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REVIEW



Shigella-mediated immunosuppression in the human gut: subversion extends from innate to adaptive immune responses

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ABSTRACT

The enteropathogen, *Shigella*, is highly virulent and remarkably adjusted to the intestinal environment of its almost exclusive human host. Key for *Shigella* pathogenicity is the injection of virulence effectors into the host cell via its type three secretion system (T3SS), initiating disease onset and progression by the vast diversity of the secreted T3SS effectors and their respective cellular targets. The multifaceted modulation of host signaling pathways exerted by *Shigella* T3SS effectors, which include the subversion of host innate immune defenses and the promotion of intracellular bacterial survival and dissemination, have been extensively reviewed in the recent past. This review focuses on the human species specificity of *Shigella* by discussing some possible evasion mechanisms towards the human, but not non-human or rodent gut innate defense barrier, leading to the lack of a relevant animal infection model. In addition, subversion mechanisms of the adaptive immune response are highlighted summarizing research advances of the recent years. In particular, the new paradigm of *Shigella* pathogenicity constituted of invasion-independent T3SS effector-mediated targeting of activated, human lymphocytes is discussed. Along with consequences on vaccine development, these findings offer new directions for future research endeavors towards a better understanding of immunity to *Shigella* infection.

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Exploring *Shigella* specificity towards the human innate immune barrier in the gut

The striking selectivity of *Shigella* as a human-restricted pathogen relies on its evasion mechanisms of host innate defenses during gut transit and mucosal invasion, which are highly adapted to the human digestive tract. The significance of this species specificity becomes evident when considering that up-to-now there is no experimental animal model available, which convincingly mimics human infection, *i.e.* the onset of dysentery after oral inoculum.¹ As an exception, naturally acquired *Shigella* infection has been reported for some, non-human primates, including Rhesus macaques.^{2–4} Nevertheless, under laboratory conditions, the Rhesus macaque model requires an inoculum dose of about 10^8 – 10^9 bacteria,⁵ whereas as few as 10^2 – 10^3 suffice to cause disease in humans.^{6,7} While sharing common features, there is increasing evidence suggesting for important species differences in the innate defense systems – in particular between primates and rodents⁸ – which may provide important leads as to understanding susceptibility *versus* resistance to infection observed in these species. For instance, after surviving the acidic environment of the stomach,⁹ *Shigella* reaches the small intestine where it has to resist to degradation by bile salts that commonly vary in composition between humans, non-human primates, and rodents.¹⁰ Interestingly, exposure to human-type bile salts was recently shown to increase *Shigella* virulence *via* the upregulation of survival genes as well as the

induction of biofilm formation, hence facilitating better resistance to the challenging environmental conditions of the gut.¹¹ Upon entering the colon, *Shigella* is faced with the great abundance of resident microbes, the gut microbiota, which naturally varies significantly in their composition across species.^{12,13} How *Shigella* establishes its niche in such an adverse environment is subject of further investigations but of importance is its binding to the mucus layer, which is at its greatest thickness in the colon in comparison to other sections of the digestive tract.^{14,15} Immune properties of the mucus barrier present with marked differences between location and species and are associated with host selectivity. The absence of *Shigella* binding to the small intestinal mucus^{16,17} suggests selectivity of the bacterial/mucin interactions which is possibly related to the specific glycan composition present in the colonic mucosa. *Shigella* binds with high affinity to the heavily glycosylated mucins isolated from the human colon, as opposed to those from the guinea pig (less binding) and rat (no binding).¹⁶ In addition, the inner mucus layer contains antimicrobial peptides (AMPs), which are important mediators in keeping the epithelial lining sterile. Interestingly, AMPs also underlie species-specificity presumably having adapted to the diverse ecological niches inhabited by mammals and the multitude of microbial challenges faced within.¹⁸ Here, the rate of similarities and differences across species are also dependent on the type of AMPs. For instance, divergence exists for α -defensins when comparing mouse and human,¹⁸

while in contrast Rhesus monkey β -defensins and cathelicidins are close homologs to the human molecules.¹⁹ Considering this homology, the specific down-regulation of cathelicidin and human β -defensin 1 expression by *Shigella* during natural and experimental infection which contributes to bacterial survival in the intestine^{20,21} might also happen in Rhesus monkeys, thus contributing to their susceptibility to *Shigella* oral infection.

Another key point in the inter-species variation of innate immune system components includes the sensing of microbial associated molecular patterns (MAMPs) by host pattern recognition receptors (PRRs). PRR activation triggers the transcription of IL-8 (also known as CXCL-8), which serves as a potent chemoattractant responsible for the recruitment of polymorphonuclear neutrophils (PMNs), a hallmark of *Shigella* infection in humans. In contrast, mice, which show resistance to oral infection by *Shigella* fail to elicit PMN recruitment to the intestinal mucosa. However, if infection is performed together with recombinant human IL-8, PMN infiltration and subsequent mucosal inflammation and invasion occur.²² This suggests that murine resistance to *Shigella* infection might be related to a defective pathogen sensing, needed for the initiation of an inflammatory response, which promotes the disruption of the epithelial integrity and further bacterial translocation and epithelial invasion.^{23–25} In other words, one might speculate that suboptimal MAMP/PRR interactions are involved in the lack of inflammation observed in response to *Shigella* infection in the mouse. Of note, in guinea pigs on the contrary, intrarectal administration of a dose of 10^9 *Shigella* induces rectocolitis accompanied by diarrhea similarly to human disease, suggesting for further differences between mice and guinea pigs in triggering gut inflammation.²⁶ Important is the notion of organ-specificity for such a differential bacterial sensing, since mice infected intranasally with *Shigella* develop an acute pulmonary infection characterized by a massive PMN infiltration with extensive tissular destruction.^{27,28}

In summary, inter-species differences in key components of the human innate defense barrier present throughout the gastrointestinal tract including the colon are likely to reflect the discrepancies in the human susceptibility to *Shigella* infection *versus* the reduced sensitivity or resistance observed in non-human primates, guinea pig or mouse infection models.

T3SS-mediated subversion of innate immunity: a brief reminder

The main weaponry of *Shigella* to dampen host defenses, *i.e.* the type three secretion system (T3SS), has been extensively studied and its composition and structure, as well as regulation and mode of action have been recently reviewed.^{29–31} In brief, the T3SS mediates the delivery of virulence effectors from the bacterial cytoplasm into the targeted host cell. It is composed of a type three secretion apparatus (T3SA), the secreted effectors, translocators, chaperones and transcription regulators, which are all encoded on the *Shigella* virulence plasmid.³² The syringe-like cylindrical T3SA spans both bacterial membranes and is composed of the protruding needle complex supported by the basal body.³³ The assembly of the

T3SA is temperature-dependent and only triggered at 37°C,³⁴ the human body temperature, in order to keep the energetic costs related to its expression to a minimum. After its assembly, the T3SA remains inactive, *i.e.* translocators and several effectors remain associated to chaperons in the bacterial cytoplasm, until sensing of the host cell membrane occurs at the needle tip complex.³⁵ This acts as activation signal³⁶ and initiates the secretion of the stored translocators and effectors into the host cell cytoplasm^{37–39} and additionally triggers the transcription of genes encoding for a second set of bacterial effectors. After host cell invasion and vacuolar escape, cytosolic *Shigella* down-regulate the T3SA activity^{40,41} in order to replenish the bacterial effector pool required for its subsequent dissemination into neighboring cells.^{41–43}

It is now well-established that *Shigella* T3SS effectors, similarly to those of other T3SS-carrying enteropathogens, target key cellular pathways of enterocytes and gut resident macrophages, leading to the modulation of important host cell functions.⁴⁴ Numerous *Shigella* T3SS effectors, listed in recent reviews,^{44–46} have been shown to affect signaling pathways involved in host cell actin cytoskeleton dynamics, trafficking, cell viability, and NF- κ B-mediated inflammatory pathways. The system is amazingly efficient. On one hand, one given effector can ensure a diversity of actions towards different cell types while infection proceeds. On the other hand, different effectors can target multiple host proteins in a given pathway. In addition, effectors with antagonistic effects towards a given pathway ensure a timely efficient modulation of host innate immune responses as infection proceeds.^{44–46} In a nutshell, as depicted in Figure 1, upon crossing the colonic mucosa, *Shigella* exerts a multitude of deregulatory mechanisms leading to the initiation of a pro-inflammatory milieu, accompanied by massive immune cell death, including macrophage pyroptosis, as well as facilitating its intra-epithelial survival and spreading along the colonic epithelium.

