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Integrated frontiers in mucosal defense: Evaluating next-generation vaccination strategies against *Shigella* infection

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ABSTRACT

Shigella invades the colonic epithelium through mucosal surfaces, where low infectious doses enable rapid intracellular spread and severe inflammatory damage before systemic immunity becomes effective. Consequently, effective vaccination requires localized mucosal immune responses capable of preventing epithelial adhesion, invasion, and bacterial dissemination. Current mucosal vaccine strategies target the induction of secretory IgA (SIgA), which mediates immune exclusion by neutralizing bacteria within the mucus layer and limiting epithelial attachment. Simultaneously, mucosal vaccination promotes the formation of $CD69^{+}CD103^{+}$ tissue-resident memory T cells that provide rapid local recall responses independent of circulating lymphocytes. Recent evidence also demonstrates that *Shigella* exposure induces trained innate immunity through pattern recognition receptor (PRR)-mediated epigenetic and metabolic reprogramming, including H3K4me3-associated chromatin remodeling, enhanced glycolysis, and amplified IL-1β signaling, resulting in increased phagocytic and bactericidal activity. To induce these coordinated responses, next-generation vaccine platforms increasingly employ GMMA-based formulations, InvaplexAR-Detox systems, bacterial endotoxin conditioning center -engineered live-attenuated strains, double-mutant heat-labile toxin adjuvants, PRR agonists, and nanoparticle-mediated mucosal delivery systems. Oral and intranasal administration remain the most effective routes for inducing integrated mucosal immunity, including SIgA production, tissue-resident memory T-cell establishment, and innate immune imprinting. Together, these advances support the development of mechanistically integrated mucosal vaccines capable of providing durable protection against shigellosis.

1. INTRODUCTION

Shigella is a major global pathogen responsible for bacillary dysentery. *Shigella flexneri*, *Shigella sonnei*, and *Shigella dysenteriae* are highly infectious, requiring only 10–100 organisms to initiate disease [1,2]. This pathogen invades colonic epithelium, replicates intracellularly, and spreads laterally through actin-based motility, enabling rapid mucosal destruction, intense inflammation, and dysentery [1]. Its invasion and inflammatory activity are largely confined to the mucosal surfaces of the gastrointestinal tract. Thus, the mucosal surfaces represent the front line of defense against this pathogen and, therefore, a key target for vaccination strategies [1]. Classic systemic vaccines often fail to induce effective immune protection at the mucosal portals of entry where *Shigella* initiates infection, despite their ability to elicit very strong systemic immunity [3]. This constitutes a real need for effective mucosal vaccines capable of intercepting the pathogen early, blocking its adhesion and invasion, and conferring long-lasting protection to reduce the burden of disease [4]. Mucosal vaccines are designed to stimulate the production of antigen-specific secretory immunoglobulin A at mucosal surfaces, where it serves as a key component of frontline immune defense. secretory IgA (SIgA) mediates immune exclusion by limiting microbial attachment to epithelial cells, thereby preventing epithelial invasion and early infection progression [5]. In addition to SIgA-mediated humoral protection, growing evidence highlights the importance of tissue-resident memory T cells established within mucosal tissues. These tissue-resident populations are characterized by expression of retention markers such as CD69 and CD103. The induction of such localized adaptive immune populations contributes substantially to the strength and durability of vaccine-induced mucosal immunity [6].

Progress in mucosal vaccine development against *Shigella* has increasingly centered on the use of advanced adjuvants and delivery platforms that enhance immune activation without compromising safety. In particular, the inclusion of toll-like receptor (TLR) agonists, STING agonists, and double-mutant heat-labile toxin (dmLT)-based mucosal adjuvants and mucosal permeation enhancers has

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demonstrated considerable potential by improving antigen uptake and facilitating targeted immune cell recruitment to mucosal tissues [7,8]. Of these, the *Shigella* invasin complexes, such as Invaplex, have proven to be effective mucosal adjuvants. Invaplex mimics the distribution of antigens across epithelial barriers in a very efficient sequence to induce not only systemic immune responses but also mucosal immune responses [9]. These formulations tend to induce antigen-specific Th2-type immune responses characterized by the production of increased levels of cytokines (including IL-4 and IL-5) with potent antigen-specific IgG and IgA responses [10]. In addition to classical adaptive immunity, trained innate immunity is now known as being an important factor in the efficiency of mucosal vaccines [11]. The trained innate immune system is an epigenetic and metabolic program in innate immune cells that is initiated by vaccination involving H3K4me3-associated chromatin remodeling, enhanced glycolytic activation, and amplified IL-1β signaling [12]. This is an innate immune training that works alongside adaptive mechanisms and leads to an extended, more long-lasting degree of protection at mucosal areas [11]. The implementation of this approach is further promoted by the development of mucosal viral vector-based vaccines, which mimic the natural routes of infection and activate a variety of immune compartments in a coordinated manner [13].

