

**Molecular Pathogenesis of *Shigella* spp.:
Controlling Host Cell Signaling, Invasion,
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Molecular Pathogenesis of *Shigella* spp.: Controlling Host Cell Signaling, Invasion, and Death by Type III Secretion

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INTRODUCTION

Diarrheal diseases caused by bacterial, viral, or parasitic pathogens are a major public health problem. Estimations by the World Health Organization (WHO) indicate that the world population suffered from 4.5 billion incidences of diarrhea causing 1.8 million deaths in the year 2002 (334). Approximately 99% of the cases occurred in developing countries, where poor hygiene and limited access to clean drinking water promote the spread of enteric diseases. Malnutrition and the lack of appropriate medical intervention contribute to the high mortality rate, especially for young children.

Species of the genus *Shigella* are among the bacterial pathogens most frequently isolated from patients with diarrhea. Five to fifteen percent of all diarrheal episodes worldwide can be attributed to an infection with *Shigella*, including 1.1 million fatal cases (136). Two-thirds of all episodes and deaths occur in children under 5 years. A recent multicenter study of the epidemiology and microbiology of shigellosis in Asia revealed that the incidence of this disease might even exceed previous estimations, as *Shigella* DNA could also be detected in up to one-third of culture-negative specimens (323). A general trend toward less severe clinical manifestations of shigellosis and fewer fatal cases was observed in this study. Despite these encouraging observations, the emergence of

multidrug-resistant *Shigella* strains and a continuous high disease incidence imply that shigellosis is an unsolved global health problem (248).

Shigellosis is an acute intestinal infection, the symptoms of which can range from mild watery diarrhea to severe inflammatory bacillary dysentery characterized by strong abdominal cramps, fever, and stools containing blood and mucus. The disease is usually self-limiting but may become life-threatening if patients are immunocompromised or if adequate medical care is not available. A combination of oral rehydration and antibiotics leads to the rapid resolution of infection. Currently, there is no protective *Shigella* vaccine available, but several vaccines using bacterial components or killed or live-attenuated bacteria for immunization are under development and are being tested in different clinical phases (335). Excellent recent reviews provide further information on the clinical symptoms, diagnostics, and treatment of shigellosis and give an overview on the different approaches currently employed to develop a protective *Shigella* vaccine (123, 189, 321). Here, we cover recent progress in our understanding of shigellosis on a cellular level and on a molecular level. Specifically, we focus on the type III secretion system (T3SS) of *Shigella flexneri*, secreted effector proteins, and the responses that the effectors elicit from host cells.

CELLULAR PATHOGENESIS OF SHIGELLOSIS

Shigella spp. are transmitted by the fecal-oral route and enter the human body via the ingestion of contaminated food

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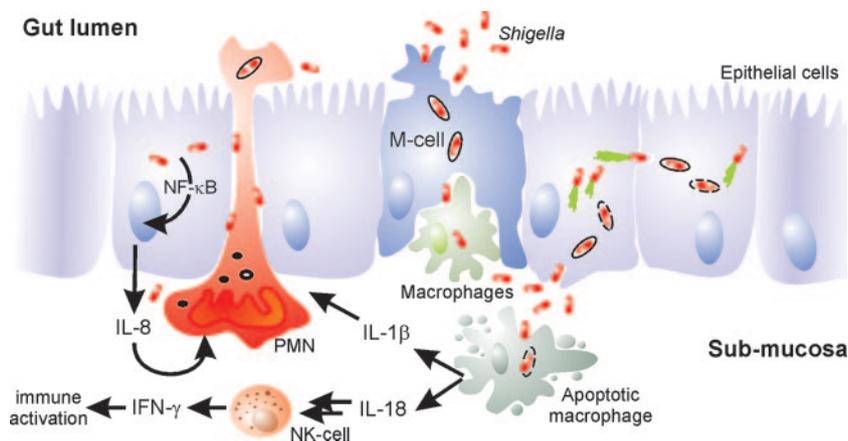


FIG. 1. Cellular pathogenesis of *Shigella* spp. *S. flexneri* passes the EC barrier by transcytosis through M cells and encounters resident macrophages. The bacteria evade degradation in macrophages by inducing an apoptosis-like cell death, which is accompanied by proinflammatory signaling. Free bacteria invade the EC from the basolateral side, move into the cytoplasm by vectorial actin polymerization, and spread to adjacent cells. Proinflammatory signaling by macrophages and EC further activates the innate immune response involving NK cells and attracts PMN. The influx of PMN disintegrates the EC lining, which initially exacerbates the infection and tissue destruction by facilitating the invasion of more bacteria. Ultimately, PMN phagocytose and kill *Shigella*, thus contributing to the resolution of the infection.

or water. The bacteria are highly infectious, since as few as 10 to 100 microorganisms are sufficient to cause disease (61). This low infectious dose can at least partially be attributed to the presence of effective acid resistance systems, which enable *S. flexneri* to survive the acidic environment in the stomach (88). Furthermore, it was shown that *Shigella* spp. are able to down-regulate the expression of antimicrobial peptides, which are important antibacterial effectors constantly released from the mucosal surfaces of the intestinal tract (118). After passage through the stomach and small intestine, the bacteria reach the large intestine, where they establish an infection.

Most of the current knowledge on mechanisms underlying *Shigella* pathogenesis is derived from studies of *S. flexneri*. Infection with the invasive pathogen *S. flexneri* is a multistep process (Fig. 1). To gain access to the intestinal mucosa, *S. flexneri* crosses the intestinal epithelium, which evolved as a physical and functional barrier to protect the body against the invasion of commensal and pathogenic bacteria (249). In the initial phase of infection, *S. flexneri* apparently does not invade the epithelial barrier from the apical side but instead triggers its uptake into microfold cells (M cells) and is transcytosed across the epithelial layer (250, 325). M cells are specialized epithelial cells (EC), which continuously sample particles from the gut lumen and deliver them to the underlying mucosal lymphoid tissue, where immune responses can be initiated (157). The use of M cells as an entry port is consistent with the in vitro observation that *S. flexneri* barely interacts with the apical surface of polarized EC and enters these cells preferentially through the basolateral pole (179).

After transcytosis, *S. flexneri* is released into an intraepithelial pocket, where the bacteria encounter resident macrophages that engulf and degrade incoming material. *S. flexneri* ensures its survival in macrophages by rapidly inducing apoptosis (119, 347, 348). Macrophage cell death is accompanied by the release of the proinflammatory cytokines interleukin-1 β (IL-1 β) and IL-18 (256, 345). Both cytokines are critical mediators of the acute and massive inflammatory response elicited by *S. flexneri* (Fig. 1). While IL-1 β signaling triggers the

strong intestinal inflammation characteristic of shigellosis, IL-18 is involved in the generation of an effective antibacterial response (251, 256). IL-18 activates natural killer (NK) cells and promotes the production of gamma interferon (IFN- γ), thus amplifying innate immune responses (148, 328). However, the role of this cytokine in shigellosis has not been completely elucidated.

Once released from the dying macrophage, *S. flexneri* is able to invade EC from the basolateral side, escapes from the phagosome, and replicates in the cytoplasm (Fig. 1) (257). Cytoplasmic *S. flexneri* moves by directed polymerization of actin, which allows the bacteria to spread to adjacent EC, avoiding exposure to extracellular components of the host immune defense (22, 174, 291). Nevertheless, this strategy is a double-edged sword, as the invasion of EC also elicits a strong inflammatory response. The Nod1-mediated intracellular surveillance system senses bacterial peptidoglycan fragments released by *S. flexneri* and activates nuclear factor κ B (NF- κ B), which triggers the up-regulation and secretion of the chemokine IL-8 (83, 211, 217, 252). IL-8 mediates a massive recruitment of polymorphonuclear neutrophil leukocytes (PMN) to the site of infection (252, 286). Furthermore, recent findings indicate that *S. flexneri* secretes effector proteins that actively shape the transcriptional response of infected EC to promote PMN migration (169, 344).

Infiltrating PMN destroy the integrity of the epithelial lining, thus enabling more luminal bacteria to reach the submucosa without the need of M cells (214, 215) (Fig. 1). In addition, *S. flexneri* seems to weaken the sealing of the EC tight junctions by altering the tight-junction protein composition (242). Thus, macrophage killing, destruction of the epithelial layer, and the massive influx of PMN exacerbate the bacterial infection and tissue lesion. These processes are essential for the development of diarrhea and the characteristic pathology of shigellosis. Ultimately, however, the PMN recruited to the site of infection entrap and kill the bacteria, thereby resolving the infection (29, 158, 342). In addition, IFN- γ plays a crucial role for the innate resistance to *S. flexneri* infection (328). The

activation of macrophages and their protection from *S. flexneri*-triggered cell death likely account for the effects of IFN- γ at least partially (108).

The severe tissue destruction caused by *Shigella* spp. results in an impaired adsorption of water, nutrients, and solutes, which might cause the watery diarrhea as well as the blood and mucus in stools characteristic of shigellosis. A disturbance of electrolyte homeostasis and changes in membrane transport processes, such as uncontrolled ion and fluid secretion, are typical of diarrheal diseases (147). However, the exact mechanism underlying the onset of diarrhea during shigellosis is still poorly defined. Notably, *Shigella* enterotoxin 1 (ShET1) and ShET2, which are produced by several *Shigella* strains, were found to induce fluid secretion into the intestine, thus accounting for the watery phase of diarrhea (70, 183). Moreover, Shiga toxin, which is produced only by *Shigella dysenteriae* serotype 1, is cytotoxic for a variety of cell types and is responsible for the development of vascular lesions in the colon, the kidney, and the central nervous system (37). Due to the high toxicity of Shiga toxin, infections with *S. dysenteriae* serotype 1 are frequently associated with life-threatening complications (37, 199).

Even though massive inflammation promotes the initial infection by *S. flexneri*, recent reports provide evidence that bacteria secrete effectors that actively downregulate proinflammatory signals, perhaps to balance the severity of the inflammation on a level beneficial for the bacteria (14, 117, 131, 197). It is becoming increasingly clear that *S. flexneri* skillfully exploits and evades the potentially harmful responses of the immune system (216).

EVOLUTION OF SHIGELLA VIRULENCE

Shigella spp. are gram-negative, nonsporulating, rod-shaped bacteria that belong to the family *Enterobacteriaceae*. The bacteria are facultative intracellular pathogens that show a high specificity for human or primate hosts. The first report on the isolation and characterization of bacteria causing bacillary dysentery, later named *Shigella*, was published by Kiyoshi Shiga at the end of the 19th century (280). Descriptions of numerous differing strains followed over the next decades, with all of them being closely related to the nonpathogenic bacterium *Escherichia coli*. To distinguish the pathogenic strains of high clinical relevance from less-pathogenic or nonpathogenic strains, the genus *Shigella* was defined based on biochemical, serological, and clinical phenotypes (69). The genus *Shigella* includes the four species *S. dysenteriae* (serogroup A), *S. flexneri* (serogroup B), *S. boydii* (serogroup C), and *S. sonnei* (serogroup D). According to variations in their O antigens, the species were further divided into several serotypes. *S. flexneri* and, to a lesser extent, *S. sonnei* are endemic and cause the majority of all infections (136). *S. dysenteriae* accounts for epidemic disease outbreaks and the most severe form of dysentery, which causes the majority of fatal shigellosis cases.

Today, this traditional classification is challenged from results obtained by comparative genomics. Several studies using different technical approaches clearly prove that *Shigella* spp. belong to the species *E. coli*, rather than forming a separate genus (79, 192, 225, 240). The sequence divergence between *S. flexneri* and *E. coli* K-12 is about 1.5% (146). This is marginal

compared to, e.g., 15% in the case of pathogenic *Salmonella enterica*, which is also closely related to *E. coli*. Moreover, diarrheagenic enteroinvasive *E. coli* (EIEC) strains share biochemical characteristics, essential virulence factors, and clinical symptoms with *Shigella* spp. While EIEC does not completely fulfill the definition of the genus *Shigella*, genome analysis revealed a closer relationship to *Shigella* spp. than to commensal *E. coli* strains (144, 337).

Comparative genomics clearly indicates that *Shigella* spp. and EIEC evolved from multiple *E. coli* strains by convergent evolution (226, 337). The two different phylogenetic trees of *Shigella* deduced either from numerical and phenotypic taxonomy or from comparative genomics are shown in Fig. 2. In the latter, three main *Shigella* clusters, each containing strains from the traditionally defined species, are identified. *S. sonnei* and some *S. dysenteriae* strains are more distantly related to these main clusters but still group with *E. coli*. The three main *Shigella* clusters started to diverge from *E. coli* 35,000 to 270,000 years ago (146). *S. sonnei* is of more recent origin and separated from the other strains about 10,000 years ago (277). Since EIEC retained more characteristics of commensal *E. coli* than *Shigella* spp., these strains apparently acquired the virulence machinery more recently and might reflect an earlier stage of the evolutionary process undergone by *Shigella* spp. (144, 336). As there is currently no new nomenclature for *Shigella* that reflects the evolutionary as well as the phenotypic classifications, the traditional nomenclature for *Shigella* is used in this review.

In addition to a reassignment of the phylogenetic relationships between *Shigella* strains, comparative genomics provides insight into the genetic basis of *Shigella* virulence. The genetic information constituting the phenotypes of *Shigella* spp. is encoded on the bacterial chromosome and on a large virulence plasmid. The virulence plasmid is an essential virulence determinant of all *Shigella* spp. and encodes the molecular machinery necessary for tissue invasion and the intracellular lifestyle (253, 254, 259). The central element of this machinery is a T3SS. The T3SS enables the bacteria to translocate a set of approximately 25 proteins from the bacterial cytoplasm directly into the eukaryotic host cell, where these "effector" proteins interfere with various host cell processes (193, 206, 310).

The complete sequences of the virulence plasmids and chromosomes of several *Shigella* strains including all four species are currently available (30, 124, 125, 186, 320, 329, 336). Furthermore, the genomes of a strain collection representing all serotypes of *Shigella* were characterized by comparative genomic hybridization (213). This vast amount of genetic information allows the identification of the successive genetic events that led to the evolution of pathogenic *Shigella* from nonpathogenic *E. coli* and provides insight into how variations in the virulence traits of different *Shigella* strains developed. Figure 3 illustrates the events that are considered to be most important for the evolution of pathogenic *Shigella* spp.

Bacterial evolution is a dynamic process that is accelerated by the gain of phenotypic traits through the acquisition of genes by horizontal gene transfer between distantly related microorganisms as well as through the loss of genes by deletion (191). The transfer and chromosomal incorporation of large mobile genetic entities carrying one or more virulence-associ-

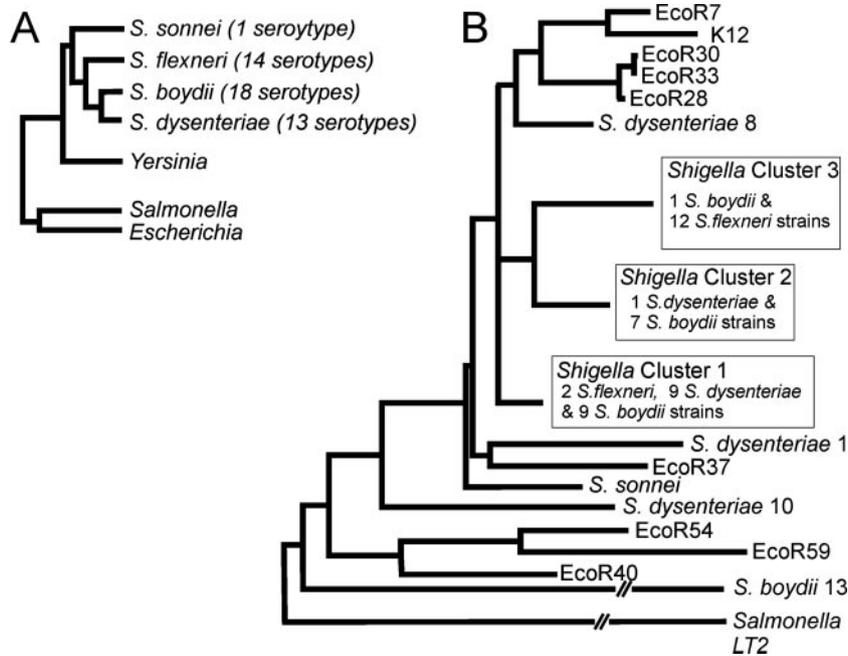


FIG. 2. Phylogenetic trees of *Shigella* and related enterobacteria. (A) Classical taxonomy of *Shigella* spp. based on phenotypic and numerical classification. (B) Evolutionary tree of *Shigella* and nonpathogenic *E. coli* strains (EcoR strains and K-12) based on comparative genomics. *Shigella* spp. fall into three main clusters, wherein the traditionally classified species are intermingled. The distribution of several *E. coli* EcoR strains between the *Shigella* strains indicates that the *Shigella* strains evolved from multiple ancestral origins. (Adapted from references 57a and 146 with permission from the Society for General Microbiology [United Kingdom] and Elsevier, respectively.)

ated genes, termed pathogenicity islands (PAI), are important steps for the rapid evolution of pathogenic bacteria from non-pathogenic progenitors (57, 266). Besides the presence of virulence genes, PAI are characterized by nucleotide composition and codon usage differing from those of the rest of the genome, a mosaic-like structure, association with mobile genetic elements, and genetic instability, which is reflected by an increased deletion frequency. Both the *Shigella* chromosome and the virulence plasmid reflect these dynamics by containing numerous insertion sequences (IS) and markers of genomic rearrangements (30, 125). The distribution of IS types and the phylogenetic grouping of chromosome- and plasmid-encoded genes indicate that the *Shigella* chromosome and the virulence

plasmid coevolved (67, 145, 337). Moreover, these findings suggest that the acquisition and transfer of plasmids between the strains were early steps in the diversification from *E. coli* ancestors.

In addition to pathogenicity islands on the virulence plasmid, *Shigella* pathogenicity islands (SHI) were identified in the chromosome (115, 266). The presence and genomic localization of SHI differ between *Shigella* strains and may contribute to the variation of virulence phenotypes (213, 336). A specific virulence function has been determined for only some of these genes. The immunoglobulin A-like cytotoxic protease SigA and the enterotoxin ShET1, encoded in SHI-1, were found to induce intestinal fluid accumulation in the rabbit ileal loop

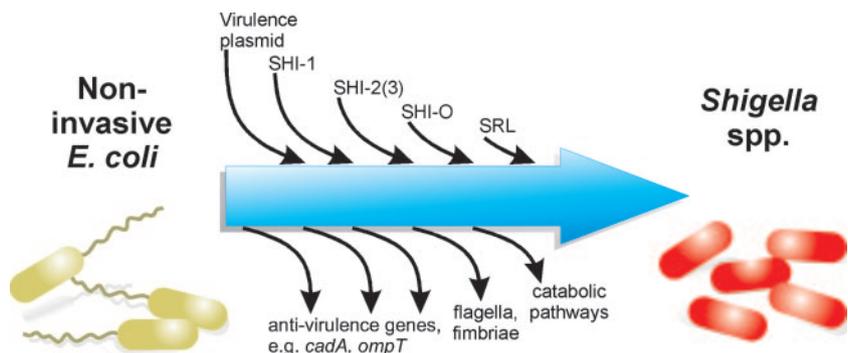


FIG. 3. Genetic events contributing to the evolution of *Shigella* spp. from nonpathogenic *E. coli* ancestors. *Shigella* spp. evolved from nonpathogenic *E. coli* through the acquisition of a large virulence plasmid and chromosomal pathogenicity islands as well as through the loss of genetic loci, which are not functional intracellularly or impede virulence. SRL, *Shigella* resistance locus. (Modified from reference 191 with permission from Macmillan Publishers Ltd.)

TABLE 1. Virulence factors encoded by chromosomal *Shigella* PAIs

PAI	Gene(s)	Biochemical activity	Virulence function(s)	Reference(s)
SHI-1	<i>sigA</i> <i>pic</i>	Immunoglobulin A-like cytopathic protease Serine protease, mucinase	Intestinal fluid accumulation, cytopathic toxin Mucus permeabilization, serum resistance, hemagglutination	5 105
	<i>set1A</i> , <i>set1B</i>	Enterotoxin ShET1	Intestinal fluid accumulation, development of watery diarrhea	70, 71
SHI-2	<i>iucA</i> to <i>iucD</i> , <i>iutA</i>	Siderophore (aerobactin) production and transport	Iron acquisition	177, 182, 322
	<i>shiD</i>	Unknown	Colicin I and colicin V immunity	116, 117
	<i>shiA</i>	Unknown	Downregulation of inflammation by suppression of T-cell signaling	
SHI-3	<i>iucA</i> to <i>iucD</i> , <i>iutA</i>	Aerobactin production and transport	Iron acquisition (found only in <i>S. boydii</i>)	227
SHI-O	<i>gtrA</i> , <i>gtrB</i> , <i>gtrV</i>	Modification of O antigens, serotype conversion	Evasion of host immune response	113, 115
SRL	<i>fecA</i> to <i>fecE</i> , <i>fecI</i> , <i>fecR</i>	Ferric dicitrate uptake	Iron acquisition	154, 314
	<i>tetA</i> to <i>tetD</i> , <i>tetR</i>	Efflux system	Tetracycline resistance	
	<i>cat</i>	Acetyltransferase	Chloramphenicol resistance	
	<i>oxa-1</i>	β -Lactamase	Ampicillin resistance	
	<i>aadA1</i>	Adenylyltransferase	Streptomycin resistance	

model of shigellosis (5, 70, 71), and a role for the serine protease Pic in mucus degradation and tissue invasion was postulated based on in vitro studies (105). Moreover, the SHI-2-encoded aerobactin iron acquisition system was found to contribute to virulence in vivo, and *shiA* attenuates *Shigella*-induced inflammation by suppression of T-cell signaling (116, 117, 182). Table 1 summarizes the SHI virulence factors that were identified and further characterized.

