14 Dysentery

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Introduction ***309***

Taxonomy ***309***

Habitat ***310***

Epidemiology ***310***

Evolution and Genomics ***310***

Pathogenesis and Virulence Factors ***311***

Shigella ***and the Immune Response*** ***315***

Clinical Disease Due to **Shigella** ***spp*** ***316***

Laboratory Identification, Isolation, and Clinical Diagnosis of **Shigella** ***Infection*** ***316***

Treatment and Vaccine Development ***317***

Conclusion ***317***

Abstract
Dysentery is bloody diarrhea caused by infection with certain bacteria or parasites. The most common bacterial causes are members of the Genus *Shigella*. *Shigella* are Gram-negative intracellular bacterial pathogens that cause diarrheal disease by infecting intestinal epithelial cells. Following invasion of intestinal cells, *Shigella* induce host cell cytoskeletal rearrangements and interfere with host cell signal transduction cascades. These effects are mediated by multiple different effector proteins that are translocated from the bacterial cell into the host cell through a type three secretion system. Translocated *Shigella* effector proteins modulate the host immune response, which contributes to inflammation during infection and to clearance of the organism. Antibiotics are available and effective against *Shigella* infection; however, isolates resistant to routine antibiotics are increasingly frequent in many areas of the world. Vaccine development is an ongoing area of research.

Introduction

Dysentery is bloody diarrhea caused by infection with certain bacteria or parasites. The most common bacterial causes are members of the genus *Shigella*, the biology of which is discussed in this chapter. The most common parasitic causes of dysentery are the amoebae.

Shigella spp. are nonmotile Gram-negative, nonspore forming, non-lactose fermenting, facultative anaerobic bacillus-shaped bacterium that are very closely related to *Escherichia coli*. Within the genus *Shigella* are four species (*S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*) and multiple serotypes of each species. *S. dysenteriae* is the most virulent pathogen within this genus; it was first discovered and isolated by the Japanese microbiologist Kiyoshi Shiga in 1898. The epidemiology and pathology of this microbe is of particular clinical significance as *Shigella* is associated with severe diarrheal disease and dysentery in humans. The organism is spread from person to person through contact with contaminated food and water products.

The complete genome of *Shigella* includes a single circular chromosome, one large virulence plasmid, and a variable number of small plasmids, which collectively encode genes for a type three secretion system and multiple virulence factors that enable this pathogen to invade epithelial cells, manipulate the host cytoskeleton, spread through tissue, and modulate the innate immune response.

Taxonomy

The genus *Shigella* is very closely related to the genus *Escherichia*; both belong to the family Enterobacteriaceae. In the current classification, within the genus *Shigella* are four species: *S. dysenteriae* (serogroup A), *S. flexneri* (serogroup B), *S. boydii* (serogroup C), and *S. sonnei* (serogroup D). The most virulent of the *Shigella* species, *S. dysenteriae*, was discovered by the Japanese microbiologist Kiyoshi Shiga during a severe outbreak in 1898. Serogroups A and C (*S. flexneri* and *S. sonnei*) are most commonly associated with clinical disease. Within each serogroup are multiple serotypes: serogroup A (*S. dysenteriae*), 12 serotypes; serogroup B (*S. flexneri*), 6 serotypes; serogroup C (*S. boydii*), 23 serotypes; and serogroup D (*S. sonnei*), 1 serotype (*Table 14.1*). The four species share several key

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Table 14.1
Classification of *Shigella* serotypes

Serogroup	Species	No. of serotypes	Phylogenetic cluster	Serotype designation
A	*S. dysenteriae*	13	C1	D3, D4, D5, D6, D9, D11, D12, D13
			C2	D2
			C3	None
			Outliers	D1, D8, D10
B	*S. flexneri*	6	C1	F6
			C2	none
			C3	F1a, F1b, F2a, F2b, F3, F4a, F4b, F5, Fx, & Fy
			Outliers	None
C	*S. boydii*	23	C1	B1, B2, B3, B4, B6, B8, B10, B14, B18
			C2	B5, B7, B9, B11, B15, B16, B17
			C3	B12
			Outliers	B13
D	*S. sonnei*	1	Outlier	SS

Peng et al. (2009), Yang et al. (2007)

features, including lack of motility, inability to form spores, and inability to ferment lactose, and all four species are facultative anaerobes. *Shigella* species are differentiated from one another using a method of serotyping, which is based on antigen type.

Habitat

Humans are the only natural host for *Shigella*. Monkeys and certain small animals can be infected in the laboratory, but are not natural hosts. In most cases, spread of disease from one individual to another occurs via the fecal-oral route, typically via contamination of the hand. However, with increasing frequency, spread involves ingestion of contaminated foodstuffs or contaminated water.

Epidemiology

Humans are the only natural reservoir of *Shigella*. Annually in the United States, *Shigella* are estimated to cause approximately 450,000 cases (Mead et al. 1999), with about four cases per 100,000 population (2009). Annually worldwide, they are estimated to cause 165 million infections (Kotloff et al. 1999). The species most commonly associated with sporadic infections and outbreaks are *S. flexneri* and *S. sonnei*. *S. flexneri* is overall the most common serogroup isolated from clinical infections worldwide. It is most prevalent in the developing world, whereas *S. sonnei* is the most prevalent serogroup in Europe and the United States. *S. flexneri* and *S. sonnei* are associated with endemic forms of the disease, while *S. dysenteriae* serotype 1 is responsible for most epidemics. *S. dysenteriae* infections in North America are most commonly due to serotype 1, whereas in other areas of the world, other serotypes of *S. dysenteriae* have largely replaced serotype 1. Infections due to *Shigella* typically occur in situations of overcrowding or poor hygiene and sanitation, such as day care centers, institutions for the mentally disabled, and cruise ships.