The route of vaccine delivery plays a critical role in shaping the immune response. Intranasal and oral administration have proven particularly effective in inducing both mucosal and systemic immunity [11]. Intranasal vaccines, in particular, have been extensively investigated and shown to elicit strong humoral and cellular immune responses at mucosal barriers, including the respiratory and gastrointestinal tracts, offering protection against *Shigella* and other mucosal pathogens [14]. Oral vaccines offer complementary advantages by directly stimulating gut-associated immune responses, which is especially relevant given the intestinal tropism of *Shigella*. A central challenge in the development of mucosal vaccines lies in achieving an optimal balance between immune activation and immune tolerance [15]. Mucosal tissues are continuously exposed to diverse antigens, and their immune systems are tightly regulated to prevent excessive inflammation that could lead to tissue damage. Consequently, vaccine formulations must be carefully optimized to elicit robust yet controlled immune responses. This requires a detailed understanding of mucosal immunology, along with the strategic selection of adjuvants and delivery systems capable of promoting protective immunity without inducing tolerance or pathological inflammation [14]. Future *Shigella* mucosal vaccines are therefore likely to integrate multiple complementary strategies, including the induction of strong antigen-specific SIgA responses, establishment of tissue-resident memory T cells, and modulation of trained innate immunity. Although several recent reviews have discussed general principles of mucosal vaccination and delivery technologies [16–18], a mechanistic *Shigella*-focused synthesis integrating SIgA-mediated immune exclusion, tissue-resident memory T-cell responses, and trained innate immune mechanisms remains limited [19,20]. In particular, the contribution of epigenetic and metabolic reprogramming to vaccine-induced trained immunity against *Shigella* has not been extensively synthesized in the context of mucosal vaccine design [12]. Therefore, the present review aims to critically examine the coordinated roles of SIgA-mediated immune exclusion, tissue-resident memory T-cell responses, trained innate immunity, and advanced mucosal delivery systems in shaping durable protective immunity against *Shigella*.

2. REVIEW METHODOLOGY

A bibliometric analysis was carried out using the Scopus database to systematically map research trends in the areas of mucosal vaccination, *Shigella*, adaptive immune responses, and trained innate immunity. Literature searches were conducted using the combined query “Mucosal Vaccination OR *Shigella* OR Adaptive Immunity OR Trained Innate Responses,” without restrictions on study design, in order to capture a comprehensive range of experimental, clinical, and translational studies. Only articles published in English were included in the analysis. Retrieved records were initially screened based on titles, abstracts, and keywords to eliminate duplicates, grey literature (grey literature was excluded from the bibliometric dataset, but selected trial/industry sources were used for contextual discussion), and publications outside the scope of the review. Articles that passed this stage underwent full-text assessment to determine their relevance. Studies were retained if they directly addressed mucosal immunization approaches, immune mechanisms at mucosal sites, or *Shigella*-specific host immune responses (Fig. 1). The initial search retrieved 1100 records; after screening and eligibility assessment, 1025 records were included in the bibliometric dataset however, only the most relevant studies directly related to *Shigella* mucosal vaccination and immune mechanisms were retained for detailed discussion in the review. Bibliometric visualization and network analyses were subsequently performed using VOSviewer to identify dominant research clusters, collaboration patterns, and thematic relationships within the field (Fig. 2).

