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Current aspects of *Shigella* pathogenesis

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ABSTRACT. Bacillary dysentery (shigellosis) is a severe human disease caused by Shigellae. In recent years, a large amount of information has been generated regarding the host, pathogen and environmental factors that impact the pathogenesis of shigellosis at the cellular and molecular level. This review summarizes what is currently known about *Shigella*, detailing those factors that contribute to pathogenesis and examining the current progress in the development of a vaccine.

Key words: *Shigella*, pathogenicity island, invasion, virulence plasmid.

RESUMEN. Disentería bacilar (shigellosis) es una enfermedad severa en humanos causada por Shigellae. En años recientes, una gran cantidad de información se ha generado respecto a aquellos factores del hospedero, en el patógeno y ambientales; que tienen impacto en la patogénesis de shigellosis a nivel molecular y celular. Esta revisión engloba todos los aspectos relacionados con *Shigella*, detallando aquellos factores que contribuyen a la patogénesis y examina el progreso actual en el desarrollo de una vacuna.

Palabras clave: *Shigella*, isla de patogenicidad, invasión, plásmido de virulencia.

INTRODUCTION

Shigella are Gram-negative, non-spore forming, facultative anaerobic bacilli closely related biochemically and antigenically to *E. coli*. Most fail to ferment lactose or mucate and lysine is not decarboxylated. Furthermore, gas is not produced when fermenting glucose and all are nonmotile.[38] They cause a disease called dysentery (bacillary dysentery or shigellosis), an infection of the large bowel characterized by abdominal cramps, diarrhea, and fever. Initially, the diarrhea may be copious and the liquid stools often contain blood and mucus. It is sometimes accompanied by other symptoms such as vomiting and headache and the infection rarely involves other parts of the body.[39]

Shigella spp. are usually acquired by drinking water contaminated with human feces or by eating food washed with contaminated water. The organisms invade the cells lining the colonic mucosa and multiply there, killing the cell; this is the cause of the symptoms produced. However, it occasionally invades the bowel beyond the surface lining. At least one species, *Shigella dysenteriae*, also secretes a toxin that most likely plays a role in tissue destruction and more serious systemic disease.[11]

Shigella spp., continue to have an important global impact, causing an estimated 1 million deaths and 163 million cases of dysentery annually. The organisms have demonstrated extraordinary competence for acquiring plasmid-encoded multi-antibiotic resistance previously used as first-line therapy.[19] This finding, in addition to the low infectivity and potential complications with complex or often unexplained pathogenesis, have led several laboratories to try to understand the pathogenesis of shigellosis, with the aim of developing a vaccine against the disease.

Complete overviews on the pathogenesis, epidemiology, clinical significance, detection and diagnosis of *Shigella* strains have been published elsewhere.[10,11,23,40,41,46] This review focuses on the recent progress to understand the genetic and molecular basis of shigellosis.

THE GENUS *SHIGELLA*

There are four different species of *Shigella*, divided on the basis of differences in O antigen of their lipopolysaccharide and some biochemical reactions, such as indole production or mannitol fermentation. These are named *S. dysenteriae* (13 serotypes), *S. flexneri* (15 serotypes), *S. boydii* (18 serotypes), and *S. sonnei* (1 serotype).[38] In general, *S. dysenteriae* accounts for deadly epidemics in developing countries, *S. flexneri* and *S. sonnei* are responsible for endemic disease, the former being prevalent in the developing world, the latter in developed countries, and *S. boydii* accounts for most cases of infection in India and neighboring countries. From these species, the most extensively studied is *S. flexneri*.

OVERVIEW OF PATHOGENESIS

The fundamental event in the pathogenesis of *Shigella* is the ability to invade and colonize the human intestinal epi-

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thelium. This triggers an intense acute inflammatory response with infiltration by polymorphonuclear leukocytes. The pathogenesis of *Shigella* is a multi-step process which depends on the capacity of the bacteria to cross the colonic mucosa via M cells associated with Gastrointestinal Associated Lymphoid Tissue (GALT). The bacteria then invade epithelial cells and have the capacity to reprogram these cells to produce pro-inflammatory mediators, such as interleukin 8, which play a major role in the strong inflammatory response facilitating further bacterial invasion. Most of the virulence determinants responsible for invasion of epithelial cells are encoded on a 213 kilobase (kb) plasmid that is unique to virulent *Shigella* and enteroinvasive *E. coli* (EIEC) strains.