Evolution and Genomics

The genome of *Shigella* consists of a single circular chromosome, a virulence plasmid, and multiple smaller "cryptic" plasmids. The sequence of the entire *Shigella* genome, including the chromosome, the virulence plasmid, and the cryptic plasmids, was completed in the early 2000s by several independent groups of researchers (Jin et al. 2002; Nie et al. 2006; Wei et al. 2003; Yang et al. 2005, Venkatesan et al. 2001, Buchrieser et al. 2000). The genomes of five different strains, representing all four species of *Shigella*, *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*, are currently available (Jin et al. 2002; Nie et al. 2006; Wei et al. 2003; Yang et al. 2005). The circular chromosome of *S. flexneri* is 4.6 Mbp (4,599,354) with a G+C content of 50.9 % and 4,084 predicted genes (Wei et al. 2003). The large virulence plasmid, which is present in all isolates of *Shigella* and is required for virulence, is 0.220 Mbp (220 kbp), while the size and number of additional plasmids vary depending on the isolate.

Several studies have shown high sequence similarity between the genomes of *Shigella* spp. and *Escherichia coli*. Early studies using DNA hybridization revealed that these two microbes are taxonomically indistinguishable (Brenner et al. 1972). More recent studies using multilocus enzyme electrophoresis, comparative genomic hybridization, and multilocus sequence typing have confirmed early predictions of the high sequence similarity between *Shigella* spp. and *E. coli* (Lan and Reeves 2002; Pupo et al. 1997, 2000).

Sequence analysis of eight housekeeping genes in four different regions of the chromosome of multiple species of *Shigella*, *S. boydii*, *S. dysenteriae*, *S. flexneri*, and *S. sonnei*, revealed that *Shigella* spp. evolved from *E. coli* at least 35,000–270,000 years ago. Sequence variation of these eight genes and more extensive sequence analysis of housekeeping genes suggest multiple independent lines of evolution (Pupo et al. 2000; Yang et al. 2007). Based on these studies, it is now well accepted that *Shigella* belongs to the species *E. coli*, instead of belonging to its own separate genus (Schroeder and Hilbi 2008) and that pathogenic strains that are commonly known as *Shigella* spp. emerged from *E. coli* at least seven times during evolution (*Fig. 14.1*).

Comparative genomics studies further substantiate the genetic and taxonomic relationship between *Shigella* and

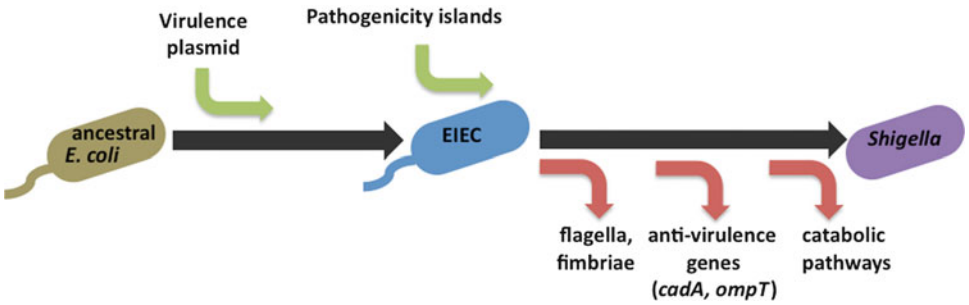

Fig. 14.1

Sequence of evolutionary events that result in the classification of *Shigella* as a distinct genus. Through a series of gene acquisition and gene loss events, *Shigella* acquired a virulence plasmid and multiple pathogenicity islands and lost genes required for flagella and fimbriae synthesis, genes encoding proteins whose activity inhibits virulence (e.g., CadA and OmpT), and genes for catabolic pathways (Adapted from Peng et al. (2009), Schroeder and Hilbi (2008)).

E. coli. The genomes of *Shigella* and *E. coli* are only 1.5 % divergent (Fukushima et al. 2002; Lan and Reeves 2002; Pupo et al. 1997, 2000). The chromosome of *S. flexneri* is slightly smaller (4,599,354 bp) than that of enterohemorrhagic *E. coli* (4,639,221 bp) (Perna et al. 2001; Wei et al. 2003). The overall organization of the two chromosomes is similar, consisting of large regions of backbone with islands. *S. flexneri* has a slighter larger amount of backbone (82 %) than enterohemorrhagic and uropathogenic *E. coli* (75 %) and 200 more pseudogenes than these *E. coli* (Wei et al. 2003).

Shigella spp. are most closely related to enteroinvasive *E. coli* (EIEC) as opposed to other strains of *E. coli* (Lan et al. 2004; Yang et al. 2007). *Shigella* spp. and EIEC evolved from other *E. coli* via convergent evolution (Lan et al. 2004; Pupo et al. 2000) involving multiple events of gene acquisitions, horizontal gene transfer, and genetic loss, through gene deletion (Yang et al. 2007). *Shigella* spp. acquired the large virulence plasmid and five chromosomal pathogenicity islands (SHI-1, SHI-2, SHI-3, SHI-O, and SRL) (Ingersoll et al. 2002; Luck et al. 2001; Ochman et al. 2000; Peng et al. 2009; Purdy and Payne 2001; Rajakumar et al. 1997; Schroeder and Hilbi 2008; Vokes et al. 1999). *Shigella* spp. lost genes for flagella synthesis, rendering the organism nonmotile, and for fimbriae synthesis (Al Mamun et al. 1996; Hacker et al. 1990; Tominaga et al. 2005). *Shigella* spp. also lost the gene encoding the outer membrane protein OmpT, a protease that can cleave the outer membrane protein IcsA (VirG) at the bacterial surface, and acquired on the virulence plasmid the gene encoding a similar yet more highly regulated protease (IcsP, SopA). IcsA, described below, is required for actin polymerization and intracellular spread (Bernardini et al. 1989; Lett et al. 1989). The genes involved in the biosynthesis of cadaverine, the small polyamine product of lysine decarboxylation, were lost during the evolution of both *Shigella* spp. and EIEC from *E. coli* (Casalino et al. 2003; Maurelli et al. 1998). The presence of cadaverine during *Shigella* infection leads to delayed lysis of the phagocytic vacuole by intracellular bacteria, decreased transmigration of polymorphonuclear leukocytes across the infected epithelium, and consequent attenuation of the infection (Fernandez et al. 2001; Maurelli et al. 1998; McCormick et al. 1999). *Shigella* spp. also lost genes for the L-aspartate-dihydroxyacetone and lactose fermentation pathways (Ito et al. 1991; Prunier et al. 2007a, b; Yang et al. 2005).