Bibliometric analysis identified major interconnected research themes involving mucosal immune responses, vaccine adjuvants, trained

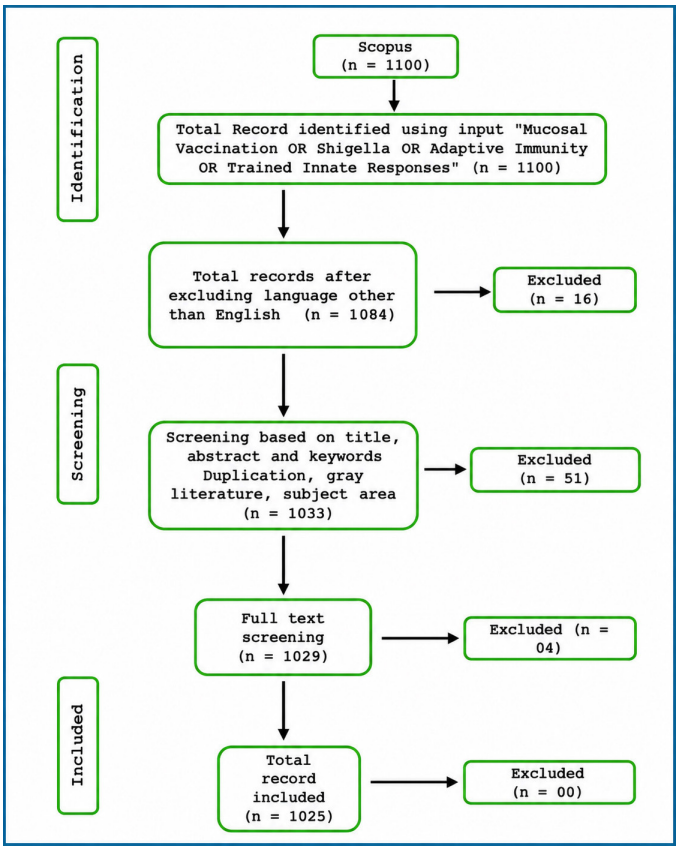


Figure 1. Literature retrieval, screening, and study selection workflow used for bibliometric mapping and qualitative synthesis of research related to mucosal vaccination and *Shigella*.

innate immunity, and advanced delivery systems associated with *Shigella* mucosal vaccination. Keyword co-occurrence mapping revealed strong associations among SIgA-mediated immunity, tissue-resident memory T cells, and mucosal vaccine platforms, reflecting growing scientific emphasis on integrated mucosal protection strategies. Collaboration network analysis further revealed active international research interactions among countries contributing prominently to vaccine immunology and infectious disease research. Publication output peaked in 2021 and subsequently declined through 2025.

3. EXISTING AND EMERGING VACCINES AGAINST *SHIGELLA*

The development of *Shigella* vaccines over the past few years has made further advances beyond the classical platform of whole cells, conjugates, and recombinant vaccines. The GMMA-based vaccines have also continued to progress, with clinical trials demonstrating that parenteral vaccines inoculated at the IM site can elicit serum IgG antibodies, and even α4β7 integrin-expressing antibody-secreting cells that primarily seed the gut, to some extent, even following intramuscular inoculation [21]. The candidate altSonflex1-2-3 for GMMA has been successfully tested in an EU clinical trial in adults (Phase 1/2) and demonstrated a favorable safety profile and inducibility of strong serotype-specific functional antibody responses, paving the way for progression to dose optimization clinical trials in targeted pediatric populations [21]. This program is in-licensed to Bharat Biotech for further development and deployment in LMICs in 2025, reflecting its perceived suitability as a scalable, low-cost platform for endemic settings [22]. To test the potential of inducing gut-homing integrin α4β7 + plasmablasts against *S. sonnei* LPS, an earlier vaccination study with the GAHB GMMA vaccine showed efficient induction of both the mucosal homing integrin α4β7 + IgG and intestinal-preferring integrin α4β7+ IgA plasmablasts, even after parenteral vaccination (intramuscular) [23]. Conjugate and subunit strategies have also continued to evolve toward broader coverage and improved mucosal relevance. Synthetic or bioconjugate O-antigen vaccines (such as newer constructs, Ac3-S-LPS) have been reported to induce polyvalent responses in the mucosal supply of antibodies against several LPS antigens of the GIT, indicating that optimized conjugation of O-antigens might induce both mucosal and systemic immunity [24]. Furthermore, conserved-protein strategies are gaining in force with next-generation fusion subunits such as L-DBF, which conferred both cross-protection against multiple *Shigella* serotypes (and protection against both young and aged mice) and was recently proposed for cross-serotype, age-independent protection [25]. New, more complex mucosal subunit vaccines are being introduced into clinical trials. InvaplexAR-Detox is specifically developed to examine the effect of incorporating a potent mucosal adjuvant, such as the dmLT, on the simultaneous boosting of systemic and mucosal

