy of rabbit ileal loops suggests that *Shigella* does not access the M cell cytosol but rather traverses M cells in endocytic vacuoles (36). *Shigella* transcytosis is not generally associated with

significant cytotoxicity to the M cells and thus leaves the entry portal intact (36). After transcytosis, *Shigella* is captured by macrophages residing in the M cell pocket and in the subepithelial dome of the Peyer's patches. Macrophages are, however, largely unable to contain *Shigella* in the phagosomal compartment. Instead, *Shigella* reaches the macrophage cytosol and the macrophage undergoes pyroptotic death, allowing *Shigella* to escape to the lamina propria.

SHIGELLA ESCAPES MACROPHAGES BY INDUCING PYROPTOSIS

The main functions of macrophages are to detect and phagocytose microbes, to kill them, and to coordinate an inflammatory response to regain sterility. Due to their central role in clearing infections, pathogens have devised strategies to subvert them. Macrophages are used by some pathogens, such as *Listeria monocytogenes*, as replicative niches and Trojan horses to reach deeper tissues. *Listeria* therefore evolved to support macrophage survival in order to safeguard its replicative cytosolic niche. Unlike *Listeria*, *Shigella* does not generally disseminate into deeper tissues and instead aims to rapidly escape from the macrophage to reach and invade its preferred replicative niche, the colonic epithelium. *Shigella* escapes from macrophages by triggering and actively promoting pyroptotic macrophage death (Fig. 3) (47).

Pyroptosis is a highly inflammatory type of programmed cell death that links the detection of danger signals known as microbe-associated molecular patterns (MAMPs) and danger-associated molecular patterns (DAMPs) by cytosolic pattern recognition receptors (PRRs) to the release of proinflammatory cytokines and lytic cell death (48). Rupture of the infected cell in turn releases cytokines and DAMPs such as ATP and DNA, which further augment the proinflammatory environment and lead to the recruitment of immune cells. Pyroptosis is therefore an antimicrobial response to intracellular pathogens activated when the host cell has been compromised and MAMPs are sensed in the host cell cytoplasm. It thereby serves to release the invading bacteria into the extracellular space, making them accessible for killing by the more bactericidal neutrophils while concomitantly alerting the immune system to the intruder.

Pyroptosis, like apoptosis, is mediated through the controlled activation of caspases, which are aspartate-specific cysteine proteases that cleave cellular targets to ultimately kill the cell (48, 49). Unlike apoptosis, a noninflammatory programmed cell death, pyroptosis involves the activation of inflammatory caspases, such as caspase-1 and caspase-11 in mice and caspase-4 and -5 in humans. The canonical inflammasome is a multiprotein complex composed of activated PRRs and, depending on the PRR, the adaptor protein-associated speck-like protein and caspase-1, previously known as IL-1β-converting enzyme (50). Three types of cytosolic PRRs sense a wide variety of MAMPS: the nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family, the Aim2 (absent in melanoma 2)-like receptor family, and the retinoic acid-inducible gene-related I-like receptor family. Activation of a subset of these cytosolic PRRs leads to inflammasome formation and caspase-1 activation.

Direct sensing of bacterial compounds is achieved by Aim2, a bacterial double-stranded DNA sensor, and NLRC4/IPAF, a PRR coreceptor that links the recognition of conserved bacterial proteins such as flagella and T3SS rod and needle proteins by neuronal apoptosis inhibitory proteins (NAIPs) to caspase-1 activation. Conversely, indirect sensing of bacterial infection through sensing of pathogenicity patterns is achieved by NLRP1B, a sensor for the proteolytic activity of bacterial toxins (e.g., *Bacillus anthracis* lethal factor); by NLRP3, a PRR activated by a wide range of MAMPs and DAMPs that lead to host cell changes such as potassium efflux, production of reactive oxygen species, and lysosomal destabilization; and by pyrin, a sensor for the deactivation of a subset of small GTPases (RhoA, -B, and -C) involved in cytoskeleton regulation and a frequent target of bacterial effectors.

Upon its activation by the inflammasome, caspase-1 cleaves and activates the proinflammatory cytokines IL-1β and IL-18 and the cellular protein gasdermin D (49). Activated gasdermin D oligomerizes and forms pores in host membranes that allow the release of the proinflammatory cytokine IL-1β but eventually lead to water influx, cell swelling, host cell lysis, and release of the cellular contents. Gasdermin D-mediated host cell death can also be induced by the activation of caspase-11, or its human homolog, through direct sensing of the cytosolic hexaacyl lipid A moiety of LPS. The secreted proinflammatory cytokines IL-1β and IL-18 bind to the IL-1 and related IL-18 receptors, respectively, to trigger NF-κB- and mitogen-activated protein kinase (MAPK)-signaling pathways in a MyD88- and TRAF-dependent manner, leading to release of additional proinflammatory cytokines such as tumor necrosis factor alpha and IL-6. IL-1β also functions to recruit neutrophils and to stimulate a Th17 response, while IL-18 stimulates T and NK cells to produce gamma interferon, which enhances the antimicrobial activity of macrophages by inducing nitric oxide production.

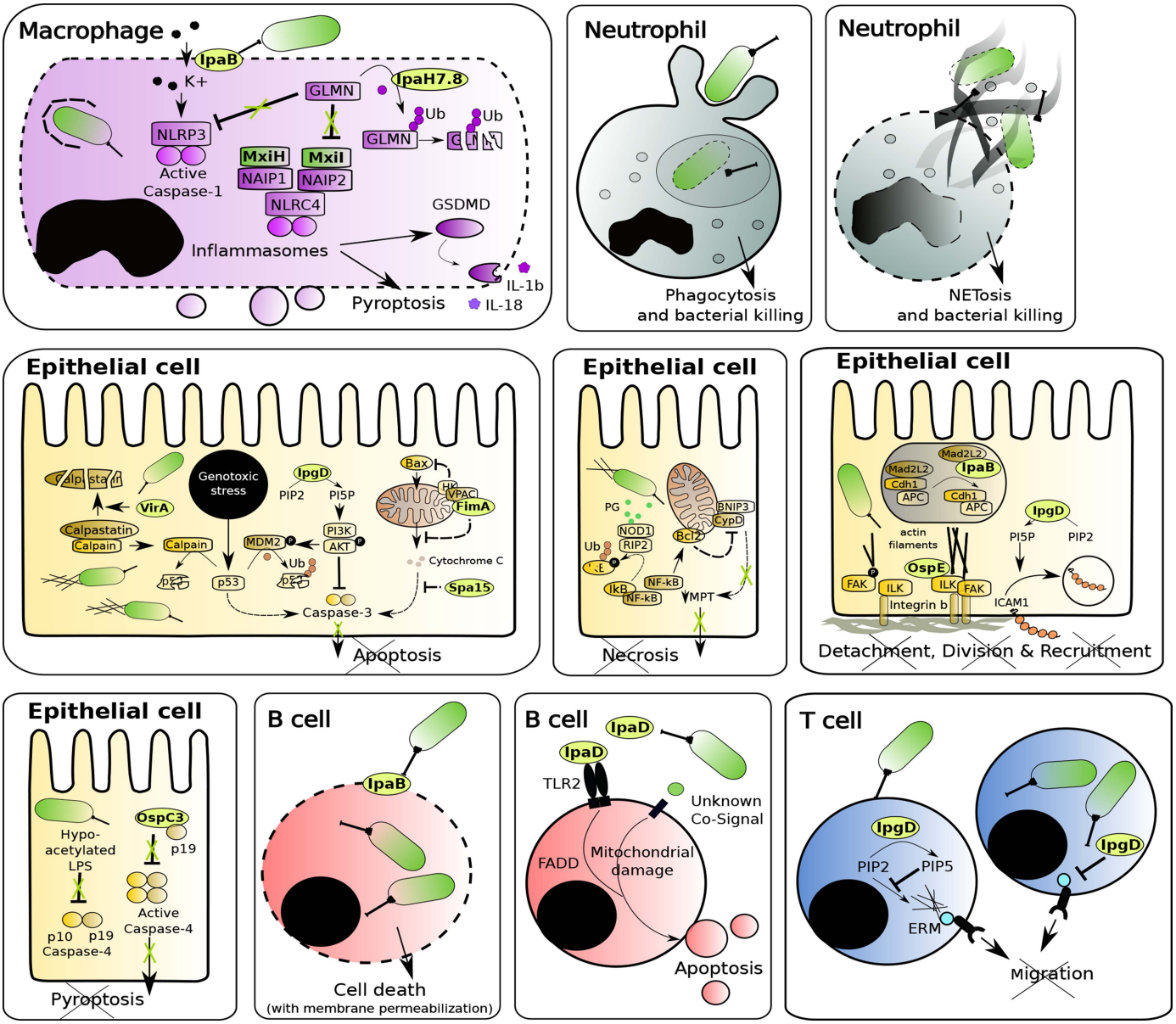


FIGURE 3 *Shigella* subversion of host cell survival, integrity, and function. *Shigella* produces numerous effectors that subvert various host cell processes to promote its virulence. Upon invasion of epithelial cells, numerous effectors function to protect the cytosolic replicative niche of *Shigella* by antagonizing host cell death (apoptosis, pyroptosis, and necrosis), promoting host cell integrity, and inhibiting the recruitment of neutrophils, which kill *Shigella*. Conversely, *Shigella* actively promotes host cell death in infected macrophages. For immune cells, *Shigella* mediates B cell death and the inhibition of T cell migration in infected and noninfected cells.

In *Shigella*-infected macrophages, pyroptotic host cell death requires phagosomal escape of *Shigella* and is mediated by the NLRC4 inflammasome, with some contribution from the NLRP3 inflammasome (Fig. 3) (47, 51). *Shigella* first escapes the phagosome with the help of the pore-forming activity of the IpaC-IpaB translocon at the tip of the T3SS. Pore formation by IpaB allows influx of potassium, and phagosomal rupture releases vacuolar contents and results in membrane damage that may be recognized and serves to activate the NLRC4 inflam-

masome (47). In addition, during cytosolic replication, the T3SS rod and needle proteins MxiH and MxiI are sensed by NAIP1 and NAIP2 of the NLR family and activate NAIP-dependent NLRC4 inflammasomes, triggering caspase-1 activation and pyroptosis (52–55). *Shigella* LPS is also sensed by human caspase-4, exacerbating the pro-death response.

Notably, *Shigella* is not an innocent bystander in triggering macrophage cell death but actively promotes caspase-1 activation and pyroptosis, as the T3SS-secreted E3 ubiquitin ligase IpaH7.8 was shown to ubiquitinate glomulin, an inhibitor of NALP3/NLRC4 inflammasome activity, and target it for degradation by host proteasomes (26). Loss of glomulin inhibition leads to inflammasome activation and pyroptosis. Notably, in the absence of IpaH7.8, intranasal challenge of mice with *Shigella* leads to a reduction in macrophage cell death, reduced IL-1β production, and less extensive colonization. Thus, although pyroptosis serves as an antimicrobial defense mechanism of the host, *Shigella* actively promotes it and, at least in the early stages of the infection, uses it to its advantage. Pyroptosis of infected macrophages releases *Shigella* near the basolateral side of the epithelium, which *Shigella* invades to reach its replicative niche, the epithelial cell cytosol.

ADHESION AND FORCED ENTRY INTO NONPHAGOCYTIC HOST CELLS

Shigella interacts with several host receptors to adhere to epithelial cells and to favor insertion of the T3SS tip complex, which is required for epithelial cell invasion. The needle complex components IpaB, IpaC, and IpaD can bind $\alpha_5\beta_1$ integrins, and their overexpression in Chinese hamster ovary cells increases invasion efficiency of *Shigella* in these cells (56). In addition, IpaB can bind to CD44, a hyaluronic acid receptor, to mediate entry. Expression of CD44 in host cells increases *Shigella* adhesion, while treatment of CD44-expressing cells with a monoclonal antibody directed against CD44 decreases *Shigella* invasion (57). Both $\alpha_5\beta_1$ integrins and CD44 are surface receptors that interact with the extracellular matrix and, as expected, are located on the basolateral side of polarized epithelial cells, supporting a role for these receptors in the basolateral invasion of *Shigella*. *Shigella* may also use IcsA-mediated adhesion to a yet-undefined receptor to facilitate entry during basolateral invasion of epithelial cells.

Unlike *L. monocytogenes*, which induces a zipper-type uptake into epithelial cells, *Shigella* uses a trigger mechanism that results in extensive ruffle formation at the cell surface. Ruffle formation and invasion are mediated by a combination of changes in membrane tension, host cell signaling, and hijacking of the host actin cytoskeleton, although other cytoskeletal components, such as intermediate filaments, are also implicated. Cellular actin cytoskeletal rearrangements are largely regulated through p21 Rho-family small GTPases, including Rho, Rac, and Cdc42 GTPases. These master regulators of actin dynamics are molecular switches. In their GTP-bound "on" state, they activate a range of host factors to promote actin dynamics. When the intrinsic GTPase activity of the small GTPases is stimulated by GTPase-activating proteins (GAPs), GTP is hydrolyzed to GDP and renders the small GTPase in its "off" state. The replacement of GDP with GTP is then promoted by guanine nucleotide exchange factors. Through this cycling between the GTP- and GDP-bound states, these small GTPases drive actin dynamics.