A late but important event in the diversification of *Shigella* strains was the acquisition of SHI-O. This PAI harbors genes modifying the structure of the bacterial lipopolysaccharide (LPS) O antigens, thus accounting for the large variety of *Shigella* serotypes (186). LPS is a crucial virulence factor, and the host immune response to *Shigella* is serotype specific (153, 216a, 343). The most recent acquisition of genes with new functions is reflected by the emergence of multidrug-resistant *Shigella* strains. A PAI, designated the *Shigella* resistance locus, confers resistance to streptomycin, ampicillin, chloramphenicol, and tetracycline (154, 313, 314).

Besides the extensive gain of virulence factors, the evolution of *Shigella* was shaped by a substantial loss of gene function due to deletion or gene inactivation (Fig. 3). Compared to *E. coli* K-12, an average of 726 genes are missing, and over 200 pseudogenes are found per *Shigella* strain (213, 336). Genome reduction due to the loss of functional genes is characteristic of the adaptation of bacteria to an intracellular pathogenic lifestyle (164, 175). Different factors drive this reductive evolution. The arrival of new virulence factors does not enhance bacterial virulence per se, as their expression and function are modulated by the existing genomic background. To be beneficial and therefore retained, virulence factors might require a specific genomic background (66). Consequently, genes that attenuate or interfere with virulence are prone to be inactivated.

Shigella prominently exemplifies the inactivation of so-called "antivirulence genes" (Fig. 3). The outer membrane protease

OmpT, which is present in various nonpathogenic *E. coli* strains but deleted in all *Shigella* strains, was found to inhibit the spread and virulence of *Shigella* by interfering with the polar localization of the actin nucleator protein IcsA (VirG) (180, 213). Virulence attenuation was also observed after the introduction of the *cadA* gene into *Shigella*. *cadA* encodes a lysine decarboxylase, which catalyzes the production of the polyamine cadaverine (166). Cadaverine inhibits the activity of the *Shigella* enterotoxins, thus causing a less severe disease. Genome analyses confirmed that *cadA*, present in ancestral *E. coli* strains, was deleted in most of the *Shigella* strains (46, 213).

Colonizing a host exerts strong selective pressure on the optimization of virulence traits. In parallel, continuous interaction with a host reduces the pressure to be able to respond to environmental changes encountered by free-living bacteria. Nutrients are abundant within hosts, which renders many bacterial metabolic pathways dispensable. Hence, catabolic pathways, e.g., lactose fermentation, represent a substantial proportion of genes inactivated or eliminated from the *Shigella* genome (120, 336). Furthermore, recent reports suggest that de novo synthesis of NAD is inactivated in *Shigella* spp. and EIEC, because the NAD precursor quinolinate strongly inhibits bacterial virulence (223, 224). These and other intermediates of antivirulence pathways might hold a potential for novel virulence inhibitors.

Shigella spp. have also lost the ability to synthesize functional flagella. Comparisons of different *Shigella* strains revealed that the genetic basis of this motility defect varies, indicating that the loss of motility is a result of convergent evolution (11, 307). It is tempting to speculate that flagellar motility was abandoned because it was not suitable as a mode of intracellular movement and therefore was substituted by actin-based motility, which exploits host resources. In addition, flagella and other surface structures like fimbriae, which are also degenerated in *Shigella*, are potent activators of the host immune

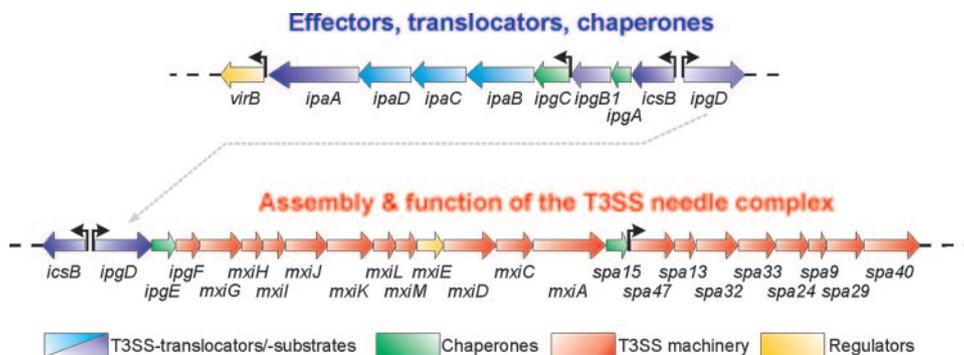


FIG. 4. Map of the 31-kb “entry region” on the *S. flexneri* virulence plasmid pWR100. The genes indicated encode structural components of the Mxi-Spa T3SS, secreted translocator and effector proteins, chaperones, and regulatory proteins. The region shown is essential and sufficient to invade EC and induce macrophage cell death.

system (21, 232, 244). The loss of these surface structures might therefore help to minimize the exposure to the host defense system.

MOLECULAR DETERMINANTS OF *SHIGELLA* PATHOGENESIS

The *Shigella* Virulence Plasmid

The cellular pathogenesis and clinical presentation of shigellosis are the sum of the complex action of a large number of bacterial virulence factors. The essential part of the molecular machinery required for bacterial invasion and intracellular survival is encoded on the large *S. flexneri* virulence plasmid (247, 253, 254, 259). Sequencing of virulence plasmids from different *Shigella* strains revealed that these plasmids of approximately 200 kb contain a mosaic of around 100 genes and a comparable number of IS (30, 124, 320, 336). The core of the plasmid is the conserved 31-kb entry region, which was shown to be necessary and sufficient for EC invasion and macrophage killing (129, 165, 260–262). The PAI-like region consists of 34 genes that are organized into two clusters transcribed in opposite directions (Fig. 4). Based on their functions, these genes can be divided into four different groups.

The first group consists of proteins secreted by the *S. flexneri* T3SS that act as effectors manipulating host cell processes in favor of the bacteria (see Table 2 and sections below). Among these proteins are the dominant immunogenic antigens of *S. flexneri*, the invasion plasmid antigens IpaA to IpaD (32, 190). Three of them, IpaB to IpaD, are the key virulence factors of *S. flexneri*. Apart from having effector functions that are essential for host cell invasion and intracellular survival, these proteins also control the secretion and translocation of other effector proteins into eukaryotic host cells (23, 170, 171).

Genes of the second group comprise more than half of the entry region and are required for the secretion of the Ipa proteins and other effector proteins. These genes were designated membrane expression of *ipa* (*mxi*) and surface presentation of *ipa* (*spa*) antigens (112, 319). The *mxi-spa* locus encodes the components needed for the assembly and function of a T3SS, which, together with IpaB, IpaC, and IpaD, allows the direct translocation of effector proteins from the bacterial cytoplasm into the host cell (23). Approximately 25 proteins

encoded in different locations on the virulence plasmid are secreted via this system (30).

In addition to the fundamental bacterial weaponry, the entry region contains the two transcriptional activators VirB and MxiE, representing group 3, which regulate T3SS-associated genes located in the entry region or scattered throughout the remainder of the virulence plasmid (2, 59, 128, 167). Finally, four genes, classified as group 4, encode chaperones (*ipgA*, *ipgC*, *ipgE*, and *spa15*) and are also located within the entry region. These chaperones stabilize T3SS substrates in the bacterial cytoplasm, and at least two of them, IpgC and Spa15, participate in the transcriptional regulation of T3SS effector genes located outside of the entry region (167, 201, 207, 208, 316).

Most of the T3SS effector proteins encoded outside of the entry region adopt postinvasion virulence functions. Several members of the outer *Shigella* protein (Osp) family shape the transcriptional response of EC invaded by *S. flexneri*, thus modulating the immune response in favor of the bacteria (14, 30, 131, 344). A role in the modulation of the immune response was also established for one member of the multigene IpaH family (102, 197). Members of the IpaH family share a conserved C-terminal domain and a variable N-terminal leucine-rich repeat (LRR) domain (318). LRR domains are involved in the sensing of pathogen-associated molecular patterns (PAMPs) by host cells and the subsequent induction of immune responses (19, 78, 163). Five IpaH genes have been identified on the virulence plasmid, and, depending on the *Shigella* strain, up to seven genes have been identified in the chromosome (125). The chromosomal IpaH genes seem to be regulated by MxiE/IpgC and are the only chromosomally encoded T3SS substrates (15, 168).

The dissemination of *S. flexneri* in the epithelial layer crucially depends on the ability to move by directed actin polymerization. The essential mediators of efficient movement by actin nucleation at one pole of the bacterium are IcsA/VirG, SopA/IscP, VirA, and PhoN2 (apyrase). The corresponding genes are also located outside of the entry region on the virulence plasmid (22, 63, 150, 156, 258, 338, 339). Among these proteins, only the secretion of VirA depends on the T3SS. In contrast, IcsA and SepA belong to the family of autotransporter proteins, which possess a C-terminal domain mediating

TABLE 2. Virulence factors encoded on the *S. flexneri* virulence plasmid

Effector	Biochemical activity	Host cell target(s)	Virulence function and/or phenotype	Reference(s) ^b
IpaA	Vinculin activation	Vinculin, β_1 -integrins, Rho signaling	Efficient invasion, actin cytoskeleton rearrangements, disassembly of cell-matrix adherence	52, 96, 121, 311
IpaB	Membrane fusion	Cholesterol, CD44, caspase-1	Control of type III secretion, translocon formation, phagosome escape, macrophage apoptosis	23, 36, 103, 109, 114, 170
IpaC	Actin polymerization	Actin, β -catenin	Translocon formation, filopodium formation, phagosome escape, disruption of EC tight junctions	23, 100, 171, 312
IpaD			Control of type III secretion, membrane insertion of translocon	68, 170, 317
IpaH7.8 IpaH9.8	E3 ubiquitin ligase	Splicing factor U2AF, MAPK kinase	Efficient phagosome escape Host cell transcriptome modulation, reduction of inflammation	73 197, 236, 309
IcsA ^a (VirG)		N-WASP, vinculin	Recruitment of actin-nucleating complex required for actin-based motility and intercellular spread	22, 84, 296, 298
IcsB			Camouflage of IcsA to prevent autophagic recognition	7, 194, 195
IcsP ^a	Serine protease		Cleavage of IcsA, modulation of actin-based motility	63, 278
IpgB1	RhoG mimicry	ELMO protein	Induction of Rac1-dependent membrane ruffling	12, 99, 196
IpgB2	RhoA mimicry	RhoA ligands	Induction of actin stress fiber-dependent membrane ruffling	12
IpgD	Phosphoinositide 4-phosphatase	Phosphatidylinositol 4,5-bisphosphate	Facilitation of entry, promotion of host cell survival	187, 188, 212
OspB			T3SS substrate, unknown function	258
OspC1		Nucleus and cytoplasm	Induction of PMN migration	344
OspD1			T3SS substrate, unknown function in host cells, antiactivator of MxiE	207
OspE2		Focal contacts	Maintenance of EC morphology, efficient intercellular spread	173
OspF	Phosphothreonine lyase	MAPKs Erk and p38	Inhibition of histone phosphorylation and NF- κ B-dependent gene expression, reduction of PMN recruitment	14, 137, 151, 344
OspG	Protein kinase, ubiquitination inhibitor	Ubiquitin-conjugating enzymes	Downregulation of NF- κ B activation, reduction of inflammation	131
PhoN2 ^a	Apyrase		Unipolar localization of IcsA	258
SepA ^a	Serine protease		Promotion of intestinal tissue invasion and destruction	20
VirA	Cysteine protease	α -Tubulin	Facilitation of entry and intracellular motility by degradation of microtubules	338, 339

^a These proteins are not substrates of the Mxi-Spa T3SS.

^b For the sake of clarity, only the most relevant references are cited.

secretion (20, 56, 185). The secreted serine protease SepA was found to enhance tissue destruction and inflammation in a rabbit ligated ileal loop model.

Finally, besides the large repertoire of genes implicated in bacterial virulence, the virulence plasmid encodes systems to ensure its replication and propagation. These systems include a postsegregational killing system, ensuring plasmid maintenance, particularly at 37°C, the temperature of infection (228, 263, 264).

Regulation of Virulence Plasmid Gene Expression

The function of the plasmid-encoded virulence machinery requires the complex interaction of several dozen proteins, the

permanent expression of which would be energetically costly. Consequently, all virulence genes are under the tight control of a regulatory network, which responds to environmental changes encountered upon the entry of the bacteria into the host (58, 59, 222, 308) (Fig. 5). The major trigger inducing the expression of the virulence plasmid entry region genes is a temperature shift to 37°C after uptake by the host (305). Additional factors that influence virulence plasmid gene expression are pH, osmolarity, and iron concentration (181, 210, 221).

The primary elements of the regulatory cascade, which sense and react to environmental changes, are encoded on the *S. flexneri* chromosome. Global regulators control the expression of chromosomal as well as virulence plasmid genes (58, 222).

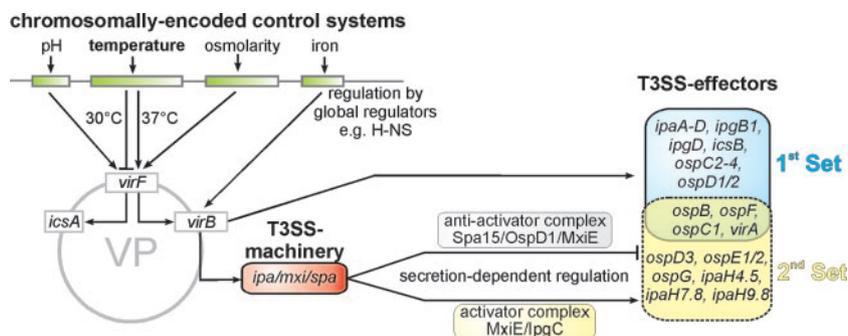


FIG. 5. Regulatory elements controlling the expression of the T3SS and its substrates on the *S. flexneri* virulence plasmid. The major virulence plasmid (VP) activator VirF is induced by environmental stimuli and triggers the expression of the central transcriptional activator VirB. VirB promotes the expression of the entry region genes and some additional effector genes scattered on the virulence plasmid. The secretion of the “first-set” T3SS effectors stored in the bacterial cytoplasm enables MxiE/IpgC-controlled induction of a discrete set of already-induced effector proteins and expression of the “second-set” effectors.

The virulence plasmid-encoded transcriptional activator VirF is induced at 37°C (305). VirF activates the second essential virulence plasmid regulator VirB and the actin nucleator protein IcsA (2, 220, 243, 306). VirB in turn induces the transcription of the entry region operons and some additional genes of the Osp family (149). Thus, the bacteria are equipped with the T3SS and a first set of effector proteins, allowing the invasion of host cells (Fig. 5).

The secretion of these “first-set” effector proteins leads to the increased transcription of a subset of already-induced proteins and a “second set” of additional T3SS effectors (53, 149, 167). The expression of the “second-set” effectors is controlled by the transcriptional activator MxiE, the activity of which is blocked by an antiactivator complex consisting of OspD1 and the chaperone Spa15 (207). Upon the induction of type III secretion, the “first-set” effector OspD1 is secreted along with other substrates like IpaB and IpaC, thus liberating the cognate chaperone IpgC. Consequently, MxiE associates with the coactivator IpgC (167), and the MxiE-IpgC complex activates the transcription of the “second-set” effectors (Fig. 5), which are secreted by intracellular bacteria.

The Mxi-Spa T3SS

S. flexneri, as well as other gram-negative pathogenic or symbiotic bacteria, manipulates host cell processes to establish a successful infection. The bacteria directly target intracellular host cell signaling pathways by transporting effector proteins across three membranes: the bacterial inner membrane (IM) and outer membrane (OM) as well as the host plasma membrane. The *S. flexneri* Mxi-Spa T3SS is a representative of sophisticated molecular export machineries that translocate proteins into the host cell cytoplasm in one step (43, 82). T3SSs are found in more than 25 bacterial species interacting with animal or plant hosts. It is noteworthy that several of these species encode two T3SSs, which usually belong to different subfamilies of T3SSs. As an example, *Salmonella enterica* serovar Typhimurium harbors two T3SSs encoded in the *Salmonella* pathogenicity island 1 (SPI-1) or SPI-2. More than 100 different T3SS substrates with versatile biological activities are currently known (1, 90, 265, 340). The architecture of T3SSs from different bacterial species shows some adaptation to the

specific hosts but shares a conserved main structure, which is closely related to those of the flagellar T3SS (25, 43, 301). Based on this conserved core structure, T3SSs can be distinguished from type IV secretion systems (T4SSs), which also permit the secretion and direct translocation of proteins from the bacteria into the host cell cytoplasm. T4SSs are found in many gram-negative bacteria, e.g., *Legionella* spp., and are related to bacterial DNA conjugation systems (16, 38, 275).

Architecture of the Mxi-Spa T3SS. The visualization of major parts of the closely related T3SSs encoded by *S. flexneri* and *Salmonella* SPI-1 by transmission electron microscopy revealed the ultrastructure of this multiprotein complex and allowed the determination of its dimensions (23, 24, 138, 245, 299). The observed macromolecular structure, termed the needle complex, is comprised of a seven-ringed basal body with an average length of 32 nm and a protruding needle with a length of 45 to 60 nm. The rings of the basal body have a diameter of 20 to 40 nm, whereas the outer diameter of the needle is approximately 7 nm. Consistent with a model of a one-step shuttling system, the needle complex contains a central 2- to 3-nm-wide channel. The narrow channel size does not permit the transport of fully folded proteins. Indeed, it was shown that the *Salmonella* serovar Typhimurium T3SS substrate SptP is separated from its cognate chaperone and largely unfolded prior to its export via the T3SS (3). However, structural models of the T3SS needle and T3SS substrates suggest that some secondary structures such as alpha helices, which are retained after partial unfolding, might be able to pass the needle (47, 65).

The molecular architecture, assembly, and function of the *S. flexneri* Mxi-Spa T3SS involves more than 25 proteins encoded in the entry region of the virulence plasmid (43) (Fig. 6). The first step of the assembly of the T3SS is the formation of the seven-ringed basal body, which spans the bacteria IM, periplasm, peptidoglycan layer, and OM. The major constituent of the OM rings of the basal body is the secretin protein MxiD, which is transported to the periplasm by the Sec-dependent secretion pathway (9, 24, 299). Membrane insertion and stabilization of the homo-oligomeric MxiD ring is essentially mediated by the lipoprotein MxiM, also referred to as pilot protein, which is anchored in the periplasmic leaflet of the OM by a lipid moiety (270, 271). The formation of the OM rings requires the penetration of the peptidoglycan meshwork un-

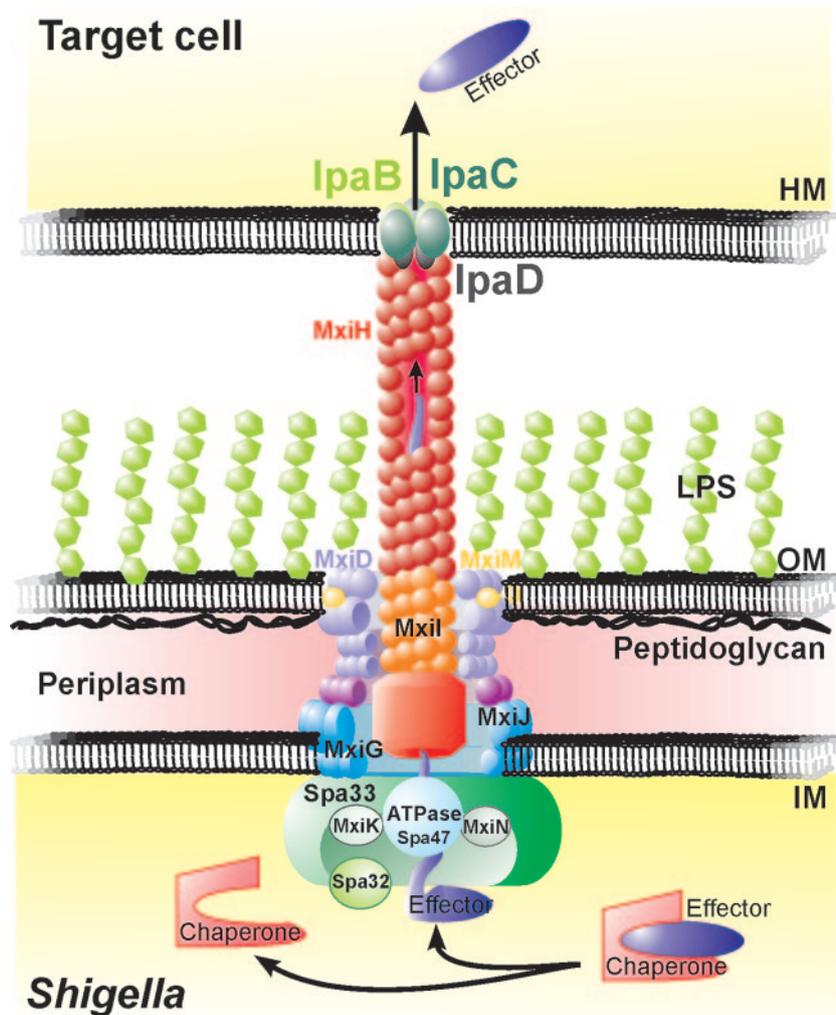


FIG. 6. Architecture of the *S. flexneri* Mxi-Spa T3SS. The *S. flexneri* Mxi-Spa T3SS consists of four main parts. The seven-ringed basal body spans the bacterial IM, the periplasm, and the OM. The hollow needle is attached to a socket and protrudes from the basal body to the bacterial surface. Contact with host cell membranes (HM) triggers the IpaD-guided membrane insertion of the IpaB-IpaC translocon at the needle tip. The T3SS is completed by the cytoplasmic C ring, which is comprised of proteins that energize the transport process and mediate the recognition of substrates, chaperone release, and substrate unfolding.

derlying the OM. The efficient assembly and function of T3SSs or T4SSs of different bacterial species, e.g., *Citrobacter rodentium* or *Helicobacter pylori*, depends on the peptidoglycan-degrading activity of specialized lytic transglycosylases (54, 77). Interestingly, the protein IpgF encoded next to the *mxi-spa* locus belongs to this enzyme family and exhibits peptidoglycan-degrading activity *in vitro*, yet a deletion of this gene does not attenuate virulence *in vivo* (6, 341). This might indicate that IpgF is not required or functionally redundant for efficient T3SS formation in *Shigella*.