The chromosomal loci that have been acquired during evolution are designated chromosomal pathogenicity islands SHI-1, SHI-2, SHI-3, SHI-O, and the *Shigella* resistance locus (SRL) (Ingersoll et al. 2002). Pathogenicity islands are large genomic regions that encode virulence factors and are typically characterized by a G+C content and codon usage that are distinct from the chromosome. Acquired by horizontal gene transfer events, pathogenicity islands are often associated with mobile genetic elements and insertion sequences (Dobrindt et al. 2004). SHI-1 encodes the immunoglobulin A-like protease SigA (Al-Hasani et al. 2000), the serine protease Pic (Henderson et al. 1999), and the enterotoxin ShET1 (Fasano et al. 1995, 1997). SHI-2 encodes ShiD and ShiA, which has been shown to interfere with the T-cell immune response during infection (Ingersoll et al. 2003; Ingersoll and Zychlinsky 2006). SHI-2 and SHI-3 encode factors involved in iron acquisition, including the siderophore aerobactin and enterochelin receptors (Luck et al. 2001; Nassif et al. 1987; Purdy and Payne 2001; Vokes et al. 1999). SHI-O, which is present in a subset of strains, contains genes that modify the O-antigen of lipopolysaccharide in ways that contribute to virulence (Huan et al. 1997; Lindberg et al. 1991; Zhong 1999). SRL encodes genes for antibiotic resistance, including tetracycline, chloramphenicol, ampicillin, and streptomycin (Luck et al. 2001; Turner et al. 2001, 2003). Collectively, these genetic acquisition and loss events have lead to the evolution of *Shigella* spp. from *E. coli* as a discrete pathogen adapted to a distinct, predominantly intracellular, lifestyle.

Pathogenesis and Virulence Factors

A key distinguishing feature of *Shigella* is its ability to invade host intestinal epithelial cells. The factors required for invasion are encoded on a large plasmid, known as the "virulence plasmid" or "invasion plasmid," which is present in all virulent

strains (Parsot 2009; Sansonetti et al. 1982). Two adjacent loci on the virulence plasmid confer invasion capabilities: the *mxi-spa* locus, which encodes the structural components of the type three secretion system (T3SS), and the *ipa* (*i*nvasion-related *p*lasmid-encoded *a*ntigens) locus, which encodes multiple different factors, including those required for delivery of effector proteins into host cells, transcriptional regulators, chaperones, and effector proteins (Schroeder and Hilbi 2008). The T3SS is essential for *Shigella* invasion, as plasmid-cured strains and strains carrying disruptions or deletions of any of the T3SS structural genes are unable to invade (Sansonetti et al. 1982). Expression of the genes encoding the structural proteins and of many of the effectors is regulated by two virulence plasmid-encoded transcription activators, VirB and VirF (Le Gall et al. 2005; Schroeder and Hilbi 2008). VirF, a member of the AraC family of transcription activators, activates transcription of *virB* and *icsA* (*virG*) in response to increase in temperature to 37 °C (Hale 1991; Tobe et al. 1993). VirB activates transcription of the T3SS structural proteins and the type three secreted invasion proteins (Porter and Dorman 1997).

The *Shigella* spp. T3SS is a multi-protein apparatus in the bacterial cell envelope that allows for the transport of effector proteins from the bacterial cell cytoplasm across both the bacterial cell envelope and the host epithelial cell plasma membrane into the host epithelial cell cytoplasm (Blocker et al. 2001). The apparatus consists of a gated channel that traverses the inner membrane, the periplasm, and the outer membrane and extends in the form of a long needle into the extracellular space. Activation of secretion is initiated upon contact with host epithelial cells (Enninga et al. 2005). Upon activation, three translocators and 25 or more effector proteins are delivered through the T3SS apparatus into the host epithelial cells (*Table 14.2*) (Enninga et al. 2005; Parsot 2009).

Delivery of proteins through the T3SS occurs in an orderly fashion, with the proteins involved in the formation of a pore in the host cell membrane being delivered first, followed by the effector proteins involved in the entry process, and lastly by the effector proteins that modulate later stages of infection, including those that participate in bacterial intercellular spread and those that manipulate the innate immune response. The genes encoding the proteins that are secreted early, including IpaB, IpaC, and IpaD, which are involved in pore formation, IpaA and IpgB1, which participate in entry, and IcsB, which functions in avoidance of autophagy, along with their chaperones, are transcribed independent of MxiE (see below) (Le Gall et al. 2005; Parsot 2009). Consequently, they are preformed in the bacterial cell and, upon contact with the host cell, are ready to be secreted.