Overall, *Shigella* promotes ruffle formation and entry by stimulating actin cytoskeleton remodeling and actin polymerization through a complex but coordinated process involving numerous effectors, including IpaC, IpaA, VirA, IpgD, and the guanine nucleotide exchange factors IpgB1 and IpgB2. Upon insertion into the host membrane, the C terminus of IpaC faces the cytoplasm and directly binds to the intermediate filaments vimentin and keratin 18 (58). The IpaC-vimentin interaction is necessary for stable docking of *Shigella* with the host cell membrane and a requirement for the secretion of effectors (58). The IpaC C terminus also stimulates actin polymerization and actin remodeling through Cdc42, Rac1, and the activation of the tyrosine kinase Src (59). The role of these host factors has been addressed through targeted inhibition in host cells. Thus, inhibition of Cdc42 blocks *Shigella*-induced membrane extensions and inhibition of Rac activity interferes with lamellae formation, while inhibition of the Src pathway blocks cellular extensions and *Shigella* engulfment and reduces entry by a factor of 10. Src activation by IpaC leads to the phosphorylation and activation of the actin-binding protein cortactin, which in turn interacts with the Arp2/3 complex and the Src-related tyrosine kinase CrK to stimulate actin polymerization and actin remodeling around the entry site.

IpaA is also a critical effector for entry, as an IpaA deletion mutant exhibits a 10-fold entry defect (60). IpaA regulates formation of actin protrusions and counteracts the uncontrolled formation of microspike structures induced by IpaC by promoting actin filament depolymerization (60). IpaA directly binds vinculin, a cytosolic actin-binding protein that is enriched in focal adhesions and links integrin adhesion molecules to the actin cyto-

skeleton (61). Binding of vinculin to the C-terminal domain of IpaA enhances vinculin affinity for F-actin, thereby partially capping the barbed end of actin filaments to inhibit monomer addition and promoting F-actin depolymerization. At the same time, IpaA promotes actin cytoskeletal rearrangements by targeting β1 integrins and stimulating the GTPase activity of the small GTPase RhoA, rendering RhoA inactive. Through the ROCK (Rho-associated protein kinase)–myosin II pathway, this leads to stress fiber disassembly and the freeing up of actin for membrane ruffle formation.

IpgB1, like IpaC, stimulates Rac1 and Cdc42 activity and, by stabilizing them in their GTP-bound state, promotes ruffle formation in an Arp2/3 complex-dependent manner. In the absence of IpgB1, invasion is reduced twofold. In addition, VirA may indirectly destabilize microtubules to promote Rac1 activity (62, 63). In contrast to IpaA, IpgB2 stimulates actin nucleation and stress fiber formation by stimulating GDP-GTP exchange in RhoA. *Shigella* thereby targets stress fibers for disassembly and formation in a coordinated fashion to promote bacterial entry. Entry is also supported by the hydrolysis of phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2] by the effector IpgD. This activity loosens the connection between cortical actin and the membrane to facilitate actin dynamics at the invasion site (64).

Entry is furthermore dependent on local calcium signaling induced by *Shigella* (65). T3SS translocators, likely through their pore-forming activity, stimulate the activity of host phospholipases C-β1 and C-γ1, leading to the production of InsP3 through the hydrolysis of PIP2. A local increase in InsP3 at the invasion site and its binding to recruited InsP3 receptors on the endoplasmic reticulum (ER) leads to an atypical and highly localized Ca^{2+} response. Fluorescent recovery after photobleaching revealed that bacterially induced actin reorganization at the site of entry hinders diffusion of small molecules, thereby restricting diffusion of InsP3, enriching InsP3 receptors, and favoring sustained local Ca^{2+} increases that are of long (tens of seconds) duration, peaking 15 minutes after *Shigella* makes contact with the host cell (66). In addition, long-lasting local Ca^{2+} signals are promoted by hydrolysis of PI(4,5)P2 through IpgD, which effectively limits the substrate pool available to host phospholipases and thereby restricts the generation, and subsequent diffusion, of InsP3 (67). Low InsP3 diffusion curbs the release of Ca^{2+} stores from the ER, thereby also maintaining cell integrity by preventing Ca^{2+}-mediated disassembly of focal adhesions (67). These atypical local Ca^{2+} responses are critical for entry; more global Ca^{2+} responses with slower dynamics follow and, while not critical to entry, facilitate *Shigella* invasion by filopodial capture. *Shigella* mediates these atypical Ca^{2+} responses through IpgD.

PHAGOSOMAL ESCAPE OF *SHIGELLA FLEXNERI*

Shigella invasion of epithelial cells and phagosomal escape are difficult processes to investigate because they are rapid events that last less than 15 minutes. Phagosomal rupture is dependent on a functional T3SS and is facilitated by a number of *Shigella* effectors, including IpgB1, IpgB2, and IpgD, that each contribute a small amount to the efficiency of phagosomal lysis (68, 69). Small interfering RNA screens and advanced large-volume correlative light electron microscopy have recently yielded new insights into the invasion and phagosomal escape process of *Shigella* (68, 70). Despite the massive ruffling induced upon invasion, *Shigella* is found in a tight vacuole where the bacteria are in close contact with the phagosomal membrane. Ruffling leads to the formation of macropinosomes surrounding the entry site, but while macropinosomes take up fluid-phase markers, such as fluorescently labeled dextran, these markers are excluded from the *Shigella*-containing vacuole.

IpgD activity promotes ruffle formation and fosters the formation of macropinosomes and recruitment of the GTPase Rab11 to macropinosomes in the vicinity of bacterial entry (Fig. 4A) (70). Although Rab11 is not required for macropinosome formation, inhibition of Rab11 by expression of a dominant negative mutant delays vacuolar rupture and interferes with the entry process (70). How exactly Rab11 macropinosomes foster phagosomal lysis remains unclear. Rab11-positive macropinosomes come into direct contact with the *Shigella*-containing vacuole but do not fuse or deliver fluid-phase markers to the phagosome (68). In nonphagocytic cells, macropinosomes recycle back to the plasma membrane with minimal interaction with the endosomal pathway and are not known to recruit Rab11, a marker of the endosomal recycling compartment. *Shigella* thereby triggers macropinosome formation but through the action of IpgD initiates a novel vesicular trafficking pathway that is dependent on the formation of modified macropinosomes to mediate phagosomal lysis.

SURVIVAL OF *SHIGELLA FLEXNERI* IN EPITHELIAL CELLS

Shigella uses the epithelial host cell cytosol as its main replicative niche and therefore has evolved mechanisms to escape host antimicrobial defenses and to safeguard its

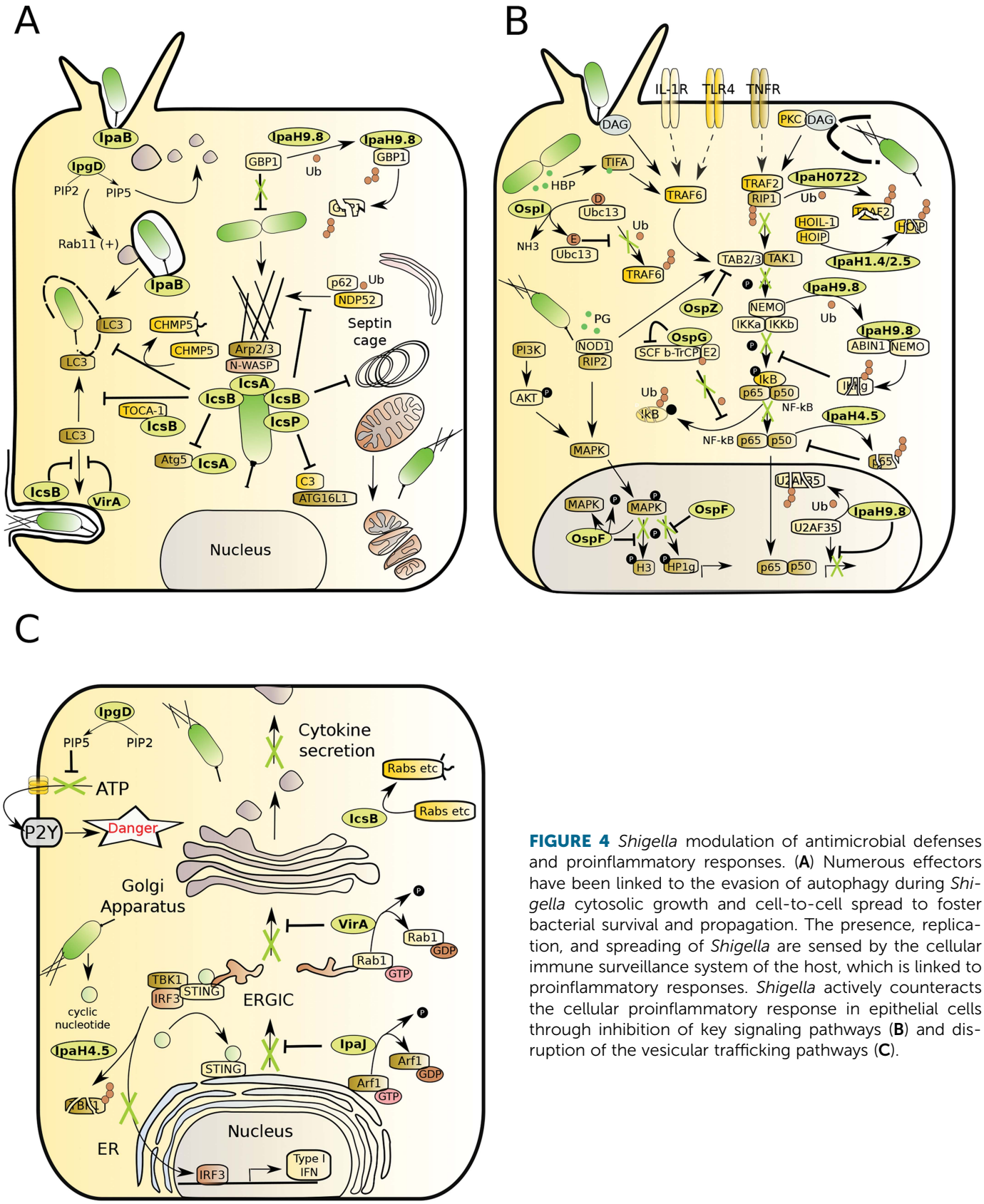


FIGURE 4 *Shigella* modulation of antimicrobial defenses and proinflammatory responses. (**A**) Numerous effectors have been linked to the evasion of autophagy during *Shigella* cytosolic growth and cell-to-cell spread to foster bacterial survival and propagation. The presence, replication, and spreading of *Shigella* are sensed by the cellular immune surveillance system of the host, which is linked to proinflammatory responses. *Shigella* actively counteracts the cellular proinflammatory response in epithelial cells through inhibition of key signaling pathways (**B**) and disruption of the vesicular trafficking pathways (**C**).

replicative niche from destruction. One major host defense mechanism that is activated and that *Shigella* must subvert in order to survive in host cells is autophagy (Fig. 4A) (71). Canonical autophagy involves around 40 autophagy-related proteins that act together in a multistep process to sequester cytoplasmic cargo, including large structures such as whole organelles, in newly generated double-membrane vacuoles. Autophagosomes mature along the endocytic pathway into autolysosomes, where lysosomal enzymes degrade their contents. The main function of this process is to recycle cellular components for the host cell and maintain cellular homeostasis.

A second critical role of autophagy is in cellular defense, whereby intracellular bacteria are targeted and delivered to autolysosomes for killing and degradation in a process termed xenophagy. During xenophagy, cytosolic bacteria are recognized by autophagy sensor proteins such as p62 and nuclear dot protein 52 (NDP52), which bind ubiquitinated proteins and the major autophagy marker LC3 to initiate autophagosome formation. Conversely, pathogens surrounded by phagosomal membranes may be targeted for lysosomal degradation by noncanonical autophagy that involves only a subset of the autophagy machinery, including the autophagy markers LC3 and Atg5. In this noncanonical pathway, phagosomes containing bacterial pathogens are distinguished by the host from endogenous vesicles through the detection of phagosomal membrane damage. Membrane damage associated with the insertion of the secretion system pores of the T3SS exposes the sugar-coated luminal side of the vacuole, which can be recognized by carbohydrate-binding proteins such as galectin-3 and galectin-8, leading to recruitment of autophagy-related proteins.

Shigella is susceptible to recognition by both the noncanonical and canonical autophagy pathways but counteracts autophagic capture through at least three effectors, IcsP, IcsB, and VirA (Fig. 4A). During initial entry, recognition of *Shigella* peptidoglycan by the cytosolic sensor NOD1 at the bacterial entry site leads to the recruitment of the autophagic factor ATG16L and stimulation of autophagy (72). In addition, *Shigella* induces a nutrient stress response that blocks mTOR signaling and leads to a stimulation of autophagy (73).