Periplasmic and IM rings consist of the lipoprotein MxiJ and the membrane protein MxiG (Fig. 6). These proteins are anchored or inserted into the IM and interact with MxiD and MxiM through periplasmic domains (8, 10, 245, 271). Furthermore, based on sequence similarities with components of the flagellar apparatus, the Mxi-Spa proteins MxiA, Spa9, Spa24, Spa29, and Spa40 are proposed to be associated with the IM ring of the basal body (25, 43). Once the basal body is com-

pleted, the T3SS needle is assembled. The needle is composed of the major subunit MxiH and the minor component MxiI, which form a helical polymer extruding into the extracellular space (24, 42, 299). The assembly of the T3SS needle is controlled by cytoplasmic proteins associated with the IM ring of the basal body. The backbone of this cytoplasmic interface of the secretion apparatus, also called the C ring, is the Spa33 protein, which interacts with several T3SS effectors and a set of essential T3SS components (176, 272). This set includes the MxiN and MxiK proteins, which are required for the externalization of the needle subunits but not effector proteins, and Spa32, which determines the length of the T3SS needle (127, 155, 300). The control of the needle length seems to be of high importance for *S. flexneri*, as single isolated needles are of a strikingly uniform length (24, 299). In fact, it was shown that the needle length coevolved with the length of the *S. flexneri* LPS O antigens and is adjusted to allow maximal infectivity along with optimal protection against innate immune effectors

(330). It is not fully understood how Spa32 regulates needle length, but the protein is proposed to switch substrate specificity of the growing T3SS from the major needle subunit MxiH to the Ipa proteins (155).

Substrate recognition and energetics of type III secretion.

The assembly of the T3SS needle and the subsequent effector secretion also depend on the C-ring ATPase Spa47 (299, 319). T3SS-associated ATPases, such as Spa47, are believed to provide the energy for the unfolding of T3SS substrates, chaperone release, and transmembrane transport (3, 331). The transport of substrates across biological membranes is a thermodynamically unfavorable process and has to be energized by the hydrolysis of ATP as well as the transmembrane proton motive force (4). Accordingly, type III secretion of *Yersinia enterocolitica* effector proteins consumes ATP and requires the proton motive force (332). In agreement with these findings, the protonophore carbonyl cyanide *m*-chlorophenylhydrazone blocked *S. flexneri* type III secretion in infected macrophages as well as in vitro, triggered by the dye Congo red (269).

T3SS ATPases occupy a central position in the secretion process and, together with other C-ring proteins, are also involved in T3SS effector recognition (155, 288). An N-terminal secretion signal targets T3SS effectors to the secretion system, yet it is currently not clear if there is a hierarchy in the release of effector proteins and how intrinsic properties of the effectors contribute to controlling their export (93, 101, 218). Evidence from several bacterial T3SSs indicates an important role for the chaperones of T3SS effectors in orchestrating secretion (31, 72, 202). The *S. flexneri* T3SS chaperone Spa15 is necessary for the secretion of IpaA stored in the bacterial cytoplasm (203).

Host cell membrane insertion of the translocon. The complex formed by the C ring, basal body, and needle is sufficient to release proteins into the extracellular space, but a controlled secretion and translocation of effector proteins require the transport of the translocator proteins IpaB, IpaC, and IpaD to the tip of the T3SS needle (170, 317). The translocator proteins are expressed alongside the T3SS components and stored in the cytoplasm. To avoid premature association, IpaB and IpaC are bound by the chaperone IpgC (172), and in addition, IpaD has self-chaperoning activity (126). The exact mechanism of how secretion is controlled by the translocator proteins is not understood. Several reports indicate that in the absence of a host cell, the hydrophilic protein IpaD localizes to the tip of the T3SS needle and blocks secretion through interactions with IpaB (68, 198, 245, 246, 317). In the absence of a secretion trigger, IpaD seems to maintain a conformation and position that retain the hydrophobic protein IpaB inside the needle channel. Upon host cell contact, the T3SS is activated (326). The contact might trigger a conformational change in the “plug” protein IpaD, which also leads to the repositioning of IpaB and allows its passage and membrane insertion along with the second hydrophobic translocator protein IpaC, thus forming the multimeric translocation pore (23, 68, 317). Even though IpaB and IpaC interact and insert into artificial membranes in vitro, the efficient formation of the T3SS translocation pore in vivo strictly depends on IpaD, which might act as a scaffold for the correct translocon assembly (45, 49, 114, 172, 219).

Recent structural and mutational analyses further suggested

that, in addition to the translocator proteins IpaB and IpaD, the needle protein MxiH actively participates in the regulation of secretion and host cell sensing (49, 130, 317). As proposed for the tip protein IpaD, MxiH might undergo a subtle conformational change upon host cell contact, which contributes to the correct formation of the needle tip and is relayed through the needle to open the T3SS channel for additional effector proteins. However, structural analysis of MxiH mutants, which display different levels of secretion activity, did not reveal changes in the needle structure, indicating that these changes are too small to be detected or that MxiH controls secretion through a novel mechanism (41). Once the translocation pore is established and the needle is in the “open” conformation, additional Ipa proteins and other effector proteins can directly pass the T3SS channel and reach their targets inside the host cell.

SUBVERSION OF EPITHELIAL CELL SIGNALING

Adherence and Induction of Type III Secretion

While recent insight in the molecular architecture of the Mxi-Spa T3SS led to a model on how conformational changes in the translocator and needle tip open the transport channel (47, 68, 130), how host cell contact triggers translocation under natural conditions (327) or how the dye Congo red induces secretion in vitro (17) is not well defined.

The initial interactions of *S. flexneri* and its close relative *Salmonella* with a host cell occur at cholesterol-rich microdomains in the plasma membrane (80, 142) (Fig. 7). Cholesterol is a ubiquitous component of eukaryotic membranes and strongly influences their biophysical properties (283, 285). The majority of cholesterol is found in the plasma membrane of the eukaryotic cell, where it is not distributed evenly but rather accumulates in microdomains enriched in sphingolipids and proteins, so-called lipid rafts (98, 282, 285). A consequence of the unique raft lipid composition is the preferential recruitment or exclusion of membrane proteins, thus creating clusters of specific proteins and an asymmetric distribution of these clusters on the cell surface (238). Raft protein clusters are intimately connected to the actin cytoskeleton and to proteins localizing at the cytoplasmic leaflet of the plasma membrane (152). These exceptional properties predispose rafts as versatile signaling platforms, controlling signal transduction pathways such as endocytosis, intracellular vesicle trafficking, and activation of immune responses and apoptosis (86, 87, 104, 209, 230, 284). The central role of lipid rafts in the signaling networks of eukaryotic cells renders them a prime target for bacterial pathogens. Specifically, the unique protein and lipid composition, especially the high cholesterol content, might be a trigger to release bacterial effectors in close proximity to these signaling hot spots (Fig. 7).

The T3SS-mediated effector translocation by *Shigella*, *Salmonella*, and enteropathogenic *E. coli* requires the presence of membrane cholesterol (103). Interestingly, purified lipid rafts or liposomes, which mimic raft lipid composition but do not contain proteins, trigger effector secretion by the Mxi-Spa T3SS (315). A biochemical analysis revealed that the translocator protein IpaB and its *Salmonella* homologue SipB directly bind cholesterol with high affinity and insert into

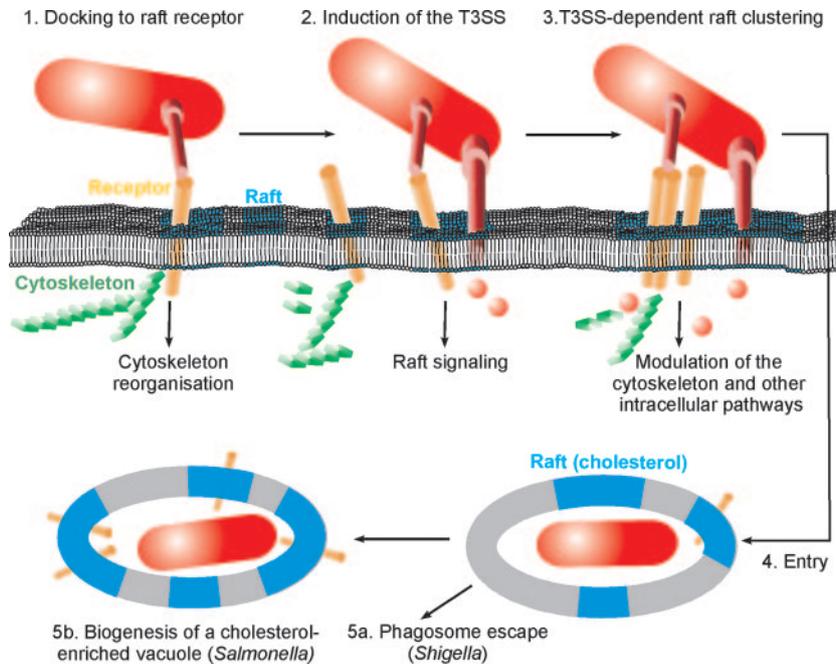


FIG. 7. Cholesterol and lipid rafts as triggers of type III secretion and targets of effectors. (1) The initial contact of bacteria with cholesterol-rich membrane microdomains is mediated by receptors, which partition to rafts. Receptor binding might already induce raft signaling, e.g., trigger the reorganization of the cytoskeleton. (2) Raft contact is followed by the cholesterol-dependent induction of effector protein secretion. (3) Receptor binding and effector secretion lead to the clustering of rafts and the subsequent uptake of the bacteria as well as the activation of other signaling pathways. (4) After uptake, the bacteria reside in a raft-containing vacuole, which is lysed (5a) or modified to create a replication-permissive niche (5b). Modification of the vacuole leads to a further enrichment of rafts (cholesterol), thus permitting bacterial manipulation of raft-associated signaling pathways.

membranes in a cholesterol-dependent manner. The translocon protein IpaC also integrates into cholesterol-containing membranes, but a high cholesterol content strongly reduces its membrane disruption activity (100). However, the *in vivo* significance of this observation and whether the needle tip protein IpaD also interacts with cholesterol remain unclear. It is noteworthy that a large number of secreted bacterial pore-forming or enzymatically active toxins, such as perfringolysin O and Shiga toxin, also target lipid rafts and require cholesterol for membrane insertion, oligomerization, and activity (204, 235).

The induction of the *S. flexneri* Mxi-Spa T3SS or *Salmonella* SPI-1 T3SS at cholesterol-rich membrane microdomains triggers the accumulation of cholesterol and lipid raft markers at the bacterial entry sites (80, 142). This observation indicates that the bacteria promote the aggregation and fusion of rafts at the entry site, thus creating an extended raft signaling platform with a high density of receptors and putative signaling molecules. Several other pathogens, e.g., *Mycobacterium* spp., *Brucella* spp., or *Chlamydia* spp., enter their host cells via rafts (143, 159). *S. flexneri* adheres to host cells by binding to two receptors, the hyaluronan receptor CD44 and $\alpha_5\beta_1$ integrin, which both partition to lipid rafts and accumulate at the bacterial entry site (287, 326). Binding to CD44 apparently occurs through IpaB, while the $\alpha_5\beta_1$ integrin receptor is bound by a IpaBCD complex. These interactions might precede the induction of the T3SS and direct the bacteria to lipid rafts. Finally, binding to either receptor induces actin

cytoskeleton reorganization and promotes invasion by *S. flexneri* (142, 326).

Entry via lipid rafts not only remodels the actin cytoskeleton but also critically influences the fate of the intracellular bacteria (Fig. 7). Lipid rafts modulate intracellular vesicle trafficking, and raft-derived endosomes do not enter the lysosomal degradation pathway (104). Accordingly, uptake via lipid rafts protects some bacteria, including *Mycobacterium* spp., against lysosomal degradation (48, 75). Along the same line, the formation of replication-permissive vacuoles by invading bacteria, e.g., the *Salmonella*-containing vacuole or the parasitophorous vacuole of the obligate intracellular pathogen *Coxiella burnetii*, coincides with an enrichment of cholesterol and raft proteins on the vacuolar membranes (33, 111). In *Salmonella* infections, cholesterol acquisition correlates with bacterial replication and seems to depend on the SPI-2 T3SS, which is activated in the intracellular environment. It is tempting to speculate that *S. flexneri*, which escapes from the phagosome into the host cell cytoplasm, uses the signaling potential of lipid rafts for the manipulation of host cell processes other than the initial steps of invasion. Recently, cholesterol depletion by the compound methyl- β -cyclodextrin after macrophage invasion by *S. flexneri* was found to inhibit macrophage cell death and the activation of caspase-1 (268). Furthermore, the amount of membrane-bound IpaB in *S. flexneri*-infected macrophages was reduced under these conditions. These findings indicate that cholesterol-dependent signaling might be required for the proapoptotic cascade induced by intracellular *S. flexneri*.

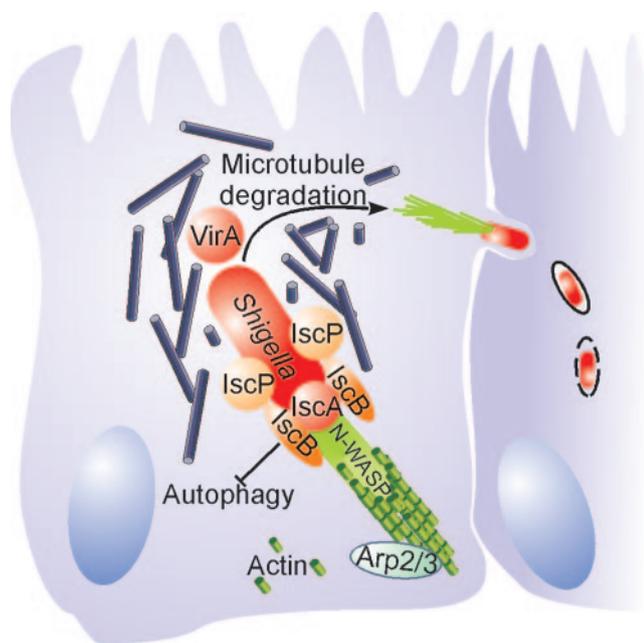


FIG. 9. Intracellular movement of *S. flexneri* by directed actin polymerization. Due to the activity of the serine protease SopA/IcsP, *S. flexneri* IcsA localizes to one pole of the bacterium, where it interacts with the host cell N-WASP protein. The IcsA/N-WASP complex recruits and activates the Arp2/Arp3 complex, thereby mediating actin nucleation. Elongation of the actin tail pushes *S. flexneri* through the cytoplasm. The movement is facilitated by VirA, which opens a path by degradation of the microtubule network. To avoid sequestration by the autophagy defense system, an autophagy recognition site on IcsA is masked by the protein IcsB.

closely related IpaC homologue SipC not only complements the SipC deletion phenotype but also leads to the lysis of the *Salmonella*-containing vacuole (200).

In addition to the translocator proteins, the T3SS effector IpaH7.8 was found to promote bacterial phagosome escape in macrophages by an unknown mechanism (73). IpaH7.8 belongs to the “second-set” T3SS effectors, which are expressed after the activation of the T3SS and the translocation of “first-set” effectors (Fig. 5).

Intracellular Motility and Intercellular Dissemination

The cytoplasm of EC is the main replicative niche for *S. flexneri*. Here, the bacteria are protected from the immune system components present in the extracellular environment. Nevertheless, bacteria have to evade intracellular host defense mechanisms and prepare to spread to new replicative niches. The intracellular survival and intercellular spread of *S. flexneri* are linked to the machinery allowing the bacteria to move by directed actin polymerization.

The molecular mechanisms mediating the actin-based motility of *S. flexneri* and other intracellular pathogens have been studied extensively (89, 291) (Fig. 9). The central bacterial mediator of actin polymerization is the IcsA (intracellular spread) protein, which localizes to one pole of the bacterium (22, 84, 85) (Table 2). The unipolar localization and activity of IcsA are controlled by the serine protease SopA/IcsP and the

apyrase PhoN2 (63, 239, 258, 278, 290). Surface-bound IcsA recruits and activates host cell factors, including the neuronal Wiskott-Aldrich syndrome protein (N-WASP) and the Arp2/Arp3 complex (64, 97, 294–296). The complex thus formed serves as an actin nucleator and catalyzes the directed elongation of an actin tail, which propels *S. flexneri* through the cytoplasm. In addition to the actin-nucleating complex, the T3SS-secreted cysteine protease VirA was recently identified to be pivotal for efficient intracellular movement and subsequent intercellular spread (338). VirA degrades α -tubulin, thus creating a “tunnel” in the dense intracellular microtubule network.

Intracellular motility, replication, and survival further depend on the T3SS substrate IcsB (194), which protects the bacteria from being recognized and entrapped by the host cell autophagy machinery (195). Autophagy is a host cell recycling and defense system, which engulfs and sequesters cytoplasmic components in double-membrane-bound compartments (55, 132). Interestingly, IcsA contains an autophagy-inducing recognition site, which has to be masked by IcsB to prevent engulfment by autophagic vacuoles and to ensure the intracellular survival of *S. flexneri* (195).

Due to propulsion through the host cell cytoplasm by actin polymerization, moving *S. flexneri* eventually impinges on the plasma membrane at the tight junctions of adjacent EC. The arising protrusions can be endocytosed by the neighboring cell in a process requiring host cell factors like myosin light-chain kinase (233). After lysis of the surrounding double membrane, which depends on the T3SS and the translocator proteins IpaB, IpaC, and IpaD, *S. flexneri* is free in the cytoplasm, and a new cycle of replication and cell-to-cell spread can start (201, 219, 234, 273).

Modulation of Host Cell Survival and Immune Signaling

The intracellular lifestyle protects *Shigella* spp. from immune system effector cells present in the subepithelial layer. *S. flexneri* secretes at least two different type III effectors, which promote the survival of the invaded host cells. In addition to being involved in the uptake of the bacteria, IpgD triggers the activation of the phosphatidylinositol 3-kinase/Akt antiapoptotic signaling pathway. The activation of this pathway requires the formation of PtdIns(5)*P* and involves host cell protein phosphorylation events (212). Moreover, intracellular *S. flexneri* secretes an MxiE-activated effector protein, which blocks cell death triggered by the apoptosis inducer staurosporine (39). The identity of this antiapoptotic activity and its intracellular target currently remain unknown.