A second group of proteins secreted by the T3SS are those that modulate later stages of infection. As a rule, transcription of these effectors is dependent on MxiE, an AraC family transcription activator encoded within the T3SS locus, whose transcription is regulated by VirB. Transcription by MxiE is intricately co-regulated by IpgC, the chaperone for the translocases IpaB and IpaC. Prior to host cell contact, IpgC is bound to IpaB and IpaC, preventing their premature association (Menard et al. 1994). Upon contact, IpaB and IpaC are dissociated from IpgC in the bacterial cytoplasm and are secreted, whereupon they interact with each other to form a pore in the host plasma membrane (Menard et al. 1994). Concurrently, IpgC becomes available to serve as co-activator of MxiE-mediated transcription (Mavris et al. 2002a, b). Prior to MxiE-IpgC assembly, MxiE is in complex with OspD1 and the chaperone Spa15, which effectively inhibit the activity of MxiE (Page et al. 2002; Parsot et al. 2005). Four effectors secreted by the T3SS, VirA, OspB, OspC1, and OspF, are partially regulated by MxiE-mediated transcriptional activation (Parsot 2009).

In addition to the transcriptional regulation described above, Spa32 negatively regulates the length of the T3SS apparatus needle that extends from the bacterial surface and controls the selection of substrates for secretion, whereas Spa33 regulates Ipa protein secretion (Magdalena et al. 2002; Schuch and Maurelli 2001). Under anaerobic conditions, such as those present in the lumen of the human intestine, transcription of *spa32* and *spa33* are repressed by FNR, a conserved regulator of anaerobic metabolism, and the needles become long (Marteyn et al. 2010). Close to the epithelial surface, however, oxygen tension increases sufficiently to de-repress transcription of *spa32* and *spa33*, triggering molecular events necessary for cellular entry (Marteyn et al. 2010).

The best-described functions of IpaB, IpaC, and IpaD are as translocators that form a pore in the host plasma membrane (Menard et al. 1994). In addition, they are thought to possibly gate the pore and anchor the T3SS needle to the plasma membrane. IpaB and IpaC are positioned at the tip of the T3SS apparatus from where they integrate into cholesterol-rich domains of the plasma membrane (De Geyter et al. 1997; Harrington et al. 2006; Lafont et al. 2002), creating a pore and a conduit between the bacterial and host cells that allows for the subsequent delivery of effector proteins (Blocker et al. 1999; Espina et al. 2006; Menard et al. 1993, 1994; Veenendaal et al. 2007). IpgD is also positioned at the tip of the T3SS apparatus, where in addition to providing scaffolding, it also regulates secretion (Picking et al. 2005; Schroeder and Hilbi 2008). IpaD is anchored directly to MxiH, the protein that forms the needle of the T3SS apparatus (Zhang et al. 2007). The precise signals responsible for activating secretion and the molecular mechanisms by which these signals trigger the assembly of the T3SS apparatus tip upon epithelial cell contact are unknown and yet are active areas of research. In addition to their roles as translocators, IpaB and IpaC have also been shown to have effector-like activities.

The early effector IpaA causes localized actin depolymerization at the sites of bacterial invasion, leading to enhanced bacterial uptake (Bourdet-Sicard et al. 1999). The effects of IpaA activity are mediated by its direct interaction with the host actin cytoskeletal protein vinculin. The C-terminal domain of IpaA binds to the amino-terminal head domain of vinculin (Bourdet-Sicard et al. 1999; Demali et al. 2006; Ramarao et al. 2007; Tran Van Nhieu et al. 1997). Independent of its interaction with vinculin, IpaA induces weakening of cellular adhesion to the extracellular matrix (Demali et al. 2006). Spa15 serves as a chaperone for IpaA (Page et al. 2002).

Table 14.2
Known functions of *Shigella* virulence proteins

Effector	Expression	Function
IpaB	Early effector; not regulated by MxiE	Translocator; essential for pore formation and delivery of effectors; integrates into cholesterol-rich domains of the plasma membrane
IpaC	Early effector; not regulated by MxiE	Translocator; essential for pore formation and delivery of effectors; integrates into cholesterol-rich domains of the plasma membrane
IpaD	Early effector; not regulated by MxiE	Translocator; essential for pore formation and delivery of effectors; provides scaffolding and regulates secretion; anchored to MxiH
IpaA	Early effector; not regulated by MxiE	Involved in entry into host epithelial cells; causes localized actin depolymerization; interacts with vinculin; weakens integrin interactions with extracellular matrix
IpgB1	Early effector; not regulated by MxiE	Involved in entry into host epithelial cells; causes membrane ruffling; serves as a GTP exchange factor (GEF) for the Rho GTPase RhoG; interacts with ELMO-Dock180, which results in activation of Rac1 and Cdc42
IpgB2	Not regulated by MxiE	Homologue of IpgB1; serves as a GEF for the Rho GTPase RhoA
IcsA	Activated by VirF	Required for actin tail polymerization via N-WASP-Toca-1-Arp2/3; activates autophagy through Atg5
IcsB	Early effector; not regulated by MxiE	Evasion of autophagy through Atg5
MxiE		AraC family transcriptional activator; regulated by VirB and IpgC
IpgC		Transcriptional co-activator of MxiE; chaperone for IpaB and IpaC
Spa15		Chaperone for multiple effectors
OspD1	Not regulated by MxiE	MxiE anti-activator; function in host cell unknown
VirA	Partially regulated by MxiE	Function in host cell unknown
OspB	Partially regulated by MxiE	Function in host cell unknown
OspC1	Partially regulated by MxiE	Function in host cell unknown
Spa32		Negatively regulates the length of the T3SS apparatus needle Spa33
Spa33		Regulates Ipa protein secretion
MxiH		Constitutes the needle of the T3SS apparatus
IpgD	Early effector; not regulated by MxiE	An inositol phosphatase; mediates the dephosphorylation of PI-(4,5)P_2 into PI-(5)P, which leads to PI3-kinase activation of Akt that results in decreased lysosomal degradation and increased host cell survival
OspC2 OspC3 OspC4		Function in host cell unknown
OspE1		Interact with integrin-linked kinases (ILKs) to stabilize cell adhesion to the substratum
OpsE2		
OspD2	OspD2 is not regulated by MxiE	Function in host cell unknown
OspD3		
IpaH family		E3 ligases
OspF	Partially regulated by MxiE	Phosphothreonine lyase activity; dephosphorylates and inhibits MAPK signaling
OpsZ		Inhibits NF-kB activity
OspG		Protein kinase whose function leads to inhibition of NF-κB activation