Virulence plasmid. The initial observation that established the essential role of plasmids *in Shigella* virulence was performed in *S. sonnei* and shortly thereafter in *S. flexneri*.[17,43] Non-invasive *S. sonnei* isolates obtained upon subculture in the laboratory recovered their invasive abilities when a large plasmid, normally found in clinical isolates of *Shigella*, was reintroduced.[17] Since this initial observation, the so-called virulence plasmid (Fig. 1) has been shown to encode genes for: a) the production of invasion plasmid antigens (Ipas); b) the synthesis of a type III secretion apparatus, a flagella-like structure able to deliver *Shigella* effector proteins into the eukaryotic cell; c) the induction of endocytic uptake of the bacteria and disruption of endocytic vacuoles; d) the intra- and intercellular spreading phenotype, and e) the regulation of plasmid-encoded virulence genes.

Two independent groups have recently reported the complete sequence analysis of the large virulence plasmid in *S. flexneri* serotype 5a.[5,48] The DNA sequence indicated that the genes necessary for entry of bacteria into epithelial cells are clustered within a 31-kb region of the virulence plasmid. The genes within this region have been extensively characterized.[31] The entry region is a pathogenicity island-like cluster (see below) that contains: a) the *mxi* and *spa* genes encoding components of a type III secretion apparatus; b) the *ipaA*, *B*, *C* and *D* and *ipgD* genes encoding proteins secreted by this machinery; c) the *ipgC* and *ipgE* genes encoding cytoplasmic chaperones required for stability of IpaB and IpaC, and IpgD, respectively; d) the *virB* gene encoding a protein required for transcription of the *mxi*, *spa* and *ipa* genes; and e) additional genes of unknown function.

Outside of the entry region, other genes associated with virulence have been identified. They include: a) the *icsA* (*virG*) gene encoding an outer membrane protein that is directly responsible for the ability of the bacteria to move within the cytoplasm of infected cells; b) the *virF* gene encoding a transcriptional activator that controls expression of *icsA* and *virB*; and c) the *sepA* gene, which encodes a secreted serine protease of the autotransporter family.

In addition, the virulence plasmid contains two copies of the *shet2* gene encoding a putative enterotoxin, and genes encoding several secreted proteins, which include *virA*, *ipaH4.5*, *ipaH7.8*, *ipaH9.8* and six uncharacterized genes designated (outer *Shigella* proteins): *ospB*, *ospC1*, *ospD1*, *ospE1*, *ospF* and *ospG*. The proteins encoded in this plasmid are directly involved in the entry into epithelial cells and invasive phenotypes observed in the pathogenesis of *Shigella* strains.

Entry into epithelial cells and type III secretion system. In order for *Shigella* to enter an epithelial cell, the