T3SS-mediated subversion of adaptive immunity: an as of yet underestimated implication

Upon crossing of the epithelial barrier, *Shigella* encounters *lamina propria* cells responsible for the priming of the adaptive immunity, which include dendritic cells (DCs), B and T lymphocytes. In addition, interactions with these cells may also occur in the lymphoid structures that are associated with the intestinal mucosa. In experimental infection, *Shigella* has been shown to reach the mesenteric lymph nodes (P. J. Sansonetti, personal communication), which constitutes the end of its journey since systemic dissemination is usually not observed, except for rare cases of immunocompromized, malnourished infants in endemic settings.⁴⁷

Immunity to *Shigella* is characterized by a humoral response mediated by mucosal sIgAs and systemic IgGs directed against the LPS O-antigen and some other bacterial molecules such as the Invasion plasmid Antigens (Ipa) proteins.^{48–50} Antibodies directed towards the LPS O-antigen are associated with protection in cohort studies.⁵⁰ The composition of the O-antigens differs greatly across strains and serotypes, accounting for the serotype-specificity observed in antibody-mediated protection.^{28,51}

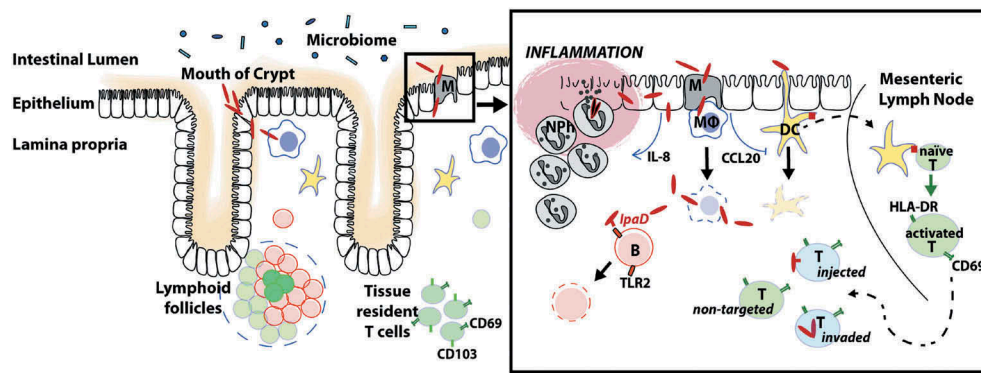


Figure 1. Model of *Shigella* pathogenicity.

Note. After its successful journey through the gut, passing a diversity of luminal innate immune components, *Shigella* eventually reaches the colonic epithelium. The apical pole of colonocytes is commonly regarded as resistant to invasion.¹⁰² Instead, *Shigella* crosses the epithelial barrier via M cells¹⁰³ or via colonocytes located at the mouth of the intestinal crypts, which has recently been described as alternative route of entry.¹⁰⁴ In case of the latter, it was hypothesized that physical forces employed by the gut peristalsis facilitate invasion by entrapping bacteria at the crypt opening and thus enforce entry of the colonocytes in close proximity. Independently of its mode of invasion, *Shigella* subsequently arrives in the subjacent lamina propria, which is densely populated with immune cells involved in the priming of both innate and adaptive immunity. The pro-inflammatory environment induced by *Shigella* in the colon is correlated with a massive cell death as observed in biopsies obtained from infected individuals.^{49,55,105} *Shigella* evades from macrophage-induced killing via the induction of pyroptosis, a caspase-1 dependent cell death associated with the release of the pro-inflammatory cytokines IL-1 β and IL-18.^{106–108} Escaping from the dying macrophage, *Shigella* invades enterocytes from their basolateral side and spreads throughout the epithelium.^{42,109–111} Intracellular bacteria and their T3SS effectors, influence the innate inflammatory response by mobilizing a cascade of immunomodulatory processes in a time-dependent manner (reviewed in Ref⁴⁴). A few hours post-infection, mucosal invasion progresses and is further exacerbated due to successive epithelial damage mediated by the arrival of PMN²⁵ following the chemoattractant, IL-8.^{57,112–114} While PMN-induced tissue damage is thought to facilitate bacterial colonization, invasion and multiplication during the early phases of bacterial infection, *Shigella* is unable to counteract the antimicrobial activity of these professional phagocytes and is ultimately eliminated.^{115,116} *Shigella* impairs adaptive immune cell priming by hampering epithelial CCL20 secretion and the subsequent recruitment of dendritic cells (DCs).²¹ Antigen-presenting DCs migrate to the mesenteric lymph nodes where they prime naïve T cells. These activated T lymphocytes then home back to the lamina propria where they render targetable by *Shigella* via injection-only or invasion mechanisms.⁵⁵ Finally, *Shigella* also inhibits B lymphocyte function by the induction of apoptosis via the T3SS needle-tip effector IpaD.⁶⁶

Shigella antigen-specific B memory cells can be primed and are associated with decreased disease severity in subjects challenged with *Shigella*.⁵² Nevertheless, the establishment of a protective adaptive immunity requires several rounds of infection and is only of short-term duration.^{50,53,54} Interference with the innate and adaptive immune response during primary infection has been associated with the higher susceptibility of children to shigellosis, as compared to adults.^{55,56} Evidence indicates that both the induced pro-inflammatory response and a direct targeting of DCs, B and T lymphocytes might contribute to an inefficient priming of the adaptive immune response upon infection. *Shigella* reprogramming of gene expression in infected enterocytes towards a pro-inflammatory profile, includes the down-regulation of CCL20 production, a chemokine mediating DCs recruitment.⁵⁷ Accordingly, in an experimental model of infection, a reduced recruitment of DCs is observed towards the lamina propria of animals infected with invasive *Shigella*, as compared to those infected with non-invasive *Shigella* harboring a non-functional T3SS.²¹ In addition, DC death occurs within a couple of hours of *Shigella* infection *in vitro* as a response to host cell caspase activation.⁵⁸ Whether recruited and gut resident DCs are susceptible to bacterial killing during the natural infection remains to be established, however the massive cell death observed in patient biopsies are supportive of this notion.⁵⁵ Another link between the host innate and adaptive immune responses is provided by the induction of *Shigella*-specific Th17 cells,⁵⁹ as a result of the pro-inflammatory environment induced upon infection in the mouse pulmonary model of infection.^{60,61} This might reflect what happens in the gut, since Th17 cells are a lineage of T helper cells abundantly present in the gut mucosa and defined by the secretion of IL-17, an important cytokine in the host immune response towards bacterial pathogens.⁶² IL-6, which is significantly induced

upon *Shigella* infection, contributes to the induction of Th17 cells while blocking the development and function of regulatory T cells (T_{reg}).⁶³ Noteworthy, antigen-specific CD8⁺ T lymphocytes fail to be primed during *Shigella* infection *in vivo*,^{59,64} suggesting that specific CD8⁺ T cell-mediated killing of invaded enterocytes is likely to be impaired.