The early effector protein IpgB1 activates a cellular pathway that induces formation of membrane ruffles, likely by serving as a GTP exchange factor (GEF) for the Rho GTPase RhoG. Like RhoG, IpgB1 interacts with the cellular protein complex ELMO-Dock180, which results in activation of the actin nucleation-promoting factors Rac1 and Cdc42 and actin-mediated formation of membrane ruffles (Handa et al. 2007; Ohya et al. 2005). IpgB2, a homologue of IpgB1, serves as a GEF for the Rho

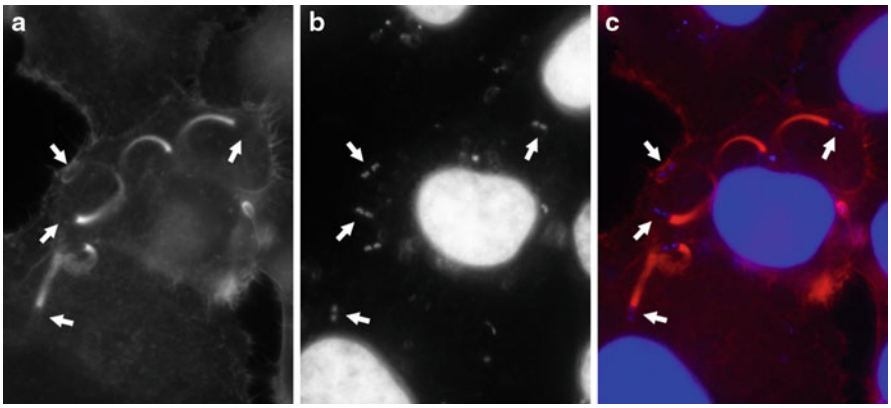

Fig. 14.2
Actin tail assembly by *Shigella* during infection. (a) Polymerized actin; (b) bacterial and cellular DNA; (c) overlay of polymerized actin (*red*) and DNA staining (*blue*). *Arrows*, bacteria at the tip of actin tails

GTPase RhoA (Klink et al. 2010) and activates the immune modulator NF-κB (Fukazawa et al. 2008), but its molecular function in *Shigella* pathogenesis remains uncertain. OspB also activates NF-κB by a mechanism that is unclear (Fukazawa et al. 2008).

IpgD, an early type three secreted effector, is an inositol phosphatase that promotes membrane ruffling during bacterial entry and alters cellular survival and lysosomal degradation pathways (Niebuhr et al. 2000; Pendaries et al. 2006; Ramel et al. 2011). IpgD specifically mediates the dephosphorylation of phosphatidylinositol 4,5-bisphosphate (PI-(4,5)P_2) to yield phosphatidylinositol 5-monophosphate (PI-(5)P) (Niebuhr et al. 2002). IpgD-induced formation of PI-(5)P results in activation of the PI3-kinase signaling pathway that leads to Akt phosphorylation in a manner that depends on the epidermal growth factor receptor (EGFR), which modulates endosomal trafficking (Pendaries et al. 2006; Ramel et al. 2011). Increased levels of PI-(5)P lead to decreased lysosomal degradation and increased host cell survival (Pendaries et al. 2006; Ramel et al. 2011).

Following *Shigella* uptake into host epithelial cells, a second wave of effectors is secreted via the T3SS into the *Shigella*-containing vacuole and, after vacuolar lysis, into the cell cytoplasm. Some of these effectors are important for pathogen survival, others are essential for *Shigella* dissemination through the epithelial cell layer, and others modulate the host immune response. Finally, the functions of other effectors are less well defined.

Shigella effectors that mediate lysis of the *Shigella*-containing vacuole are unknown. Early work suggested that IpaB is involved in this process (High et al. 1992), but given what has been learned since about the role of IpaB in secretion of other effectors, the mechanism of its involvement in vacuolar lysis is unclear. Lysis of the vacuole releases the bacterium into the cell cytoplasm, where it utilizes the cellular actin polymerization machinery to move. The bacterium polymerizes actin into a tail at one end of the bacterial body (*Fig. 14.2*). Recruitment of the actin polymerization machinery to the bacterium depends on the *Shigella* outer membrane protein IcsA (VirG) (Bernardini et al. 1989; Lett et al. 1989), which is a member of the autotransporter family of proteins and is not secreted by the T3SS. IcsA binds the cellular actin nucleation-promoting factor N-WASP, and N-WASP is activated by the cellular protein Toca-1, whereupon it recruits and activates the actin polymerizing complex Arp2/3 (Leung et al. 2008; Lommel et al. 2001; Snapper et al. 2001; Suzuki et al. 1998). Polymerization of the tail propels the bacterium to the cell periphery, whereupon through processes that are incompletely understood, it utilizes diaphanous formins to generate protrusions of the plasma membrane that enclose the bacterium (Heindl et al. 2010). Bacterium-containing protrusions are engulfed by adjacent cells, leading to spread of the bacterium into these cells.

In addition to recruiting actin polymerization machinery, IcsA can be recognized by the cellular autophagy protein Atg5. Atg5 recognition activates the autophagosome formation pathway. Autophagy is a cellular pathway that engulfs foreign objects present in the cytoplasm, such as intracellular bacteria, and kills and degrades them. The type three secreted effector IcsB shares the same binding region and has higher affinity than Atg5 for IcsA, such that IcsB binding to IcsA masks Atg5 recognition and allows the bacterium to escape detection and destruction via autophagy (Ogawa and Sasakawa 2006; Ogawa et al. 2005).