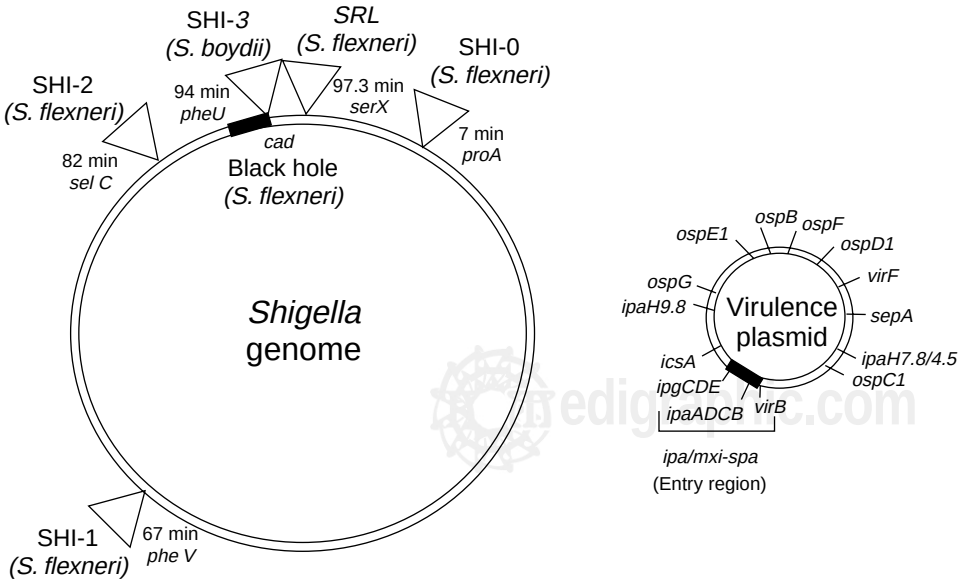

Figure 1. *Location of selected pathogenicity islands and selected plasmid-encoded genes of* Shigella. *The location in the chromosome of the pathogenicity islands (PAIs) is indicated by minutes based on the chromosomal map of* E. coli *K-12, and the genes adjacent or disrupted by the insertion of the PAI. The approximate location of the black hole is indicated with a closed square. The regions implicated in the entry into epithelial cells and genes associated with pathogenesis are indicated in the virulence plasmid.*

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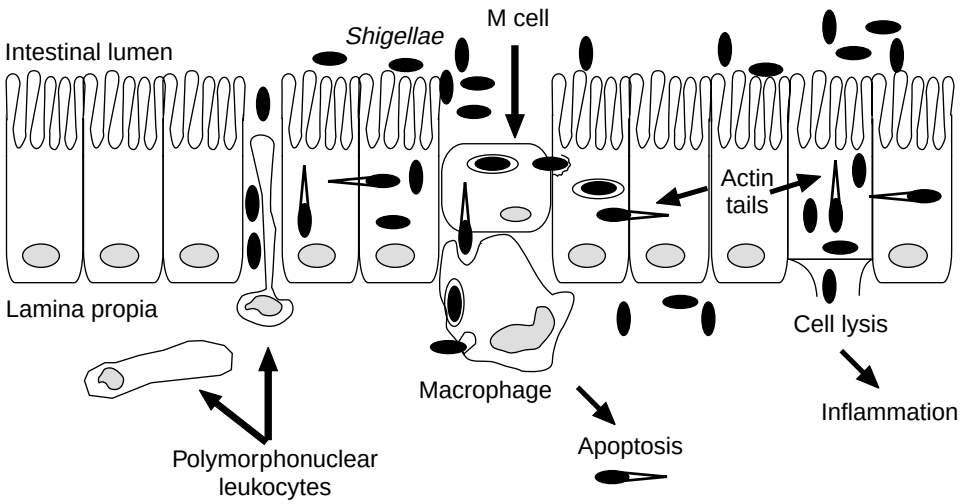

Figure 2. *Entry and dissemination of* Shigella *in epithelial cells. The bacteria do not invade the intestinal epithelium directly. Instead,* Shigella *enter the M cells by inducing their own endocytosis (see text for details).* Shigella *escapes the endocytic vacuole and uses the cell cytoskeleton actin to spread from cell to cell. Then they invade the epithelial cells and macrophages.* Shigella *invasion induces programmed cell death in macrophages which triggers the initial stages of inflammation.* Shigella *multiply in the cytoplasm and cause the subsequent death of the infected epithelial cell, inducing an intense response of inflammatory cells (polymorphonuclear leukocytes), bleeding and abscess formation.*

bacterium must adhere to its target cell. Entry into the host cell appears to be receptor-mediated, but the specific adherence factor has not been defined.[11] The bacterium crosses the epithelium in selected areas corresponding to M cells of the Follicle-Associated Epithelium (FAE) (Fig. 2).[44] More than 25 different genes are essential for *Shigella* entry and most of them are located in two adjacent operons located in an area called the entry region (Fig. 1).[40] One of these operons, the mxi-spa operon encodes a type III secretion apparatus that allows bacterial proteins to be secreted or translocated into the host cell cytosol.[46] There are at least 25 proteins secreted through the Mxi-Spa secretion apparatus.[5] The Ipa proteins (IpaA-D), which are encoded in the adjacent operon, are critical for *Shigella* entry and are among the major products secreted through the type III secretion apparatus.[46] The secretion of Ipa proteins is induced by the epithelial cell and controlled by IpaB and IpaD, which prevent secretion of proteins before the bacterium-host cell contact occurs. The type III secretion apparatus allows the insertion of a pore into the host epithelial cell membrane. This pore contains a complex of the IpaB and IpaC proteins that induce a cascade of cellular signals which result in actin polymerization and internalization of the bacterium. Among the other secreted proteins, IpaA and IpgD are required for the formation of a focal adhesion-like structure at the bacterium-cell membrane contact site,[45] whereas the function of the other secreted proteins is unknown. *Shigella* are then internalized by a process caused by the formation of finger-like extensions of the plasma membrane known as filopods that are quickly remodeled in lamellipods, resulting in an endocytic vacuole that entraps the microorganism.[40] While the type III secretion apparatus are relative complex and the secreted proteins have unique properties, sequence similarities exist between components of the flagella and those of the type III secretion assembly machineries in prokaryotes. These homologies have been used to identify proteins in different systems which may perform similar functions and their characterization will also help to understand the evolutionary strategies of protein trafficking in cells.