While intracellular *S. flexneri* is protected from patrolling immune cells, in EC, the bacteria are detected by several intracellular surveillance systems that recognize microbial components and activate the host immune defense. *S. flexneri* secretes T3SS effectors, which interfere with these host cell signaling cascades on different levels (Table 2). The effector protein OspF interferes with mitogen-activated protein kinase (MAPK) signaling pathways (14, 137, 151). OspF is the first member of a new family of phosphothreonine lyases, which irreversibly remove phosphate groups from MAPKs such as p38. Interestingly, the inhibition of MAPK signaling specifically blocks histone phosphorylation and thus impairs the ac-

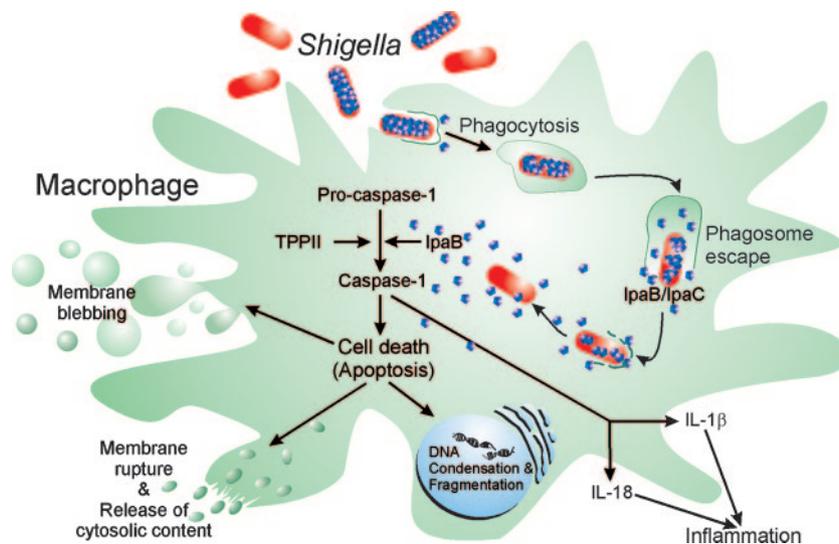


FIG. 10. *S. flexneri*-induced macrophage death. Depending on the Mxi-Spa T3SS, *S. flexneri* enhances its uptake by macrophages and secretes the translocators/ effectors IpaB and IpaC to escape from the phagosome. In the cytoplasm, the remaining bacterial pool of IpaB is gradually released. Secreted IpaB integrates into membranes in a cholesterol-dependent manner and triggers the proteolytic activation of procaspase-1, which might be further promoted by TPPII. Active caspase-1 executes cell death and cleaves the precursors of the proinflammatory cytokines IL-1 β and IL-18. The mature cytokines are released from the dying macrophage and elicit the strong inflammation characteristic of shigellosis.

cess of the transcription factor NF- κ B to its chromatin target sites (14). Therefore, genes involved in immune signaling are transcribed at a lower level. It is noteworthy that the production of the chemokine IL-8 (a potent chemoattractant for PMN) is reduced, which in turn results in a less pronounced inflammatory response. OspG is another Mxi-Spa-secreted effector protein, which blocks NF- κ B-dependent immune signaling, thus dampening intestinal inflammation. OspG is a protein kinase that targets and inhibits ubiquitinated ubiquitin-conjugating enzymes such as the stem cell factor SCF ^{β -TrCP}, which is required to activate the degradation of the phosphorylated inhibitor of NF- κ B type α (phospho-I κ B α) by the proteasome (131).

Recently, the interference of effector proteins with ubiquitination-dependent protein degradation was established as a virulence strategy of *S. flexneri* (236). The Mxi-Spa-secreted effector IpaH9.8 functions as an E3 ubiquitin ligase and inhibits MAPK kinase-dependent signaling by targeting components of this pathway for degradation by the proteasome. IpaH9.8 belongs to the IpaH multiprotein family, suggesting that other *S. flexneri* proteins also possess ubiquitin ligase activity. However, their specific intracellular protein targets have yet to be identified. Interestingly, the chromosome-encoded IpaH family protein members reduce *Shigella*-induced inflammation in a mouse pulmonary infection model (15). Several IpaH proteins might be functionally redundant, as only an *S. flexneri* strain deficient in all chromosomal *ipaH* genes displayed a detectable phenotype. Finally, apart from their ubiquitin ligase activities, the IpaH proteins might have additional functions, as IpaH9.8 localizes to the nucleus and interacts with the splicing factor U2AF³⁵ (197, 309). The reduction in the U2AF³⁵ levels resulted in a decreased expression of proinflammatory cytokines such as IL-8 or IL-1 β , thus strongly decreasing the inflammatory response upon infection with *S. flexneri*. In summary, these findings indicate that *S. flexneri*

modulates the survival and the inflammatory responses of invaded EC in intricate ways.

INDUCTION OF MACROPHAGE DEATH

Caspase-1-Dependent Macrophage Death

Macrophages are professional phagocytes that take up and degrade invading pathogens, and thus, these cells represent a fundamental component of the innate immune response (122, 229, 302). Bacterial pathogens have developed various strategies to avoid recognition and killing by macrophages. Whereas some bacteria inject effectors to prevent phagocytosis or interfere with vacuolar degradation, *S. flexneri* and other bacterial species avoid being killed by inducing macrophage cell death (34, 51, 107a, 133, 184, 241). The evasion of killing by macrophages is a crucial step of the pathogenesis of *S. flexneri*.

Macrophages are among the first cells that *S. flexneri* encounters after transcytosis through M cells from the lumen to the subepithelial dome (Fig. 10). The professional phagocytes engulf *S. flexneri*, yet the bacteria further enhance their uptake by secreting effectors via the Mxi-Spa T3SS, in particular IpaC (140). After uptake, *S. flexneri* rapidly disrupts the phagosomal membrane, thereby avoiding phagosome-lysosome fusion and degradation. Instead, the bacteria kill the macrophage (40) but only after they escaped from the phagosome (36, 108, 109, 347). Macrophage death is mediated by the Mxi-Spa-secreted translocator/effector protein IpaB (36, 109, 346). *S. flexneri* triggers an apoptotic cell death pathway characterized by chromatin condensation, DNA fragmentation, cytoplasmic blebbing and vacuolization, as well as a loss of plasma membrane integrity (40, 347). Moreover, macrophage death is paralleled by a rapid decrease of intracellular ATP and the loss of the mitochondrial membrane potential (135, 255). In *S. flexneri*-infected macrophages, caspase-1 (IL-1 β -converting enzyme

[ICE]) is activated (36, 108, 109, 345) (Fig. 10). Caspase-1 is required for this cell death pathway, and purified IpaB specifically binds to this caspase-1 (109). The inhibition of type III secretion throughout an infection of macrophages strongly reduces the cytotoxicity of *S. flexneri*, and consequently, the maximum activation of caspase-1 and macrophage cell death appear to require prolonged intracellular type III secretion of IpaB by cytosolic bacteria (269). Furthermore, cholesterol depletion after invasion and phagosome escape of *S. flexneri* inhibits caspase-1 activation as well as cell death and coincides with a reduced membrane association of IpaB (268). These findings suggest a functional role for host cell membranes, in particular cholesterol, in caspase-1 activation.

The onset of *S. flexneri*-induced macrophage death depends on caspase-1 but not other caspases, such as caspase-3, an "executioner" of cell death, or caspase-11, an activator of caspase-1 in response to LPS challenge (50, 109, 324). In addition, the cytoplasmic high-molecular-weight serine protease tripeptidyl peptidase II (TPPII) might participate upstream of caspase-1 in this cell death pathway (110). TPPII is a member of the family of self-compartmentalizing proteases involved particularly in protein degradation during the trimming of major histocompatibility complex class I antigens and cell cycle control (81, 276, 289).

The only cellular targets of activated caspase-1 that have been identified in the course of an infection with *S. flexneri* are the precursors of the proinflammatory cytokines IL-1 β and IL-18 (256, 345). The cleavage of pro-IL-1 β and pro-IL-18 generates their biologically active forms, which are released from dying macrophages. Both cytokines are central mediators of inflammatory immune signaling and the key triggers of the massive inflammation characteristic of shigellosis (251, 256). The caspase-1 substrates that execute *S. flexneri*-induced macrophage death are presently not known.

In addition to the pathway described above, *S. flexneri* triggers a caspase-1-independent cell death pathway in macrophages and dendritic cells. *S. flexneri*-induced killing of dendritic cells was found to be only partially prevented by caspase-1 inhibitors (62). This caspase-1-independent cell death is activated by the presence of bacterial LPS in the macrophage cytoplasm (297). Thus, different cell death phenotypes induced by *S. flexneri* in macrophages may be explained by the complex interplay of multiple cell death pathways (95).

Activation of Caspase-1 in Multiprotein Complexes

Caspase-1 belongs to the family of aspartate-specific cysteine proteases, which are essential mediators of apoptosis and inflammatory signaling (139, 162, 304). Based on their main cellular functions, caspases are defined as apoptotic caspases (e.g., caspase-2, caspase-3, caspase-6, caspase-8, and caspase-10) or inflammatory caspases (caspase-1, caspase-4, caspase-5, caspase-11, and caspase-12) (274, 281). However, there is increasing evidence that several caspases, e.g., caspase-1 and caspase-8, fulfill dual functions in apoptotic and immune signaling. An alternative classification distinguishes initiator caspases (e.g., caspase-1, caspase-2, and caspase-8) and executioner caspases (e.g., caspase-3, caspase-6, and caspase-7) according to their positions in cell death pathways (237).

Caspases are synthesized as inactive zymogens comprised of a prodomain and a catalytic domain. Upon extra- or intracellular stimulation, initiator procaspases are recruited into multiprotein complexes, wherein they come in close proximity and become activated by autocatalytic proteolysis (26, 279). Mature initiator caspases in turn activate downstream executioner caspases, which cleave a large number of cellular targets that ultimately kill and dismantle the cell.

The breakthrough in the understanding of how various stimuli converge in the activation of caspase-1 was the discovery of the inflammasome, the caspase-1-activating protein platform (161) (Fig. 11). Extensive research over the last years described the inflammasome as being a dynamic multiprotein complex (160, 162). The central scaffold of this complex is comprised of proteins of the nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family. NOD proteins and NLR proteins represent a class of intracellular receptors that share a conserved domain structure and sense a wide variety of PAMPs and other danger signals (78, 292). Furthermore, NLRs harbor an ATPase domain that is essential for their signaling function (60). Three major subtypes of NLRs are involved in inflammasome assembly, the NACHT-, LRR-, and pyrin-domain-containing proteins (NALPs), the neuronal apoptosis inhibitor proteins (NAIPs), and the ICE protease-activating factor (IPAF). The presence of 14 different NALPs, IPAF, and NAIP in the human genome and even more homologues in the mouse genome suggests that each NLR functions as a receptor for a specific danger signal or a small number of danger signals. Recently, several caspase-1-activating PAMPs including bacterial flagellin, RNA, and bacterial pore-forming toxins and their cognate NLRs were identified (160, 162).

The conserved modular domain structure enables NLRs to detect these multiple danger signals and integrate them in one response, namely, caspase-1 activation (78) (Fig. 11). All of these proteins consist of a mostly C-terminal variable LRR domain sensing the danger signal, a conserved NACHT oligomerization domain, and an N-terminal protein-protein interaction domain, which is involved in caspase-1 recruitment. The N-terminal domain is characteristic of each NLR subtype and consists of three repeats of a baculovirus inhibitor-of-apoptosis repeat domain in NAIPs, one caspase recruitment domain (CARD) in IPAF, and one pyrin domain (PYD) in the case of the NALPs. The CARD, which is also present in the prodomains of several caspases, e.g., caspase-1, caspase-4, and caspase-9, and the PYD belong to the death domain superfamily and fulfill critical roles in apoptotic and inflammatory signaling (205).

How NAIPs mediate caspase-1 activation is poorly understood. NALPs and IPAF become activated upon danger signal recognition by their LRR domains, oligomerize via the central NACHT domain, and expose the protein-protein interaction CARD or PYD. The formation of the inflammasome complex and caspase-1 activation require the ATPase activity of the NLR (60). The PYD of the NALPs engages an adaptor protein, the apoptosis-associated speck-like protein containing a CARD (ASC), through the homotypic binding of the PYD of ASC. Homotypic interactions between the CARDS of IPAF or ASC and the CARD of the caspase-1 zymogen recruit the proenzyme to the inflammasome complex and induce its autocatalytic activation. Finally, the proteolysis of the 45-kDa

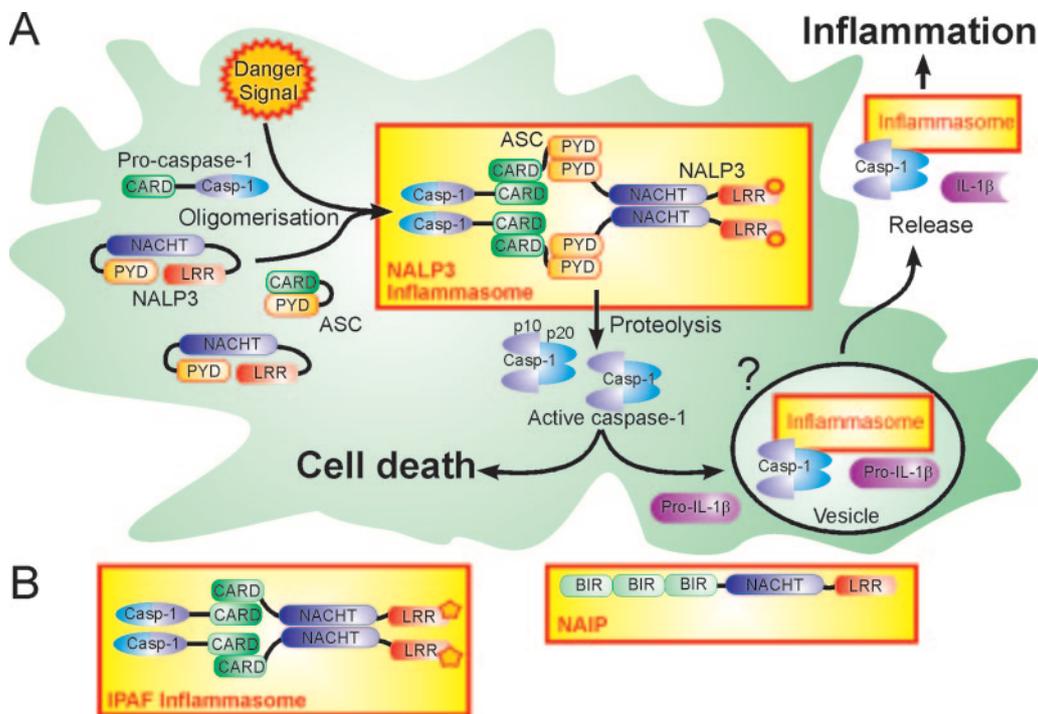


FIG. 11. Caspase-1 activation in the inflammasome. (A) Caspase-1 activation and IL-1 β processing by the NALP3 inflammasome. A danger signal(s) induces a conformational change in NALP3, which leads to oligomerization and exposure of the PYD. Through homotypic PYD-PYD interactions, ASC is bound and recruits procaspase-1 through its CARD domain. Brought into close proximity, procaspase-1 undergoes autocatalytic cleavage into p10 and p20 fragments, which form the active caspase-1 tetramer. Active caspase-1 induces cell death and processes pro-IL-1 β . The activation of caspase-1 occurs in or leads to the formation of vesicles through which caspase-1, IL-1 β , and the inflammasome are released into the extracellular space. (B) Domain structure of the IPAF inflammasome and the NAIP.

caspase-1 zymogen removes the prodomain and generates a 10-kDa (p10) subunit and a 20-kDa (p20) subunit, which associate to form the active caspase-1 heterotetramer (92, 333).

The Mxi-Spa-secreted *S. flexneri* translocator/effector protein IpaB binds caspase-1 directly in vitro (109). However, it is not clear whether IpaB has to be considered a bacterial PAMP, which induces inflammasome formation upon infection, or if IpaB directly recruits caspase-1 into the inflammasome complex. Caspase-1 activation in response to *S. flexneri* was recently shown to depend on the cytosolic pattern recognition receptors IPAF and ASC (293). While the bacterial component inducing caspase-1 activation remains unidentified, active caspase-1 was found to dampen autophagy triggered by intracellular *S. flexneri*. IPAF but not ASC is required for autophagy inhibition and the rapid induction of macrophage membrane permeabilization observed. These findings suggest that ASC might have different regulatory functions, which are dependent on the presence or absence of other inflammasome components.

Active caspase-1 cleaves the precursors of the proinflammatory cytokines IL-1 β , IL-18, and IL-33 and triggers cell death by an unknown mechanism (35, 91, 267, 303). However, the activation of caspase-1 is not only a trigger of cell death. Rather, this caspase also seems to have vital functions, e.g., the induction of membrane repair in response to bacterial pore-forming toxins (94). The factor(s), that tips the balance between cell survival and death has not been discovered. Several reports showed that mature IL-1 β , active caspase-1, and inflammasome components can be released from living cells,

possibly by exocytosis of endolysosome-related vesicles or the budding of microvesicles (13, 74, 161). How these vesicles are formed, contribute to caspase-1 activation, and are released is presently unclear. It will be interesting to determine whether and how *S. flexneri* controls the composition and secretion of vesicles containing inflammasome components to modulate macrophage death.

CONCLUDING REMARKS

More than 100 years after their discovery, *Shigella* spp. still pose a worldwide major health problem due to the devastating diarrhea that the bacteria may cause. Research performed over the last 25 years has tremendously contributed to our understanding of shigellosis on molecular, cellular, and organismic levels. Thus, we gained profound insight into the evolution of *Shigella* spp. originating from harmless enterobacterial relatives, the structure and function of the major virulence factors, including a T3SS and cognate effector proteins, as well as the interactions of *S. flexneri* with various host cells. Specifically, pathogen-triggered invasion of EC and the induction of macrophage death by *S. flexneri* have been studied in detail. Intricate mechanisms by which *S. flexneri* modulates inflammatory pathways in the host, thereby launching a successful colonization, were unraveled only recently. An in-depth understanding of the molecular mechanisms underlying these processes is a prerequisite to design a protective, live-attenuated *Shigella* vaccine strain that is safe and efficient. In concert with ongoing

efforts to improve hygiene standards, such a vaccine would offer substantial relief from what is still one of the most dreadful bacterial pathogens.