Evidence for the direct targeting of human lymphocytes by *Shigella* has been provided in the last years, predominantly conducted by our laboratory. Historically, *Shigella* is regarded as primarily intracellular bacterium able to invade a large diversity of cells following the injection of T3SS effectors *in vitro*. We recently showed that the injection of T3SS effectors does not inevitably result in cell invasion.⁶⁵ Indeed, using an optimized T3SS injection reporter, we demonstrated that effector injection without subsequent cell invasion, termed the “injection-only” mechanism, is the main route of lymphocyte targeting utilized by *Shigella*.⁶⁵ *In vitro*-activated human peripheral blood B, CD4⁺ T, and CD8⁺ T lymphocytes, as well as switched memory B cells, are primarily targeted by the injection-only mechanism, as are B and T lymphocytes extracted from human colonic tissue, *i.e.* representing the lamina propria-residing cells encountered by *Shigella* in the gut. These findings reveal that through this mechanism, *Shigella* targeting can be extended to a large diversity of host cells, including those refractory to invasion.⁶⁵ Furthermore, B cells were shown to undergo apoptosis after the activation of a TLR2-dependent signaling pathway triggered by the T3SS needle-tip protein, revealing a *Shigella* targeting mechanism that is independent of both invasion and T3SS effector injection.⁶⁶ Consequently, we propose an additional mode of *Shigella* pathogenicity, termed the “kiss-and-run” strategy, describing the ability of *Shigella* to target cells via a T3SS-dependent contact, which results in either effector interaction

with cell-surface receptors or effector delivery into the host cells not followed by cell invasion. These findings highlight an as yet underestimated facet of *Shigella*'s extracellular mode of action.

Another interesting aspect is the observation that only activated human CD4⁺ T lymphocytes are found targeted by *Shigella*, as opposed to non-activated cells. Investigating the underlying molecular mechanism, we demonstrated that non-activated human CD4⁺ T lymphocytes, which are refractory to *Shigella* invasion and effector-injection, become susceptible to targeting upon loading of their plasma membrane with sialylated glycosphingolipids (gangliosides) that are abundantly present in activated cells.⁶⁷ Interactions between the sugar polar part of gangliosides and the polysaccharide moiety of *Shigella* lipopolysaccharide (LPS) promote bacterial binding resulting in the injection of T3SS effectors. While interactions of surface glycans has been previously reported,⁶⁸ these findings suggest that bacterial-host glycan interactions are important for *Shigella* pathogenesis by driving selective interactions with host immune cells. This study also provides a new cell adherence mechanism available to *Shigella* and maybe other pathogens, which are devoid of adhesins and therefore rely on alternative strategies to effectively bind to the host cells. In this context, various molecules promoting *Shigella* binding to enterocytes have been identified including the human enteric α -defensin 5 (HD5),⁶⁹ the virulence T3SS factors OspE1/E2 and IcsA acting as adhesin-like molecule following bile salt exposure.^{70,71} Therefore, beyond its potential importance in driving host specificity, *Shigella* binding to specific cell surface glycans is likely to direct selective targeting of the different T lymphocyte subsets and, more globally, the wide-range of mucosal immune cells with their specific membrane glycosylation patterns.⁷²

The so far only example of the outcomes of direct targeting of human T lymphocytes by *Shigella* is the demonstration of the impairment of activated CD4⁺ T cell dynamics and migration, both *in vitro*⁷³ and *in vivo*,⁷⁴ which is mediated by the T3SS effector IpgD.⁷³ IpgD is a phosphoinositide 4-phosphatase mediating hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂). In T lymphocytes, the IpgD-mediated reduction of the pool of PIP₂ at the plasma membrane leads to dephosphorylation of the ERM proteins and their inability to relocalize at one T cell pole upon chemokine stimulus, likely affecting the formation of the polarized edge required for cell migration.⁷³ The impact on other key T cell functions such as synapse formation and subsequent activation are currently under investigation. An updated model of *Shigella* physiopathology comprising the recent findings on B and T lymphocyte targeting is schematized in Figure 1.

Future perspectives

The molecular mechanisms downstream of *Shigella* lymphocyte targeting and the global role of the injected T3SS effectors on adaptive immunity remain to be deciphered. While the impact of specific immune priming on infection can be accomplished by *in vitro* studies on human *lamina propria* cells, further in-depth investigations of the effector phase and *in vivo* implications are compromised by the previously

mentioned lack of a suitable animal model. The use of the controlled human infection model (CHIM) can present an important alternative, as recently exemplified by studying human immune response to *Salmonella* infection.^{75–77} However, so far CHIM for *Shigella* are restricted to efficacy tests for vaccine candidates but may present an opportunity to be further exploited.^{78,79} Another important point lays in the establishment of adult and infant cohort studies in endemic regions of shigellosis, which especially in the light of now available high throughput analysis tools would certainly provide important insights into human immune responses to natural infection.

In the context of the adaptive immunomodulation and recurrent infections frequently observed in *Shigella* infected individuals, further insights into the underlying mechanisms are needed which include a better understanding of the adaptive immune cell subsets that are targeted as well as the specific T3SS effectors involved and the thereby affected host signaling pathways. Of particular interest here is the analysis of the vast diversity of immune cells present in the human colonic mucosa at a single-cell level. It is important to note that most experiments conducted so far are based on either T cell-lines or peripheral blood lymphocytes, which do not represent the entire spectrum of lymphocyte subsets encountered by *Shigella* in the colonic *lamina propria*. Based on human peripheral blood mononuclear cells (PBMCs), T lymphocytes are classified into CD45RA⁺ naïve and CD45RO⁺ memory T cells, the latter being further subdivided into central memory T cells (T_{CM}) or effector memory T cells (T_{EM}) according to the presence or absence of the lymphoid homing factor CCR7 and CD62L on the cell surface, respectively.⁸⁰ However, more recent publications highlight the existence of a non-circulating, tissue resident memory T cell (T_{RM}) population that constitutively express the integrin CD103, while being devoid of lymphoid homing factors.^{80,81} Analysis of various tissue samples obtained from healthy individuals confirmed the presence of these specific T_{RM} cells in all mucosal sites and in particular in the colon, while being completely absent in circulating PBMCs.⁸¹ Colonic T_{RM} cells are predominantly of the CD8⁺ resident effector type as identified by the constitutive expression of CD69⁺ in the absence of CCR7, and play an important role in the local protective immunity to infection.^{80,82} Of particular interest in the light of our previous findings highlighting *Shigella* preferential targeting of activated T cells, is the “active state” of colonic T_{RM} cells, as judged by the constitutive surface expression of the activation marker CD69.⁸⁰ Therefore, it would be meaningful to investigate *Shigella* interactions with this specific non-circulating T_{RM} subset and to assess its implication on the local immunity. Another interesting point to be considered for further investigation is the observed effect of *Shigella* on the host cell metabolism by rerouting the metabolic output of intestinal epithelial cells after invasion to facilitate its energetic demands.⁸³ Interestingly, metabolic programming is also implicated in the switch from effector to memory T cell phenotype and therefore play an important role in the establishment of long-term immunity.⁸⁴