Before invasion, *Shigella* is susceptible to being recognized by the complement system, which leads to the coating of bacteria with split products of complement C3 (74). In the host cell cytosol, surface-deposited C3 targets bacteria for autophagy through a direct interaction with the autophagy marker ATG16L1 (74). *Shigella* subverts this process by the shedding of C3, in part, through the proteolytic activity of the surface protease IcsP (74). During invasion, phagosomal damage induced by the *Shigella* T3SS is recognized by galectin-3 and galectin-8, but *Shigella* escapes recruitment of the autophagic marker LC3 to the phagosome or phagosomal remnants by secreting IcsB (75). IcsB prevents LC3 and NDP52 targeting by binding to Toca-1 (transducer of CDC42-dependent actin assembly 1), a host protein necessary for efficient actin polymerization.

Biorthogonal chemical proteomic profiling, biochemical assays, and proteomic analysis using SILAC (stable isotope labelling with amino acids in cell culture) recently revealed IcsB to be an 18-carbon fatty acyltransferase catalyzing lysine N^{ε}-fatty acylation of up to 60 host proteins involved in the regulation of the actin cytoskeleton, membrane trafficking, cell-cell adherens junctions, endocytosis, and endosomal recycling (76). By stearoylating charged multivesicular body protein 5, a component of the ESCRT-III complex involved in endosomal membrane sorting, IcsB is thought to inhibit the development of the autophagic response (76).

Lysis of the phagosome allows *Shigella* access to cytosolic host factors, including those required for actin-based mobility, and enables *Shigella* to move within the cell and to ultimately reach new replicative niches in neighboring cells (77). Actin-based motility is mediated by surface-localized IcsA. IcsA acts as a mimic of the small GTPase Cdc42 to recruit neutral Wiskott-Aldrich syndrome protein, which binds actin monomers and activates the actin-related protein 2 (Arp2)–Arp3 complex to mediate actin polymerization. Arp2/Arp3 complex-mediated F-actin assembly and filament growth provide the propulsive force for *Shigella* to move in the cytosol, at speeds up to 26 μm/min, and induce membrane protrusions into neighboring cells for cell-to-cell spread. IcsP also mediates correct polar localization and cleavage of IcsA to facilitate directed motility and efficient spreading (78), which primarily occurs at multicellular junctions involving components of the clathrin endocytic pathway (79). By hijacking the host cell cytoskeletal machinery, *Shigella* gains motility in the cytosol without itself having to invest into metabolically costly motility machinery, such as flagella, which could also be recognized as a MAMP and activate the host NLRC4 inflammasome. However, while subversion of the host cytoskeleton is an evolutionarily conserved mechanism used by pathogens to gain intracellular motility, it also triggers autophagic defense mechanisms that the pathogen then must try to counter to survive and replicate (Fig. 4A).

One way the host tries to target cytosolic *Shigella* to autophagy is through septins (80). These GTP-binding proteins are part of the cytoskeleton network, as they can

form filaments that interact with cellular membranes, actin filaments, and microtubules (80). They are involved in several biological processes, including cell division, but also link the recognition of actin polymerization to the autophagic pathway. Septins are recruited to IcsA-mediated actin polymerization and form cages around cytosolic *Shigella*. Septin cages restrict *Shigella* movement and recruit the autophagic adaptors p62 and NDP52 to target *Shigella* for autophagic destruction (81). This process is antagonized by mitochondrial fragmentation through *Shigella* actin-mediated motility, as well as the effector IcsB, as wild-type cytosolic bacteria are only half as likely to show recruitment of these autophagic markers as an *icsB* mutant (82, 83). Notably, the evolutionary conservation of septin caging as an innate host defense that targets bacteria for autophagic destruction was demonstrated in a novel zebrafish *Shigella* infection model (84). In this model, as observed *in vitro* in mammalian cells, *Shigella* became trapped in p62-associated septin cages upon infection of the zebrafish, leading to p62 recruitment and autophagic restriction.

Besides the recognition of actin polymerization, the autophagic marker Atg5 directly recognizes the surface-localized protein IcsA. IcsB appears to antagonize this autophagic process by binding to IcsA, thereby masking the Atg5 recognition site and competitively inhibiting Atg5 binding to IcsA (83).

Shigella also uses the T3SS effector VirA to interfere with autophagy. Besides being important during the entry process (85), VirA counteracts autophagosome formation during cytosolic growth of *Shigella* by disrupting ER-to-Golgi vesicular trafficking. VirA activates the GTPase activity of the small GTPase Rab1, rendering Rab1 inactive and unable to direct vesicle traffic from the ER to the Golgi apparatus (86, 87). Finally, VirA and IcsB also have important functions in preventing autophagy by mediating phagosomal escape from the double-membrane phagosome formed during cell-to-cell spread. In the absence of VirA and/or IcsB, intercellular spreading is reduced as *Shigella* becomes impaired in escaping the secondary phagosome and becomes trapped in LC3-decorated phagosomes that mature to autolysosomes (88).

In addition to autophagy, *Shigella* replication and motility are further antagonized through guanylate-binding proteins (GBPs) (Fig. 4A) (89, 90). GBPs are induced by gamma interferon stimulation and mediate cell-autonomous antimicrobial defenses to a number of intracellular pathogens in a yet poorly characterized manner. However, during *Shigella* infection, IpaH9.8 specifically targets human GBP1 for ubiquitin-mediated degradation to promote *Shigella* replication and facilitate polymerization of host actin for actin-based motility.

MAINTENANCE OF THE EPITHELIAL CELL CYTOSOLIC REPLICATIVE NICHE

Aside from escaping the degradative autophagy system and spreading from cell to cell to extend its replicative niche, *Shigella* also needs to safeguard its cytosolic niche and uses several strategies to prevent host cell death (47). Since the infectious dose of *Shigella* can be as low as 10 to 100 bacteria, safeguarding its replicative niche is of particular importance early during the infection process in order to increase the bacterial load. *Shigella* evades host cell death by obstructing the activation of pro-death pathways and by activating pro-survival pathways (Fig. 3). The inhibition of epithelial cell death is impressive, as *Shigella* can replicate to high numbers (>100 bacteria) without lysing the epithelial cell, which is in stark contrast to the rapid cell death induced in macrophages.

As *Shigella* replicates in the epithelial cell cytosol, it is highly susceptible to recognition by cytosolic PRRs. One of the most abundant MAMPs, LPS, is recognized by human caspase-4 (caspase-11 in mice) and leads to pyroptotic host cell death. *Shigella*, however, limits recognition of its LPS during cytosolic growth by switching from a hexa-acylated LPS form, typically observed during growth in laboratory medium and highly stimulating for both TLR4 and caspase-11, to a tetra- and triacylated form, which is poorly recognized by these PRRs, upon cytosolic growth (91). In addition to the hypoacylation of its LPS, *Shigella* counteracts caspase-4 activity with the secretion of the late T3SS effector OspC3 (92). OspC3 binds to the p19 subunit of caspase-4 and thereby inhibits the assembly of the active caspase-4 tetramer, consisting of two p19 and two p10 subunits. In the absence of OspC3, *Shigella* infection leads to increased pyroptosis and enhanced IL-18 release by epithelial cells *in vitro* and, in the *in vivo* guinea pig colon infection model, to greater cell death of the colonic mucosa and a reduction in colonizing bacteria compared to wild-type *Shigella* infection.

Shigella infection of epithelial cells also leads to host DNA damage, or genotoxic stress, which triggers the proapoptotic p53 pathway (23) (Fig. 3). This pathway is antagonized by the delivery of VirA during entry into epithelial cells. VirA binds to calpastatin, the inhibitor of calpain protease, and targets it for degradation, resulting in the activation of calpain and calpain-mediated p53 degradation. However, while calpain activation limits apoptotic host cell death during the early phase of *Shigella* infection, calpain activation during later stages promotes

necrotic cell death. Strikingly, short hairpin RNA inhibition of calpain and live fluorescence video microscopy showed that in the absence of calpain, *Shigella* intracellular growth is impressively enhanced and cells resist lysis until the cells are literally bursting with bacteria (23). In addition to VirA, p53-mediated apoptosis is antagonized through the phosphoinositide phosphatase activity of IpgD. During bacterial entry, PI5P, liberated through IpgD activity on PI (4,5)P2, promotes the activation of the epidermal growth factor receptor and the downstream PI3-kinase/Akt pro-survival pathway (23, 93, 94). Akt activation inhibits p53 proapoptotic signaling through the phosphorylation and stabilization of Mdm2, an E3 ubiquitin ligase that, when activated, targets p53 for degradation.

The mitochondrion is another organelle affected by *Shigella*. Induction of mitochondrial permeability during infection releases cytochrome *c*, which is sensed by APAF-1, leading to the formation of the apoptosome, activation of caspase-9, and activation of the executioner caspase, caspase-3, resulting in apoptosis (95). *Shigella* antagonizes caspase-3-mediated apoptosis by FimA, Spa15m, and IpgD. FimA is a pilus protein that in the cytosol of infected cells antagonizes cytochrome *c* release and subsequent caspase-3 activation (96). By binding to the mitochondrial outer membrane protein VPAC, FimA strengthens the interaction of VPAC with hexokinase. This prevents hexokinase dissociation from the mitochondrial membrane, an event that triggers Bax translocation to the outer mitochondrial membrane, the formation of Bax/Bak mitochondrial outer membrane pores, loss of membrane integrity, and release of cytochrome *c*. Notably, however, *Shigella* does not have fimbriae, and FimA expression is questionable due to mutational inactivation across at least three phylogenetic groups (97).

Further down the pathway, *Shigella* inhibits caspase-3 activation in response to cytochrome *c* release and caspase-9 activation through the involvement of the T3SS chaperone Spa15, by a yet-unknown mechanism (98). In addition, secretion of the T3SS effector IpgD antagonizes caspase-3 activation again through the stimulation of the PI3-kinase/Akt pro-survival pathway (99). *Shigella* infection also leads to oxidative stress of the cell that triggers a mitochondrial permeability transition-dependent necrosis-like cell death through the signaling of the BH3-only protein BNIP3 and CypD (100). This cell death pathway is counterbalanced by the sensing of *Shigella* peptidoglycan and activation of the cytosolic PRR Nod1-mediated Rip2–IKKβ–NF-κB signaling cascade, which upregulates proinflammatory factors but also induces the expression of potent antiapoptotic factors, including Bcl-2. *Shigella* thereby uses the activation of pro-survival pathways to counteract necrotic and apoptotic cell death.

In addition to obstructing host cell death, *Shigella* also preserves its intracellular niche within the epithelial layer by promoting the adhesion of infected cells to the basement membrane and preventing cell division. To ensure adhesion, the *Shigella* T3SS effector OspE is recruited to focal adhesions, where it interacts with the integrin-like kinase ILK, reduces focal adhesion kinase phosphorylation, and increases surface β1 integrins (101). The OspE-ILK complex thereby promotes epithelial adhesion to the basal membrane by inhibiting focal adhesion disassembly (101, 102) (Fig. 3). To prevent division, and the accompanying reduction of adhesion to neighboring cells and the lamina propria, the *Shigella* effector IpaB can mediate cell cycle arrest by interfering with the binding of the anaphase-promoting complex inhibitor Mad2L2 to Cdh1 (103), which is part of the anaphase-promoting complex and is involved in preventing mitosis by suppressing mitotic cyclins needed to enter mitosis. Lack of binding of Mad2L2 to Cdh1 keeps Cdh1 constitutively active and prevents cells from undergoing mitosis. Additionally, the phosphatase activity of IpgD that generates PI5P favors the internalization and subsequent degradation of ICAM-1 in infected epithelial cells, thereby reducing the adhesion of neutrophils and the inflammation and tissue destruction associated with their recruitment (104).

Through these multiple mechanisms, *Shigella* safeguards its replicative niche and ensures sufficient time to replicate extensively in the host cell and to spread to neighboring cells.

REPLICATION OF *SHIGELLA* IN THE EPITHELIAL CELL CYTOSOL

Unlike intravacuolar pathogens, such as *Salmonella*, which need strategies to acquire nutrients in the phagosomal compartment and to subvert the endomembrane system of the host to prevent the maturation of the phagosome into hostile and degradative lysosomal compartments, *Shigella* enters and replicates in what is considered a relatively nutrient-rich environment of the cytosol. Transcriptional profiling and global proteomic analysis of *Shigella* during cytosolic growth have given a good overview of the metabolic changes that accompany *Shigella* adaptation to the cytosolic replicative niche. In contrast to its extracellular growth, *Shigella* adapts to the low-oxygen environment of the epithelial cytosol by downregulating enzymes involved in oxidative respiration and increasing expression of enzymes involved in glycolysis and mixed-acid fermentation (105). *Shigella*

also increases fructose and mannose importers as well as the dipeptide transporter DppA to scavenge nutrients from the host. Transcriptional profiling also suggests that the cytosolic environment of epithelial cells is not a particularly stressful environment as, at least in the *in vitro* system, *Shigella* does not upregulate stress response proteins, such as nitric oxide dioxygenase, during cytosolic growth compared to growth in broth (105).