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REFERENCES

- Abe, A., T. Matsuzawa, and A. Kuwae. 2005. Type-III effectors: sophisticated bacterial virulence factors. *C. R. Biol.* **328**:413–428.
- Adler, B., C. Sasakawa, T. Tobe, S. Makino, K. Komatsu, and M. Yoshikawa. 1989. A dual transcriptional activation system for the 230 kb plasmid genes coding for virulence-associated antigens of *Shigella flexneri*. *Mol. Microbiol.* **3**:627–635.
- Akeda, Y., and J. E. Galan. 2005. Chaperone release and unfolding of substrates in type III secretion. *Nature* **437**:911–915.
- Alder, N. N., and S. M. Theg. 2003. Energy use by biological protein transport pathways. *Trends Biochem. Sci.* **28**:442–451.
- Al-Hasani, K., I. R. Henderson, H. Sakellaris, K. Rajakumar, T. Grant, J. P. Nataro, R. Robins-Browne, and B. Adler. 2000. The *sigA* gene which is borne on the *she* pathogenicity island of *Shigella flexneri* 2a encodes an exported cytopathic protease involved in intestinal fluid accumulation. *Infect. Immun.* **68**:2457–2463.
- Allaoui, A., R. Ménard, P. J. Sansonetti, and C. Parsot. 1993. Characterization of the *Shigella flexneri* *ipgD* and *ipgF* genes, which are located in the proximal part of the *mxi* locus. *Infect. Immun.* **61**:1707–1714.
- Allaoui, A., J. Mournier, M. C. Prévost, P. J. Sansonetti, and C. Parsot. 1992. *icsB*: a *Shigella flexneri* virulence gene necessary for the lysis of protrusions during intercellular spread. *Mol. Microbiol.* **6**:1605–1616.
- Allaoui, A., P. J. Sansonetti, R. Ménard, S. Barzu, J. Mounier, A. Phalipon, and C. Parsot. 1995. MxiG, a membrane protein required for secretion of *Shigella* spp. Ipa invasins: involvement in entry into epithelial cells and in intercellular dissemination. *Mol. Microbiol.* **17**:461–470.
- Allaoui, A., P. J. Sansonetti, and C. Parsot. 1993. MxiD: an outer membrane protein necessary for the secretion of the *Shigella flexneri* Ipa invasins. *Mol. Microbiol.* **7**:59–68.
- Allaoui, A., P. J. Sansonetti, and C. Parsot. 1992. MxiI, a lipoprotein involved in secretion of *Shigella* Ipa invasins, is homologous to YscJ, a secretion factor of the *Yersinia* Yop proteins. *J. Bacteriol.* **174**:7661–7669.
- Al Mamun, A. A., A. Tominaga, and M. Enomoto. 1996. Detection and characterization of the flagellar master operon in the four *Shigella* subgroups. *J. Bacteriol.* **178**:3722–3726.
- Alto, N. M., F. Shao, C. S. Lazar, R. L. Brost, G. Chua, S. Mattoo, S. A. McMahon, P. Ghosh, T. R. Hughes, C. Boone, and J. E. Dixon. 2006. Identification of a bacterial type III effector family with G protein mimicry functions. *Cell* **124**:133–145.
- Andrei, C., C. Dazzi, L. Lotti, M. R. Torrisi, G. Chimini, and A. Rubartelli. 1999. The secretory route of the leaderless protein interleukin- β involves exocytosis of endolysosome-related vesicles. *Mol. Biol. Cell* **10**:1463–1475.
- Arbibe, L., D. W. Kim, E. Batsche, T. Pedron, B. Mateescu, C. Muchardt, C. Parsot, and P. J. Sansonetti. 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.
- Ashida, H., T. Toyotome, T. Nagai, and C. Sasakawa. 2007. *Shigella* chromosomal IpaH proteins are secreted via the type III secretion system and act as effectors. *Mol. Microbiol.* **63**:680–693.
- Backert, S., and T. F. Meyer. 2006. Type IV secretion systems and their effectors in bacterial pathogenesis. *Curr. Opin. Microbiol.* **9**:207–217.
- Bahrani, F. K., P. J. Sansonetti, and C. Parsot. 1997. Secretion of Ipa proteins by *Shigella flexneri*: inducer molecules and kinetics of activation. *Infect. Immun.* **65**:4005–4010.
- Barzu, S., Z. Benjelloun-Touimi, A. Phalipon, P. Sansonetti, and C. Parsot. 1997. Functional analysis of the *Shigella flexneri* IpaC invasin by insertional mutagenesis. *Infect. Immun.* **65**:1599–1605.
- Bell, J. K., G. E. Mullen, C. A. Leifer, A. Mazzoni, D. R. Davies, and D. M. Segal. 2003. Leucine-rich repeats and pathogen recognition in Toll-like receptors. *Trends Immunol.* **24**:528–533.
- Benjelloun-Touimi, Z., P. J. Sansonetti, and C. Parsot. 1995. SepA, the major extracellular protein of *Shigella flexneri*: autonomous secretion and involvement in tissue invasion. *Mol. Microbiol.* **17**:123–135.
- Bergsten, G., B. Wullt, and C. Svanborg. 2005. *Escherichia coli*, fimbriae, bacterial persistence and host response induction in the human urinary tract. *Int. J. Med. Microbiol.* **295**:487–502.
- Bernardini, M. L., J. Mounier, H. d'Hauteville, M. Coquis-Rondon, and P. J. Sansonetti. 1989. Identification of *icsA*, a plasmid locus of *Shigella flexneri* that governs bacterial intra- and intercellular spread through interaction with F-actin. *Proc. Natl. Acad. Sci. USA* **86**:3867–3871.
- Blocker, A., P. Gounon, E. Larquet, K. Niebuhr, V. Cabiaux, C. Parsot, and P. Sansonetti. 1999. The tripartite type III secretin of *Shigella flexneri* inserts IpaB and IpaC into host membranes. *J. Cell Biol.* **147**:683–693.
- Blocker, A., N. Jouihri, E. Larquet, P. Gounon, F. Ebel, C. Parsot, P. Sansonetti, and A. Allaoui. 2001. Structure and composition of the *Shigella flexneri* “needle complex,” a part of its type III secretin. *Mol. Microbiol.* **39**:652–663.
- Blocker, A., K. Komoriya, and S. Aizawa. 2003. Type III secretion systems and bacterial flagella: insights into their function from structural similarities. *Proc. Natl. Acad. Sci. USA* **100**:3027–3030.
- Boatright, K. M., M. Renatus, F. L. Scott, S. Sperandio, H. Shin, I. M. Pedersen, J. E. Ricci, W. A. Edris, D. P. Sutherlin, D. R. Green, and G. S. Salvesen. 2003. A unified model for apical caspase activation. *Mol. Cell* **11**:529–541.
- Boquet, P., and E. Lemichez. 2003. Bacterial virulence factors targeting Rho GTPases: parasitism or symbiosis? *Trends Cell Biol.* **13**:238–246.
- Bourdet-Sicard, R., M. Rudiger, B. M. Jockusch, P. Gounon, P. J. Sansonetti, and G. Tran Van Nhieu. 1999. Binding of the *Shigella* protein IpaA to vinculin induces F-actin depolymerization. *EMBO J.* **18**:5853–5862.
- Brinkmann, V., U. Reichard, C. Goosmann, B. Fauler, Y. Uhlemann, D. S. Weiss, Y. Weinrauch, and A. Zychlinsky. 2004. Neutrophil extracellular traps kill bacteria. *Science* **303**:1532–1535.
- Bruchrieser, C., P. Glaser, C. Rusniok, H. Nedjari, H. D'Hauteville, F. Kunst, P. Sansonetti, and C. Parsot. 2000. The virulence plasmid pWR100 and the repertoire of proteins secreted by the type III secretion apparatus of *Shigella flexneri*. *Mol. Microbiol.* **38**:760–771.
- Buttner, D., and U. Bonas. 2006. Who comes first? How plant pathogenic bacteria orchestrate type III secretion. *Curr. Opin. Microbiol.* **9**:193–200.
- Buyse, J. M., C. K. Stover, E. V. Oaks, M. Venkatesan, and D. J. Kopecko. 1987. Molecular cloning of invasion plasmid antigen (*ipa*) genes from *Shigella flexneri*: analysis of *ipa* gene products and genetic mapping. *J. Bacteriol.* **169**:2561–2569.
- Catron, D. M., M. D. Sylvester, Y. Lange, M. Kadekoppala, B. D. Jones, D. M. Monack, S. Falkow, and K. Haldar. 2002. The *Salmonella*-containing vacuole is a major site of intracellular cholesterol accumulation and recruits the GPI-anchored protein CD55. *Cell. Microbiol.* **4**:315–328.
- Celli, J., and B. B. Finlay. 2002. Bacterial avoidance of phagocytosis. *Trends Microbiol.* **10**:232–237.
- Cerretti, D. P., C. J. Kozlosky, B. Mosley, N. Nelson, K. Van Ness, T. A. Greenstreet, C. J. March, S. R. Kronheim, T. Druck, L. A. Cannizarro, K. Huebner, and R. A. Black. 1992. Molecular cloning of the interleukin-1 β converting enzyme. *Science* **256**:97–99.
- Chen, Y., M. R. Smith, K. Thirumalai, and A. Zychlinsky. 1996. A bacterial invasin induces macrophage apoptosis by directly binding ICE. *EMBO J.* **15**:3853–3860.
- Cherla, R. P., S. Y. Lee, and V. L. Tesh. 2003. Shiga toxins and apoptosis. *FEMS Microbiol. Lett.* **228**:159–166.
- Christie, P. J., K. Atmakuri, V. Krishnamoorthy, S. Jakubowski, and E. Cascales. 2005. Biogenesis, architecture, and function of bacterial type IV secretion systems. *Annu. Rev. Microbiol.* **59**:451–485.
- Clark, C. S., and A. T. Maurelli. 2007. *Shigella flexneri* inhibits staurosporine-induced apoptosis in epithelial cells. *Infect. Immun.* **75**:2531–2539.
- Clerc, P. L., A. Ryter, J. Mounier, and P. J. Sansonetti. 1987. Plasmid-mediated early killing of eukaryotic cells by *Shigella flexneri* as studied by infection of J774 macrophages. *Infect. Immun.* **55**:521–527.
- Cordes, F. S., S. Daniell, R. Kenjale, S. Saurya, W. L. Picking, W. D. Picking, F. Booy, S. M. Lea, and A. Blocker. 2005. Helical packing of needles from functionally altered *Shigella* type III secretion systems. *J. Mol. Biol.* **354**:206–211.
- Cordes, F. S., K. Komoriya, E. Larquet, S. Yang, E. H. Egelman, A. Blocker, and S. M. Lea. 2003. Helical structure of the needle of the type III secretion system of *Shigella flexneri*. *J. Biol. Chem.* **278**:17103–17107.
- Cornelis, G. R. 2006. The type III secretion injectisome. *Nat. Rev. Microbiol.* **4**:811–825.
- Cossart, P., and P. J. Sansonetti. 2004. Bacterial invasion: the paradigms of enteroinvasive pathogens. *Science* **304**:242–248.
- Davis, R., M. E. Marquart, D. Lucius, and W. D. Picking. 1998. Protein-protein interactions in the assembly of *Shigella flexneri* invasion plasmid antigens IpaB and IpaC into protein complexes. *Biochim. Biophys. Acta* **1429**:45–56.
- Day, W. A., Jr., R. E. Fernandez, and A. T. Maurelli. 2001. Pathoadaptive mutations that enhance virulence: genetic organization of the *cadA* regions of *Shigella* spp. *Infect. Immun.* **69**:7471–7480.
- Deane, J. E., P. Roversi, F. S. Cordes, S. Johnson, R. Kenjale, S. Daniell, F. Booy, W. D. Picking, W. L. Picking, A. J. Blocker, and S. M. Lea. 2006. Molecular model of a type III secretion system needle: implications for host-cell sensing. *Proc. Natl. Acad. Sci. USA* **103**:12529–12533.
- De Chastellier, C., and L. Thilo. 2006. Cholesterol depletion in *Mycobacterium avium*-infected macrophages overcomes the block in phagosomal maturation and leads to the reversible sequestration of viable mycobacteria in phagolysosome-derived autophagic vacuoles. *Cell. Microbiol.* **8**:242–256.
- De Geyter, C., B. Vogt, Z. Benjelloun-Touimi, P. J. Sansonetti, J. M.

- Ruyschaert, C. Parsot, and V. Cabaux. 1997. Purification of IpaC, a protein involved in entry of *Shigella flexneri* into epithelial cells and characterization of its interaction with lipid membranes. *FEBS Lett.* **400**:149–154.
50. Degtrev, A., M. Boyce, and J. Yuan. 2003. A decade of caspases. *Oncogene* **22**:8543–8567.
51. DeLeo, F. R. 2004. Modulation of phagocyte apoptosis by bacterial pathogens. *Apoptosis* **9**:399–413.
52. Demali, K. A., A. L. Jue, and K. Burrige. 2006. IpaA targets beta 1 integrins and rho to promote actin cytoskeleton rearrangements necessary for *Shigella* entry. *J. Biol. Chem.* **281**:39534–39541.
53. Demers, B., P. J. Sansonetti, and C. Parsot. 1998. Induction of type III secretion in *Shigella flexneri* is associated with differential control of transcription of genes encoding secreted proteins. *EMBO J.* **17**:2894–2903.
54. Deng, W., J. L. Puente, S. Gruenheid, Y. Li, B. A. Vallance, A. Vazquez, J. Barba, J. A. Ibarra, P. O'Donnell, P. Metalnikov, K. Ashman, S. Lee, D. Goode, T. Pawson, and B. B. Finlay. 2004. Dissecting virulence: systematic and functional analyses of a pathogenicity island. *Proc. Natl. Acad. Sci. USA* **101**:3597–3602.
55. Deretic, V. 2006. Autophagy as an immune defense mechanism. *Curr. Opin. Immunol.* **18**:375–382.
56. Desvaux, M., N. J. Parham, and I. R. Henderson. 2004. The autotransporter secretion system. *Res. Microbiol.* **155**:53–60.
57. Dobrindt, U., B. Hochhut, U. Hentschel, and J. Hacker. 2004. Genomic islands in pathogenic and environmental microorganisms. *Nat. Rev. Microbiol.* **2**:414–424.
- 57a. Dodd, C. E. R., and D. Jones. 1982. A numerical taxonomic study of the genus *Shigella*. *J. Gen. Microbiol.* **128**:1933–1957.
58. Dorman, C. J., S. McKenna, and C. Beloin. 2001. Regulation of virulence gene expression in *Shigella flexneri*, a facultative intracellular pathogen. *Int. J. Med. Microbiol.* **291**:89–96.
59. Dorman, C. J., and M. E. Porter. 1998. The *Shigella* virulence gene regulatory cascade: a paradigm of bacterial gene control mechanisms. *Mol. Microbiol.* **29**:677–684.
60. Duncan, J. A., D. T. Bergstralh, Y. Wang, S. B. Willingham, Z. Ye, A. G. Zimmermann, and J. P. Ting. 2007. Cryopyrin/NALP3 binds ATP/dATP, is an ATPase, and requires ATP binding to mediate inflammatory signaling. *Proc. Natl. Acad. Sci. USA* **104**:8041–8046.
61. DuPont, H. L., M. M. Levine, R. B. Hornick, and S. B. Formal. 1989. Inoculum size in shigellosis and implications for expected mode of transmission. *J. Infect. Dis.* **159**:1126–1128.
62. Edgeworth, J. D., J. Spencer, A. Phalipon, G. E. Griffin, and P. J. Sansonetti. 2002. Cytotoxicity and interleukin-1 β processing following *Shigella flexneri* infection of human monocyte-derived dendritic cells. *Eur. J. Immunol.* **32**:1464–1471.
63. Egile, C., H. d'Hauteville, C. Parsot, and P. J. Sansonetti. 1997. SopA, the outer membrane protease responsible for polar localization of IcsA in *Shigella flexneri*. *Mol. Microbiol.* **23**:1063–1073.
64. Egile, C., T. P. Loisel, V. Laurent, R. Li, D. Pantaloni, P. J. Sansonetti, and M. F. Carlier. 1999. Activation of the CDC42 effector N-WASP by the *Shigella flexneri* IcsA protein promotes actin nucleation by Arp2/3 complex and bacterial actin-based motility. *J. Cell Biol.* **146**:1319–1332.
65. Erskine, P. T., M. J. Knight, A. Ruau, H. Mikolajek, N. W. F. Sang, J. Withers, R. Gill, S. P. Wood, M. Wood, G. C. Fox, and J. B. Cooper. 2006. High resolution structure of BipD: an invasion protein associated with the type III secretion system of *Burkholderia pseudomallei*. *J. Mol. Biol.* **363**:125–136.
66. Escobar-Paramo, P., O. Clermont, A. B. Blanc-Potard, H. Bui, C. Le Bouguenec, and E. Denamur. 2004. A specific genetic background is required for acquisition and expression of virulence factors in *Escherichia coli*. *Mol. Biol. Evol.* **21**:1085–1094.
67. Escobar-Paramo, P., C. Giudicelli, C. Parsot, and E. Denamur. 2003. The evolutionary history of *Shigella* and enteroinvasive *Escherichia coli* revised. *J. Mol. Evol.* **57**:140–148.
68. Espina, M., A. J. Olive, R. Kenjale, D. S. Moore, S. F. Ausar, R. W. Kaminski, E. V. Oaks, C. R. Middaugh, W. D. Picking, and W. L. Picking. 2006. IpaD localizes to the tip of the type III secretion system needle of *Shigella flexneri*. *Infect. Immun.* **74**:4391–4400.
69. Ewing, W. H. 1949. *Shigella* nomenclature. *J. Bacteriol.* **57**:633–638.
70. Fasano, A., F. R. Noriega, F. M. Liao, W. Wang, and M. M. Levine. 1997. Effect of *Shigella* enterotoxin 1 (ShET1) on rabbit intestine in vitro and in vivo. *Gut* **40**:505–511.
71. Fasano, A., F. R. Noriega, D. R. Maneval, Jr., S. Chanasonggram, R. Russell, S. Guandalini, and M. M. Levine. 1995. *Shigella* enterotoxin 1: an enterotoxin of *Shigella flexneri* 2a active in rabbit small intestine in vivo and in vitro. *J. Clin. Invest.* **95**:2853–2861.
72. Feldman, M. F., and G. R. Cornelis. 2003. The multitalented type III chaperones: all you can do with 15 kDa. *FEMS Microbiol. Lett.* **219**:151–158.
73. Fernandez-Prada, C. M., D. L. Hoover, B. D. Tall, A. B. Hartman, J. Kopelowitz, and M. M. Venkatesan. 2000. *Shigella flexneri* IpaH_{7,8} facilitates escape of virulent bacteria from the endocytic vacuoles of mouse and human macrophages. *Infect. Immun.* **68**:3608–3619.
74. Ferrari, D., C. Pizzirani, E. Adinolfi, R. M. Lemoli, A. Curti, M. Idzko, E. Panther, and F. Di Virgilio. 2006. The P₂X₇ receptor: a key player in IL-1 processing and release. *J. Immunol.* **176**:3877–3883.
75. Ferrari, G., H. Langen, M. Naito, and J. Pieters. 1999. A coat protein on phagosomes involved in the intracellular survival of mycobacteria. *Cell* **97**:435–447.
76. Finlay, B. B., and S. Falkow. 1988. Comparison of the invasion strategies used by *Salmonella cholerae-suis*, *Shigella flexneri* and *Yersinia enterocolitica* to enter cultured animal cells: endosome acidification is not required for bacterial invasion or intracellular replication. *Biochimie* **70**:1089–1099.
77. Fischer, W., J. Puls, R. Buhrdorf, B. Gebert, S. Odenbreit, and R. Haas. 2001. Systematic mutagenesis of the *Helicobacter pylori* *cag* pathogenicity island: essential genes for CagA translocation in host cells and induction of interleukin-8. *Mol. Microbiol.* **42**:1337–1348.
78. Fritz, J. H., R. L. Ferrero, D. J. Philpott, and S. E. Girardin. 2006. Nod-like proteins in immunity, inflammation and disease. *Nat. Immunol.* **7**:1250–1257.
79. Fukushima, M., K. Kakinuma, and R. Kawaguchi. 2002. Phylogenetic analysis of *Salmonella*, *Shigella*, and *Escherichia coli* strains on the basis of the *gyrB* gene sequence. *J. Clin. Microbiol.* **40**:2779–2785.
80. Garner, M. J., R. D. Hayward, and V. Koronakis. 2002. The *Salmonella* pathogenicity island 1 secretion system directs cellular cholesterol redistribution during mammalian cell entry and intracellular trafficking. *Cell. Microbiol.* **4**:153–165.
81. Geier, E., G. Pfeifer, M. Wilm, M. Lucchiari-Hartz, W. Baumeister, K. Eichmann, and G. Niedermann. 1999. A giant protease with potential to substitute for some functions of the proteasome. *Science* **283**:978–981.
82. Ghosh, P. 2004. Process of protein transport by the type III secretion system. *Microbiol. Mol. Biol. Rev.* **68**:771–795.
83. Girardin, S. E., I. G. Boneca, L. A. Carneiro, A. Antignac, M. Jehanno, J. Viala, K. Tedin, M. K. Taha, A. Labigne, U. Zahringer, A. J. Coyle, P. S. DiStefano, J. Bertin, P. J. Sansonetti, and D. J. Philpott. 2003. Nod1 detects a unique muropeptide from gram-negative bacterial peptidoglycan. *Science* **300**:1584–1587.
84. Goldberg, M. B., O. Barzu, C. Parsot, and P. J. Sansonetti. 1993. Unipolar localization and ATPase activity of IcsA, a *Shigella flexneri* protein involved in intracellular movement. *J. Bacteriol.* **175**:2189–2196.
85. Goldberg, M. B., and J. A. Theriot. 1995. *Shigella flexneri* surface protein IcsA is sufficient to direct actin-based motility. *Proc. Natl. Acad. Sci. USA* **92**:6572–6576.
86. Golub, T., S. Wacha, and P. Caroni. 2004. Spatial and temporal control of signaling through lipid rafts. *Curr. Opin. Neurobiol.* **14**:542–550.
87. Gombos, I., E. Kiss, C. Detre, G. Laszlo, and J. Matko. 2006. Cholesterol and sphingolipids as lipid organizers of the immune cells' plasma membrane: their impact on the functions of MHC molecules, effector T-lymphocytes and T-cell death. *Immunol. Lett.* **104**:59–69.
88. Gordon, J., and P. L. Small. 1993. Acid resistance in enteric bacteria. *Infect. Immun.* **61**:364–367.
89. Gouin, E., M. D. Welch, and P. Cossart. 2005. Actin-based motility of intracellular pathogens. *Curr. Opin. Microbiol.* **8**:35–45.
90. Grant, S. R., E. J. Fisher, J. H. Chang, B. M. Mole, and J. L. Dangl. 2006. Subterfuge and manipulation: type III effector proteins of phytopathogenic bacteria. *Annu. Rev. Microbiol.* **60**:425–449.
91. Gu, Y., K. Kuida, H. Tsutsui, G. Ku, K. Hsiao, M. A. Fleming, N. Hayashi, K. Higashino, H. Okamura, K. Nakanishi, M. Kurimoto, T. Tanimoto, R. A. Flavell, V. Sato, M. W. Harding, D. J. Livingston, and M. S.-S. Su. 1997. Activation of interferon- γ inducing factor mediated by interleukin-1 β converting enzyme. *Science* **275**:206–209.
92. Gu, Y., J. Wu, C. Faucheu, J. L. Lalanne, A. Diu, D. J. Livingston, and M. S. Su. 1995. Interleukin-1 β converting enzyme requires oligomerization for activity of processed forms in vivo. *EMBO J.* **14**:1923–1931.
93. Guichon, A., D. Hersh, M. R. Smith, and A. Zychlinsky. 2001. Structure-function analysis of the *Shigella* virulence factor IpaB. *J. Bacteriol.* **183**:1269–1276.
94. Gurcel, L., L. Abrami, S. Girardin, J. Tschopp, and F. G. van der Goot. 2006. Caspase-1 activation of lipid metabolic pathways in response to bacterial pore-forming toxins promotes cell survival. *Cell* **126**:1135–1145.
95. Haimovich, B., and M. M. Venkatesan. 2006. *Shigella* and *Salmonella*: death as a means of survival. *Microbes Infect.* **8**:568–577.
96. Hamiaux, C., A. van Eerde, C. Parsot, J. Broos, and B. W. Dijkstra. 2006. Structural mimicry for vinculin activation by IpaA, a virulence factor of *Shigella flexneri*. *EMBO Rep.* **7**:794–799.
97. Hanajima-Ozawa, M., T. Matsuzawa, A. Fukui, S. Kamitani, H. Ohnishi, A. Abe, Y. Horiguchi, and M. Miyake. 2007. Enteropathogenic *Escherichia coli*, *Shigella flexneri*, and *Listeria monocytogenes* recruit a junctional protein, zonula occludens-1, to actin tails and pedestals. *Infect. Immun.* **75**:565–573.
98. Hancock, J. F. 2006. Lipid rafts: contentious only from simplistic standpoints. *Nat. Rev. Mol. Cell Biol.* **7**:456–462.
99. Handa, Y., M. Suzuki, K. Ohya, H. Iwai, N. Ishijima, A. J. Koleske, Y.