Intercellular and intracellular spread. After engulfment, *Shigella* is surrounded by a membrane-bound vacuole within the host cell (Fig. 2). *Shigella* rapidly lyses the surrounding vacuole and is released into the cytosol, where it grows and divides. Once the microorganism has escaped from the vacuole, it quickly becomes coated with filamentous actin and ultimately forms an actin tail at one pole of the bacterium.[4] *Shigella* actin-based motility is mediated exclusively by the IcsA/VirG outer membrane protein.[21] IcsA/VirG recruits host cytosolic components to induce actin nucleation. The neural Wiskoff-Aldrich syndrome protein (N-WASP), vinculin, the Arp 2/3 complex and several other host cytoskeletal proteins interact directly or indirectly with IcsA/VirG to cause actin tail formation, which propels the bacterium through the cytoplasm.[40] When the pathogen reaches the plasma membrane of the cell, it forms a finger-like projection from the surface of an infected cell to the surface of an uninfected cell. The tip of the protrusion penetrates the lateral membrane of the adjacent cell with the subsequent internalization of the microbe. *Shigella* then break out of the double membrane of the vacuole and are released into the cytoplasm, thereby starting a new cycle of infection in a new host cell. In the case of *Shigella* and other bacterial pathogens containing type III secretion systems, many of their secreted proteins interact directly with host cell components to alter host cell signal transduction. Most of the secreted proteins act inside the eukaryotic cytosol into which they are translocated to display some of

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the pathogenicity properties associated with the microorganism.

Following host cell invasion and penetration, degeneration of the epithelium and inflammation of the lamina propia characterize *Shigella* infection. After crossing M cells, *Shigella* are found in an area essentially populated by macrophages and dendritic cells. *Shigella* expressing the invasion phenotype, have another useful role, which is to cause the death of phagocytic macrophages by activating normal programmed cell death (apoptosis).[52] Macrophage apoptosis caused by the bacterial IpaB protein not only permits bacterial survival following the crossing of the FAE but is also central to the early triggering of inflammation.[53] It is likely that this apoptotic process participates in the inflammatory rupture of the epithelial barrier and facilitation of bacterial dissemination.

Invasion of epithelial cells by *Shigella* stimulates the release of proinflammatory cytokines and chemokines, such as IL-8. This event accounts for the recruitment of polymorphonuclear leukocytes (PMN) to the site of infection and their transmigration through the epithelium, which causes major tissue destruction.[31] It has been shown that IL-8 production by invaded epithelial cell results in the containment of *Shigella* infection at the epithelial level, but at the cost of massive epithelial destruction, particularly by PMNs.[42]

Shiga toxin. *Shigella dysenteriae* serotype 1 is unique among *Shigella* species in the production of a potent toxin known as the Shiga toxin (Stx).[24,27] Stx is a bipartite molecule composed of a single enzymatic A subunit and a pentamer of receptor-binding B subunits. The toxin binds to a glycolipid receptor found in target cells, globotriaosylceramide (Gb_3: Galα1-4-Galβ1-4-glucosylceramide), and it is endocytosed preferentially by the clathrin-coated pathway. The A subunit is proteolytically cleaved and reduced, generating an A_1 and an A_2 peptide. The A_1 peptide inhibits mammalian protein synthesis by cleaving the N-glycosidic bond at adenine residue 4324 in the 28S RNA of the 60S host cell ribosome. The importance of this toxin is that infections with Stx-producing bacteria may lead to hemolytic uremic syndrome (HUS), an often-fatal kidney failure condition, particularly in children. In addition to *Shigella dysenteriae* type 1, related Shiga toxins are secreted by enterohemorrhagic *E. coli* (EHEC) strains and other bacteria that are associated with cases of HUS on a worldwide basis.