Implications for vaccine development

The new paradigm of *Shigella* pathogenicity including the novel targeting mechanisms of human lymphocytes has

implications on the different *Shigella* vaccine strategies that are currently under investigation. One strategy relies on the use of orally-administered, live, rationally attenuated vaccine candidates for which T3SS activity toward B and T lymphocytes has been so-far-underestimated. The latest developed candidates of this type⁸⁵ have yet been rationally attenuated on the bases of virulence knowledge (i.e. alteration of metabolic functions essential for in vivo growth, of dissemination capacities in invaded tissues) that preceded recent deciphering of *Shigella* immunosuppressive capacities. In consequence, these candidates are likely to express a functional T3SS and secrete its dedicated effectors, thus retaining their ability to subvert human lymphocyte functions. As a matter of fact, although some of these candidates have shown decent mucosal and systemic immunogenicity in phase I-II studies, including significant protection against dysentery in a challenge study carried out in Western volunteers,⁸⁶ the performance of these candidates were far less promising in phase I-II studies carried out in endemic areas.⁸⁷ These latter studies pointed to the issue that colonization by these candidates was insufficient in level and duration in order to achieve sufficient and reproducible protective mucosal immunogenicity. This poor colonization capacity in endemic zones may be linked to several factors including a colonization barrier effect of the resident intestinal microbiota keeping attenuated candidates at bay and leading to minimal engagement with the epithelial surface and its associated immune system. Hence the joined action of persisting immunosuppressive properties and deficient colonization performance possibly prevents efficacy of current live-attenuated candidates. Trying to address these combined issues and rationally design a new generation of candidates is not impossible but sets substantial challenges and must address two main questions: (i) the mutation, both individually and collectively, of several immunosuppressive effectors and their global assessment, (ii) the yet poorly identified parameters involved in colonization and interaction with the microbiota barrier. All this has to be achieved in the context of a human-specific disease and under consideration of the low predictive value of animal models. Considering the limited resources available for research of neglected infectious diseases such as shigellosis, the time and funding scale to achieve the goal of a tetravalent vaccine on such basis seems rather challenging.

Besides orally administered, live, rationally attenuated vaccine strains, a different approach relies on the development of parenterally delivered subunit vaccines. This strategy is based on the notion that specific bacterial surface polysaccharides, i.e. the O-antigen of membrane lipopolysaccharide (LPS), are the primary targets of the antibody response associated to protection against homologous re-infection.⁵⁰ Among the different vaccine candidates,⁸⁵ only the glycoconjugate vaccine approach incorporating detoxified LPS, i.e. the O-antigen conjugated to a carrier protein, has reached phase III clinical trial testing. In particular, along with safety, protective efficacy was shown with a *S. sonnei* glycoconjugate in adults and children above, but not below three years of age.⁸⁸ Based on these promising results, several candidates are now under development. Bioconjugate vaccines, sourced from gene-edited *E. coli*, have the benefit of an easy and large-scale production and have been shown to be

safe and immunogenic.^{89,90} On the other hand, synthetic molecular glycovaccines also bear advantages for they are rationally designed and fully defined. Such a glycoconjugate vaccine incorporating a synthetic oligosaccharide mimicking the O-antigen of *S. flexneri* 2a has been shown to be safe, well tolerated and immunogenic in healthy adults⁹¹ (Cohen D. et al., in preparation). Along with the GMMA (Generalized Module for Membrane Antigens) approach based on the use of bacterial outer membrane particles,^{92,93} these three strategies are currently supported for further clinical development by the Bill and Melinda Gates Foundation⁹⁴ and the Wellcome Trust.⁹⁵ The aim is to develop a tetravalent vaccine against *S. flexneri* 2a, 3a and 6, and *S. sonnei* to cover, with some expected cross-protection, about 90–95% of the circulating *Shigella* strains. Hopefully, with the current support from funding agencies in advancing clinical studies on the already available tetravalent bioconjugates and GMMA candidates,^{94–97} a tetravalent *Shigella* vaccine might be available at the horizon 2028. Preventing shigellosis remains more than ever a public health priority.^{98–100} Indeed, as recently reviewed,¹⁰¹ there is a growing appreciation for the role of vaccines in confronting the problem of antimicrobial resistance, which includes the alarming emergence of multi-drug resistant *Shigella* strains.⁹⁸

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed

References

1. Marteyn BS. *Shigella* vaccine development : the model matters. JSM Trop Med Res. 2016;1:1011–14.
2. Banish LD, Sims R, Sack D, Montali RJ, Phillips L, Bush M. Prevalence of shigellosis and other enteric pathogens in a zoologic collection of primates. J Am Vet Med Assoc. 1993;203:126–32.
3. Black-Schultz L, Coatney RW, Warnick CL, Swif B. Lack of reactivation of shigellosis in naturally infected enrofloxacin-treated cynomolgus monkeys after exogenous immunosuppression. Lab Anim Sci. 1997;47:602–05.
4. Vickers JH. Infectious diseases of primates related to capture and transportation. Am J Phys Anthropol. 1973;38:511–13. doi:10.1002/(ISSN)1096-8644.
5. Collins TA, Barnoy S, Baqar S, Ranallo RT, Nemelka KW, Venkatesan MM. Safety and colonization of two novel virG(icsA)-based live *Shigella sonnei* vaccine strains in Rhesus macaques (*Macaca mulatta*). Comp Med. 2008;58:88–94.
6. Dupont HL, Levine MM, Hornick RB, Formal SB. Inoculum size in shigellosis and implications for expected mode of transmission. J Infect Dis. 1989;159:1126–28.
7. Islam MS, Hasan MK, Khan SI. Growth and survival of *Shigella flexneri* in common bangladeshi foods under various conditions of time and temperature. Appl Environ Microbiol. 1993;59:652–54.
8. Mestas J, Hughes CCW. Of mice and not men: differences between mouse and human immunology. J Immunol. 2004;175:2731–38. doi:10.4049/jimmunol.172.5.2731.
9. Gorden J, Small PLC. Acid resistance in enteric bacteria. Infect Immun. 1993;61:364–67.
10. Kararli TT. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. Biopharm Drug Dispos. 1995;16:351–80. doi:10.1002/(ISSN)1099-081X.
11. Nickerson KP, Chanin RB, Sistrunk JR, Rasko DA, Fink PJ, Barry EM, Nataro JP, Faherty CS. Analysis of *Shigella flexneri*