Shigella spp. encode two copies of the type three secreted effectors OspE, OspE1, and OspE2, which have nearly identical protein sequences. OspE proteins interact with integrin-linked kinase (ILK) within sites of cellular attachment to the extracellular matrix, causing stabilization of these attachment sites and preventing cell release from the substratum during infection (Kim et al. 2009).

The type three secreted effector VirA is homologous and structurally similar and can partially functionally complement EspG, a type three secreted effector of enterohemorrhagic *Escherichia coli* (EHEC) and enteropathogenic *E. coli* (EPEC) (Davis et al. 2008; Elliott et al. 2001; Germane and Spiller 2011; Selyunin et al. 2011). EspG regulates endomembrane trafficking through interactions with ADP-ribosylation factor GTPases and

p21-activated kinases (Germane and Spiller 2011; Selyunin et al. 2011). However, the specific function of VirA remains uncertain, as a possible role in endomembrane trafficking has not been examined and data indicating a role in microtubule destabilization and protease activity are conflicting (Germane et al. 2008; Yoshida et al. 2006).

Among the type three secreted effectors of *Shigella* spp. is a family of 5–7 effector proteins designated IpaH, including some encoded on the *Shigella* chromosome (Ashida et al. 2007). IpaH proteins contain a conserved C-terminal domain and a variable N-terminal domain. The C-terminal domains function as E3 ligases (Rhode et al. 2007; Singer et al. 2008; Zhu et al. 2008), proteins that target specific substrates for degradation via the cellular ubiquitination pathway. The N-terminal domain is the site of a leucine-rich repeat domain, which is a classical pathogen-associated molecular pattern recognition site involved in the host epithelial cell immune response during pathogen infection (Bell et al. 2003; Hartman et al. 1990; Okuda et al. 2005; Parsot 2009; Venkatesan et al. 1991). The substrate specificity of the IpaH proteins is determined by the N-terminal domain and has been identified for only one, IpaH9.8, which targets NEMO/IKKγ, a host inflammatory response modulator, for degradation, thereby dampening the NF-κB regulated inflammatory response (Ashida et al. 2010). In the same pathway, OspG, a type three effector protein that is not a member of the IpaH family, binds ubiquitinated ubiquitin-conjugating enzymes, thereby preventing the degradation of the NF-kB inhibitor IκBα (Kim et al. 2005).

Two other type three secreted effector proteins involved in modulation of the host inflammatory response are OspF and OspZ. OspF possesses phosphothreonine lyase activity, an unusual enzymatic activity. It irreversibly dephosphorylates components of the mitogen-activated protein kinase (MAPK) signaling pathway, leading to inhibition of this pathway (Arbibe et al. 2007; Kramer et al. 2007). OspZ inhibits the nuclear translocation of NF-kB (Newton et al. 2010). As for the activity of IpaH9.8 and OspG, the activities of OspF and OspZ attenuate the host inflammatory response. The functions of several type three effectors, including OspC1, OspC2, OspC3, OspD2, and OspD3, are currently unknown (*Table 14.2*).

Shigella and the Immune Response

Shigella infection is generally restricted to the mucosal layer of the large intestine. The organism is able to survive the environment of the stomach due to acid resistance mechanisms (Gorden and Small 1993). Once at the epithelial lining of the large intestine, *Shigella* may be taken up by M-cells, which are specialized in gut-lumen sampling; uptake by M-cells leads to transcytosis of the bacteria across the epithelial layer (Sansonetti et al. 1996; Wassef et al. 1989). Transcytosis enables *Shigella* to enter the epithelial cell lining at the basolateral surface, instead of at the apical surface, and it also enables bacterial interactions with macrophages and dendritic cells within the mucosa (Mounier et al. 1992; Sansonetti et al. 1999). *Shigella* may also enter cells by disrupting epithelial intercellular junctions (Perdomo et al. 1994a; Sakaguchi et al. 2002). Whether organisms also enter cells from the apical side of the epithelium in vivo is uncertain.

When phagocytosed by macrophages, *Shigella* evades killing by triggering apoptosis, which is accompanied by the release of massive amounts of the pro-inflammatory cytokines interleukin (IL)-1β and IL-18 (Islam et al. 1997; Sansonetti et al. 2000; Zychlinsky et al. 1992; Zychlinsky et al. 1996). Release of IL-1β triggers intestinal inflammation (Sansonetti et al. 1995). Release of IL-18 is associated with an antimicrobial response that involves NK (natural killer) cell activation and the production of interferon (IFN)-γ, which is critical for mounting an innate immune response against microbial infection (Hilbi et al. 1997; Le-Barillec et al. 2005; Sansonetti et al. 2000; Way et al. 1998).

Shigella enters the intestinal epithelium by the basolateral surface of cells (Sansonetti et al. 1986). Following entry, internalized bacteria escape the uptake vacuole, replicate within the cytoplasm, and utilize the host cytoskeleton to move to the cell periphery and into adjacent cells. All eukaryotic cells possess mechanisms for eliminating intracellular foreign bodies, including autophagy and activation of the innate immune response. *Shigella* has evolved mechanisms to evade each of these host responses.

Eukaryotic cells possess a lysosomal degradation pathway called autophagy that serves both to recover nutrients during periods of starvation and to rid the cell of undesirable particles, including invading pathogens. Upon entry into cells, *Shigella* is surrounded by a vacuolar membrane, which it rapidly lyses. The remnants of the vacuolar membrane are degraded by the autophagy pathway (Dupont et al. 2009). Then, a tug-of-war is staged between the intracytoplasmic bacteria and the innate immune response, in which some of the intracytoplasmic bacteria succumb to autophagy and some escape. Those that are destined to succumb are ubiquitinated and may be surrounded by a scaffold of the cytoskeletal protein septin, before being engulfed in cellular membranes and degraded (Mostowy et al. 2010; Ogawa et al. 2005). Escape from autophagy is mediated at least in part by the type three secreted effector protein IcsB, which blocks binding of the autophagy protein Atg5 to the surface of *Shigella* (Ogawa et al. 2005).