Pathogenicity islands and "black holes". In general, pathogenicity islands (PAIs) are large and unstable genetic elements, acquired by lateral gene transfer, with different G + C content, often associated with tRNA genes, which contribute to the virulence of bacterial pathogens. The concept of PAIs was developed on the basis of data on genome structure and pathogenicity of enteric organisms, especially pathogenic *E. coli*. However, this concept is now used broadly in other gram-negative and gram-positive pathogens. In this section, the recent progress in the identification and characterization of different PAIs in *Shigella species* will be discussed and the new concept known as "black holes" introduced.

Pathogenicity islands. In *Shigella spp*., five distinct PAIs have been identified (Fig. 1). These include SHI-O, containing the genes for determination of the Lipopolysaccharide (LPS) O-antigen; SHI-1, with genes encoding an enterotoxin and several autotransporter proteins; SHI-2 and SHI-3, encoding aerobactin iron-uptake systems; and SRL, carrying genes for antibiotic resistance and a ferric dicitrate system.

In *S. flexneri* serotype 1, the genes encoding the enzymes causing glycosylation and O acetylation of the O-antigen determinant, are located in the SHI-O PAI.[14] The O-specific polysaccharide domain of LPS is both an essential virulence factor and a serotype-specific protective antigen.[36] Its importance in virulence was initially inferred from the avirulence phenotype of rough *Shigella* in animal models and the inability of these strains to spread from cell to cell in tissue culture monolayers.[11,28] More recently, it has been shown that both the length distribution and the number of LPS O-antigen molecules on cell surface are important for *S. flexneri* invasion and full virulence.[12,47] The SHI-O PAI is derived from an ancestral bacteriophage which has lost its ability to be excised from the bacterial chromosome. A larger part of the phage genome is deleted. The remaining genome is flanked by two typical phage attachment sites which are located in an unusually short distance of 6.5-kb. Besides phage gene remnants and insertion elements, the SHI-O PAI contains three ORFs whose products have sequence identity to proteins encoded by other serotype-converting bacteriophages.

The SHI-1 PAI (previously named "*she* PAI" because it contains the *she* gene) is 46-kb unstable chromosomal locus inserted next to the *pheV* tRNA gene in *S. flexneri* serotype 2a.[1,34] This PAI carries the *set1A* and *set1B* genes encoding the two subunits of ShET1 enterotoxin and the genes encoding the autotransporter proteins SigA, Pic and Sap. In addition, this PAI includes genes commonly found in PAIs of pathogenic *E. coli* strains, and several open reading frames (ORFs) of unknown function. ShET1 production results in fluid accumulation in a rabbit ileal loop model. Pic, is a secreted protease implicated in mucinase activity, serum resistance, and hemagglutination. Finally, SigA, is a cytopathic protease that contributes to intestinal fluid accumulation. The precise contribution in pathogenesis and epidemiology of the *she* PAI is under further investigation.

The siderophore aerobactin is a low molecular weight iron chelator with high affinity for iron frequently found among *Shigella* and EIEC strains that has been associated with increased virulence.[30] The genes involved in synthesis and transport of aerobactin, that are often plasmid-encoded, were recently found within a pathogenicity island in two *S. flexneri* strains from serotypes 2a and 5.[25,49] This island, designated SHI-2 (*Shigella* pathogenicity island 2), is located downstream of *selC*, and occupies 23.8-kb in *S. flexneri* serotype 5 and 30-kb in *S. flexneri* serotype 2a. The G+C content of the island is slightly lower than that of the rest of the *Shigella* chromosome (51%) and varies from 48.5% in serotype 5 to 46% in serotype 2. In addition to the aerobactin transport and synthesis genes, SHI-2 encodes other genes found in the PAI of enterohemorrhagic *E. coli* O157:H7, and genes encoding proteins which confer immunity to colicins I and V as well as ShiA, a protein that allows *Shigella* to attenuate the host inflammatory response.[15]

A fourth island has been characterized in *S. boydii* strain O-1392.[33] SHI-3 is located within a 21-kb region between the *lysU* and *pheU* genes. The island contains the aerobactin operon flanked by genes found in mobile genetic elements. The fact that SHI-3 was found in other locations among closely related species, suggests that this PAI is an unstable and mobile genetic element associated with virulent *Shigella* species, although its role in virulence has not been established.