- resistance, biofilm formation, and transcriptional profile in response to bile salts. *Infect Immun.* 2017;85:e01067–16. doi:10.1128/IAI.01067-16.
12. Lagkouvardos I, Pukall R, Abt B, Foessel BU, Meier-Kolthoff JP, Kumar N, Bresciani A, Martínez I, Just S, Ziegler C, et al. The Mouse Intestinal Bacterial Collection (mIBC) provides host-specific insight into cultured diversity and functional potential of the gut microbiota. *Nat Microbiol.* 2016;1:16131. doi:10.1038/nmicrobiol.2016.131.
 13. Spor A, Koren O, Ley R. Unravelling the effects of the environment and host genotype on the gut microbiome. *Nat Rev Microbiol.* 2011;9:279–90. doi:10.1038/nrmicro2540.
 14. Mowat AM, Agace WW. Regional specialization within the intestinal immune system. *Nat Rev Immunol.* 2014;14:667–85. doi:10.1038/nri3738.
 15. McGuckin MA, Lindén SK, Sutton P, Florin TH. Mucin dynamics and enteric pathogens. *Nat Rev Microbiol.* 2011;9:265–78. doi:10.1038/nrmicro2538.
 16. Sai Sudha P, Devaraj H, Devaraj N. Adherence of *Shigella dysenteriae* 1 to human colonic mucin. *Curr Microbiol.* 2001;42:381–87. doi:10.1007/s002840010234.
 17. Prakash R, Raja S, Devaraj H, Devaraj SN. Up-regulation of MUC2 and IL-1 β expression in human colonic epithelial cells by *Shigella* and its interaction with mucins. *PLoS One.* 2011;6:e27046. doi:10.1371/journal.pone.0027046.
 18. Patil A, Hughes AL, Zhang G. Rapid evolution and diversification of mammalian α -defensins as revealed by comparative analysis of rodent and primate genes. *Physiol Genomics.* 2004;20:1–11. doi:10.1152/physiolgenomics.00150.2004.
 19. Bals R, Lang C, Weiner DJ, Vogelmeier C, Welsch U, Wilson JM. Rhesus monkey (*Macaca mulatta*) mucosal antimicrobial peptides are close homologues of human molecules. *Clin Diagnostic Lab Immunol.* 2001;8:370–75.
 20. Islam D, Bandholtz L, Nilsson J, Wigzell H, Christensson B, Agerberth B, Gudmundsson GH. Downregulation of bactericidal peptides in enteric infections: A novel immune escape mechanism with bacterial DNA as a potential regulator. *Nat Med.* 2001;7:180–85. doi:10.1038/84627.
 21. Sperandio B, Regnault B, Guo J, Zhang Z, Stanley SL, Sansonetti PJ, Pédrón T. Virulent *Shigella flexneri* subverts the host innate immune response through manipulation of antimicrobial peptide gene expression. *J Exp Med.* 2008;205:1121–32. doi:10.1084/jem.20071698.
 22. Singer M, Sansonetti PJ. IL-8 is a key chemokine regulating neutrophil recruitment in a new mouse model of *Shigella*-induced colitis. *J Immunol.* 2004;173:4197–206. doi:10.4049/jimmunol.173.6.4197.
 23. Perdomo JJ, Gounon P, Sansonetti PJ. Polymorphonuclear leukocyte transmigration promotes invasion of colonic epithelial monolayer by *Shigella flexneri*. *J Clin Invest.* 1994;93:633–43. doi:10.1172/JCI117015.
 24. Sansonetti PJ, Arondel J, Cavaillon JM, Huerre M. Role of interleukin-1 in the pathogenesis of experimental shigellosis. *J Clin Invest.* 1995;96:884–92. doi:10.1172/JCI118135.
 25. Sansonetti PJ, Arondel J, Huerre M, Harada A, Matsushima K. Interleukin-8 controls bacterial transepithelial translocation at the cost of epithelial destruction in experimental shigellosis. *Infect Immun.* 1999;67:1471–80.
 26. Shim D-H, Suzuki T, Chang S-Y, Park S-M, Sansonetti PJ, Sasakawa C, Kweon M-N. New animal model of shigellosis in the guinea pig: its usefulness for protective efficacy studies. *J Immunol.* 2007;178:2476–82. doi:10.4049/jimmunol.178.4.2476.
 27. Voino-Yasenetsky MV, Voino-Yasenetskaya MK. Experimental pneumonia caused by bacteria of the *Shigella* group. *Acta Morphol Acad Sci Hung.* 1962;11:439–54.
 28. Phalipon A, Kaufmann M, Michetti P, Cavaillon J-M, Huerre M, Sansonetti PJ, Kraehenbuhl J-P. Monoclonal immunoglobulin a antibody directed against serotype-specific epitope of *Shigella flexneri* lipopolysaccharide protects against murine experimental shigellosis. *J Exp Med.* 1995;182:769–78. doi:10.1084/jem.182.3.769.
 29. Galán JE, Waksman G. Protein-injection machines in bacteria. *Cell.* 2018;172:P1306–1318. doi:10.1016/j.cell.2018.01.034.
 30. de Nisco NJ, Rivera-Cancel G, Orth K. The biochemistry of sensing: enteric pathogens regulate type III secretion in response to environmental and host cues. *MBio.* 2018;9:e02122–17. doi:10.1128/mBio.02122-17.
 31. Gunasinghe SD, Webb CT, Elgass KD, Hay ID, Lithgow T. Super-resolution imaging of protein secretion systems and the cell surface of gram-negative bacteria. *Front Cell Infect Microbiol.* 2017;7:220. doi:10.3389/fcimb.2017.00517.
 32. Sansonetti PJ, Kopecko DJ, Formal SB. Involvement of a plasmid in the invasive ability of *Shigella flexneri*. *Infect Immun.* 1982;35:852–60.
 33. Galán JE, Lara-Tejero M, Marlovits TC, Wagner S. Bacterial type III secretion systems: specialized nanomachines for protein delivery into target cells. *Annu Rev Microbiol.* 2014;68:415–38. doi:10.1146/annurev-micro-092412-155725.
 34. Maurelli AT, Blackmon B, Curtis R III. Temperature-Dependent Expression of virulence genes in *Shigella* species. *Infect Immun.* 1984;43:195–201.
 35. Veenendaal AKJ, Hodgkinson JL, Schwarzer L, Stabat D, Zenk SF, Blocker AJ. The type III secretion system needle tip complex mediates host cell sensing and translocon insertion. *Mol Microbiol.* 2007;63:1719–30. doi:10.1111/j.1365-2958.2006.05509.x.
 36. Ménard R, Sansonetti PJ, Parsot C. The secretion of the *Shigella flexneri* Ipa invasins is activated by epithelial cells and controlled by IpaB and IpaD. *Embo J.* 1994;13:5293–302. doi:10.1002/j.1460-2075.1994.tb06863.x.
 37. Enninga J, Mounier J, Sansonetti P, Tran Van Nhieu G. Secretion of type III effectors into host cells in real time. *Nat Methods.* 2005;2:959–65. doi:10.1038/nmeth738.
 38. Marlovits TC, Kubori T, Sukhan A, Galán JE, Unger VM. Structural insights into the assembly of the type III secretion needle complex. *Science.* 2004;306:1040–42. doi:10.1126/science.1102610.
 39. Martinez-Argudo I, Blocker AJ. The *Shigella* T3SS needle transmits a signal for MxiC release, which controls secretion of effectors. *Mol Microbiol.* 2010;78:1365–78. doi:10.1111/j.1365-2958.2010.07413.x.
 40. Demers B, Sansonetti PJ, Parsot C. Induction of type III secretion in *Shigella flexneri* is associated with differential control of transcription of genes encoding secreted proteins. *Embo J.* 1998;17:2894–903. doi:10.1093/emboj/17.11.3016.
 41. Campbell-Valois FX, Schnupf P, Nigro G, Sachse M, Sansonetti PJ, Parsot C. A fluorescent reporter reveals on/off regulation of the *Shigella* type III secretion apparatus during entry and cell-to-cell spread. *Cell Host Microbe.* 2014;15:177–89. doi:10.1016/j.chom.2014.01.005.
 42. Page AL, Ohayon H, Sansonetti PJ, Parsot C. The secreted IpaB and IpaC invasins and their cytoplasmic chaperone IpgC are required for intercellular dissemination of *Shigella flexneri*. *Cell Microbiol.* 1999;1:183–93.
 43. Schuch R, Sandlin RC, Maurelli AT. 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.* 1999;34:675–89. doi:10.1046/j.1365-2958.1999.01627.x.
 44. Pinaud L, Sansonetti PJ, Phalipon A. Host cell targeting by enteropathogenic bacteria T3SS effectors. *Trends Microbiol.* 2018;26:266–83. doi:10.1016/j.tim.2018.01.010.
 45. de Jong MF, Alto NM. Cooperative immune suppression by *Escherichia coli* and *Shigella* effector proteins. *Infect Immun.* 2018;86:e00560–17. doi:10.1128/IAI.00560-17.
 46. Ashida H, Mimuro H, Sasakawa C. *Shigella* manipulates host immune responses by delivering effector proteins with specific roles. *Front Immunol.* 2015;6:219. doi:10.3389/fimmu.2015.00219.
 47. Jain S, Sharma M, Gupta R, Shree N, Kumar M. Multidrug resistant *Shigella flexneri*: A rare case of septicemia in an infant. *J Clin Diagnostic Res.* 2014;8:DD03–DD04.
 48. Islam D, Wretling B, Hammarström L, Christensson B, Lindberg AA. Semiquantitative estimation of *Shigella*