A notable exception is iron stress. Due to the inherent toxicity of iron, the host limits intracellular iron concentrations and sequesters free iron by expressing iron-binding proteins. Iron is an essential micronutrient for *Shigella* growth, and *Shigella* can capture intracellular iron through multiple routes, including the expression of siderophores and uptake of heme or ferric and ferrous iron through dedicated iron transport systems, which *Shigella* upregulates in response to the low iron accessibility in the cytosolic milieu (106). However, overall, nutrients are not limiting for *Shigella* growth in the host cell cytosol, as *Shigella* maintains an extremely high growth rate during cytosolic growth, doubling around every 37 minutes, similar to what is seen in rich nutrient broth (107). Analysis of auxotrophic mutants of *Shigella* also indicates that *Shigella* has access to diverse amino acids in sufficient quantities, while availability of host fatty acids, purine nucleosides, and some amino acids, such as asparagine and proline, is limiting.

While *Shigella* obtains a substantial amount of biomass through uptake of nutrients, metabolite mass spectrometry using ^{13}C glucose isotype tracking revealed that *Shigella* derives its energy almost exclusively from pyruvate during cytosolic growth in HeLa cells (107). Pyruvate is a particularly abundant metabolite in HeLa cells, as these cancer cells mostly rely on glycolytic pathways rather than the oxidative tricarboxylic acid cycle for energy production. *Shigella* metabolizes pyruvate into acetate using the low-yield but high-speed acetate pathway. The acetate pathway is a typical pathway used during rapid growth under nutrient-rich conditions in *Enterobacteriaceae* because although it produces only one ATP per pyruvate molecule, it also uses only two enzymes and can therefore rapidly generate energy to sustain rapid growth. Infected host cells are therefore not compromised for energy output, as *Shigella* infection does not affect the host's main energy pathways. Rather, *Shigella* efficiently uses the waste product of the host energy production to power its replication.

Shigella infection of epithelial cells does, however, activate metabolic stress responses, as metabolic labeling of *de novo* protein synthesis shows a global shutdown of host protein synthesis, particularly early within the first two hours during infection (108). *Shigella* infection triggers the host amino acid starvation response whereby activation of the sensor kinase GCN2 (general control nonderepressible 2) leads to downstream phosphorylation of the master regulator eIF2α (eukaryotic initiation factor 2α) (73). Phosphorylated eIF2α blocks translation initiation on a global scale while selectively enhancing translation of the transcription factor ATF4, which together with ATF3 enhances transcription of stress-related genes involved in amino acid metabolism and protection against oxidative damage. Nutrient limitation is also sensed through the mechanistic target of rapamycin (mTOR) pathway and leads to its inhibition. Inhibition of the mTOR pathway during *Shigella* infection stimulates autophagy and contributes to the general inhibition of translation. To support the metabolic needs of its rapid replication, *Shigella* may benefit from the amino acid resources made available through the reduction in overall host translation and stress-induced stimulation of amino acid metabolism. However, *Shigella* also actively antagonizes the downregulation of the mTOR pathway through the secretion of the T3SS effector OspB (109). OspB acts early during infection by turning on the mTOR complex 1 through the scaffolding protein IQ motif-containing GAP1, possibly to prevent the activation of the autophagy pathway.

SHIGELLA-MEDIATED SUBVERSION OF HOST INFLAMMATORY RESPONSES

While inflammation can support *Shigella* virulence early during infection by facilitating *Shigella* invasion of the colonic epithelium, *Shigella* has devised multiple strategies to limit the inflammatory response of infected epithelial cells to promote its virulence. This includes targeted inhibition of factors involved in proinflammatory pathways, interference in transcriptional activation of proinflammatory cytokines, inhibition of the endomembrane system required for cytokine secretion, and prevention of the release of endogenous danger signals (Fig. 4) (12).

Epithelial cells, similar to professional immune cells, are alerted to the presence of microbes through sensing of conserved MAMPs by various PRRs. Sensing of bacterial compounds in the extracellular environment is largely mediated by the membrane-anchored Toll-like receptor (TLR) family, while cytosolic sensing is mediated by cytosolic PRRs, including NOD1 and -2 and the family of NLRs. The host response to PRR activation depends on the type of PRR activated, its location, and the danger it represents (110). Thus, CpG activation of TLR9 on the apical side of differentiated epithelial cells leads to a

cytoprotective and tolerogenic host response, while engagement of TLR9 located at the basolateral side leads to expression of proinflammatory genes, as it signals a breach in the epithelial barrier (111). In addition, sensing of the same MAMP by different receptors can lead to different host responses. For example, while sensing of extracellular LPS by membrane-bound TLR4 triggers the activation of two major proinflammatory pathways, NF-κB and MAPKs, cytosolic LPS is sensed by caspase-11 (and caspase-4 in humans), which leads to gasdermin D-mediated host cell lysis, potentially because the presence of a pathogen in the cytosol is a greater threat to the cell than sensing of the microbe extracellularly.

Extracellular and intracellular sensing of MAMPs is also intimately linked to the cellular response (112). Thus, TLR sensing, through for example TLR-3 or -4, activates the NF-κB pathway and leads to transcriptional upregulation and synthesis of IL-1β, IL-18, and caspase-1. Under these circumstances, whereby the host cell is first primed, activation of caspase-11 leads to pyroptotic host cell death, as gasdermin D-mediated membrane damage is sensed by the NLRP3 inflammasome and leads to caspase-1 activation and proteolytic activation of the proinflammatory cytokines IL-1β and IL-18.

The NF-κB and MAPK pathway are two major proinflammatory pathways activated by epithelial cells in response to PRR recognition, danger sensing, and cytokine stimulation. Both pathways use the activation of presynthesized transcriptional activators, allowing a rapid transcriptional response to harmful stimuli. The MAPK pathway involves the activation of MAPKs ERK, JNK, and p38 through a phosphorelay system of upstream kinases and the downstream activation of transcription factors. Conversely, the transcription factor NF-κB, composed of a p50 and p65 subunit in its canonical state, is maintained in an inactive state in the cytoplasm through direct interaction with the inhibitor IκBα. Phosphorylation of IκBα by the upstream IκB kinase (IKK), composed of the master regulator NEMO (also called IKKγ) and the catalytic heterodimer IKKα and IKKβ, targets IκBα for ubiquitination and degradation by host proteasomes, releasing NF-κB from its inhibition and freeing it to move into the nucleus to drive transcription of target genes.

Early during *Shigella* invasion, recognition of the bacterial MAMP diaminopimelic acid-containing peptidoglycan fragments by the PRR NOD1 drives a robust inflammatory response through the activation of the JNK MAPK pathway and a RIP2-dependent NF-κB activation (113, 114). NOD1-independent activation of NF-κB also occurs in response to sensing of DAMPs generated during *Shigella* invasion and phagosomal escape. Notably, later during infection, active replication of intracellular *Shigella* is sensed through the TRAF-interacting forkhead-associated protein A (TIFA)-dependent cytosolic surveillance pathway (Fig. 4B). The TIFA pathway senses cytosolic levels of the bacterial metabolite heptose-1,7-bisphosphate, a MAMP specific to Gram-negative-specific bacteria, leading to TIFA oligomerization, recruitment, and activation of the E3 ubiquitin ligase TRAF6 and NF-κB (115).

Attesting to the central role of NF-κB in host defense, *Shigella* employs at least nine effectors to inhibit the NF-κB pathway. *Shigella* effectors inhibit steps upstream of IKK activation, at the level of IKK and the NF-κB complex, and at the level of NF-κB transcription factor trafficking and target promoter binding, as well as downstream of NF-κB-mediated transcription (Fig. 4B).

During *Shigella* entry, the accumulation and liberation of diacylglycerol at the entry site are sensed as a danger signal and lead to TRAF6-dependent activation of NF-κB signaling. This pathway is inhibited by OspI, a glutamine deaminase, which targets UBC13, an E2 ubiquitin-conjugating enzyme (116, 117). Binding of OspI to UBC13-ubiquitin leads to deamination of UBC13 at Gln-100 and inactivation of its E2 activity. This prevents ubiquitination of TRAF6, which is necessary for the activation of NF-κB signaling. Lysis of the phagosomal membrane is also sensed by the host cell and leads to activation of the protein kinase C pathway, which activates NF-κB through TRAF2. This pathway is antagonized by IpaH0722, which targets TRAF2 for degradation by host proteasomes and thereby damps NF-κB activation (118). In addition, IpaH1.4 and IpaH2.5 ubiquitinate and target for proteasome degradation HOIP, a component of the linear ubiquitin chain assembly complex that generates Met1-linked linear ubiquitin chains involved in activating NF-κB signaling downstream of TRAF2 signaling (119).

The methyltransferase OspZ interferes with NF-κB signaling by modifying the zinc finger cysteines of TAK1-binding proteins 2 and 3 in order to prevent ubiquitin chain binding and subsequent NF-κB activation (120, 121). Conversely, IpaH9.8 is particularly important for blocking NF-κB activation through Nod1 sensing of *Shigella* peptidoglycan fragments. IpaH9.8 targets NEMO for degradation through its interaction with the IKK complex and ABIN-1, a ubiquitin-binding adaptor protein that further promotes NEMO degradation by stimulating NEMO polyubiquitination (122). Acting at the level of the NF-κB complex, the secreted kinase OspG prevents ubiquitination and subsequent degradation of phosphorylated IκBα by inhibiting the E3 ubiquitin ligase $SCF^{b\text{-}TrCP}$ through its interaction with the ubiquitinated

E2 ubiquitin-conjugating enzyme, which is part of the $SCF^{b\text{-}TrCP}$ complex (123). OspG thereby inhibts NF-κB activation within the first hour of infection. In addition, IpaH4.5 interferes with NF-κB activation by targeting the p64 subunit of the NF-κB complex for ubiquitination and subsequent degradation (124).

In the nucleus, NF-κB-dependent and -independent transcription of proinflammatory genes is inhibited through several mechanisms involving OspF. OspF has a phosphothreonine lyase activity, a rare enzymatic activity that irreversibly dephosphorylates threonine through beta elimination (125). OspF specifically targets and inactivates MAPKs (Erk1/2 and p38), thereby preventing transcription of MAPK-dependent proinflammatory genes (126). OspF-mediated MAPK inactivation also inhibits NF-κB -mediated transcriptional activation through at least two epigenetic modifications downstream of MAPK activation. OspF indirectly reduces phosphorylation of histone H3 (H3pS10) and heterochromatin protein 1γ (HP1γS83) at proinflammatory target genes, including the IL-8 gene (127). Reduced phosphorylation of histone H3 and HP1γ promotes chromatin condensation and a transcriptionally inactive chromatin state, thereby preventing access of NF-κB to the gene promoters, leading to repression of proinflammatory-cytokine transcription. Notably, phosphoproteomics showed that OspF affects the phosphorylation status of several hundred host proteins, highlighting the extensive effect a single effector can have, directly and indirectly, on host signaling networks (128). In addition, IpaH9.8 interferes with proinflammatory gene activation at the posttranscriptional level (129). After nuclear translocation, IpaH9.8 interacts with the mRNA splicing factor $U2AF^{35}$ and inhibits $U2AF^{35}$-mediated splicing reactions, resulting in reduced proinflammatory gene transcripts.

Vesicular trafficking, required for cytokine secretion, is also subverted by *Shigella* with the injection of the effectors IpaJ and VirA, and likely IcsB, as IcsB targets and modifies numerous host proteins involved in vesicular trafficking (76) (Fig. 4C). Vesicular trafficking is regulated by small GTPases of the Ras superfamily, such as Rabs and ADP-ribosylation factors (ARFs). These GTPases regulate successive steps in endomembrane trafficking from the ER to the Golgi apparatus and beyond, as well as endocytic trafficking, by mediating the formation, trafficking, and docking of vesicles from donor membranes to specific target membranes. IpaJ is a cysteine protease that preferentially cleaves *N*-myristoylated proteins but specifically targets ARFs, and notably ARF1, during infection. IpaJ cleavage of the myristoyl moiety from ARF1 irreversibly releases ARF1 from the Golgi membrane, inhibiting vesicular trafficking from the ER to the ER-Golgi intermediate compartments (ERGIC) (130). Conversely, as a GAP, VirA can interact with and inactivate many Rab GTPases, but through its preferred activity on Rab1, which localizes on the ERGIC, it mainly disrupts vesicular trafficking from the ERGIC to the Golgi (87). The activity of IpaJ, and to a lesser degree VirA, thereby leads to a striking fragmentation of the Golgi membrane.

Functionally, the disruption of vesicular trafficking during *Shigella* infection inhibits the general secretory pathway used to secrete cytokines and also interferes with the innate immune sensor STING (87, 130–132). STING senses the cytosolic presence of cyclic dinucleotides released from intracellular bacteria or generated from cytosolic double-stranded DNA by the nucleotidyltransferase cyclic GMP-AMP synthase. Recognition of cyclic nucleotides by the ER transmembrane protein STING triggers its activation and relocation from the ER to the ERGIC, where it recruits TBK1 and the transcription factor IRF3 to initiate IRF3-mediated transcriptional activation of an antiviral interferon response. By interfering with ER-to-ERGIC vesicular trafficking, IpaJ effectively blocks type I interferon induction by STING in response to *Shigella* infection (131). In addition, *Shigella* interferes with TBK1-mediated immune activation with its effector IpaH4.5, which targets TBK1 for ubiquitination and proteasomal degradation (133).