- Fukui, and C. Sasakawa. 2007. *Shigella* IpgB1 promotes bacterial entry through the ELMO-Dock180 machinery. *Nat. Cell Biol.* **9**:121–128.
100. Harrington, A., N. Darboe, R. Kenjale, W. L. Picking, C. R. Middaugh, S. Birket, and W. D. Picking. 2006. Characterization of the interaction of single tryptophan containing mutants of IpaC from *Shigella flexneri* with phospholipid membranes. *Biochemistry* **45**:626–636.
 101. Harrington, A. T., P. D. Hearn, W. L. Picking, J. R. Barker, A. Wessel, and W. D. Picking. 2003. Structural characterization of the N terminus of IpaC from *Shigella flexneri*. *Infect. Immun.* **71**:1255–1264.
 102. Hartman, A. B., M. Venkatesan, E. V. Oaks, and J. M. Buysse. 1990. Sequence and molecular characterization of a multicopy invasion plasmid antigen gene, *ipaH*, of *Shigella flexneri*. *J. Bacteriol.* **172**:1905–1915.
 103. Hayward, R. D., R. J. Cain, E. J. McGhie, N. Phillips, M. J. Garner, and V. Koronakis. 2005. Cholesterol binding by the bacterial type III translocator is essential for virulence effector delivery into mammalian cells. *Mol. Microbiol.* **56**:590–603.
 104. Helms, J. B., and C. Zurzolo. 2004. Lipids as targeting signals: lipid rafts and intracellular trafficking. *Traffic* **5**:247–254.
 105. Henderson, I. R., J. Czczulnic, C. Eslava, F. Noriega, and J. P. Nataro. 1999. Characterization of Pic, a secreted protease of *Shigella flexneri* and enteroaggregative *Escherichia coli*. *Infect. Immun.* **67**:5587–5596.
 106. High, N., J. Mounier, M. C. Prevost, and P. J. Sansonetti. 1992. IpaB of *Shigella flexneri* causes entry into epithelial cells and escape from the phagocytic vacuole. *EMBO J.* **11**:1991–1999.
 107. Hilbi, H. 2006. Modulation of phosphoinositide metabolism by pathogenic bacteria. *Cell. Microbiol.* **8**:1697–1706.
 - 107a. Hilbi, H. 1999. Host responses to secreted *Shigella* virulence factors. *Curr. Opin. Infect. Dis.* **12**:221–228.
 108. Hilbi, H., Y. Chen, K. Thirumalai, and A. Zychlinsky. 1997. The interleukin-1 β converting enzyme, caspase-1, is activated during *Shigella flexneri*-induced apoptosis in human monocyte-derived macrophages. *Infect. Immun.* **65**:5165–5170.
 109. Hilbi, H., J. E. Moss, D. Hersh, Y. Chen, J. Arondel, S. Banerjee, R. A. Flavell, J. Yuan, P. J. Sansonetti, and A. Zychlinsky. 1998. *Shigella*-induced apoptosis is dependent on caspase-1 which binds to IpaB. *J. Biol. Chem.* **273**:32895–32900.
 110. Hilbi, H., R. J. Puro, and A. Zychlinsky. 2000. Tripeptidyl peptidase II promotes maturation of caspase-1 in *Shigella flexneri*-induced macrophage apoptosis. *Infect. Immun.* **68**:5502–5508.
 111. Howe, D., and R. A. Heinzen. 2006. *Coxiella burnetii* inhabits a cholesterol-rich vacuole and influences cellular cholesterol metabolism. *Cell. Microbiol.* **8**:496–507.
 112. Hromockyj, A. E., and A. T. Maurelli. 1989. Identification of *Shigella* invasion genes by isolation of temperature-regulated *inv::lacZ* operon fusions. *Infect. Immun.* **57**:2963–2970.
 113. Huan, P. T., D. A. Bastin, B. L. Whittle, A. A. Lindberg, and N. K. Verma. 1997. Molecular characterization of the genes involved in O-antigen modification, attachment, integration and excision in *Shigella flexneri* bacteriophage SfV. *Gene* **195**:217–227.
 114. Hume, P. J., E. J. McGhie, R. D. Hayward, and V. Koronakis. 2003. The purified *Shigella* IpaB and *Salmonella* SipB translocators share biochemical properties and membrane topology. *Mol. Microbiol.* **49**:425–439.
 115. Ingersoll, M., E. A. Groisman, and A. Zychlinsky. 2002. Pathogenicity islands of *Shigella*. *Curr. Top. Microbiol. Immunol.* **264**:49–65.
 116. Ingersoll, M. A., J. E. Moss, Y. Weinrauch, P. E. Fisher, E. A. Groisman, and A. Zychlinsky. 2003. The ShiA protein encoded by the *Shigella flexneri* SHI-2 pathogenicity island attenuates inflammation. *Cell. Microbiol.* **5**:797–807.
 117. Ingersoll, M. A., and A. Zychlinsky. 2006. ShiA abrogates the innate T-cell response to *Shigella flexneri* infection. *Infect. Immun.* **74**:2317–2327.
 118. Islam, D., L. Bandholtz, J. Nilsson, H. Wigzell, B. Christensson, B. Agerberth, and G. Gudmundsson. 2001. Downregulation of bactericidal peptides in enteric infections: a novel immune escape mechanism with bacterial DNA as a potential regulator. *Nat. Med.* **7**:180–185.
 119. Islam, D., B. Veress, P. K. Bardhan, A. A. Lindberg, and B. Christensson. 1997. In situ characterization of inflammatory responses in the rectal mucosae of patients with shigellosis. *Infect. Immun.* **65**:739–749.
 120. Ito, H., N. Kido, Y. Arakawa, M. Ohta, T. Sugiyama, and N. Kato. 1991. Possible mechanisms underlying the slow lactose fermentation phenotype in *Shigella* spp. *Appl. Environ. Microbiol.* **57**:2912–2917.
 121. Izard, T., G. Tran Van Nhieu, and P. R. Bois. 2006. *Shigella* applies molecular mimicry to subvert vinculin and invade host cells. *J. Cell Biol.* **175**:465–475.
 122. Janeway, C. A., Jr., and R. Medzhitov. 2002. Innate immune recognition. *Annu. Rev. Immunol.* **20**:197–216.
 123. Jennison, A. V., and N. K. Verma. 2004. *Shigella flexneri* infection: pathogenesis and vaccine development. *FEMS Microbiol. Rev.* **28**:43–58.
 124. Jiang, Y., F. Yang, X. Zhang, J. Yang, L. Chen, Y. Yan, H. Nie, Z. Xiong, J. Wang, J. Dong, Y. Xue, X. Xu, Y. Zhu, S. Chen, and Q. Jin. 2005. The complete sequence and analysis of the large virulence plasmid pSS of *Shigella sonnei*. *Plasmid* **54**:149–159.
 125. Jin, Q., Z. Yuan, J. Xu, Y. Wang, Y. Shen, W. Lu, J. Wang, H. Liu, J. Yang, F. Yang, X. Zhang, J. Zhang, G. Yang, H. Wu, D. Qu, J. Dong, L. Sun, Y. Xue, A. Zhao, Y. Gao, J. Zhu, B. Kan, K. Ding, S. Chen, H. Cheng, Z. Yao, B. He, R. Chen, D. Ma, B. Qiang, Y. Wen, Y. Hou, and J. Yu. 2002. Genome sequence of *Shigella flexneri* 2a: insights into pathogenicity through comparison with genomes of *Escherichia coli* K12 and O157. *Nucleic Acids Res.* **30**:4432–4441.
 126. Johnson, S., P. Rovarsi, M. Espina, A. Olive, J. E. Deane, S. Birket, T. Field, W. D. Picking, A. J. Blocker, E. E. Galyov, W. L. Picking, and S. M. Lea. 2007. Self-chaperoning of the type III secretion system needle tip proteins IpaD and BipD. *J. Biol. Chem.* **282**:4035–4044.
 127. Jouihri, N., M. P. Sory, A. L. Page, P. Gounon, C. Parsot, and A. Allaoui. 2003. MxiK and MxiN interact with the Spa47 ATPase and are required for transit of the needle components MxiH and MxiI, but not of Ipa proteins, through the type III secretion apparatus of *Shigella flexneri*. *Mol. Microbiol.* **49**:755–767.
 128. Kane, C. D., R. Schuch, W. A. Day, Jr., and A. T. Maurelli. 2002. MxiE regulates intracellular expression of factors secreted by the *Shigella flexneri* 2a type III secretion system. *J. Bacteriol.* **184**:4409–4419.
 129. Kato, J.-I., K.-I. Ito, A. Nakamura, and H. Watanabe. 1989. Cloning of regions required for contact hemolysis and entry into LLC-MK2 cells from *Shigella sonnei* form I plasmid: *virF* is a positive regulator gene for these phenotypes. *Infect. Immun.* **57**:1391–1398.
 130. Kenjale, R., J. Wilson, S. F. Zenk, S. Saurya, W. L. Picking, W. D. Picking, and A. Blocker. 2005. The needle component of the type III secretion of *Shigella* regulates the activity of the secretion apparatus. *J. Biol. Chem.* **280**:42929–42937.
 131. Kim, D. W., G. Lenzen, A. L. Page, P. Legrain, P. J. Sansonetti, and C. Parsot. 2005. The *Shigella flexneri* effector OspG interferes with innate immune responses by targeting ubiquitin-conjugating enzymes. *Proc. Natl. Acad. Sci. USA* **102**:14046–14051.
 132. Kirkegaard, K., M. P. Taylor, and W. T. Jackson. 2004. Cellular autophagy: surrender, avoidance and subversion by microorganisms. *Nat. Rev. Microbiol.* **2**:301–314.
 133. Kirschnek, S., J. Scheffel, U. Heinzmann, and G. Hacker. 2004. Necrosis-like cell death induced by bacteria in mouse macrophages. *Eur. J. Immunol.* **34**:1461–1471.
 134. Knodler, L. A., and O. Steele-Mortimer. 2003. Taking possession: biogenesis of the *Salmonella*-containing vacuole. *Traffic* **4**:587–599.
 135. Koterski, J. F., M. Nahvi, M. M. Venkatesan, and B. Haimovich. 2005. Virulent *Shigella flexneri* causes damage to mitochondria and triggers necrosis in infected human monocyte-derived macrophages. *Infect. Immun.* **73**:504–513.
 136. Kotloff, K. L., J. P. Winickoff, B. Ivanoff, J. D. Clemens, D. L. Swerdlow, P. J. Sansonetti, G. K. Adak, and M. M. Levine. 1999. Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies. *Bull. W. H. O.* **77**:651–666.
 137. Kramer, R. W., N. L. Slagowski, N. A. Eze, K. S. Giddings, M. F. Morrison, K. A. Siggers, M. N. Starnbach, and C. F. Lesser. 2007. Yeast functional genomic screens lead to identification of a role for a bacterial effector in innate immunity regulation. *PLoS Pathog.* **3**:e21.
 138. Kubori, T., Y. Matsushima, D. Nakamura, J. Uralil, M. Lara-Tejero, A. Sukhan, J. E. Galan, and S. I. Aizawa. 1998. Supramolecular structure of the *Salmonella typhimurium* type III protein secretion system. *Science* **280**:602–605.
 139. Kumar, S. 2007. Caspase function in programmed cell death. *Cell Death Differ.* **14**:32–43.
 140. Kuwae, A., S. Yoshida, K. Tamano, H. Mimuro, T. Suzuki, and C. Sasakawa. 2001. *Shigella* invasion of macrophage requires the insertion of IpaC into the host plasma membrane. Functional analysis of IpaC. *J. Biol. Chem.* **276**:32230–32239.
 141. Kwiatkowska, K., and A. Sobota. 1999. Signaling pathways in phagocytosis. *Bioessays* **21**:422–431.
 142. Lafont, F., G. Tran Van Nhieu, K. Hanada, P. Sansonetti, and F. G. van der Goot. 2002. Initial steps of *Shigella* infection depend on the cholesterol/sphingolipid raft-mediated CD44-IpaB interaction. *EMBO J.* **21**:4449–4457.
 143. Lafont, F., and F. G. van der Goot. 2005. Bacterial invasion via lipid rafts. *Cell. Microbiol.* **7**:613–620.
 144. Lan, R., M. C. Alles, K. Donohoe, M. B. Martinez, and P. R. Reeves. 2004. Molecular evolutionary relationships of enteroinvasive *Escherichia coli* and *Shigella* spp. *Infect. Immun.* **72**:5080–5088.
 145. Lan, R., B. Lumb, D. Ryan, and P. R. Reeves. 2001. Molecular evolution of large virulence plasmid in *Shigella* clones and enteroinvasive *Escherichia coli*. *Infect. Immun.* **69**:6303–6309.
 146. Lan, R., and P. R. Reeves. 2002. *Escherichia coli* in disguise: molecular origins of *Shigella*. *Microbes Infect.* **4**:1125–1132.
 147. Laohachai, K. N., R. Bahadi, M. B. Hardo, P. G. Hardo, and J. I. Kourie. 2003. The role of bacterial and non-bacterial toxins in the induction of changes in membrane transport: implications for diarrhea. *Toxicol* **42**:687–707.
 148. Le-Barillec, K., J. G. Magalhaes, E. Corcuff, A. Thuizat, P. J. Sansonetti, A. Phalipon, and J. P. Di Santo. 2005. Roles for T and NK cells in the innate immune response to *Shigella flexneri*. *J. Immunol.* **175**:1735–1740.

149. Le Gall, T., M. Mavris, M. C. Martino, M. L. Bernardini, E. Denamur, and C. Parsot. 2005. Analysis of virulence plasmid gene expression defines three classes of effectors in the type III secretion system of *Shigella flexneri*. *Microbiology* **151**:951–962.
150. Lett, M.-C., C. Sasakawa, N. Okada, T. Sakai, S. Makino, M. Yamada, K. Komatsu, and M. Yoshikawa. 1989. *virG*, a plasmid-coded virulence gene of *Shigella flexneri*: identification of the VirG protein and determination of the complete coding sequence. *J. Bacteriol.* **171**:353–359.
151. Li, H., H. Xu, Y. Zhou, J. Zhang, C. Long, S. Li, S. Chen, J. M. Zhou, and F. Shao. 2007. The phosphothreonine lyase activity of a bacterial type III effector family. *Science* **315**:1000–1003.
152. Lillemeier, B. F., J. R. Pfeiffer, Z. Surviladze, B. S. Wilson, and M. M. Davis. 2006. Plasma membrane-associated proteins are clustered into islands attached to the cytoskeleton. *Proc. Natl. Acad. Sci. USA* **103**:18992–18997.
153. Lindberg, A. A., A. Karnell, and A. Weintraub. 1991. The lipopolysaccharide of *Shigella* bacteria as a virulence factor. *Rev. Infect. Dis.* **13**(Suppl. 4):S279–S284.
154. Luck, S. N., S. A. Turner, K. Rajakumar, H. Sakellaris, and B. Adler. 2001. Ferric citrate transport system (Fec) of *Shigella flexneri* 2a YSH6000 is encoded on a novel pathogenicity island carrying multiple antibiotic resistance genes. *Infect. Immun.* **69**:6012–6021.
155. Magdalena, J., A. Hachani, M. Chamekh, N. Jouihri, P. Gounon, A. Blocker, and A. Allaoui. 2002. Spa32 regulates a switch in substrate specificity of the type III secretion of *Shigella flexneri* from needle components to Ipa proteins. *J. Bacteriol.* **184**:3433–3441.
156. Makino, S., C. Sasakawa, K. Kamata, T. Kurata, and M. Yoshikawa. 1986. A genetic determinant required for continuous reinfection of adjacent cells on the large plasmid in *S. flexneri* 2a. *Cell* **46**:551–555.
157. Man, A. L., M. E. Prieto-Garcia, and C. Nicoletti. 2004. Improving M cell-mediated transport across mucosal barriers: do certain bacteria hold the keys? *Immunology* **113**:15–22.
158. Mandic-Mulec, I., J. Weiss, and A. Zychlinsky. 1997. *Shigella flexneri* is trapped in polymorphonuclear leukocyte vacuoles and efficiently killed. *Infect. Immun.* **65**:110–115.
159. Manes, S., G. del Real, and A. C. Martinez. 2003. Pathogens: raft hijackers. *Nat. Rev. Immunol.* **3**:557–568.
160. Mariathasan, S., and D. M. Monack. 2007. Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation. *Nat. Rev. Immunol.* **7**:31–40.
161. Martinon, F., K. Burns, and J. Tschopp. 2002. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-1 β . *Mol. Cell* **10**:417–426.
162. Martinon, F., and J. Tschopp. 2007. Inflammatory caspases and inflammasomes: master switches of inflammation. *Cell Death Differ.* **14**:10–22.
163. Martinon, F., and J. Tschopp. 2005. NLRs join TLRs as innate sensors of pathogens. *Trends Immunol.* **26**:447–454.
164. Maurelli, A. T. 2007. Black holes, antivirulence genes, and gene inactivation in the evolution of bacterial pathogens. *FEMS Microbiol. Lett.* **267**:1–8.
165. Maurelli, A. T., B. Baudry, H. d'Hauteville, T. L. Hale, and P. J. Sansonetti. 1985. Cloning of plasmid DNA sequences involved in invasion of HeLa cells by *Shigella flexneri*. *Infect. Immun.* **49**:164–171.
166. Maurelli, A. T., R. E. Fernandez, C. A. Bloch, C. K. Rode, and A. Fasano. 1998. "Black holes" and bacterial pathogenicity: a large genomic deletion that enhances the virulence of *Shigella* spp. and enteroinvasive *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **95**:3943–3948.
167. Mavris, M., A. L. Page, R. Tournebise, B. Demers, P. Sansonetti, and C. Parsot. 2002. Regulation of transcription by the activity of the *Shigella flexneri* type III secretion apparatus. *Mol. Microbiol.* **43**:1543–1553.
168. Mavris, M., P. J. Sansonetti, and C. Parsot. 2002. Identification of the *cis*-acting site involved in activation of promoters regulated by activity of the type III secretion apparatus in *Shigella flexneri*. *J. Bacteriol.* **184**:6751–6759.
169. McCormick, B. A., A. M. Siber, and A. T. Maurelli. 1998. Requirement of the *Shigella flexneri* virulence plasmid in the ability to induce trafficking of neutrophils across polarized monolayers of the intestinal epithelium. *Infect. Immun.* **66**:4237–4243.
170. Ménard, R., P. Sansonetti, and C. Parsot. 1994. The secretion of the *Shigella flexneri* Ipa invasins is activated by epithelial cells and controlled by IpaB and IpaD. *EMBO J.* **13**:5293–5302.
171. Ménard, R., P. J. Sansonetti, and C. Parsot. 1993. Nonpolar mutagenesis of the *ipa* genes defines IpaB, IpaC, and IpaD as effectors of *Shigella flexneri* entry into epithelial cells. *J. Bacteriol.* **175**:5899–5906.
172. Ménard, R., P. J. Sansonetti, C. Parsot, and T. Vasselton. 1994. Extracellular association and cytoplasmic partitioning of the IpaB and IpaC invasins of *S. flexneri*. *Cell* **79**:515–525.
173. Miura, M., J. Terajima, H. Izumiya, J. Mitobe, T. Komano, and H. Watanabe. 2006. OspE2 of *Shigella sonnei* is required for the maintenance of cell architecture of bacterium-infected cells. *Infect. Immun.* **74**:2587–2595.
174. Monack, D. M., and J. A. Theriot. 2001. Actin-based motility is sufficient for bacterial membrane protrusion formation and host cell uptake. *Cell. Microbiol.* **3**:633–647.
175. Moran, N. A. 2002. Microbial minimalism: genome reduction in bacterial pathogens. *Cell* **108**:583–586.
176. Morita-Ishihara, T., M. Ogawa, H. Sagara, M. Yoshida, E. Katayama, and C. Sasakawa. 2006. *Shigella* Spa33 is an essential C-ring component of type III secretion machinery. *J. Biol. Chem.* **281**:599–607.
177. Moss, J. E., T. J. Cardozo, A. Zychlinsky, and E. A. Groisman. 1999. The *selC*-associated SHI-2 pathogenicity island of *Shigella flexneri*. *Mol. Microbiol.* **33**:74–83.
178. Mounier, J., V. Laurent, A. Hall, P. Fort, M. F. Carlier, P. J. Sansonetti, and C. Egile. 1999. Rho family GTPases control entry of *Shigella flexneri* into epithelial cells but not intracellular motility. *J. Cell Sci.* **112**:2069–2080.
179. Mounier, J., T. Vasselton, R. Hellio, M. Lesourd, and P. J. Sansonetti. 1992. *Shigella flexneri* enters human colonic Caco-2 epithelial cells through the basolateral pole. *Infect. Immun.* **60**:237–248.
180. Nakata, N., T. Tobe, I. Fukuda, T. Suzuki, K. Komatsu, M. Yoshikawa, and C. Sasakawa. 1993. The absence of a surface protease, OmpT, determines the intercellular spreading ability of *Shigella*: the relationship between the *ompT* and *kcpA* loci. *Mol. Microbiol.* **9**:459–468.
181. Nakayama, S., and H. Watanabe. 1995. Involvement of CpxA, a sensor of a two-component regulatory system, in the pH-dependent regulation of expression of *Shigella sonnei virF* gene. *J. Bacteriol.* **177**:5062–5069.
182. Nassif, X., M. C. Mazert, J. Mounier, and P. J. Sansonetti. 1987. Evaluation with an *iac::Tn10* mutant of the role of aerobactin production in the virulence of *Shigella flexneri*. *Infect. Immun.* **55**:1963–1969.
183. Nataro, J. P., J. Seriwatana, A. Fasano, D. R. Maneval, L. D. Guers, F. Noriega, F. Dubovsky, M. M. Levine, and J. G. Morris, Jr. 1995. Identification and cloning of a novel plasmid-encoded enterotoxin of enteroinvasive *Escherichia coli* and *Shigella* strains. *Infect. Immun.* **63**:4721–4728.
184. Navarre, W. W., and A. Zychlinsky. 2000. Pathogen-induced apoptosis of macrophages: a common end for different pathogenic strategies. *Cell. Microbiol.* **2**:265–273.
185. Newman, C. L., and C. Stathopoulos. 2004. Autotransporter and two-partner secretion: delivery of large-size virulence factors by gram-negative bacterial pathogens. *Crit. Rev. Microbiol.* **30**:275–286.
186. Nie, H., F. Yang, X. Zhang, J. Yang, L. Chen, J. Wang, Z. Xiong, J. Peng, L. Sun, J. Dong, Y. Xue, X. Xu, S. Chen, Z. Yao, Y. Shen, and Q. Jin. 2006. Complete genome sequence of *Shigella flexneri* 5b and comparison with *Shigella flexneri* 2a. *BMC Genomics* **7**:173.
187. Niebuhr, K., S. Giuriato, T. Pedron, D. J. Philpott, F. Gaits, J. Sable, M. P. Sheetz, C. Parsot, P. J. Sansonetti, and B. Payrastré. 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.
188. Niebuhr, K., N. Jouihri, A. Allaoui, P. Gounon, P. J. Sansonetti, and C. Parsot. 2000. IpgD, a protein secreted by the type III secretion machinery of *Shigella flexneri*, is chaperoned by IpgE and implicated in entry focus formation. *Mol. Microbiol.* **38**:8–19.
189. Niyogi, S. K. 2005. Shigellosis. *J. Microbiol.* **43**:133–143.
190. Oaks, E. V., T. L. Hale, and S. B. Formal. 1986. Serum immune response to *Shigella* protein antigens in rhesus monkeys and humans infected with *Shigella* spp. *Infect. Immun.* **53**:57–63.
191. Ochman, H., J. G. Lawrence, and E. A. Groisman. 2000. Lateral gene transfer and the nature of bacterial innovation. *Nature* **405**:299–304.
192. Ochman, H., T. Whittam, D. A. Caugant, and R. K. Selander. 1983. Enzyme polymorphism and genetic population structure in *Escherichia coli* and *Shigella*. *J. Gen. Microbiol.* **129**:2715–2726.
193. Ogawa, M., and C. Sasakawa. 2006. Intracellular survival of *Shigella*. *Cell. Microbiol.* **8**:177–184.
194. Ogawa, M., T. Suzuki, I. Tatsuno, H. Abe, and C. Sasakawa. 2003. IcsB, secreted via the type III secretion system, is chaperoned by IpgA and required at the post-invasion stage of *Shigella* pathogenicity. *Mol. Microbiol.* **48**:913–931.
195. Ogawa, M., T. Yoshimori, T. Suzuki, H. Sagara, N. Mizushima, and C. Sasakawa. 2005. Escape of intracellular *Shigella* from autophagy. *Science* **307**:727–731.
196. Ohya, K., Y. Handa, M. Ogawa, M. Suzuki, and C. Sasakawa. 2005. IpgB1 is a novel *Shigella* effector protein involved in bacterial invasion of host cells. Its activity to promote membrane ruffling via Rac1 and Cdc42 activation. *J. Biol. Chem.* **280**:24022–24034.
197. Okuda, J., T. Toyotome, N. Kataoka, M. Ohno, H. Abe, Y. Shimura, A. Seyedarabi, R. Pickersgill, and C. Sasakawa. 2005. *Shigella* effector IpaH9.8 binds to a splicing factor U2AF(35) to modulate host immune responses. *Biochem. Biophys. Res. Commun.* **333**:531–539.
198. Olive, A. J., R. Kenjale, M. Espina, D. S. Moore, W. L. Picking, and W. D. Picking. 2007. Bile salts stimulate recruitment of IpaB to the *Shigella flexneri* surface, where it colocalizes with IpaD at the tip of the type III secretion needle. *Infect. Immun.* **75**:2626–2629.
199. O'Loughlin, E. V., and R. M. Robins-Browne. 2001. Effect of Shiga toxin and Shiga-like toxins on eukaryotic cells. *Microbes Infect.* **3**:493–507.
200. Oscecki, J. C., J. Barker, W. L. Picking, A. B. Serfis, E. Berring, S. Shah, A. Harrington, and W. D. Picking. 2001. IpaC from *Shigella* and SipC from *Salmonella* possess similar biochemical properties but are functionally distinct. *Mol. Microbiol.* **42**:469–481.