Finally, the SRL (*Shigella* resistance locus) PAI, which was discovered following the spontaneous loss of multiple antibiotic resistance by *S. flexneri* 2a and that is accompanied by the deletion of a chromosomal region of about 99-kb. Within this PAI, a 66-kb element harboring 59 ORFs, carries the multiple antibiotics resistance determinants.[20] SRL PAI is integrated downstream of the *serX* tRNA gene and contains genes encoding resistance to streptomycin, ampicillin, and tetracycline, and a complete ferric dicitrate uptake system.

Black holes. One common biochemical property of *Shigella* and EIEC strains is their lack of lysine decarboxylase (LDC) activity. The LDC^- phenotype has been proposed to be associated with virulence and Maurelli et al. found that introduction of *cadA* (the gene for LDC) in *Shigella flexneri* 2a causes attenuation in the virulence and inhibition of enterotoxin production.[22] Failure to transduce *cadA* into the chromosome of *Shigella* led to the analysis of the chromosomal region flanking this gene in *S. flexneri* and EIEC. Comparison of the chromosomal region flanking *cadA* in *S. flexneri* and EIEC with *E. coli* K-12 revealed a large chromosomal deletion up to 90-kb in the vicinity of the cadA gene (Fig. 1). This novel concept called "black holes" suggests that deleting genes that are detrimental to the pathogenesis of the organism provides an evolutionary advantage that enables *S. flexneri* and EIEC to enhance virulence.

Other potential virulence factors. Sequencing of the *S. flexneri* 2a genome has given new insights into the genome structure and the presence of further putative PAIs.[16,50] Several chromosomal genes have been implicated in *Shigella* virulence and in strain SF301, at least 64 Shigella-specific islands (S-islands), larger than 1-kb, have been identified. In addition to the SHI-1 and SHI-2 PAIs, the S-islands include five large regions containing seven copies of the *ipaH* gene (*ipaH* islands). *ipaH* island 2 may be associated with iron uptake and two other islands, the sci and SfII islands share similarities to the Salmonella *sciCDEF* operon, involved in the expression of a type 1 fimbriae.

Finally, in *S. dysenteriae* type 1, the utilization of heme as an iron source is associated with the *shu* locus.[51] The genes encoding the *shu* heme iron transport system are contained on a region found in two distantly related lineages of pathogenic *E. coli* and *S. dysenteriae* strains, but not in other *Shigella* species, suggesting acquisition by horizontal gene transfer. In *S. dysenteriae*, however, a mutation in the heme uptake system did not reduce the virulence, suggesting that the presence of additional iron transport systems in *S. dysenteriae* complement the function of the heme uptake system.[35] The role in virulence of the multiple *S. dysenteriae* iron transport systems is currently under investigation.

SHIGELLA AND THE ENVIRONMENT

Regulation of *Shigella* virulence is subject to tight control by several mechanisms involving many proteins and an array of environmental signals (Fig. 3). It is thought that the tight control by environmental factors prevents inappropriate expression of the virulence genes in or outside the host. The expression of *S. flexneri* virulence genes is activated when bacteria are shifted from 30 to 37ºC, in medium of moderate level of osmotic stress (similar to that of physiological saline) and a pH of 7.4.[9] Temperature is a key environmental cue exploited by *S. flexneri* to sense passage into the human gut.[13] The factors controlling the thermal regulation have been identified and include chromosomally encoded H-NS, which represses virulence gene expression at 30ºC, and the virulence plasmid-encoded VirF and VirB proteins, which are transcription factors required for the expression of the invasive phenotype at 37ºC.[9,32]

A second global regulator, the integration host factor (IHF), also contributes to the expression of virulence genes of *S. flexneri*. IHF is required for the positive activation of