A separate mechanism used by *Shigella* to limit inflammation is preventing the release of ATP by connexin hemichannels. Connexin hemichannels are gated pores formed at the plasma membrane through the assembly of six connexins into a ring structure. Connexin channels allow the flow of ions and signaling molecules either to the extracellular milieu in the case of hemichannels or between neighboring cells through the stacking of hemichannels at sites of cell-cell contact. Release of ATP into the extracellular environment by connexin hemichannels is sensed by the host as a danger signal and promotes inflammatory responses by binding to purinergic receptors on both professional and nonprofessional immune cells. The rapid release of cellular ATP, the ubiquitous expression of ATP-sensing receptors, and a positive feedback loop of ATP release upon ATP sensing make extracellular ATP a highly potent immune mediator that rapidly amplifies the response to a local cellular insult.

Downstream signaling to ATP depends on the cell type, but in addition to enhancing filopodium formation, it includes phagocytosis and motility, the activation of NF-κB signaling, expression of adhesion molecules,

induction of proinflammatory mediators, and activation of the NLRP3 inflammasome. ATP release is a common response of epithelial cells to challenge with enteric pathogens, but *Shigella* subverts this innate immune response and limits inflammation at the early stages of infection by injecting the T3SS effector IpgD (134). IpgD phosphatase activity on PI(4,5)P2 increases the levels of PI5P, which blocks hemichannel opening and ATP release in a poorly understood manner. However, as PI5P is a relatively rare lipid in the host cell, small changes in its cellular abundance may have a rapid and large impact.

The multiple mechanisms used by *Shigella* and the many host pathways targeted during infection of epithelial cells to limit inflammation clearly demonstrate the crucial role innate immune evasion plays in promoting infection.

SHIGELLA SUBVERTS HOST ADAPTIVE IMMUNITY

In recent years, evasion of acquired immune cell responses and function has become an exciting new area of investigation, driven by new methods and applications of new technologies. The main portal of entry for *Shigella* during its crossing of the epithelial barrier is the specialized lymphoid follicle-associated epithelium, bringing *Shigella* into early contact with both innate and acquired immune cells. At the same time, *Shigella* can migrate to the draining mesenteric lymph nodes using the host lymphatic system. In these secondary lymphoid tissues, *Shigella* encounters not only macrophages but also dendritic cells (DCs), B cells, and T cells. DCs are specialized antigen-presenting cells that stimulate T cell responses, while both DCs and T cells support B cell responses, including memory B cell responses. Shigellosis is characterized by massive T and B cell death in rectal biopsy samples and by poor long-lived B cell immunity, requiring multiple infections for the host to become more resistant to infection. *Shigella* is therefore effective in inhibiting the generation of long-lived immunity and likely does this through several mechanisms.

Shigella interactions with DCs are similar to those of macrophages, whereby invasion leads to pyroptotic host cell death, limiting DC-mediated immune activation, while the interaction of *Shigella* with B and T cells has only recently been highlighted. In *in vivo* models, *Shigella* has been demonstrated to invade both T and B cells, while *in vitro*, *Shigella* is capable of invading B cells and activated, but not unactivated, T cells (44, 135, 136). Real-time two-photon microscopy of popliteal lymph nodes after footpad inoculation with *Shigella* showed that *Shigella* invasion of $CD4^+$ T cells arrested T cell migration, while T cell mobility patterns were also dramatically affected independently of *Shigella* invasion (136).

Mechanistic insights were unraveled *in vitro*, where *Shigella* inhibited T cell motility towards a chemokine attractant in an IpgD-dependent manner (135). IpgD injected into T cells hydrolyzed PIP2 and reduced the pool of PIP2 at the plasma membrane, leading to a reduction in phosphorylated active ezrin, radixin, and myosin proteins, which are important for the early steps of cell cortex organization during T cell polarization in response to a chemokine stimulus, effectively impairing T cell function (Fig. 3). Notably, a FACS (fluorescence-activated cell sorting)-based analysis of a FRET (fluorescence resonance energy transfer) pair-based approach for monitoring effector injection revealed that IpgD can be injected into activated T cells and can inhibit T cell migration in the absence of invasion (135). In this assay, activated T cells are loaded with the FRET pair CCF4 and then challenged with *Shigella* in the presence of cytochalasin D, a drug that inhibits actin polymerization and prevents invasion. T3SS-dependent injection of an IpgD–β-lactamase fusion construct into T cells is then monitored through the shift in fluorescent emission upon β-lactamase cleavage of CCF4 in the host cytosol. Inhibition of T cell migration may interfere with T-cell-mediated immunity by preventing T cell interactions with DCs and B cells and thereby preventing priming of an effective protective response. Notably, improvements in the sensitivity of this *in vitro* reporter system, coupled to the use single-cell FACS imaging, suggests that injection of effectors in the absence of invasion is the main mechanism *Shigella* uses to target T cells (137).

Conversely, *Shigella* interactions with B cells lead to both necrotic and apoptotic cell death depending on the interaction pattern (44). While invasion of B cells leads to necrotic cell death, noninfected B cells primarily undergo apoptosis. During apoptosis, B cells are first sensitized via a yet-unidentified bacterial cosignal, leading to upregulation of TLR2, which recognizes the *Shigella* translocator protein IpaD, a novel TLR2 ligand, and triggers apoptosis. Induction of B cell death in both invaded and noninvaded cells antagonizes antibody-mediated immune responses and likely contributes to the poor priming of B cell immunity to *Shigella* infection.

THE COMPLEXITY OF *SHIGELLA* PATHOGENESIS

The pathogenesis of *Shigella* is a highly complex and multifaceted process at the tissue, cellular, and molecular

levels, underscored by the multitude of effectors *Shigella* uses to subvert host processes. However, the context of timing and cell specificity are also important considerations. Thus, *Shigella* benefits from inflammation early during infection and actively promotes the pro-inflammatory pyroptosis of macrophages while using a multitude of effectors to damp the host inflammatory responses in epithelial cells. Having evolved to utilize the host cell cytosol as its main replicative niche, *Shigella* targets numerous host cell processes to ensure its own survival and the integrity of the infected cell. These host cell processes may be targeted by a single effector or by many, and an effector may have one or multiple cellular targets or an enzymatic activity that can affect a wide range of processes. In addition, while most investigations have centered on elucidating effector function in infected cells, recent advances in our understanding of interaction of *Shigella* with cells of the adaptive immune system have already highlighted the role that some *Shigella* effectors can play independently of cell invasion to subvert host cell function. This new "kiss and run" model adds an additional layer of complexity to the study of *Shigella* pathogenesis and, like many other areas of *Shigella* pathogenesis, has strongly benefited from the development of novel methods and new techniques.

ACKNOWLEDGMENTS

We apologize to investigators whose work on *Shigella* pathogenesis was not included in the review or only cited through other reviews due to space limitations. We thank Mariana Ferrari and Nathalie Sauvonnet for critical reading of the manuscript. Work in the group of P.S. is supported by Gates Foundation Grand Challenge grant OPP1141322, while P.J.S. is a Howard Hughes International Scholar and supported by the ERC Advance grant DECRYPT (339579).

REFERENCES

1. Trofa AF, Ueno-Olsen H, Oiwa R, Yoshikawa M. 1999. Dr. Kiyoshi Shiga: discoverer of the dysentery bacillus. *Clin Infect Dis* **29:**1303–1306 http://dx.doi.org/10.1086/313437.

2. Anderson M, Sansonetti PJ, Marteyn BS. 2016. Shigella diversity and changing landscape: insights for the twenty-first century. *Front Cell Infect Microbiol* **6:**45 http://dx.doi.org/10.3389/fcimb.2016.00045.

3. Schroeder GN, Hilbi H. 2008. Molecular pathogenesis of Shigella spp.: controlling host cell signaling, invasion, and death by type III secretion. *Clin Microbiol Rev* **21:**134–156 http://dx.doi.org/10.1128/CMR.00032-07.

4. Muthuirulandi Sethuvel DP, Devanga Ragupathi NK, Anandan S, Veeraraghavan B. 2017. Update on: *Shigella* new serogroups/serotypes and their antimicrobial resistance. *Lett Appl Microbiol* **64:**8–18 http://dx.doi.org/10.1111/lam.12690.

5. Kotloff KL, Riddle MS, Platts-Mills JA, Pavlinac P, Zaidi AKM. 2018. Shigellosis. *Lancet* **391:**801–812 http://dx.doi.org/10.1016/S0140-6736(17)33296-8.

6. Hosangadi D, Smith PG, Giersing BK. 2017. Considerations for using ETEC and *Shigella* disease burden estimates to guide vaccine development strategy. *Vaccine* S0264-410X(17)31343-9.

7. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, Wu Y, Sow SO, Sur D, Breiman RF, Faruque AS, Zaidi AK, Saha D, Alonso PL, Tamboura B, Sanogo D, Onwuchekwa U, Manna B, Ramamurthy T, Kanungo S, Ochieng JB, Omore R, Oundo JO, Hossain A, Das SK, Ahmed S, Qureshi S, Quadri F, Adegbola RA, Antonio M, Hossain MJ, Akinsola A, Mandomando I, Nhampossa T, Acácio S, Biswas K, O'Reilly CE, Mintz ED, Berkeley LY, Muhsen K, Sommerfelt H, Robins-Browne RM, Levine MM. 2013. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet* **382:**209–222 http://dx.doi.org/10.1016/S0140-6736(13)60844-2.

8. Kasper CA, Sorg I, Schmutz C, Tschon T, Wischnewski H, Kim ML, Arrieumerlou C. 2010. Cell-cell propagation of NF-κB transcription factor and MAP kinase activation amplifies innate immunity against bacterial infection. *Immunity* **33:**804–816 http://dx.doi.org/10.1016/j.immuni.2010.10.015.

9. Raqib R, Ekberg C, Sharkar P, Bardhan PK, Zychlinsky A, Sansonetti PJ, Andersson J. 2002. Apoptosis in acute shigellosis is associated with increased production of Fas/Fas ligand, perforin, caspase-1, and caspase-3 but reduced production of Bcl-2 and interleukin-2. *Infect Immun* **70:**3199–3207 http://dx.doi.org/10.1128/IAI.70.6.3199-3207.2002.

10. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A. 2004. Neutrophil extracellular traps kill bacteria. *Science* **303:**1532–1535 http://dx.doi.org/10.1126/science.1092385.

11. Parsot C. 2009. Shigella type III secretion effectors: how, where, when, for what purposes? *Curr Opin Microbiol* **12:**110–116 http://dx.doi.org/10.1016/j.mib.2008.12.002.

12. Mattock E, Blocker AJ. 2017. How do the virulence factors of *Shigella* work together to cause disease? *Front Cell Infect Microbiol* **7:**64 http://dx.doi.org/10.3389/fcimb.2017.00064.

13. Demers J-P, Habenstein B, Loquet A, Kumar Vasa S, Giller K, Becker S, Baker D, Lange A, Sgourakis NG. 2014. High-resolution structure of the *Shigella* type-III secretion needle by solid-state NMR and cryo-electron microscopy. *Nat Commun* **5:**4976 http://dx.doi.org/10.1038/ncomms5976.

14. Dohlich K, Zumsteg AB, Goosmann C, Kolbe M. 2014. A substrate-fusion protein is trapped inside the type III secretion system channel in *Shigella flexneri*. *PLoS Pathog* **10:**e1003881 http://dx.doi.org/10.1371/journal.ppat.1003881.

15. Epler CR, Dickenson NE, Bullitt E, Picking WL. 2012. Ultrastructural analysis of IpaD at the tip of the nascent MxiH type III secretion apparatus of *Shigella flexneri*. *J Mol Biol* **420:**29–39 http://dx.doi.org/10.1016/j.jmb.2012.03.025.

16. Barta ML, Guragain M, Adam P, Dickenson NE, Patil M, Geisbrecht BV, Picking WL, Picking WD. 2012. Identification of the bile salt binding site on IpaD from *Shigella flexneri* and the influence of ligand binding on IpaD structure. *Proteins* **80:**935–945 http://dx.doi.org/10.1002/prot.23251.

17. van der Goot FG, Tran Van Nhieu G, Allaoui A, Sansonetti P, Lafont F. 2004. Rafts can trigger contact-mediated secretion of bacterial effectors via a lipid-based mechanism. *J Biol Chem* **279:**47792–47798 http://dx.doi.org/10.1074/jbc.M406824200.

18. Hayward RD, Cain RJ, McGhie EJ, Phillips N, Garner MJ, Koronakis V. 2005. Cholesterol binding by the bacterial type III translocon is essential for virulence effector delivery into mammalian cells. *Mol Microbiol* **56:**590–603 http://dx.doi.org/10.1111/j.1365-2958.2005.04568.x.

19. Roehrich AD, Bordignon E, Mode S, Shen D-K, Liu X, Pain M, Murillo I, Martinez-Argudo I, Sessions RB, Blocker AJ. 2017. Steps for *Shigella* gatekeeper protein MxiC function in hierarchical type III secretion regulation. *J Biol Chem* **292:**1705–1723 http://dx.doi.org/10.1074/jbc.M116.746826.