201. Page, A. L., H. Ohayon, P. J. Sansonetti, and C. Parsot. 1999. The secreted IpaB and IpaC invasins and their cytoplasmic chaperone IpgC are required for intercellular dissemination of *Shigella flexneri*. *Cell. Microbiol.* **1**:183–193.
202. Page, A. L., and C. Parsot. 2002. Chaperones of the type III secretion pathway: jacks of all trades. *Mol. Microbiol.* **46**:1–11.
203. Page, A. L., P. Sansonetti, and C. Parsot. 2002. Spa15 of *Shigella flexneri*, a third type of chaperone in the type III secretion pathway. *Mol. Microbiol.* **43**:1533–1542.
204. Palmer, M. 2004. Cholesterol and the activity of bacterial toxins. *FEMS Microbiol. Lett.* **238**:281–289.
205. Park, H. H., Y. C. Lo, S. C. Lin, L. Wang, J. K. Yang, and H. Wu. 2007. The death domain superfamily in intracellular signaling of apoptosis and inflammation. *Annu. Rev. Immunol.* **25**:561–586.
206. Parsot, C. 2005. *Shigella* spp. and enteroinvasive *Escherichia coli* pathogenicity factors. *FEMS Microbiol. Lett.* **252**:11–18.
207. Parsot, C., E. Ageron, C. Penno, M. Mavris, K. Jamoussi, H. d'Hauteville, P. Sansonetti, and B. Demers. 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.
208. Parsot, C., C. Hamiaux, and A. L. Page. 2003. The various and varying roles of specific chaperones in type III secretion systems. *Curr. Opin. Microbiol.* **6**:7–14.
209. Parton, R. G., and A. N. Richards. 2003. Lipid rafts and caveolae as portals for endocytosis: new insights and common mechanisms. *Traffic* **4**:724–738.
210. Payne, S. M., E. E. Wyckoff, E. R. Murphy, A. G. Oglesby, M. L. Boulette, and N. M. Davies. 2006. Iron and pathogenesis of *Shigella*: iron acquisition in the intracellular environment. *Biomaterials* **19**:173–180.
211. Pedron, T., C. Thibault, and P. J. Sansonetti. 2003. The invasive phenotype of *Shigella flexneri* directs a distinct gene expression pattern in the human intestinal epithelial cell line Caco-2. *J. Biol. Chem.* **278**:33878–33886.
212. Pendaries, C., H. Tronchere, L. Arbibe, J. Mounier, O. Gozani, L. Cantley, M. J. Fry, F. Gaits-Iacovoni, P. J. Sansonetti, and B. Payrastrre. 2006. PtdIns5P activates the host cell PI3-kinase/Akt pathway during *Shigella flexneri* infection. *EMBO J.* **25**:1024–1034.
213. Peng, J., X. Zhang, J. Yang, J. Wang, E. Yang, W. Bin, C. Wei, M. Sun, and Q. Jin. 2006. The use of comparative genomic hybridization to characterize genome dynamics and diversity among the serotypes of *Shigella*. *BMC Genomics* **7**:218.
214. Perdomo, J. J., P. Gounon, and P. J. Sansonetti. 1994. Polymorphonuclear leukocyte transmigration promotes invasion of colonic epithelial monolayer by *Shigella flexneri*. *J. Clin. Investig.* **93**:633–643.
215. Perdomo, O. J., J. M. Cavillon, M. Huerre, H. Ohayon, P. Gounon, and P. J. Sansonetti. 1994. Acute inflammation causes epithelial invasion and mucosal destruction in experimental shigellosis. *J. Exp. Med.* **180**:1307–1319.
216. Phalipon, A., and P. J. Sansonetti. 2007. *Shigella's* ways of manipulating the host intestinal innate and adaptive immune system: a tool box for survival? *Immunol. Cell Biol.* **85**:119–129.
- 216a. Phalipon, A., M. Kaufmann, J. M. Cavillon, M. Huerre, P. Sansonetti, and J. P. Kraehenbuhl. 1995. Monoclonal immunoglobulin A antibody directed against serotype-specific epitope of *Shigella flexneri* lipopolysaccharide protects against murine experimental shigellosis. *J. Exp. Med.* **182**:769–778.
217. Philpott, D. J., S. Yamaoka, A. Israel, and P. J. Sansonetti. 2000. Invasive *Shigella flexneri* activates NF- κ B through a lipopolysaccharide-dependent innate intracellular response and leads to IL-8 expression in epithelial cells. *J. Immunol.* **165**:903–914.
218. Picking, W. L., L. Coye, J. C. Osiecki, A. B. Serfis, E. Schaper, and W. D. Picking. 2001. Identification of functional regions within invasion plasmid antigen C (IpaC) of *Shigella flexneri*. *Mol. Microbiol.* **39**:100–111.
219. Picking, W. L., H. Nishioka, P. D. Hearn, M. A. Baxter, A. T. Harrington, A. Blocker, and W. D. Picking. 2005. IpaD of *Shigella flexneri* is independently required for regulation of Ipa protein secretion and efficient insertion of IpaB and IpaC into host membranes. *Infect. Immun.* **73**:1432–1440.
220. Porter, M. E., and C. J. Dorman. 1997. Differential regulation of the plasmid-encoded genes in the *Shigella flexneri* virulence regulon. *Mol. Gen. Genet.* **256**:93–103.
221. Porter, M. E., and C. J. Dorman. 1994. A role for H-NS in the thermosensitive regulation of virulence gene expression in *Shigella flexneri*. *J. Bacteriol.* **176**:4187–4191.
222. Prosseda, G., M. Falconi, M. Nicoletti, M. Casalino, G. Micheli, and B. Colonna. 2002. Histone-like proteins and the *Shigella* invasivity regulon. *Res. Microbiol.* **153**:461–468.
223. Prunier, A.-L., R. Schuch, R. E. Fernández, and A. T. Maurelli. 2007. Genetic structure of the *nadA* and *nadB* antivirulence loci in *Shigella* spp. *J. Bacteriol.* **189**:6482–6486.
224. Prunier, A. L., R. Schuch, R. E. Fernandez, K. L. Mumy, H. Kohler, B. A. McCormick, and A. T. Maurelli. 2007. *nadA* and *nadB* of *Shigella flexneri* 5a are antivirulence loci responsible for the synthesis of quinolinate, a small molecule inhibitor of *Shigella* pathogenicity. *Microbiology* **153**:2363–2372.
225. Pupo, G. M., D. K. Karaolis, R. Lan, and P. R. Reeves. 1997. Evolutionary relationship among pathogenic and nonpathogenic *Escherichia coli* strains inferred from multilocus enzyme electrophoresis and *mdh* sequence studies. *Infect. Immun.* **65**:2685–2692.
226. Pupo, G. M., R. Lan, and P. R. Reeves. 2000. Multiple independent origins of *Shigella* clones of *Escherichia coli* and convergent evolution of many of their characteristics. *Proc. Natl. Acad. Sci. USA* **97**:10567–10572.
227. Purdy, G. E., and S. M. Payne. 2001. The SHI-3 iron transport island of *Shigella boydii* 0-1392 carries the genes for aerobactin synthesis and transport. *J. Bacteriol.* **183**:4176–4182.
228. Radnedge, L., M. A. Davis, B. L. Youngren, and S. J. Austin. 1997. Plasmid maintenance functions of the large virulence plasmid of *Shigella flexneri*. *J. Bacteriol.* **197**:3670–3675.
229. Radtke, A. L., and M. X. O'Riordan. 2006. Intracellular innate resistance to bacterial pathogens. *Cell. Microbiol.* **8**:1720–1729.
230. Rajendran, L., and K. Simons. 2005. Lipid rafts and membrane dynamics. *J. Cell Sci.* **118**:1099–1102.
231. Ramarao, N., C. Le Clainche, T. Izard, R. Bourdet-Sicard, E. Ageron, P. J. Sansonetti, M. F. Carlier, and G. Tran Van Nhieu. 2007. Capping of actin filaments by vinculin activated by the *Shigella* IpaA carboxyl-terminal domain. *FEBS Lett.* **581**:853–857.
232. Ramos, H. C., M. Rumbo, and J. C. Sirard. 2004. Bacterial flagellins: mediators of pathogenicity and host immune responses in mucosa. *Trends Microbiol.* **12**:509–517.
233. Rathman, M., P. de Lancerolle, H. Ohayon, P. Gounon, and P. Sansonetti. 2000. Myosin light chain kinase plays an essential role in *S. flexneri* dissemination. *J. Cell Sci.* **113**:3375–3386.
234. Rathman, M., N. Jourih, A. Allaoui, P. Sansonetti, C. Parsot, and G. Tran Van Nhieu. 2000. The development of a FACS-based strategy for the isolation of *Shigella flexneri* mutants that are deficient in intercellular spread. *Mol. Microbiol.* **35**:974–990.
235. Reig, N., and F. G. van der Goot. 2006. About lipids and toxins. *FEBS Lett.* **580**:5572–5579.
236. Rhode, J. R., A. Breitreutz, A. Chenal, P. J. Sansonetti, and C. Parsot. 2007. Type III secretion effectors of the IpaH family are E3 ubiquitin ligases. *Cell Host Microbe* **1**:77–83.
237. Riedl, S. J., and Y. Shi. 2004. Molecular mechanisms of caspase regulation during apoptosis. *Nat. Rev. Mol. Cell Biol.* **5**:897–907.
238. Rietveld, A., and K. Simons. 1998. The differential miscibility of lipids as the basis for the formation of functional membrane rafts. *Biochim. Biophys. Acta* **1376**:467–479.
239. Robbins, J. R., D. Monack, S. J. McCallum, A. Vegas, E. Pham, M. B. Goldberg, and J. A. Theriot. 2001. The making of a gradient: IcsA (VirG) polarity in *Shigella flexneri*. *Mol. Microbiol.* **41**:861–872.
240. Rolland, K., N. Lambert-Zechovsky, B. Picard, and E. Denamur. 1998. *Shigella* and enteroinvasive *Escherichia coli* strains are derived from distinct ancestral strains of *E. coli*. *Microbiology* **144**:2667–2672.
241. Rosenberger, C. M., and B. B. Finlay. 2003. Phagocyte sabotage: disruption of macrophage signalling by bacterial pathogens. *Nat. Rev. Mol. Cell Biol.* **4**:385–396.
242. Sakaguchi, T., H. Kohler, X. Gu, B. A. McCormick, and H. C. Reinecker. 2002. *Shigella flexneri* regulates tight junction-associated proteins in human intestinal epithelial cells. *Cell. Microbiol.* **4**:367–381.
243. Sakai, T., C. Sasakawa, and M. Yoshikawa. 1988. Expression of four virulence antigens of *Shigella flexneri* is positively regulated at the transcriptional level by the 30 kilodalton VirF protein. *Mol. Microbiol.* **2**:589–597.
244. Sakellaris, H., N. K. Hannink, K. Rajakumar, D. Bulach, M. Hunt, C. Sasakawa, and B. Adler. 2000. Curli loci of *Shigella* spp. *Infect. Immun.* **68**:3780–3783.
245. Sani, M., A. Allaoui, F. Fusetti, G. T. Oostergetel, W. Keestra, and E. J. Boekema. 2007. Structural organization of the needle complex of the type III secretion apparatus of *Shigella flexneri*. *Micron* **38**:291–301.
246. Sani, M., A. Botteaux, C. Parsot, P. Sansonetti, E. J. Boekema, and A. Allaoui. 2007. IpaD is localized at the tip of the *Shigella flexneri* type III secretion apparatus. *Biochim. Biophys. Acta* **1770**:307–311.
247. Sansonetti, P. J. 2001. Rupture, invasion and inflammatory destruction of the intestinal barrier by *Shigella*, making sense of prokaryote-eukaryote cross-talks. *FEMS Microbiol. Rev.* **25**:3–14.
248. Sansonetti, P. J. 2006. Shigellosis: an old disease in new clothes? *PLoS Med.* **3**:e354.
249. Sansonetti, P. J. 2004. War and peace at mucosal surfaces. *Nat. Rev. Immunol.* **4**:953–964.
250. Sansonetti, P. J., J. Arondel, R. Cante, M. C. Prevost, and M. Huerre. 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.
251. Sansonetti, P. J., J. Arondel, J.-M. Cavillon, and M. Huerre. 1995. Role of IL-1 in the pathogenesis of experimental shigellosis. *J. Clin. Investig.* **96**:884–892.
252. Sansonetti, P. J., J. Arondel, M. Huerre, A. Harada, and K. Matsushima. 1999. Interleukin-8 controls bacterial transepithelial translocation at the cost of epithelial destruction in experimental shigellosis. *Infect. Immun.* **67**:1471–1480.