Peptidoglycan fragments from intracellular bacteria are sensed by the pattern recognition receptor Nod1, the activation of which results in NF-kB activation and subsequent release of the pro-inflammatory cytokine IL-8 (Girardin et al. 2003; Pedron et al. 2003; Philpott et al. 2000; Sansonetti et al. 1999). IL-8 is responsible for the recruitment of polymorphonuclear leukocytes (PMNs) to the sites of *Shigella* infection (Sansonetti et al. 2000; Singer and Sansonetti 2004). Infiltrating PMNs entrap and kill invading bacteria but also contribute to the destruction of the epithelial cell lining, which further enables the entry and invasion of more *Shigella* into intestinal epithelial cells (Perdomo et al. 1994a; Perdomo et al. 1994b). The ability of PMNs to destroy invading bacteria contributes to the resolution of infection (Brinkmann et al. 2004; Mandic-Mulec et al. 1997; Zhang et al. 2001).

The adaptive immune response to *Shigella* provides partial protection against subsequent infection (Taylor et al. 1989). Particularly surprising is the observation that although *Shigella* are intracellular pathogens, individuals who have been infected are protected in a serotype-specific manner (Ferreccio et al. 1991; Lerman et al. 1994; Mel et al. 1965, 1968, 1971), suggesting that protection is mediated by the humoral immune response and not the cellular immune response. In animal models, serotype-specific IgA can provide protection (Phalipon et al. 1995), yet is not required for protective immunity (Way et al. 1999), suggesting that IgG is the protective isotype. Whereas it is known that *Shigella* blocks aspects of the adaptive cellular immune response (Jehl et al. 2011), how it does so is unclear.

Clinical Disease Due to *Shigella* spp

Shigella causes diarrhea and dysentery, a diarrheal syndrome characterized by blood and white blood cells in the stool. *Shigella* is a human pathogen, with no reservoir in other animals. In the majority of cases, the organism is acquired from an infected individual by direct human-to-human spread. In other cases, the organism is acquired from food or water that has been contaminated by an infected individual. The incubation period averages 3 days, with a range of 1–7 days.

The infectious inoculum is as few as 10–100 bacteria (Dupont et al. 1989), in large part because the organism is relatively resistant to stomach acid, such that even when only a small number of organisms are ingested, a sufficient number gain access to the intestine, where they replicate and cause disease. As a consequence, outbreaks are common in day care centers, mental institutions, and other settings where housing is crowded or hygiene suboptimal, and secondary infection rates among family members are reported as high as 20 %.

Shigella infect intestinal epithelial cells of the sigmoid colon and rectum, the distal most segments of the colon. The organism is thought to enter the epithelium largely by transcytosis of microfold (M) cells, whose normal function is to sample antigens from the intestinal lumen. Following transcytosis to the subepithelium, *Shigella* enter into the epithelial cells using a type three secretion system apparatus (T3SS). Once within the cells, the organism spreads into adjacent cells. The release of pro-inflammatory cytokines leads to an acute inflammatory cell infiltrate. The combination of bacterial spread through the epithelium and the inflammatory response leads to local destruction of the epithelium with ulceration and abscess formation and, in many cases, blood and white blood cells in the stool. Symptoms characteristically include severe abdominal cramping, rectal urgency (tenesmus), frequent small loose stools, general malaise, and fever. In the absence of antibiotic therapy, the diarrhea is typically self-limited and resolves in 7 or fewer days.

Significant complications are uncommon. Approximately 4 % of infected individuals will have transient seeding of the bloodstream (bacteremia), 2.5 % will experience obstruction of the intestine, and a small percentage of children will develop rectal prolapse or seizures. In infection due to *S. dysenteriae* 1, 3 % of individuals will develop a severe dilatation of the colon, called toxic megacolon, which is treated with surgery.

Two uncommon, yet important, complications of *Shigella* infection are post-infectious arthritis (formerly Reiter syndrome) and hemolytic-uremic syndrome. Occurring in a small percentage of cases, post-infectious arthritis develops 1–2 weeks after the diarrhea and may be accompanied by conjunctivitis and painful urination (urethritis). Seventy percent of patients who develop this syndrome have the haplotype HLA-B27. Post-infectious arthritis can occur following infection with any of several enteric and urethral bacterial pathogens, including *Campylobacter*, *Salmonella*, and *Yersinia* spp.

Hemolytic-uremic syndrome is a potentially life-threatening complication that is characterized by the combination of anemia due to hemolysis of red blood cells, decreased platelets, and kidney failure due to injury of the renal glomeruli. Most commonly affected are children under the age of 5, with 5–25 % suffering from some degree of permanent kidney dysfunction. The damage is mediated by Stx toxin (formerly Shiga toxin), which among *Shigella* spp. is encoded only by *S. dysenteriae* 1.

Treatment with antibiotics is recommended for all individuals infected with *Shigella*. Prognosis is excellent, with nearly all individuals recovering fully. No *Shigella* vaccines are currently approved for use, although both live attenuated vaccines and subunit vaccines that combine purified protein and lipopolysaccharide (LPS) are under development. Prevention of spread of *Shigella* depends on meticulous hand hygiene.