20. Parsot C, Ageron E, Penno C, Mavris M, Jamoussi K, d'Hauteville H, Sansonetti P, Demers B. 2005. A secreted anti-activator, OspD1, and its

chaperone, Spa15, are involved in the control of transcription by the type III secretion apparatus activity in *Shigella flexneri*. *Mol Microbiol* **56:**1627–1635 http://dx.doi.org/10.1111/j.1365-2958.2005.04645.x.

21. Campbell-Valois F-X, Schnupf P, Nigro G, Sachse M, Sansonetti PJ, Parsot C. 2014. A fluorescent reporter reveals on/off regulation of the *Shigella* type III secretion apparatus during entry and cell-to-cell spread. *Cell Host Microbe* **15:**177–189 http://dx.doi.org/10.1016/j.chom.2014.01.005.

22. Mou X, Souter S, Du J, Reeves AZ, Lesser CF. 2018. Synthetic bottom-up approach reveals the complex interplay of *Shigella* effectors in regulation of epithelial cell death. *Proc Natl Acad Sci USA* **115:**6452–6457 http://dx.doi.org/10.1073/pnas.1801310115.

23. Bergounioux J, Elisee R, Prunier A-L, Donnadieu F, Sperandio B, Sansonetti P, Arbibe L. 2012. Calpain activation by the *Shigella flexneri* effector VirA regulates key steps in the formation and life of the bacterium's epithelial niche. *Cell Host Microbe* **11:**240–252 http://dx.doi.org/10.1016/j.chom.2012.01.013.

24. Ashida H, Sasakawa C. 2016. *Shigella* IpaH family effectors as a versatile model for studying pathogenic bacteria. *Front Cell Infect Microbiol* **5:**100 http://dx.doi.org/10.3389/fcimb.2015.00100.

25. Rohde JR, Breitkreutz A, Chenal A, Sansonetti PJ, Parsot C. 2007. Type III secretion effectors of the IpaH family are E3 ubiquitin ligases. *Cell Host Microbe* **1:**77–83 http://dx.doi.org/10.1016/j.chom.2007.02.002.

26. Ashida H, Sasakawa C. 2017. Bacterial E3 ligase effectors exploit host ubiquitin systems. *Curr Opin Microbiol* **35:**16–22 http://dx.doi.org/10.1016/j.mib.2016.11.001.

27. Martino MC, Rossi G, Martini I, Tattoli I, Chiavolini D, Phalipon A, Sansonetti PJ, Bernardini ML. 2005. Mucosal lymphoid infiltrate dominates colonic pathological changes in murine experimental shigellosis. *J Infect Dis* **192:**136–148 http://dx.doi.org/10.1086/430740.

28. Anderson MC, Vonaesch P, Saffarian A, Marteyn BS, Sansonetti PJ. 2017. *Shigella sonnei* encodes a functional T6SS used for interbacterial competition and niche occupancy. *Cell Host Microbe* **21:**769–776.e3 http://dx.doi.org/10.1016/j.chom.2017.05.004.

29. Yang G, Wang L, Wang Y, Li P, Zhu J, Qiu S, Hao R, Wu Z, Li W, Song H. 2015. *hfq* regulates acid tolerance and virulence by responding to acid stress in *Shigella flexneri*. *Res Microbiol* **166:**476–485 http://dx.doi.org/10.1016/j.resmic.2015.06.007.

30. Brotcke Zumsteg A, Goosmann C, Brinkmann V, Morona R, Zychlinsky A. 2014. IcsA is a *Shigella flexneri* adhesin regulated by the type III secretion system and required for pathogenesis. *Cell Host Microbe* **15:**435–445 http://dx.doi.org/10.1016/j.chom.2014.03.001.

31. Faherty CS, Redman JC, Rasko DA, Barry EM, Nataro JP. 2012. *Shigella flexneri* effectors OspE1 and OspE2 mediate induced adherence to the colonic epithelium following bile salts exposure. *Mol Microbiol* **85:**107–121 http://dx.doi.org/10.1111/j.1365-2958.2012.08092.x.

32. Vergara-Irigaray M, Fookes MC, Thomson NR, Tang CM. 2014. RNA-seq analysis of the influence of anaerobiosis and FNR on *Shigella flexneri*. *BMC Genomics* **15:**438 http://dx.doi.org/10.1186/1471-2164-15-438.

33. Marteyn B, West NP, Browning DF, Cole JA, Shaw JG, Palm F, Mounier J, Prévost M-C, Sansonetti P, Tang CM. 2010. Modulation of *Shigella* virulence in response to available oxygen in vivo. *Nature* **465:**355–358 http://dx.doi.org/10.1038/nature08970.

34. Sansonetti PJ, Arondel J, Fontaine A, d'Hauteville H, Bernardini ML. 1991. *Omp*B (osmo-regulation) and *ics*A (cell-to-cell spread) mutants of *Shigella flexneri*: vaccine candidates and probes to study the pathogenesis of shigellosis. *Vaccine* **9:**416–422 http://dx.doi.org/10.1016/0264-410X(91)90128-S.

35. Mathan MM, Mathan VI. 1991. Morphology of rectal mucosa of patients with shigellosis. *Rev Infect Dis* **13**(Suppl 4)**:**S314–S318 http://dx.doi.org/10.1093/clinids/13.Supplement_4.S314.

36. 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:**2752–2764.

37. Wassef JS, Keren DF, Mailloux JL. 1989. Role of M cells in initial antigen uptake and in ulcer formation in the rabbit intestinal loop model of shigellosis. *Infect Immun* **57:**858–863.

38. Perdomo OJ, Cavaillon JM, Huerre M, Ohayon H, Gounon P, Sansonetti PJ. 1994. Acute inflammation causes epithelial invasion and mucosal destruction in experimental shigellosis. *J Exp Med* **180:**1307–1319 http://dx.doi.org/10.1084/jem.180.4.1307.

39. Shim D-H, Suzuki T, Chang S-Y, Park S-M, Sansonetti PJ, Sasakawa C, Kweon M-N. 2007. New animal model of shigellosis in the guinea pig: its usefulness for protective efficacy studies. *J Immunol* **178:**2476–2482 http://dx.doi.org/10.4049/jimmunol.178.4.2476.

40. Romero S, Grompone G, Carayol N, Mounier J, Guadagnini S, Prevost M-C, Sansonetti PJ, Tran Van Nhieu GT. 2011. ATP-mediated Erk1/2 activation stimulates bacterial capture by filopodia, which precedes *Shigella* invasion of epithelial cells. *Cell Host Microbe* **9:**508–519 http://dx.doi.org/10.1016/j.chom.2011.05.005.

41. Romero S, Quatela A, Bornschlögl T, Guadagnini S, Bassereau P, Tran Van Nhieu G. 2012. Filopodium retraction is controlled by adhesion to its tip. *J Cell Sci* **125:**4999–5004. *ERRATUM J Cell Sci* **125:**5587 http://dx.doi.org/10.1242/jcs.104778.

42. Xu D, Liao C, Zhang B, Tolbert WD, He W, Dai Z, Zhang W, Yuan W, Pazgier M, Liu J, Yu J, Sansonetti PJ, Bevins CL, Shao Y, Lu W. 2018. Human enteric α-defensin 5 promotes *Shigella* infection by enhancing bacterial adhesion and invasion. *Immunity* **48:**1233–1244.e6 http://dx.doi.org/10.1016/j.immuni.2018.04.014.

43. Arena ET, Campbell-Valois F-X, Tinevez J-Y, Nigro G, Sachse M, Moya-Nilges M, Nothelfer K, Marteyn B, Shorte SL, Sansonetti PJ. 2015. Bioimage analysis of *Shigella* infection reveals targeting of colonic crypts. *Proc Natl Acad Sci USA* **112:**E3282–E3290 http://dx.doi.org/10.1073/pnas.1509091112.

44. Nothelfer K, Arena ET, Pinaud L, Neunlist M, Mozeleski B, Belotserkovsky I, Parsot C, Dinadayala P, Burger-Kentischer A, Raqib R, Sansonetti PJ, Phalipon A. 2014. B lymphocytes undergo TLR2-dependent apoptosis upon *Shigella* infection. *J Exp Med* **211:**1215–1229 http://dx.doi.org/10.1084/jem.20130914.

45. Miller H, Zhang J, Kuolee R, Patel GB, Chen W. 2007. Intestinal M cells: the fallible sentinels? *World J Gastroenterol* **13:**1477–1486 http://dx.doi.org/10.3748/wjg.v13.i10.1477.

46. Jensen VB, Harty JT, Jones BD. 1998. Interactions of the invasive pathogens *Salmonella typhimurium*, *Listeria monocytogenes*, and *Shigella flexneri* with M cells and murine Peyer's patches. *Infect Immun* **66:**3758–3766.

47. Ashida H, Kim M, Sasakawa C. 2014. Manipulation of the host cell death pathway by *Shigella*. *Cell Microbiol* **16:**1757–1766 http://dx.doi.org/10.1111/cmi.12367.

48. Jorgensen I, Miao EA. 2015. Pyroptotic cell death defends against intracellular pathogens. *Immunol Rev* **265:**130–142 http://dx.doi.org/10.1111/imr.12287.

49. Shi J, Gao W, Shao F. 2017. Pyroptosis: gasdermin-mediated programmed necrotic cell death. *Trends Biochem Sci* **42:**245–254 http://dx.doi.org/10.1016/j.tibs.2016.10.004.

50. Storek KM, Monack DM. 2015. Bacterial recognition pathways that lead to inflammasome activation. *Immunol Rev* **265:**112–129 http://dx.doi.org/10.1111/imr.12289.

51. Hermansson A-K, Paciello I, Bernardini ML. 2016. The orchestra and its maestro: *Shigella*'s fine-tuning of the inflammasome platforms. *Curr Top Microbiol Immunol* **397:**91–115 http://dx.doi.org/10.1007/978-3-319-41171-2_5.

52. Suzuki S, Franchi L, He Y, Muñoz-Planillo R, Mimuro H, Suzuki T, Sasakawa C, Núñez G. 2014. *Shigella* type III secretion protein MxiI is recognized by Naip2 to induce Nlrc4 inflammasome activation independently of Pkcδ. *PLoS Pathog* **10:**e1003926 http://dx.doi.org/10.1371/journal.ppat.1003926.

53. Miao EA, Mao DP, Yudkovsky N, Bonneau R, Lorang CG, Warren SE, Leaf IA, Aderem A. 2010. Innate immune detection of the type III secretion apparatus through the NLRC4 inflammasome. *Proc Natl Acad Sci USA* **107:**3076–3080 http://dx.doi.org/10.1073/pnas.0913087107.

54. Yang J, Zhao Y, Shi J, Shao F. 2013. Human NAIP and mouse NAIP1 recognize bacterial type III secretion needle protein for inflammasome activation. *Proc Natl Acad Sci USA* **110:**14408–14413 http://dx.doi.org/10.1073/pnas.1306376110.

55. Rayamajhi M, Zak DE, Chavarria-Smith J, Vance RE, Miao EA. 2013. Cutting edge: mouse NAIP1 detects the type III secretion system needle protein. *J Immunol* **191:**3986–3989 http://dx.doi.org/10.4049/jimmunol.1301549.

56. Watarai M, Funato S, Sasakawa C. 1996. Interaction of Ipa proteins of *Shigella flexneri* with α5β1 integrin promotes entry of the bacteria into mammalian cells. *J Exp Med* **183:**991–999 http://dx.doi.org/10.1084/jem.183.3.991.

57. Skoudy A, Mounier J, Aruffo A, Ohayon H, Gounon P, Sansonetti P, Tran Van Nhieu G. 2000. CD44 binds to the *Shigella* IpaB protein and participates in bacterial invasion of epithelial cells. *Cell Microbiol* **2:**19–33 http://dx.doi.org/10.1046/j.1462-5822.2000.00028.x.

58. Russo BC, Stamm LM, Raaben M, Kim CM, Kahoud E, Robinson LR, Bose S, Queiroz AL, Herrera BB, Baxt LA, Mor-Vaknin N, Fu Y, Molina G, Markovitz DM, Whelan SP, Goldberg MB. 2016. Intermediate filaments enable pathogen docking to trigger type 3 effector translocation. *Nat Microbiol* **1:**16025 http://dx.doi.org/10.1038/nmicrobiol.2016.25.

59. Tran Van Nhieu G, Caron E, Hall A, Sansonetti PJ. 1999. IpaC induces actin polymerization and filopodia formation during *Shigella* entry into epithelial cells. *EMBO J* **18:**3249–3262 http://dx.doi.org/10.1093/emboj/18.12.3249.

60. Tran Van Nhieu G, 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:**2717–2729 http://dx.doi.org/10.1093/emboj/16.10.2717.

61. Izard T, Tran Van Nhieu G, Bois PRJ. 2006. *Shigella* applies molecular mimicry to subvert vinculin and invade host cells. *J Cell Biol* **175:**465–475 http://dx.doi.org/10.1083/jcb.200605091.

62. Yoshida S, Handa Y, Suzuki T, Ogawa M, Suzuki M, Tamai A, Abe A, Katayama E, Sasakawa C. 2006. Microtubule-severing activity of *Shigella* is pivotal for intercellular spreading. *Science* **314:**985–989 http://dx.doi.org/10.1126/science.1133174.