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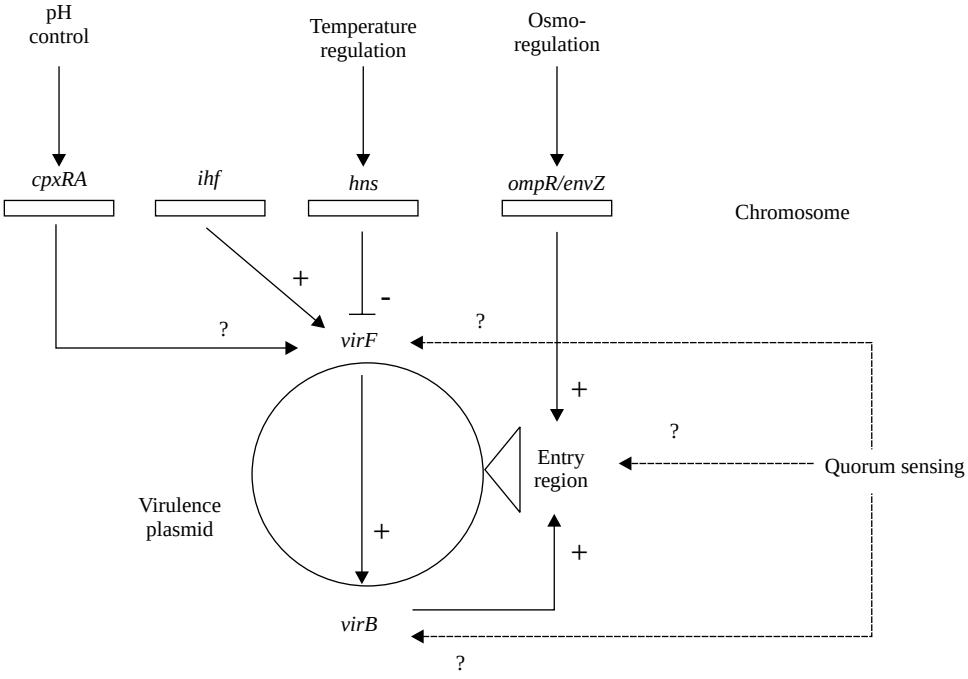

Figure 3. *Schematic representation of the key elements in the* Shigella *virulence gene regulatory cascade. For simplicity, only the entry region is shown as target of regulation in this figure. The environmental factors are shown controlling the expression of chromosomal genes. The proteins encoded by these genes activate or repress the plasmid-encoded virulence genes. The question marks refer to those mechanisms of regulation of virulence genes that have not been totally defined (+, positive regulation; -, negative regulation).*

virF in both exponential and stationary phase cultures and for the activation of *virB* in stationary phase.[9,32]

Transcription of *virF* is subject to pH regulation. In *S. sonnei*, the pH control is dependent on the chromosomally located *cpxRA* genes, a two component regulatory system.[26] The control by *cpxRA* can be overruled by thermal regulation and so far, the direct binding of CpxR to the promoter region of *virF* has not been demonstrated. A second two component regulatory system has been implicated in the control of *S. flexneri* virulence.[3] The osmotic stress sensor protein EnvZ and the response regulator OmpR have been shown to control the expression of plasmid-located virulence genes, but direct evidence of this regulation has not been obtained.

It has been proposed that an additional environmental factor, cell density, is also involved in the control of virulence gene expression in *Shigella*.[2] Bahrani *et al*. showed that the expression and secretion of Ipa proteins is increased with cell density, peaking in stationary-phase cultures. This data suggests that the quorum sensing molecules present in stationary-phase cultures (known as autoinducers), influence *Shigella* virulence gene expression. Recently, Day *et al*. demonstrated that the expression of *ipa*, *mxi* and *spa* invasion operons is maximal in stationary-phase bacteria and that conditioned media derived from stationary-phase cultures enhanced the expression of these loci.[8] This report also showed that expression of *virB*, a gene encoding the transcriptional factor essential for the expression of the invasion loci, peaks in late log phase. Their data showed that the quorum sensing autoinducer molecule active in late log phase does not influence the expression of the invasion operon and is not required for *Shigella* virulence.[8] Further experiments are required to define the role of quorum sensing in the expression of virulence factors in *Shigella* strains.

VACCINE DEVELOPMENT

Although *Shigella* species are important agents of diarrheal epidemics worldwide, causing an estimated 164.7 million cases of dysentery and approximately 1.5 million deaths annually,[19] there is not a current licensed vaccine to prevent shigellosis. There are three factors that have restricted the development of such vaccine, including: a) the inability to raise serum antibodies which confer immunity after parentally injected inactivated whole-cell vaccines; b) the lack of a good animal model; and c) only indirect evidence of the immune mechanism(s) in humans activated after infection exists.[29]

Despite these factors, a new generation of candidate vaccines has shown great promise for the prevention of shigellosis.[6,7,18] The primary role of a *Shigella* vaccine would be to protect against clinical disease. An additional benefit would be to interfere with infection and colonization. The most important *Shigella* strains to be targeted for vaccine development are *S. flexneri 2a*, *S. dysenteriae* type 1, and *S. sonnei*. However, the possible emergence of new serotypes should be emphasized. The emergence of *S. flex-*

neri serotypes 1, 3, 4 and 6 has been observed in several countries as well as a switch towards a predominance of *S. sonnei* in developing countries.[19]