253. Sansonetti, P. J., T. L. Hale, G. J. Dammin, C. Kapfer, H. H. Collins, and S. B. Formal. 1983. Alterations in the pathogenicity of *Escherichia coli* K-12 after transfer of plasmid and chromosomal genes from *Shigella flexneri*. *Infect. Immun.* **39**:1392–1402.
254. Sansonetti, P. J., D. J. Koepcke, and S. B. Formal. 1982. Involvement of a plasmid in the invasive ability of *Shigella flexneri*. *Infect. Immun.* **35**:852–860.
255. Sansonetti, P. J., and J. Mounier. 1987. Metabolic events mediating early killing of host cells infected by *Shigella flexneri*. *Microb. Pathog.* **3**:53–61.
256. Sansonetti, P. J., A. Phalipon, J. Arondel, K. Thirumalai, S. Banerjee, S. Akira, K. Takeda, and A. Zychlinsky. 2000. Caspase-1 activation of IL-1 β and IL-18 are essential for *Shigella flexneri*-induced inflammation. *Immunity* **12**:581–590.
257. Sansonetti, P. J., A. Ryter, P. Clerc, A. T. Maurelli, and J. Mounier. 1986. Multiplication of *Shigella flexneri* within HeLa cells: lysis of the phagocytic vacuole and plasmid-mediated contact hemolysis. *Infect. Immun.* **51**:461–469.
258. Santapaola, D., F. Del Chierico, A. Petrucca, S. Uzzau, M. Casalino, B. Colonna, R. Sessa, F. Berlutti, and M. Nicoletti. 2006. Apyrase, the product of the virulence plasmid-encoded *phoN2* (*apy*) gene of *Shigella flexneri*, is necessary for proper unipolar IcsA localization and for efficient intercellular spread. *J. Bacteriol.* **188**:1620–1627.
259. Sasakawa, C., K. Kamata, T. Sakai, S. Y. Murayama, S. Makino, and M. Yoshikawa. 1986. Molecular alteration of the 140-megadalton plasmid associated with loss of virulence and Congo red binding activity in *Shigella flexneri*. *Infect. Immun.* **54**:470–475.
260. Sasakawa, C., K. Kamata, T. Sakai, S. Makino, M. Yamada, N. Okada, and M. Yoshikawa. 1988. Virulence-associated genetic regions comprising 31 kilobases of the 230-kilobase plasmid in *Shigella flexneri* 2a. *J. Bacteriol.* **170**:2480–2484.
261. Sasakawa, C., K. Komatsu, T. Tobe, T. Suzuki, and M. Yoshikawa. 1993. Eight genes in region 5 that form an operon are essential for invasion of epithelial cells by *Shigella flexneri* 2a. *J. Bacteriol.* **175**:2334–2346.
262. Sasakawa, C., S. Makino, K. Kamato, and M. Yoshikawa. 1986. Isolation, characterization, and mapping of Tn5 insertions into the 140-megadalton invasion plasmid defective in the mouse Sereny test in *Shigella flexneri* 2a. *Infect. Immun.* **54**:32–36.
263. Sayeed, S., T. Brendler, M. Davis, L. Reaves, and S. Austin. 2005. Surprising dependence on postsegregational killing of host cells for maintenance of the large virulence plasmid of *Shigella flexneri*. *J. Bacteriol.* **187**:2768–2773.
264. Sayeed, S., L. Reaves, L. Radnedge, and S. Austin. 2000. The stability region of the large virulence plasmid of *Shigella flexneri* encodes an efficient post-segregational killing system. *J. Bacteriol.* **182**:2416–2421.
265. Schlumberger, M. C., and W. D. Hardt. 2006. *Salmonella* type III secretion effectors: pulling the host cell's strings. *Curr. Opin. Microbiol.* **9**:46–54.
266. Schmidt, H., and M. Hensel. 2004. Pathogenicity islands in bacterial pathogenesis. *Clin. Microbiol. Rev.* **17**:14–56.
267. Schmitz, J., A. Owyang, E. Oldham, Y. Song, E. Murphy, T. K. McClanahan, G. Zurawski, M. Moshrefi, J. Qin, X. Li, D. M. Gorman, J. F. Bazan, and R. A. Kastelein. 2005. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* **23**:479–490.
268. Schroeder, G. N., and H. Hilbi. 2007. Cholesterol is required to trigger caspase-1 activation and macrophage apoptosis after phagosomal escape of *Shigella*. *Cell. Microbiol.* **9**:265–278.
269. Schroeder, G. N., N. J. Jann, and H. Hilbi. 2007. Intracellular type III secretion by cytosolic *Shigella flexneri* promotes caspase-1-dependent macrophage cell death. *Microbiology* **153**:2862–2876.
270. Schuch, R., and A. T. Maurelli. 1999. The Mxi-Spa type III secretory pathway of *Shigella flexneri* requires an outer membrane lipoprotein, MxiM, for invasin translocation. *Infect. Immun.* **67**:1982–1991.
271. Schuch, R., and A. T. Maurelli. 2001. MxiM and MxiJ, base elements of the Mxi-Spa type III secretion system of *Shigella*, interact with and stabilize the MxiD secretin in the cell envelope. *J. Bacteriol.* **183**:6991–6998.
272. Schuch, R., and A. T. Maurelli. 2001. Spa33, a cell surface-associated subunit of the Mxi-Spa type III secretory pathway of *Shigella flexneri*, regulates Ipa protein traffic. *Infect. Immun.* **69**:2180–2189.
273. Schuch, R., R. C. Sandlin, and A. T. Maurelli. 1999. A system for identifying post-invasion functions of invasion genes: requirements for the Mxi-Spa type III secretion pathway of *Shigella flexneri* in intercellular dissemination. *Mol. Microbiol.* **34**:675–689.
274. Scott, A. M., and M. Saleh. 2007. The inflammatory caspases: guardians against infections and sepsis. *Cell Death Differ.* **14**:23–31.
275. Segal, G., M. Feldman, and T. Zusman. 2005. The Icm/Dot type-IV secretion systems of *Legionella pneumophila* and *Coxiella burnetii*. *FEMS Microbiol. Rev.* **29**:65–81.
276. Seifert, U., C. Maranon, A. Shmueli, J. F. Desoutter, L. Wesoloski, K. Janek, P. Henklein, S. Diescher, M. Andrieu, H. de la Salle, T. Weinschenk, H. Schild, D. Laderach, A. Galy, G. Haas, P. M. Kloetzl, Y. Reiss, and A. Hosmalin. 2003. An essential role for tripeptidyl peptidase in the generation of an MHC class I epitope. *Nat. Immunol.* **4**:375–379.
277. Shepherd, J. G., L. Wang, and P. R. Reeves. 2000. Comparison of O-antigen gene clusters of *Escherichia coli* (*Shigella*) Sonnei and *Plesiomonas shig-*
- elloides* O17: Sonnei gained its current plasmid-borne O-antigen genes from *P. shigelloides* in a recent event. *Infect. Immun.* **68**:6056–6061.
278. Shere, K. D., S. Sallustio, A. Manassis, T. G. D'Aversa, and M. B. Goldberg. 1997. Disruption of IcsP, the major *Shigella* protease that cleaves IcsA, accelerates actin-based motility. *Mol. Microbiol.* **25**:451–462.
279. Shi, Y. 2004. Caspase activation: revisiting the induced proximity model. *Cell* **117**:855–858.
280. Shiga, K. 1897. Sekiri byogen kenkyu hokoku dai-ichi (first report on etiologic research on dysentery). *Saikingaku Zasshi* **25**:790–810.
281. Siegel, R. M. 2006. Caspases at the crossroads of immune-cell life and death. *Nat. Rev. Immunol.* **6**:308–317.
282. Simons, K., and E. Ikonen. 1997. Functional rafts in cell membranes. *Nature* **387**:569–572.
283. Simons, K., and E. Ikonen. 2000. How cells handle cholesterol. *Science* **290**:1721–1726.
284. Simons, K., and D. Toomre. 2000. Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell Biol.* **1**:31–39.
285. Simons, K., and W. L. Vaz. 2004. Model systems, lipid rafts, and cell membranes. *Annu. Rev. Biophys. Biomol. Struct.* **33**:269–295.
286. Singer, M., and P. J. Sansonetti. 2004. IL-8 is a key chemokine regulating neutrophil recruitment in a new mouse model of *Shigella*-induced colitis. *J. Immunol.* **173**:4197–4206.
287. Skoudy, A., J. Mounier, A. Aruffo, H. Ohayon, P. Gounon, P. Sansonetti, and G. Tran Van Nhieu. 2000. CD44 binds to the *Shigella* IpaB protein and participates in bacterial invasion of epithelial cells. *Cell. Microbiol.* **2**:19–33.
288. Sorg, J. A., B. Blaylock, and O. Schneewind. 2006. Secretion signal recognition by YscN, the *Yersinia* type III secretion ATPase. *Proc. Natl. Acad. Sci. USA* **103**:16490–16495.
289. Stavropoulos, V., V. Vasquez, B. Cereser, E. Freda, and M. G. Masucci. 2006. TPPII promotes genetic instability by allowing the escape from apoptosis of cells with activated mitotic checkpoints. *Biochem. Biophys. Res. Commun.* **346**:415–425.
290. Steinhauer, J., R. Agha, T. Pham, A. W. Varga, and M. B. Goldberg. 1999. The unipolar *Shigella* surface protein IcsA is targeted directly to the bacterial old pole: IcsP cleavage of IcsA occurs over the entire bacterial surface. *Mol. Microbiol.* **32**:367–377.
291. Stevens, J. M., E. E. Galyov, and M. P. Stevens. 2006. Actin-dependent movement of bacterial pathogens. *Nat. Rev. Microbiol.* **4**:91–101.
292. Strober, W., P. J. Murray, A. Kitani, and T. Watanabe. 2006. Signalling pathways and molecular interactions of NOD1 and NOD2. *Nat. Rev. Immunol.* **6**:9–20.
293. Suzuki, T., L. Franchi, C. Toma, H. Ashida, M. Ogawa, Y. Yoshikawa, H. Mimuro, N. Inohara, C. Sasakawa, and G. Nunez. 2007. Differential regulation of caspase-1 activation, pyroptosis, and autophagy via IpaF and ASC in *Shigella*-infected macrophages. *PLoS Pathog.* **3**:e111.
294. Suzuki, T., H. Miki, T. Takenawa, and C. Sasakawa. 1998. Neural Wiskott-Aldrich syndrome protein is implicated in the actin-based motility of *Shigella flexneri*. *EMBO J.* **17**:2767–2776.
295. Suzuki, T., H. Mimuro, H. Miki, T. Takenawa, T. Sasaki, H. Nakanishi, Y. Takai, and C. Sasakawa. 2000. Rho family GTPase Cdc42 is essential for the actin-based motility of *Shigella* in mammalian cells. *J. Exp. Med.* **191**:1905–1920.
296. Suzuki, T., H. Mimuro, S. Suetsugu, H. Miki, T. Takenawa, and C. Sasakawa. 2002. Neural Wiskott-Aldrich syndrome protein (N-WASP) is the specific ligand for *Shigella* VirG among the WASP family and determines the host cell type allowing actin-based spreading. *Cell. Microbiol.* **4**:223–233.
297. Suzuki, T., K. Nakanishi, H. Tsutsui, H. Iwai, S. Akira, N. Inohara, M. Chamailard, G. Nunez, and C. Sasakawa. 2005. A novel caspase-1/Toll-like receptor 4-independent pathway of cell death induced by cytosolic *Shigella* in infected macrophages. *J. Biol. Chem.* **280**:14042–14050.
298. Suzuki, T., S. Saga, and C. Sasakawa. 1996. Functional analysis of *Shigella* VirG domains essential for interaction with vinculin and actin-based motility. *J. Biol. Chem.* **271**:21878–21885.
299. Tamano, K., S. Aizawa, E. Katayama, T. Nonaka, S. Imajoh-Ohmi, A. Kuwae, S. Nagai, and C. Sasakawa. 2000. Supramolecular structure of the *Shigella* type III secretion machinery: the needle part is changeable in length and essential for delivery of effectors. *EMBO J.* **19**:3876–3887.
300. Tamano, K., E. Katayama, T. Toyotome, and C. Sasakawa. 2002. *Shigella* Spa32 is an essential secretory protein for functional type III secretion machinery and uniformity of its needle length. *J. Bacteriol.* **184**:1244–1252.
301. Tampakaki, A. P., V. E. Fadoulglou, A. D. Gazi, N. J. Panopoulos, and M. Kokkinidis. 2004. Conserved features of type III secretion. *Cell. Microbiol.* **6**:805–816.
302. Taylor, P. R., L. Martinez-Pomares, M. Stacey, H. H. Lin, G. D. Brown, and S. Gordon. 2005. Macrophage receptors and immune recognition. *Annu. Rev. Immunol.* **23**:901–944.
303. Thornberry, N. A., H. G. Bull, J. R. Calayac, K. T. Chapman, A. D. Howard, M. J. Kostura, D. K. Miller, S. M. Molineaux, J. R. Weidner, J. Aunins, K. O. Elliston, J. M. Ayala, F. J. Casano, J. Chin, J.-F. Ding, L. A. Egger, E. P. Gaffney, G. Limjuco, P. O. C., S. M. Raju, A. M. Rolando, J. P. Salley, T. T. Yamin, T. D. Lee, J. E. Shively, M. MacCross, R. A. Mumford,

- J. A. Schmidt, and M. J. Tocci. 1992. A novel heterodimeric cysteine protease is required for interleukin-1 β -processing in monocytes. *Nature* **356**:768–774.
304. Thornberry, N. A., and Y. Lazebnik. 1998. Caspases: enemies within. *Science* **281**:1312–1316.
305. Tobe, T., S. Nagai, N. Okada, B. Adler, M. Yoshikawa, and C. Sasakawa. 1991. Temperature-regulated expression of invasion genes in *Shigella flexneri* is controlled through the transcriptional activation of the *virB* gene on the large plasmid. *Mol. Microbiol.* **5**:887–893.
306. Tobe, T., M. Yoshikawa, T. Mizuno, and C. Sasakawa. 1993. Transcriptional control of the invasion regulatory gene *virB* of *Shigella flexneri*: activation by VirF and repression by H-NS. *J. Bacteriol.* **175**:6142–6149.
307. Tominaga, A., R. Lan, and P. R. Reeves. 2005. Evolutionary changes of the *flhDC* flagellar master operon in *Shigella* strains. *J. Bacteriol.* **187**:4295–4302.
308. Torres, A. G. 2004. Current aspects of *Shigella* pathogenesis. *Rev. Latinoam. Microbiol.* **46**:89–97.
309. Toyotome, T., T. Suzuki, A. Kuwae, T. Nonaka, H. Fukuda, S. Imajoh-Ohmi, T. Toyofuku, M. Hori, and C. Sasakawa. 2001. *Shigella* protein IpaH(9.8) is secreted from bacteria within mammalian cells and transported to the nucleus. *J. Biol. Chem.* **276**:32071–32079.
310. Tran Van Nhieu, G., J. Enninga, P. Sansonetti, and G. Grompone. 2005. Tyrosine kinase signaling and type III effectors orchestrating *Shigella* invasion. *Curr. Opin. Microbiol.* **8**:16–20.
311. Tran Van Nhieu, G., A. Ben-Ze'ev, and P. Sansonetti. 1997. Modulation of bacterial entry into epithelial cells by association between vinculin and the *Shigella* IpaA invasin. *EMBO J.* **16**:2717–2729.
312. Tran Van Nhieu, G., E. Caron, A. Hall, and P. J. Sansonetti. 1999. IpaC induces actin polymerization and filopodia formation during *Shigella* entry into epithelial cells. *EMBO J.* **18**:3249–3262.
313. Turner, S. A., S. N. Luck, H. Sakellaris, K. Rajakumar, and B. Adler. 2003. Molecular epidemiology of the SRL pathogenicity island. *Antimicrob. Agents Chemother.* **47**:727–734.
314. Turner, S. A., S. N. Luck, H. Sakellaris, K. Rajakumar, and B. Adler. 2001. Nested deletions of the SRL pathogenicity island of *Shigella flexneri* 2a. *J. Bacteriol.* **183**:5535–5543.
315. van der Goot, F. G., G. Tran Van Nhieu, A. Allaoui, P. Sansonetti, and F. Lafont. 2004. Rafts can trigger contact-mediated secretion of bacterial effectors via a lipid-based mechanism. *J. Biol. Chem.* **279**:47792–47798.
316. van Eerde, A., C. Hamiaux, J. Perez, C. Parsot, and B. W. Dijkstra. 2004. Structure of Spa15, a type III secretion chaperone from *Shigella flexneri* with broad specificity. *EMBO Rep.* **5**:477–483.
317. Veenendaal, A. K., J. L. Hodgkinson, L. Schwarzer, D. Stabat, S. F. Zenk, and A. J. Blocker. 2007. The type III secretion system needle tip complex mediates host cell sensing and translocon insertion. *Mol. Microbiol.* **63**:1719–1730.
318. Venkatesan, M. M., J. M. Buysse, and A. B. Hartman. 1991. Sequence variation in two *ipaH* genes of *Shigella flexneri* 5 and homology to the LRG-like family of proteins. *Mol. Microbiol.* **5**:2435–2446.
319. Venkatesan, M. M., J. M. Buysse, and E. V. Oaks. 1992. Surface presentation of *Shigella flexneri* invasion plasmid antigens requires the products of the *spa* locus. *J. Bacteriol.* **174**:1990–2001.
320. Venkatesan, M. M., M. B. Goldberg, D. J. Rose, E. J. Grotbeck, V. Burland, and F. R. Blattner. 2001. Complete DNA sequence and analysis of the large virulence plasmid of *Shigella flexneri*. *Infect. Immun.* **69**:3271–3285.
321. Venkatesan, M. M., and R. T. Ranallo. 2006. Live-attenuated *Shigella* vaccines. *Expert Rev. Vaccines* **5**:669–686.
322. Vokes, S. A., S. A. Reeves, A. G. Torres, and S. M. Payne. 1999. The aerobactin iron transport system genes in *Shigella flexneri* are present within a pathogenicity island. *Mol. Microbiol.* **33**:63–73.
323. von Seidlein, L., D. R. Kim, M. Ali, H. Lee, X. Wang, V. D. Thiem, D. G. Canh, W. Chaicumpa, M. D. Agtini, A. Hossain, Z. A. Bhutta, C. Mason, O. Sethabutr, K. Talukder, G. B. Nair, J. L. Deen, K. Kotloff, and J. Clemens. 2006. A multicentre study of *Shigella* diarrhoea in six Asian countries: disease burden, clinical manifestations and microbiology. *PLoS Med.* **3**:e353.
324. Wang, S., M. Miura, Y. K. Jung, H. Zhu, E. Li, and J. Yuan. 1998. Murine caspase-11, an ICE-interacting protease, is essential for the activation of ICE. *Cell* **92**:501–509.
325. Wassef, J. S., D. F. Keren, and J. L. Mailloux. 1989. Role of M cells in initial antigen uptake and in ulcer formation in rabbit intestinal loop model of shigellosis. *Infect. Immun.* **57**:858–863.
326. Watarai, M., S. Funato, and C. Sasakawa. 1996. Interaction of Ipa proteins of *Shigella flexneri* with $\alpha_5\beta_1$ integrin promotes entry of the bacteria into mammalian cells. *J. Exp. Med.* **183**:991–999.
327. Watarai, M., T. Tobe, M. Yoshikawa, and C. Sasakawa. 1995. Contact of *Shigella* with host cells triggers release of Ipa invasins and is an essential function of invasiveness. *EMBO J.* **14**:2461–2470.
328. Way, S. S., A. C. Borczuk, R. Dominitz, and M. B. Goldberg. 1998. An essential role for gamma interferon in innate resistance to *Shigella flexneri* infection. *Infect. Immun.* **66**:1342–1348.
329. Wei, J., M. B. Goldberg, V. Burland, M. M. Venkatesan, W. Deng, G. Fournier, G. F. Mayhew, G. Plunkett III, D. J. Rose, A. Darling, B. Mau, N. T. Perna, S. M. Payne, L. J. Runyen-Janecky, S. Zhou, D. C. Schwartz, and F. R. Blattner. 2003. Complete genome sequence and comparative genomics of *Shigella flexneri* serotype 2a strain 2457T. *Infect. Immun.* **71**:2775–2786.
330. West, N. P., P. Sansonetti, J. Mounier, R. M. Exley, C. Parsot, S. Guadagnini, M. C. Prevost, A. Prochnicka-Chalouf, M. Delepiere, M. Tanguy, and C. M. Tang. 2005. Optimization of virulence functions through glucosylation of *Shigella* LPS. *Science* **307**:1313–1317.
331. Wilharm, G., S. Dittmann, A. Schmid, and J. Heesemann. 2006. On the role of specific chaperones, the specific ATPase, and the proton motive force in type III secretion. *Int. J. Med. Microbiol.* **297**:27–36.
332. Wilharm, G., V. Lehmann, K. Krauss, B. Lehnert, S. Richter, K. Ruckdeschel, J. Heesemann, and K. Trulzsch. 2004. *Yersinia enterocolitica* type III secretion depends on the proton motive force but not on the flagellar motor components MotA and MotB. *Infect. Immun.* **72**:4004–4009.
333. Wilson, K. P., J. A. Black, J. A. Thomson, E. E. Kim, J. P. Griffith, M. A. Navia, M. A. Murcko, S. P. Chambers, R. A. Aldape, S. A. Raybuck, et al. 1994. Structure and mechanism of interleukin-1 β converting enzyme. *Nature* **370**:270–275.
334. World Health Organization. 2004. Global burden of disease (GBD) 2002 estimates. World Health Organization, Geneva, Switzerland. http://www.who.int/topics/global_burden_of_disease/en/.
335. World Health Organization. 2006. Initiative for vaccine research (IVR). New vaccines against infectious diseases: research and development status. World Health Organization, Geneva, Switzerland. http://www.who.int/vaccine_research/en/.
336. Yang, F., J. Yang, X. Zhang, L. Chen, Y. Jiang, Y. Yan, X. Tang, J. Wang, Z. Xiong, J. Dong, Y. Xue, Y. Zhu, X. Xu, L. Sun, S. Chen, H. Nie, J. Peng, J. Xu, Y. Wang, Z. Yuan, Y. Wen, Z. Yao, Y. Shen, B. Qiang, Y. Hou, J. Yu, and Q. Jin. 2005. Genome dynamics and diversity of *Shigella* species, the etiologic agents of bacillary dysentery. *Nucleic Acids Res.* **33**:6445–6458.
337. Yang, J., H. Nie, L. Chen, X. Zhang, F. Yang, X. Xu, Y. Zhu, J. Yu, and Q. Jin. 2007. Revisiting the molecular evolutionary history of *Shigella* spp. *J. Mol. Evol.* **64**:71–79.
338. Yoshida, S., Y. Handa, T. Suzuki, M. Ogawa, M. Suzuki, A. Tamai, A. Abe, E. Katayama, and C. Sasakawa. 2006. Microtubule-severing activity of *Shigella* is pivotal for intercellular spreading. *Science* **314**:985–989.
339. Yoshida, S., E. Katayama, A. Kuwae, H. Mimuro, T. Suzuki, and C. Sasakawa. 2002. *Shigella* delivers effector proteins to trigger host microtubule destabilization, which promotes Rac1 activity and efficient bacterial internalization. *EMBO J.* **21**:2923–2935.
340. Zaharik, M. L., S. Gruenheid, A. J. Perrin, and B. B. Finlay. 2002. Delivery of dangerous goods: type III secretion in enteric pathogens. *Int. J. Med. Microbiol.* **291**:593–603.
341. Zahrl, D., M. Wagner, K. Bischof, M. Bayer, B. Zavec, A. Beranek, C. Ruckenstein, G. E. Zarfel, and G. Koraimann. 2005. Peptidoglycan degradation by specialized lytic transglycosylases associated with type III and type IV secretion systems. *Microbiology* **151**:3455–3467.
342. Zhang, Z., L. Jin, G. Champion, K. B. Seydel, and S. L. Stanley, Jr. 2001. *Shigella* infection in a SCID mouse-human intestinal xenograft model: role for neutrophils in containing bacterial dissemination in human intestine. *Infect. Immun.* **69**:3240–3247.
343. Zhong, Q. P. 1999. Pathogenic effects of O-polysaccharide from *Shigella flexneri* strain. *World J. Gastroenterol.* **5**:245–248.
344. Zurawski, D. V., C. Mitsuhashi, K. L. Mumy, B. A. McCormick, and A. T. Maurelli. 2006. OspF and OspC1 are *Shigella flexneri* type III secretion system effectors that are required for postinvasion aspects of virulence. *Infect. Immun.* **74**:5964–5976.
345. Zychlinsky, A., C. Fitting, J. M. Cavailon, and P. J. Sansonetti. 1994. Interleukin-1 is released by murine macrophages during apoptosis induced by *Shigella flexneri*. *J. Clin. Invest.* **94**:1328–1332.
346. Zychlinsky, A., B. Kenny, R. Ménard, M. C. Prevost, I. B. Holland, and P. J. Sansonetti. 1994. IpaB mediates macrophage apoptosis induced by *Shigella flexneri*. *Mol. Microbiol.* **11**:619–627.
347. Zychlinsky, A., M. C. Prevost, and P. J. Sansonetti. 1992. *Shigella flexneri* induces apoptosis in infected macrophages. *Nature* **358**:167–168.
348. Zychlinsky, A., K. Thirumalai, J. Arondel, J. R. Cantey, A. Aliprantis, and P. J. Sansonetti. 1996. In vivo apoptosis in *Shigella flexneri* infections. *Infect. Immun.* **64**:5357–5365.