63. Germane KL, Ohi R, Goldberg MB, Spiller BW. 2008. Structural and functional studies indicate that *Shigella* VirA is not a protease and does not directly destabilize microtubules. *Biochemistry* **47:**10241–10243 http://dx.doi.org/10.1021/bi801533k.

64. Niebuhr K, Giuriato S, Pedron T, Philpott DJ, Gaits F, Sable J, Sheetz MP, Parsot C, Sansonetti PJ, Payrastre B. 2002. Conversion of PtdIns(4,5)P (2) into PtdIns(5)P by the *S.flexneri* effector IpgD reorganizes host cell morphology. *EMBO J* **21:**5069–5078 http://dx.doi.org/10.1093/emboj/cdf522.

65. Bonnet M, Tran Van Nhieu G. 2016. How *Shigella* utilizes Ca(2+) jagged edge signals during invasion of epithelial cells. *Front Cell Infect Microbiol* **6:**16 http://dx.doi.org/10.3389/fcimb.2016.00016.

66. Tran Van Nhieu G, Kai Liu B, Zhang J, Pierre F, Prigent S, Sansonetti P, Erneux C, Kuk Kim J, Suh P-G, Dupont G, Combettes L. 2013. Actin-based confinement of calcium responses during *Shigella* invasion. *Nat Commun* **4:**1567 http://dx.doi.org/10.1038/ncomms2561.

67. Sun CH, Wacquier B, Aguilar DI, Carayol N, Denis K, Boucherie S, Valencia-Gallardo C, Simsek C, Erneux C, Lehman A, Enninga J, Arbibe L, Sansonetti P, Dupont G, Combettes L, Tran Van Nhieu G. 2017. The *Shigella* type III effector IpgD recodes Ca^{2+} signals during invasion of epithelial cells. *EMBO J* **36:**2567–2580 http://dx.doi.org/10.15252/embj.201696272.

68. Weiner A, Mellouk N, Lopez-Montero N, Chang Y-Y, Souque C, Schmitt C, Enninga J. 2016. Macropinosomes are key players in early *Shigella* invasion and vacuolar escape in epithelial cells. *PLoS Pathog* **12:**e1005602 http://dx.doi.org/10.1371/journal.ppat.1005602.

69. High N, Mounier J, Prévost MC, Sansonetti PJ. 1992. IpaB of *Shigella flexneri* causes entry into epithelial cells and escape from the phagocytic vacuole. *EMBO J* **11:**1991–1999 http://dx.doi.org/10.1002/j.1460-2075.1992.tb05253.x.

70. Mellouk N, Weiner A, Aulner N, Schmitt C, Elbaum M, Shorte SL, Danckaert A, Enninga J. 2014. *Shigella* subverts the host recycling compartment to rupture its vacuole. *Cell Host Microbe* **16:**517–530 http://dx.doi.org/10.1016/j.chom.2014.09.005.

71. Krokowski S, Mostowy S. 2016. Interactions between *Shigella flexneri* and the autophagy machinery. *Front Cell Infect Microbiol* **6:**17 http://dx.doi.org/10.3389/fcimb.2016.00017.

72. Travassos LH, Carneiro LAM, Ramjeet M, Hussey S, Kim Y-G, Magalhães JG, Yuan L, Soares F, Chea E, Le Bourhis L, Boneca IG, Allaoui A, Jones NL, Nuñez G, Girardin SE, Philpott DJ. 2010. Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. *Nat Immunol* **11:**55–62 http://dx.doi.org/10.1038/ni.1823.

73. Tattoli I, Sorbara MT, Vuckovic D, Ling A, Soares F, Carneiro LAM, Yang C, Emili A, Philpott DJ, Girardin SE. 2012. Amino acid starvation induced by invasive bacterial pathogens triggers an innate host defense program. *Cell Host Microbe* **11:**563–575 http://dx.doi.org/10.1016/j.chom.2012.04.012.

74. Sorbara MT, Foerster EG, Tsalikis J, Abdel-Nour M, Mangiapane J, Sirluck-Schroeder I, Tattoli I, van Dalen R, Isenman DE, Rohde JR, Girardin SE, Philpott DJ. 2018. Complement C3 drives autophagy-dependent restriction of cyto-invasive bacteria. *Cell Host Microbe* **23:**644–652.e5 http://dx.doi.org/10.1016/j.chom.2018.04.008.

75. Baxt LA, Goldberg MB. 2014. Host and bacterial proteins that repress recruitment of LC3 to *Shigella* early during infection. *PLoS One* **9:**e94653 http://dx.doi.org/10.1371/journal.pone.0094653.

76. Liu W, Zhou Y, Peng T, Zhou P, Ding X, Li Z, Zhong H, Xu Y, Chen S, Hang HC, Shao F. 2018. N^{ε}-fatty acylation of multiple membrane-associated proteins by *Shigella* IcsB effector to modulate host function. *Nat Microbiol* **3:**996–1009 http://dx.doi.org/10.1038/s41564-018-0215-6.

77. Agaisse H. 2016. Molecular and cellular mechanisms of *Shigella flexneri* dissemination. *Front Cell Infect Microbiol* **6:**29 http://dx.doi.org/10.3389/fcimb.2016.00029.

78. Monack DM, Theriot JA. 2001. Actin-based motility is sufficient for bacterial membrane protrusion formation and host cell uptake. *Cell Microbiol* **3:**633–647 http://dx.doi.org/10.1046/j.1462-5822.2001.00143.x.

79. Fukumatsu M, Ogawa M, Arakawa S, Suzuki M, Nakayama K, Shimizu S, Kim M, Mimuro H, Sasakawa C. 2012. *Shigella* targets epithelial tricellular junctions and uses a noncanonical clathrin-dependent endocytic pathway to spread between cells. *Cell Host Microbe* **11:**325–336 http://dx.doi.org/10.1016/j.chom.2012.03.001.

80. Torraca V, Mostowy S. 2016. Septins and bacterial infection. *Front Cell Dev Biol* **4:**127 http://dx.doi.org/10.3389/fcell.2016.00127.

81. Mostowy S, Sancho-Shimizu V, Hamon MA, Simeone R, Brosch R, Johansen T, Cossart P. 2011. p62 and NDP52 proteins target intracytosolic *Shigella* and *Listeria* to different autophagy pathways. *J Biol Chem* **286:**26987–26995 http://dx.doi.org/10.1074/jbc.M111.223610.

82. Sirianni A, Krokowski S, Lobato-Márquez D, Buranyi S, Pfanzelter J, Galea D, Willis A, Culley S, Henriques R, Larrouy-Maumus G, Hollinshead M, Sancho-Shimizu V, Way M, Mostowy S. 2016. Mitochondria mediate septin cage assembly to promote autophagy of *Shigella*. *EMBO Rep* **17:**1029–1043 http://dx.doi.org/10.15252/embr.201541832.

83. Ogawa M, Yoshimori T, Suzuki T, Sagara H, Mizushima N, Sasakawa C. 2005. Escape of intracellular *Shigella* from autophagy. *Science* **307:**727–731 http://dx.doi.org/10.1126/science.1106036.

84. Mostowy S, Boucontet L, Mazon Moya MJ, Sirianni A, Boudinot P, Hollinshead M, Cossart P, Herbomel P, Levraud J-P, Colucci-Guyon E. 2013. The zebrafish as a new model for the in vivo study of *Shigella flexneri*

interaction with phagocytes and bacterial autophagy. *PLoS Pathog* **9:** e1003588 http://dx.doi.org/10.1371/journal.ppat.1003588.

85. Uchiya K, Tobe T, Komatsu K, Suzuki T, Watarai M, Fukuda I, Yoshikawa M, Sasakawa C. 1995. Identification of a novel virulence gene, *virA*, on the large plasmid of *Shigella*, involved in invasion and intercellular spreading. *Mol Microbiol* **17:**241–250 http://dx.doi.org/10.1111/j.1365-2958.1995.mmi_17020241.x.

86. Huang J, Brumell JH. 2014. Bacteria-autophagy interplay: a battle for survival. *Nat Rev Microbiol* **12:**101–114 http://dx.doi.org/10.1038/nrmicro3160.

87. Dong N, Zhu Y, Lu Q, Hu L, Zheng Y, Shao F. 2012. Structurally distinct bacterial TBC-like GAPs link Arf GTPase to Rab1 inactivation to counteract host defenses. *Cell* **150:**1029–1041 http://dx.doi.org/10.1016/j.cell.2012.06.050.

88. Campbell-Valois F-X, Sachse M, Sansonetti PJ, Parsot C. 2015. Escape of actively secreting *Shigella flexneri* from ATG8/LC3-positive vacuoles formed during cell-to-cell spread is facilitated by IcsB and VirA. *mBio* **6:** e02567-14 http://dx.doi.org/10.1128/mBio.02567-14.

89. Li P, Jiang W, Yu Q, Liu W, Zhou P, Li J, Xu J, Xu B, Wang F, Shao F. 2017. Ubiquitination and degradation of GBPs by a *Shigella* effector to suppress host defence. *Nature* **551:**378–383.

90. Piro AS, Hernandez D, Luoma S, Feeley EM, Finethy R, Yirga A, Frickel EM, Lesser CF, Coers J. 2017. Detection of cytosolic *Shigella flexneri* via a C-terminal triple-arginine motif of GBP1 inhibits actin-based motility. *mBio* **8:**e01979-17 http://dx.doi.org/10.1128/mBio.01979-17.

91. Paciello I, Silipo A, Lembo-Fazio L, Curcurù L, Zumsteg A, Noël G, Ciancarella V, Sturiale L, Molinaro A, Bernardini ML. 2013. Intracellular *Shigella* remodels its LPS to dampen the innate immune recognition and evade inflammasome activation. *Proc Natl Acad Sci USA* **110:**E4345–E4354. *CORRECTION Proc Natl Acad Sci USA* **110:**20843 http://dx.doi.org/10.1073/pnas.1303641110.

92. Kobayashi T, Ogawa M, Sanada T, Mimuro H, Kim M, Ashida H, Akakura R, Yoshida M, Kawalec M, Reichhart J-M, Mizushima T, Sasakawa C. 2013. The *Shigella* OspC3 effector inhibits caspase-4, antagonizes inflammatory cell death, and promotes epithelial infection. *Cell Host Microbe* **13:** 570–583 http://dx.doi.org/10.1016/j.chom.2013.04.012.

93. Mayo LD, Donner DB. 2001. A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. *Proc Natl Acad Sci USA* **98:**11598–11603 http://dx.doi.org/10.1073/pnas.181181198.

94. Ramel D, Lagarrigue F, Pons V, Mounier J, Dupuis-Coronas S, Chicanne G, Sansonetti PJ, Gaits-Iacovoni F, Tronchère H, Payrastre B. 2011. *Shigella flexneri* infection generates the lipid PI5P to alter endocytosis and prevent termination of EGFR signaling. *Sci Signal* **4:**ra61 http://dx.doi.org/10.1126/scisignal.2001619.

95. Bhola PD, Letai A. 2016. Mitochondria—judges and executioners of cell death sentences. *Mol Cell* **61:**695–704 http://dx.doi.org/10.1016/j.molcel.2016.02.019.

96. Sukumaran SK, Fu NY, Tin CB, Wan KF, Lee SS, Yu VC. 2010. A soluble form of the pilus protein FimA targets the VDAC-hexokinase complex at mitochondria to suppress host cell apoptosis. *Mol Cell* **37:** 768–783 http://dx.doi.org/10.1016/j.molcel.2010.02.015.

97. Bravo V, Puhar A, Sansonetti P, Parsot C, Toro CS. 2015. Distinct mutations led to inactivation of type 1 fimbriae expression in *Shigella* spp. *PLoS One* **10:**e0121785 http://dx.doi.org/10.1371/journal.pone.0121785.

98. Faherty CS, Maurelli AT. 2009. Spa15 of *Shigella flexneri* is secreted through the type III secretion system and prevents staurosporine-induced apoptosis. *Infect Immun* **77:**5281–5290 http://dx.doi.org/10.1128/IAI.00800-09.

99. Pendaries C, Tronchère H, Arbibe L, Mounier J, Gozani O, Cantley L, Fry MJ, Gaits-Iacovoni F, Sansonetti PJ, Payrastre B. 2006. PtdIns5P activates the host cell PI3-kinase/Akt pathway during *Shigella flexneri* infection. *EMBO J* **25:**1024–1034 http://dx.doi.org/10.1038/sj.emboj.7601001.

100. Carneiro LAM, Travassos LH, Soares F, Tattoli I, Magalhaes JG, Bozza MT, Plotkowski MC, Sansonetti PJ, Molkentin JD, Philpott DJ, Girardin SE. 2009. *Shigella* induces mitochondrial dysfunction and cell death in nonmyeloid cells. *Cell Host Microbe* **5:**123–136 http://dx.doi.org/10.1016/j.chom.2008.12.011.

101. Kim M, Ogawa M, Fujita Y, Yoshikawa Y, Nagai T, Koyama T, Nagai S, Lange A, Fässler R, Sasakawa C. 2009. Bacteria hijack integrin-linked kinase to stabilize focal adhesions and block cell detachment. *Nature* **459:**578–582 http://dx.doi.org/10.1038/nature07952.