Several approaches for the development of *Shigella* vaccines have been attempted. Two of the most promising approaches in development of candidate vaccines include the use of live attenuated strains and acellular vaccines based on lipopolysaccharide/polysaccharide antigens. Several attenuated vaccine candidates have been derived from *S. flexneri* 2a. Recently, CVD 1207, a derivative from the wild-type *S. flexneri* 2a strain, was tested in a phase 1 clinical human trial. This live attenuated vaccine candidate retains the ability to invade epithelial cells but cannot effectively spread intercellularly after invasion, does not produce enterotoxin, and has limited proliferation *in vivo*. CVD 1207 was shown to be highly attenuated and well tolerated at dosage levels that produce adverse effects with earlier invasive *S. flexneri* 2a vaccine candidates, but showed modest serum antibody responses and was insufficiently immunogenic after a single dose.[18] Another attenuated *S. flexneri* 2a vaccine candidate has been successfully tested in human trials. Strain SC602 carries deletions of the plasmid-borne virulence gene *icsA* and the chromosomal aerobactin locus *iuc*, which result in a double attenuation of its capacity to move intra- and intercellularly, and of its survival within tissues. Double-blind, placebo-controlled studies on the safety and the immunogenicity of this candidate vaccine indicated that SC602 is the first attenuated candidate vaccine that provides protection against shigellosis in a stringent, challenge model.[7]

Shigella polysaccharide-protein conjugates are vaccine candidates consisting of proteins that elicit enhanced antibody responses against the poorly immunogenic polysaccharide.[37] A phase II (double-blind, randomized, vaccine-controlled) study of a conjugate vaccine composed of *Shigella sonnei* O-specific polysaccharide bound to *Pseudomonas aeruginosa* recombinant exoprotein A (*S. sonnei*-rEPA) has been conducted in Israel.[6] The study revealed that the vaccine was safe and highly immunogenic and indicated that one injection of *S. sonnei*-rEPA confers type specific protection against *S. sonnei* shigellosis.[6] Recently, the safety and immunogenicity of *S. sonnei and S. flexneri* 2a conjugates were evaluated in a clinical trial using different carrier proteins.[29] The data suggested that these conjugates were safe and immunogenic and they can be used to induce a more complete and long-lasting immunity against shigellosis.[29]

The essential requirement for the development of a *Shigella* vaccine in the near future is the clear understanding of the host innate immune response to the organism and how this affects the adaptive immune response throughout the disease. An ideal vaccine should be easy to administer, preferably orally, although parental vaccines should not be discarded if all the following requirements are met: well-tolerated; able to induce a high-level, long-term protection after a single dose; multivalent, and easy to manufacture. In addition, is essential that the new strategies for vaccine development take into consideration most of the antigenically distinct serotypes of *Shigella* causing endemic and epidemic infections. It has been estimated by Kotloff *et al*., that if a vaccine had 70% efficacy, up to 91 million cases and more than half million deaths might be prevented each year.[19]

CONCLUDING REMARKS

Recent progress to better understand *Shigella* infection has been possible by establishing the interactions between the bacterial secreted products and their target host cellular components. The completion of the *S. flexneri* genome and virulence plasmid sequences has allowed the analysis and identification of other putative virulence genes implicated in the pathogenesis of the microorganism. These data have become particularly important to determine the contribution of previously uncharacterized chromosomally-encoded proteins in virulence that could become potential targets for vaccine development. More studies are needed to determine, for example, the regulatory networks controlling the expression of the genes in the chromosomal PAIs, and to define the global scheme of signaling pathways at the bacterial or the cellular level that coordinate the expression of the full range of virulence factors implicated in the disease.

The global understanding at the molecular and cellular levels of the pathogenesis of *Shigella* will enable the development of the ideal vaccine that covers most of the major *Shigella* serotypes and effectively provides protection against the disease. This vaccine is obviously needed, since there is a high disease burden mostly in developing countries and a tendency toward resistance to current antibiotics. The major challenge to overcome in the assessment of *Shigella* vaccine efficacy is the absence of previous candidate vaccines that develop full protection.

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