- antigen-specific antibodies: correlation with disease severity during shigellosis. *Apmis*. 1996;104:563–74. doi:10.1111/j.1699-0463.1996.tb04912.x.
49. Islam D, Veress B, Bardhan PK, Lindberg AA, Christensson B. In situ characterization of inflammatory responses in the rectal mucosae of patients with shigellosis. In situ characterization of inflammatory responses in the rectal mucosae of patients with shigellosis. *Infect Immun*. 1997;65:739–49.
 50. Barry EM, Pasetti MF, Sztein MB, Fasano A, Kotloff KL, Levine MM. Progress and pitfalls in *Shigella* vaccine research. *Nat Rev Gastroenterol Hepatol*. 2013;10:245–55. doi:10.1038/nrgastro.2013.12.
 51. Formal SB, Oaks EV, Olsen RE, Wingfield-Eggleston M, Snoy PJ, Cogan JP. Effect of prior infection with virulent *Shigella flexneri* 2a on the resistance of monkeys to subsequent infection with *Shigella sonnei*. *J Infect Dis*. 1991;164:533–37. doi:10.1093/infdis/164.3.533.
 52. Wahid R, Simon JK, Picking WL, Kotloff KL, Levine MM, Sztein MB. *Shigella* antigen-specific B memory cells are associated with decreased disease severity in subjects challenged with wild-type *Shigella flexneri* 2a. *Clin Immunol*. 2013;148:35–43. doi:10.1016/j.clim.2013.03.009.
 53. Dupont HL, Hornick RB, Snyder MJ, Libonati JP, Formal SB, Gangarosa EJ. Immunity in shigellosis. II. Protection induced by oral live vaccine or primary infection. *J Infect Dis*. 1972;125:12–16. doi:10.1093/infdis/125.1.12.
 54. Raqib R, Qadri F, Sarker P, Mia SMS, Sansonetti PJ, Albert MJ, Andersson J. Delayed and reduced adaptive humoral immune responses in children with shigellosis compared with in adults. *Scand J Immunol*. 2002;55:414–23. doi:10.1046/j.1365-3083.2002.01079.x.
 55. Raqib R, Ekberg C, Sharkar P, Bardhan PK, Zychlinsky A, Sansonetti PJ, Andersson J. Apoptosis in acute shigellosis is associated with increased production of fas/fas ligand, perforin, caspase-1, and caspase-3 but reduced production of Bcl-2 and interleukin-2. *Infect Immun*. 2002;70:3199–207. doi:10.1128/IAI.70.6.3199-3207.2002.
 56. Raqib R, Mia SMS, Qadri F, Alam TI, Alam NH, Chowdhury AK, Mathan MM, Andersson J. Innate immune responses in children and adults with shigellosis. *Infect Immun*. 2000;68:3620–29. doi:10.1128/IAI.68.6.3620-3629.2000.
 57. Pédrón T, Thibault C, Sansonetti PJ. The invasive phenotype of *Shigella flexneri* directs a distinct gene expression pattern in the human intestinal epithelial cell line Caco-2. *J Biol Chem*. 2003;278:33878–86. doi:10.1074/jbc.M303749200.
 58. Edgeworth JD, Spencer J, Phalipon A, Griffin GE, Sansonetti PJ. Cytotoxicity and interleukin-1 β processing following *Shigella flexneri* infection of human monocyte-derived dendritic cells. *Eur J Immunol*. 2002;32:1464–71. doi:10.1002/1521-4141(200205)32:5<1464::AID-IMMU1464>3.0.CO;2-G.
 59. Sellge G, Magalhaes JG, Konradt C, Fritz JH, Salgado-Pabon W, Eberl G, Bandeira A, Di Santo JP, Sansonetti PJ, Phalipon A. Th17 cells are the dominant T cell subtype primed by *Shigella flexneri* mediating protective immunity. *J Immunol*. 2010;184:2076–85. doi:10.4049/jimmunol.0902016.
 60. Raqib R, Lindberg AA, Wretling B, Bardhan PK, Andersson U, Andersson J. Persistence of local cytokine production in shigellosis in acute and convalescent stages. *Infect Immun*. 1995;63:289–96.
 61. Raqib R, Lindberg AA, Bjork L, Bardhan PK, Wretling B, Andersson U, Andersson J. Down-regulation of gamma interferon, tumor necrosis factor type I, interleukin 1 (IL-1) type I, IL-3, IL-4, and transforming growth factor beta type I receptors at the local site during the acute phase of *Shigella* infection. *Infect Immun*. 1995;63:3079–87.
 62. Curtis MM, Way SS. Interleukin-17 in host defence against bacterial, mycobacterial and fungal pathogens. *Immunology*. 2009;126:177–85. doi:10.1111/imm.2008.126.issue-2.
 63. Zhou L, Chong MW, Littman DR. Plasticity of CD4+ T cell lineage differentiation. *Immunity*. 2009;30:646–55. doi:10.1016/j.immuni.2009.05.001.
 64. Jehl SP, Doling AM, Giddings KS, Phalipon A, Sansonetti PJ, Goldberg MB, Starnbach MN. Antigen-specific CD8+ T cells fail to respond to *Shigella flexneri*. *Infect Immun*. 2011;79:2021–30. doi:10.1128/IAI.00040-11.
 65. Pinaud L, Samassa F, Porat Z, Ferrari ML, Belotserkovsky I, Parsot C, Sansonetti PJ, Campbell-Valois F-X, Phalipon A. Injection of T3SS effectors not resulting in invasion is the main targeting mechanism of *Shigella* toward human lymphocytes. *Proc Natl Acad Sci*. 2017;114:9954–59. doi:10.1073/pnas.1707098114.
 66. Nothelfer K, Arena ET, Pinaud L, Neunlist M, Mozeleski B, Belotserkovsky I, Parsot C, Dinadayala P, Burger-Kentischer A, Raqib R, et al. B lymphocytes undergo TLR2-dependent apoptosis upon *Shigella* infection. *J Exp Med*. 2014;211:1215–29. doi:10.1084/jem.20130914.
 67. Belotserkovsky I, Brunner K, Pinaud L, Rouvinski A, Dellarole M, Baron B, Dubey G, Samassa F, Parsot C, Sansonetti P, et al. Glycan-glycan interaction determines *Shigella* tropism toward human T lymphocytes. *MBio*. 2018;9:e02309–17. doi:10.1128/mBio.02309-17.
 68. Shanker S, Hu L, Ramani S, Atmar RL, Estes MK, Venkataram Prasad B V. Structural features of glycan recognition among viral pathogens. *Curr Opin Struct Biol*. 2017;44:211–18. doi:10.1016/j.sbi.2017.05.007.
 69. Xu D, Liao C, Zhang B, Tolbert WD, He W, Dai Z, Zhang W, Yuan W, Pazgier M, Liu J, et al. Human enteric α -defensin 5 promotes *Shigella* infection by enhancing bacterial adhesion and invasion. *Immunity*. 2018;48:1233–43. doi:10.1016/j.immuni.2018.04.005.
 70. Faherty CS, Redman JC, Rasko DA, Barry EM, Nataro JP. *Shigella flexneri* effectors OspE1 and OspE2 mediate induced adherence to the colonic epithelium following bile salts exposure. *Mol Microbiol*. 2012;85:107–21. doi:10.1111/j.1365-2958.2012.08092.x.
 71. Brotcke Zumsteg A, Goosmann C, Brinkmann V, Morona R, Zychlinsky A. IcsA is a *Shigella flexneri* adhesion regulated by the type III secretion system and required for pathogenesis. *Cell Host Microbe*. 2014;15:435–45. doi:10.1016/j.chom.2014.03.001.
 72. Baum LG, Crocker PR. Glycoimmunology: ignore at your peril!. *Immunol Rev*. 2009;230:5–8.
 73. Konradt C, Frigimelica E, Nothelfer K, Puhar A, Salgado-Pabon W, Bartolo V, Scott-Algara D, Rodrigues CD, Sansonetti PJ, Phalipon A. The *Shigella flexneri* type three secretion system effector IpgD inhibits T cell migration by manipulating host phosphoinositide metabolism. *Cell Host Microbe*. 2011;9:263–72. doi:10.1016/j.chom.2011.03.010.
 74. Salgado-Pabon W, Celli S, Arena ET, Nothelfer K, Roux P, Sellge G, Frigimelica E, Bouso P, Sansonetti PJ, Phalipon A. *Shigella* impairs T lymphocyte dynamics in vivo. *Proc Natl Acad Sci*. 2013;110:4458–63. doi:10.1073/pnas.1300981110.
 75. Toapanta FR, Bernal PJ, Fresnay S, Magder LS, Darton TC, Jones C, Waddington CS, Blohmke CJ, Angus B, Levine MM, et al. Oral challenge with wild-type *Salmonella typhi* induces distinct changes in B cell subsets in individuals who develop typhoid disease. *PLoS Negl Trop Dis*. 2016;10:e0004766. doi:10.1371/journal.pntd.0004766.
 76. Fresnay S, McArthur MA, Magder L, Darton TC, Jones C, Waddington CS, Blohmke CJ, Angus B, Levine MM, Pollard AJ, et al. *Salmonella typhi*-specific multifunctional CD8+ T cells play a dominant role in protection from typhoid fever in humans. *J Transl Med*. 2016;14:62. doi:10.1186/s12967-016-0867-z.
 77. Juel HB, Thomaidis-Brears HB, Darton TC, Jones C, Jones E, Shrestha S, Sie R, Eustace A, Galal U, Kurupati P, et al. *Salmonella typhi* bactericidal antibodies reduce disease severity but do not protect against typhoid fever in a controlled human infection model. *Front Immunol*. 2018;8:1916. doi:10.3389/fimmu.2017.01960.
 78. Porter CK, Louis Bourgeois A, Frenc RW, Prouty M, Maier N, Riddle MS. Developing and utilizing controlled human models of infection. *Vaccine*. 2017;35:6813–18. doi:10.1016/j.vaccine.2017.05.068.
 79. Porter CK, Lynen A, Riddle MS, Talaat K, Sack D, Gutiérrez RL, McKenzie R, DeNearing B, Feijoo B, Kaminski RW, et al. Clinical endpoints in the controlled human challenge model for *Shigella*: A call for standardization and the development of a disease severity score. *PLoS One*. 2018;13:e0194325. doi:10.1371/journal.pone.0194325.