Laboratory Identification, Isolation, and Clinical Diagnosis of *Shigella* Infection

The diagnosis of *Shigella* infection is made by culture of the organism from stool samples. *Shigella* can be isolated from stool of infected individuals. In approximately 4 % of infections, *Shigella* can also be isolated from the bloodstream. Organisms cannot be isolated from other body sites. Stool is plated both on nonselective indicator media and on selective media. The nonselective media is typically MacConkey agar, on which *Shigella* spp. grow as white colonies and *E. coli* grow as red colonies (Fig. 14.3a). Selective media include *Salmonella Shigella* agar, which, as the name suggests, is selective for growth of *Salmonella* and *Shigella* spp., and Hektoen enteric agar, which is both selective for growth of *Salmonella* and *Shigella* spp. and differentiates between the two on the basis of the appearance of the colonies. On Hektoen, *Salmonella* spp. grow as black colonies because they produce hydrogen sulfite, whereas *Shigella* spp. grow as green colonies because they do not (Fig. 14.3b). To maximize the likelihood of recovering the organism from stool, it is generally advised to initially plate it on MacConkey agar (nonselective media) and then re-streak lactose negative colonies onto selective media. Colonies are convex, with smooth edges, and translucent, with a typical diameter of 0.5–2.0 mm, and on MacConkey are white in color.

Confirmation of the genus as *Shigella* is performed using biochemical tests. The organism is oxidase negative, catalase

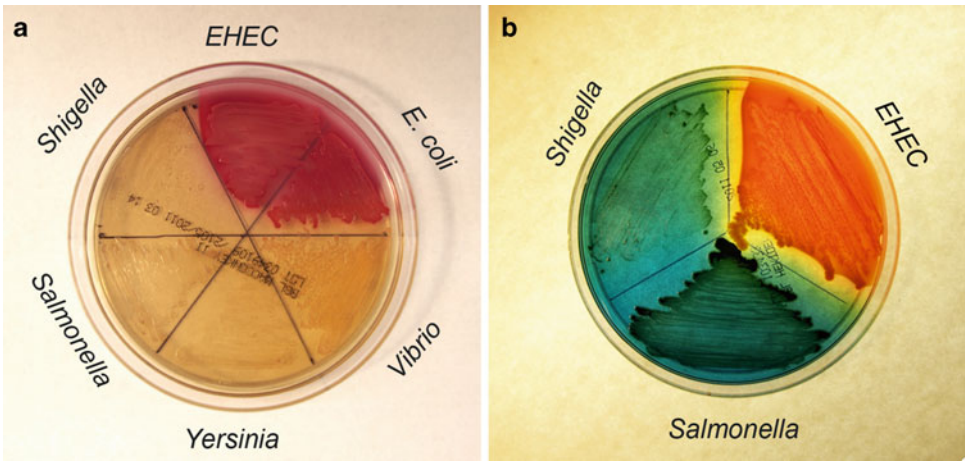

Fig. 14.3
Growth of enteric pathogens on selective agar. (a) Enteric pathogens were grown on MacConkey agar. Lactose non-fermentors, including *Shigella*, form white colonies, and lactose fermentors, such as *E. coli*, form pink colonies. EHEC, enterohemorrhagic *E. coli*. (b) On Hektoen enteric agar, *Salmonella* form *black* colonies due to production of hydrogen sulfite (H_2S), *Shigella* form green colonies, and lactose-fermenting bacteria, such as EHEC, form orange colonies

positive, Voges-Proskauer and Simmons citrate negative, lysine decarboxylase negative, arginine dihydrolase negative, and variable for indole production and ornithine reaction. It does not produce hydrogen sulfite, does not hydrolyze urea, does not utilize malonate, and does not grow on potassium cyanide (KCN) agar. *Shigella* spp. ferment glucose, but do not ferment lactose. *S. dysenteriae* can be discriminated from the other species of *Shigella* by its inability to ferment mannitol, and *S. sonnei* can be discriminated from the others by its ability to produce ornithine decarboxylase. Most clinical microbiology laboratories determine the species of a *Shigella* isolate by O-antigen typing (serotyping) using O-antigen specific antisera.

Treatment and Vaccine Development

All individuals infected with *Shigella* should receive a course of antibiotics. Agents that are recommended include the fluoroquinolones (e.g., ciprofloxacin), azithromycin, or trimethoprim-sulfamethoxazole. Resistance to ciprofloxacin, ampicillin, and trimethoprim-sulfamethoxazole is increasing worldwide, so whenever possible, the selection of an antibiotic should be based on laboratory susceptibility data. If left untreated, *Shigella* infection will resolve over 5–7 days. Antibiotic treatment has been shown to shorten the duration of illness by a couple of days (Christopher et al. 2010).

At present, no vaccine for *Shigella* is approved for use in the United States. Several distinct types of vaccines are being developed, including subunit vaccines, live attenuated vaccines, and outer membrane vesicle vaccines. The subunit vaccines under development consist of various combinations of purified IpaB and IpaC, translocases of the type three secretion system, purified IpaD, the type three secretion system needle tip, and purified lipopolysaccharide (LPS) (Martinez-Becerra et al. 2011; Riddle et al. 2011). The live attenuated vaccines under development carry deletions in genes involved in intercellular motility (*icsA*), the toxins ShET2-1 and ShET2-2 (*senA*, *senB*), acetylation of LPS (*msbB1*, *msbB2*), and guanine biosynthesis (*guaBA*) (Barnoy et al. 2010, 2011; Ranallo et al. 2010; Wu et al. 2011). Outer membrane vesicle vaccines consist of outer membrane vesicles purified from virulence strains (Camacho et al. 2011).

Conclusion

Shigella is a Gram-negative intracellular bacterial pathogen that causes diarrheal disease by infecting intestinal epithelial cells. Following invasion of intestinal cells, *Shigella* induces host cell cytoskeletal rearrangements and interferes with host cell signal transduction cascades. These effects are mediated by multiple different effector proteins that are translocated from the bacterial cell into the host cell through a type three secretion system. Translocated *Shigella* effector proteins modulate the host immune response, which contributes to inflammation during infection and to clearance of the organism. Antibiotics are available and effective against *Shigella* infection; however, isolates resistant to routine antibiotics are increasingly frequent in many areas of the world. Vaccine development is an ongoing area of research.

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