102. Miura M, Terajima J, Izumiya H, Mitobe J, Komano T, Watanabe H. 2006. OspE2 of *Shigella sonnei* is required for the maintenance of cell architecture of bacterium-infected cells. *Infect Immun* **74:**2587–2595 http://dx.doi.org/10.1128/IAI.74.5.2587-2595.2006.

103. Iwai H, Kim M, Yoshikawa Y, Ashida H, Ogawa M, Fujita Y, Muller D, Kirikae T, Jackson PK, Kotani S, Sasakawa C. 2007. A bacterial effector targets Mad2L2, an APC inhibitor, to modulate host cell cycling. *Cell* **130:**611–623 http://dx.doi.org/10.1016/j.cell.2007.06.043.

104. Boal F, Puhar A, Xuereb J-M, Kunduzova O, Sansonetti PJ, Payrastre B, Tronchère H. 2016. PI5P triggers ICAM-1 degradation in *Shigella*-infected cells, thus dampening immune cell recruitment. *Cell Reports* **14:** 750–759 http://dx.doi.org/10.1016/j.celrep.2015.12.079.

105. Pieper R, Fisher CR, Suh M-J, Huang S-T, Parmar P, Payne SM. 2013. Analysis of the proteome of intracellular *Shigella flexneri* reveals pathways important for intracellular growth. *Infect Immun* **81:**4635–4648 http://dx.doi.org/10.1128/IAI.00975-13.

106. Payne SM, Wyckoff EE, Murphy ER, Oglesby AG, Boulette ML, Davies NML. 2006. Iron and pathogenesis of *Shigella*: iron acquisition in the intracellular environment. *Biometals* **19:**173–180 http://dx.doi.org/10.1007/s10534-005-4577-x.

107. Kentner D, Martano G, Callon M, Chiquet P, Brodmann M, Burton O, Wahlander A, Nanni P, Delmotte N, Grossmann J, Limenitakis J, Schlapbach R, Kiefer P, Vorholt JA, Hiller S, Bumann D. 2014. *Shigella* reroutes host cell central metabolism to obtain high-flux nutrient supply for vigorous intracellular growth. *Proc Natl Acad Sci USA* **111:**9929–9934 http://dx.doi.org/10.1073/pnas.1406694111.

108. Vonaesch P, Campbell-Valois F-X, Dufour A, Sansonetti PJ, Schnupf P. 2016. *Shigella flexneri* modulates stress granule composition and inhibits stress granule aggregation. *Cell Microbiol* **18:**982–997 http://dx.doi.org/10.1111/cmi.12561.

109. Lu R, Herrera BB, Eshleman HD, Fu Y, Bloom A, Li Z, Sacks DB, Goldberg MB. 2015. *Shigella* effector OspB activates mTORC1 in a manner that depends on IQGAP1 and promotes cell proliferation. *PLoS Pathog* **11:**e1005200 http://dx.doi.org/10.1371/journal.ppat.1005200.

110. Yu S, Gao N. 2015. Compartmentalizing intestinal epithelial cell toll-like receptors for immune surveillance. *Cell Mol Life Sci* **72:**3343–3353 http://dx.doi.org/10.1007/s00018-015-1931-1.

111. Lee J, Mo J-H, Katakura K, Alkalay I, Rucker AN, Liu Y-T, Lee H-K, Shen C, Cojocaru G, Shenouda S, Kagnoff M, Eckmann L, Ben-Neriah Y, Raz E. 2006. Maintenance of colonic homeostasis by distinctive apical TLR9 signalling in intestinal epithelial cells. *Nat Cell Biol* **8:**1327–1336 http://dx.doi.org/10.1038/ncb1500.

112. He Y, Hara H, Núñez G. 2016. Mechanism and regulation of NLRP3 inflammasome activation. *Trends Biochem Sci* **41:**1012–1021 http://dx.doi.org/10.1016/j.tibs.2016.09.002.

113. Girardin SE, Tournebize R, Mavris M, Page AL, Li X, Stark GR, Bertin J, DiStefano PS, Yaniv M, Sansonetti PJ, Philpott DJ. 2001. CARD4/Nod1 mediates NF-κB and JNK activation by invasive *Shigella flexneri*. *EMBO Rep* **2:**736–742 http://dx.doi.org/10.1093/embo-reports/kve155.

114. Killackey SA, Sorbara MT, Girardin SE. 2016. Cellular aspects of *Shigella* pathogenesis: focus on the manipulation of host cell processes. *Front Cell Infect Microbiol* **6:**38 http://dx.doi.org/10.3389/fcimb.2016.00038.

115. Gaudet RG, Guo CX, Molinaro R, Kottwitz H, Rohde JR, Dangeard A-S, Arrieumerlou C, Girardin SE, Gray-Owen SD. 2017. Innate recog-

nition of intracellular bacterial growth is driven by the TIFA-dependent cytosolic surveillance pathway. *Cell Reports* **19:**1418–1430 http://dx.doi.org/10.1016/j.celrep.2017.04.063.

116. Sanada T, Kim M, Mimuro H, Suzuki M, Ogawa M, Oyama A, Ashida H, Kobayashi T, Koyama T, Nagai S, Shibata Y, Gohda J, Inoue J, Mizushima T, Sasakawa C. 2012. The *Shigella flexneri* effector OspI deamidates UBC13 to dampen the inflammatory response. *Nature* **483:** 623–626 http://dx.doi.org/10.1038/nature10894.

117. Nishide A, Kim M, Takagi K, Himeno A, Sanada T, Sasakawa C, Mizushima T. 2013. Structural basis for the recognition of Ubc13 by the *Shigella flexneri* effector OspI. *J Mol Biol* **425:**2623–2631 http://dx.doi.org/10.1016/j.jmb.2013.02.037.

118. Ashida H, Nakano H, Sasakawa C. 2013. *Shigella* IpaH0722 E3 ubiquitin ligase effector targets TRAF2 to inhibit PKC-NF-κB activity in invaded epithelial cells. *PLoS Pathog* **9:**e1003409 http://dx.doi.org/10.1371/journal.ppat.1003409.

119. de Jong MF, Liu Z, Chen D, Alto NM. 2016. *Shigella flexneri* suppresses NF-κB activation by inhibiting linear ubiquitin chain ligation. *Nat Microbiol* **1:**16084 http://dx.doi.org/10.1038/nmicrobiol.2016.84.

120. Zhang Y, Mühlen S, Oates CV, Pearson JS, Hartland EL. 2016. Identification of a distinct substrate-binding domain in the bacterial cysteine methyltransferase effectors NleE and OspZ. *J Biol Chem* **291:**20149–20162 http://dx.doi.org/10.1074/jbc.M116.734079.

121. Newton HJ, Pearson JS, Badea L, Kelly M, Lucas M, Holloway G, Wagstaff KM, Dunstone MA, Sloan J, Whisstock JC, Kaper JB, Robins-Browne RM, Jans DA, Frankel G, Phillips AD, Coulson BS, Hartland EL. 2010. The type III effectors NleE and NleB from enteropathogenic *E. coli* and OspZ from *Shigella* block nuclear translocation of NF-κB p65. *PLoS Pathog* **6:**e1000898 http://dx.doi.org/10.1371/journal.ppat.1000898.

122. Ashida H, Kim M, Schmidt-Supprian M, Ma A, Ogawa M, Sasakawa C. 2010. A bacterial E3 ubiquitin ligase IpaH9.8 targets NEMO/IKKγ to dampen the host NF-κB-mediated inflammatory response. *Nat Cell Biol* **12:** 66–73.

123. Kim DW, Lenzen G, Page A-L, Legrain P, Sansonetti PJ, Parsot C. 2005. The *Shigella flexneri* effector OspG interferes with innate immune responses by targeting ubiquitin-conjugating enzymes. *Proc Natl Acad Sci USA* **102:**14046–14051 http://dx.doi.org/10.1073/pnas.0504466102.

124. Wang F, Jiang Z, Li Y, He X, Zhao J, Yang X, Zhu L, Yin Z, Li X, Wang X, Liu W, Shang W, Yang Z, Wang S, Zhen Q, Zhang Z, Yu Y, Zhong H, Ye Q, Huang L, Yuan J. 2013. *Shigella flexneri* T3SS effector IpaH4.5 modulates the host inflammatory response via interaction with NF-κB p65 protein. *Cell Microbiol* **15:**474–485 http://dx.doi.org/10.1111/cmi.12052.

125. Li H, Xu H, Zhou Y, Zhang J, Long C, Li S, Chen S, Zhou J-M, Shao F. 2007. The phosphothreonine lyase activity of a bacterial type III effector family. *Science* **315:**1000–1003 http://dx.doi.org/10.1126/science.1138960.

126. Arbibe L, Kim DW, Batsche E, Pedron T, Mateescu B, Muchardt C, Parsot C, Sansonetti PJ. 2007. An injected bacterial effector targets chromatin access for transcription factor NF-kappaB to alter transcription of host genes involved in immune responses. *Nat Immunol* **8:**47–56 http://dx.doi.org/10.1038/ni1423.

127. Harouz H, Rachez C, Meijer BM, Marteyn B, Donnadieu F, Cammas F, Muchardt C, Sansonetti P, Arbibe L. 2014. *Shigella flexneri* targets the HP1γ subcode through the phosphothreonine lyase OspF. *EMBO J* **33:** 2606–2622 http://dx.doi.org/10.15252/embj.201489244.

128. Schmutz C, Ahrné E, Kasper CA, Tschon T, Sorg I, Dreier RF, Schmidt A, Arrieumerlou C. 2013. Systems-level overview of host protein phosphorylation during *Shigella flexneri* infection revealed by phosphoproteomics. *Mol Cell Proteomics* **12:**2952–2968 http://dx.doi.org/10.1074/mcp.M113.029918.

129. Okuda J, Toyotome T, Kataoka N, Ohno M, Abe H, Shimura Y, Seyedarabi A, Pickersgill R, Sasakawa C. 2005. *Shigella* effector IpaH9.8 binds to a splicing factor U2AF(35) to modulate host immune responses. *Biochem Biophys Res Commun* **333:**531–539 http://dx.doi.org/10.1016/j.bbrc.2005.05.145.

130. Burnaevskiy N, Fox TG, Plymire DA, Ertelt JM, Weigele BA, Selyunin AS, Way SS, Patrie SM, Alto NM. 2013. Proteolytic elimination of N-myristoyl modifications by the *Shigella* virulence factor IpaJ. *Nature* **496:**106–109 http://dx.doi.org/10.1038/nature12004.

131. Dobbs N, Burnaevskiy N, Chen D, Gonugunta VK, Alto NM, Yan N. 2015. STING activation by translocation from the ER is associated with infection and autoinflammatory disease. *Cell Host Microbe* **18:**157–168 http://dx.doi.org/10.1016/j.chom.2015.07.001.

132. Mounier J, Boncompain G, Senerovic L, Lagache T, Chrétien F, Perez F, Kolbe M, Olivo-Marin J-C, Sansonetti PJ, Sauvonnet N. 2012. *Shigella* effector IpaB-induced cholesterol relocation disrupts the Golgi complex and recycling network to inhibit host cell secretion. *Cell Host Microbe* **12:**381–389 http://dx.doi.org/10.1016/j.chom.2012.07.010.

133. Zheng Z, Wei C, Guan K, Yuan Y, Zhang Y, Ma S, Cao Y, Wang F, Zhong H, He X. 2016. Bacterial E3 ubiquitin ligase IpaH4.5 of *Shigella flexneri* targets TBK1 to dampen the host antibacterial response. *J Immunol* **196:**1199–1208 http://dx.doi.org/10.4049/jimmunol.1501045.

134. Puhar A, Tronchère H, Payrastre B, Tran Van Nhieu GTV, Sansonetti PJ. 2013. A *Shigella* effector dampens inflammation by regulating epithelial release of danger signal ATP through production of the lipid mediator PtdIns5P. *Immunity* **39:**1121–1131 http://dx.doi.org/10.1016/j.immuni.2013.11.013.

135. Konradt C, Frigimelica E, Nothelfer K, Puhar A, Salgado-Pabón W, di Bartolo V, Scott-Algara D, Rodrigues CD, Sansonetti PJ, Phalipon A. 2011. The *Shigella flexneri* type three secretion system effector IpgD inhibits T cell migration by manipulating host phosphoinositide metabolism. *Cell Host Microbe* **9:**263–272 http://dx.doi.org/10.1016/j.chom.2011.03.010.

136. Salgado-Pabón W, Celli S, Arena ET, Nothelfer K, Roux P, Sellge G, Frigimelica E, Bousso P, Sansonetti PJ, Phalipon A. 2013. *Shigella* impairs T lymphocyte dynamics in vivo. *Proc Natl Acad Sci USA* **110:**4458–4463 http://dx.doi.org/10.1073/pnas.1300981110.

137. Pinaud L, Samassa F, Porat Z, Ferrari ML, Belotserkovsky I, Parsot C, Sansonetti PJ, Campbell-Valois F-X, Phalipon A. 2017. Injection of T3SS effectors not resulting in invasion is the main targeting mechanism of *Shigella* toward human lymphocytes. *Proc Natl Acad Sci USA* **114:**9954–9959 http://dx.doi.org/10.1073/pnas.1707098114.