80. Gebhardt T, Mackay LK. Local immunity by tissue-resident CD8+ memory T cells. *Front Immunol.* 2012;3:340. doi:10.3389/fimmu.2012.00198.
81. Sathaliyawala T, Kubota M, Yudanin N, Turner D, Camp P, Thome JJC, Bickham KL, Lerner H, Goldstein M, Sykes M, et al. Distribution and compartmentalization of human circulating and tissue-resident memory T cell subsets. *Immunity.* 2013;38:187–97. doi:10.1016/j.immuni.2012.09.020.
82. Bergsbaken T, Bevan MJ, Fink PJ. Local inflammatory cues regulate differentiation and persistence of CD8+ tissue-resident memory T cells. *Cell Rep.* 2017;19:114–24. doi:10.1016/j.celrep.2017.03.031.
83. Kentner D, Martano G, Callon M, Chiquet P, Brodmann M, Burton O. *Shigella* reroutes host cell central metabolism to obtain high-flux nutrient supply for vigorous intracellular growth. *Proc Natl Acad Sci USA.* 2014;111:9929–34.
84. Buck MDD, O'Sullivan D, Klein Geltink RII, Curtis JDD, Chang CH, Sanin DEE, Qiu J, Kretz O, Braas D, van der Windt GJJW, et al. Mitochondrial dynamics controls T cell fate through metabolic programming. *Cell.* 2016;166:63–76. doi:10.1016/j.cell.2016.05.035.
85. Mani S, Wierzbica T, Walker RI. Status of vaccine research and development for *Shigella*. *Vaccine.* 2016;34:2887–94. doi:10.1016/j.vaccine.2016.10.045.
86. Coster TS, Hoge CW, VanDeVerg LL, Hartman AB, Oaks EV, Venkatesan MM, Cohen D, Robin G, Fontaine-Thompson A, Sansonetti PJ, et al. Vaccination against shigellosis with attenuated *Shigella flexneri* 2a strain SC602. *Infect Immun.* 1999;67:3437–43.
87. Rahman KM, Arifeen SE, Zaman K, Rahman M, Raqib R, Yunus M, Begum N, Islam MS, Sohail BM, Rahman M, et al. Safety, dose, immunogenicity, and transmissibility of an oral live attenuated *Shigella flexneri* 2a vaccine candidate (SC602) among healthy adults and school children in Matlab, Bangladesh. *Vaccine.* 2011;29:1347–54. doi:10.1016/j.vaccine.2010.10.035.
88. Passwell JH, Ashkenzi S, Banet-Levi Y, Ramon-Saraf R, Farzam N, Lerner-Geva L, Even-Nir H, Yerushalmi B, Chu C, Shiloach J, et al. Age-related efficacy of *Shigella* O-specific polysaccharide conjugates in 1–4-year-old Israeli children. *Vaccine.* 2010;28:2231–35. doi:10.1016/j.vaccine.2009.12.050.
89. Riddle MS, Kaminski RW, Di Paolo C, Porter CK, Gutierrez RL, Clarkson KA, Weerts HE, Duplessis C, Castellano A, Alaimo C, et al. Safety and immunogenicity of a candidate bioconjugate vaccine against *Shigella flexneri* 2a administered to healthy adults: A single-blind, randomized phase I study. *Clin Vaccine Immunol.* 2016;23:908–17. doi:10.1128/CVI.00224-16.
90. Hatz CFR, Bally B, Rohrer S, Steffen R, Kramme S, Siegrist CA, Wacker M, Alaimo C, Fonck VG. Safety and immunogenicity of a candidate bioconjugate vaccine against *Shigella dysenteriae* type 1 administered to healthy adults: A single blind, partially randomized Phase I study. *Vaccine.* 2015;33:4594–601. doi:10.1016/j.vaccine.2015.06.102.
91. Phalipon A, Tanguy M, Grandjean C, Guerreiro C, Belot F, Cohen D, Sansonetti PJ, Mulard LA. A synthetic carbohydrate-protein conjugate vaccine candidate against *Shigella flexneri* 2a infection. *J Immunol.* 2009;182:2241–47. doi:10.4049/jimmunol.0802775.
92. Launay O, Lewis DJM, Anemona A, Loulergue P, Leahy J, Sciré AS, Maugard A, Marchetti E, Zancan S, Huo Z, et al. Safety profile and immunologic responses of a novel vaccine against *Shigella sonnei* administered intramuscularly, intradermally and intranasally: results from two parallel randomized phase I clinical studies in healthy adult volunteers in Europe. *EBio Med.* 2017;22:164–72. doi:10.1016/j.ebiom.2017.07.013.
93. Gerke C, Colucci AM, Giannelli C, Sanzone S, Vitali CG, Sollai L, Rossi O, Martin LB, Auerbach J, Di Cioccio V, et al. Production of a *Shigella sonnei* vaccine based on generalized modules for membrane antigens (GMMA), 1790GAHB. *PLoS One.* 2015;10:e0134478. doi:10.1371/journal.pone.0134478.
94. Bill & Melinda Gates Foundation. Enteric and diarrheal diseases strategy overview [Internet]; [accessed 2018 Dec 10]. <https://www.gatesfoundation.org/What-We-Do/Global-Health/Enteric-and-Diarrheal-Diseases>.
95. Global Biodefences. Wellcome trust to finance development of *Shigella* vaccine [Internet]. [accessed 2019 Feb 11]. <https://globalbiodefense.com/2013/07/30/wellcome-trust-to-finance-development-of-shigella-vaccine/>.
96. The European & Developing Countries Clinical Trials Partnership (EDCTP). Vaccines for diarrhoeal diseases or lower respiratory tract infections: Research and Innovation Action (RIA) call budget [Internet]; [accessed 2018 Dec 10]. <http://www.edctp.org/call/vaccines-for-diarrhoeal-diseases-or-lower-respiratory-tract-infections/>.
97. PATH. Diarrheal disease [Internet]; [accessed 2018 Dec 10]. <https://www.path.org/diarrheal-disease/>.
98. Kotloff KL, Riddle MS, Platts-Mills JA, Pavlinac P, Zaidi AKM. Shigellosis. *Lancet.* 2017;17:33296–98. doi:10.1016/S1473-3099(17)30242-6.
99. Williams PCM, Berkley JA. Paediatrics and International Child Health Guidelines for the treatment of dysentery (shigellosis): a systematic review of the evidence. *Paediatr Int Child Health.* 2018;9047:1–16.
100. Chen WH, Kotloff L, Riddle MS, Kaminski RW, Di Paolo C, Porter CK, Gutierrez RL, Immu CV. *Shigella* vaccine development: finding the path of least resistance. *Clin Vaccine Immunol.* 2016;23:904–07.
101. Lipsitch M, Siber GR. How can vaccines contribute to solving the antimicrobial resistance problem? *MBio.* 2016;7:e00428–16. doi:10.1128/mBio.00428-16.
102. Mounier J, Vasselon T, Hellio R, Lesourd M, Sansonetti PJ. *Shigella flexneri* enters human colonic Caco-2 epithelial cells through the basolateral pole. *Infect Immun.* 1992;60:237–48.
103. Sansonetti PJ, Phalipon A. M cells as ports of entry for enteroinvasive pathogens: mechanisms of interaction, consequences for the disease process. *Semin Immunol.* 1999;11:193–203. doi:10.1006/smim.1999.0175.
104. Arena ET, Campbell-Valois F-X, Tinevez J-Y, Nigro G, Sachse M, Moya-Nilges M, Nothelfer K, Marteyn B, Shorte SL, Sansonetti PJ. Bioimage analysis of *Shigella* infection reveals targeting of colonic crypts. *Proc Natl Acad Sci.* 2015;112:E3282–90. doi:10.1073/pnas.1509091112.
105. Mathan MM, Mathan VI. Morphology of rectal mucosa of patients with shigellosis. *Rev Infect Dis.* 1991;13:314–18.
106. Hilbi H, Moss JE, Hersh D, Chen Y, Arondel J, Banerjee S, Flavell RA, Yuan J, Sansonetti PJ, Zychlinsky A. *Shigella*-induced apoptosis is dependent on caspase-1 which binds to IpaB. *J Biol Chem.* 1998;273:32895–900. doi:10.1074/jbc.273.49.32895.
107. Zychlinsky A, Prevost MC, Sansonetti PJ. *Shigella flexneri* induces apoptosis in infected macrophages. *Nature.* 1992;358:167–69. doi:10.1038/358167a0.
108. Arizmendi O, Picking WD, Picking WL. Macrophage apoptosis triggered by IpaD from *Shigella flexneri*. *Infect Immun.* 2016;84:1857–65. doi:10.1128/IAI.01483-15.
109. Sansonetti PJ, Ryter A, Clerc P, Maurelli AT, Mounier J. Multiplication of *Shigella flexneri* within HeLa cells: lysis of the phagocytic vacuole and plasmid-mediated contact hemolysis. *Infect Immun.* 1986;51:461–69.
110. Goldberg MB, Barzu O, Parsot C, Sansonetti PJ, De Biochimie U. Unipolar localization and ATPase activity of IcsA, a *Shigella flexneri* protein involved in intracellular movement. *J Bacteriol.* 1993;175:2189–96.
111. Bernardini ML, Mounier J, Dwhauteville HNE, Coquis-Rondont M, Sansonetti PJ. 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.* 1989;86:3867–71.
112. Girardin SE, Boneca IG, Viala J, Chamaillard M, Labigne A, Thomas G, Philpott DJ, Sansonetti PJ. Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. *J Biol Chem.* 2003;278:8869–72. doi:10.1074/jbc.C200651200.
113. Philpott DJ, Yamaoka S, Israel A, Sansonetti PJ. 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.* **2000**;165:903–14. doi:[10.4049/jimmunol.165.2.903](https://doi.org/10.4049/jimmunol.165.2.903).
114. Kasper CA, Sorg I, Schmutz C, Tschon T, Wischnewski H, Kim ML, Arrieumerlou C. Cell-cell propagation of NF- κ B transcription factor and MAP kinase activation amplifies innate immunity against bacterial infection. *Immunity.* **2010**;33:804–16. doi:[10.1016/j.immuni.2010.10.015](https://doi.org/10.1016/j.immuni.2010.10.015).
115. Lowell GH, MacDermott RP, Summers PL, Reeder AA, Bertovich MJ, Formal SB. Antibody-dependent cell-mediated antibacterial activity: K lymphocytes, monocytes, and granulocytes are effective against *Shigella*. *J Immunol.* **1980**;125:2778–84.
116. Weinrauch Y, Drujan D, Shapiro SD, Weiss J, Zychlinsky A. Neutrophil elastase targets virulence factors of enterobacteria. *Nature.* **2002**;417:91–94. doi:[10.1038/417091a](https://doi.org/10.1038/417091a).