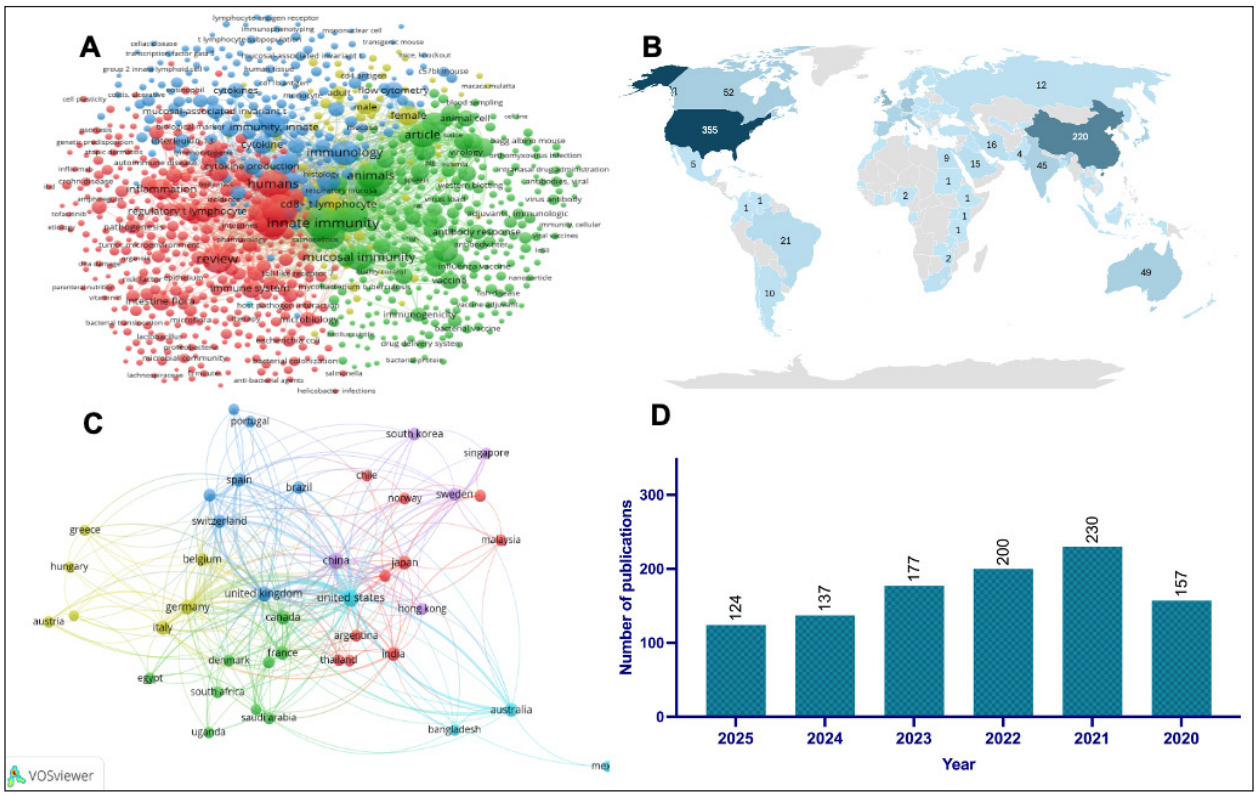


Figure 2. Bibliometric analysis of research on mucosal vaccination and *Shigella*. (A) Keyword co-occurrence network illustrating major thematic clusters and interrelationships among research topics. Node size represents keyword occurrence frequency, link thickness indicates the strength of co-occurrence relationships, and color-coded clusters represent closely associated research themes within the field. (B) Global distribution of publications showing country-wise research output, where darker color intensity corresponds to higher publication frequency. (C) International collaboration network depicting co-authorship and cross-country research interactions. Node size reflects the contribution of individual countries or institutions, while connecting lines indicate collaborative link strength. Different colors denote distinct collaboration clusters identified through network analysis. (D) Temporal distribution of publications from 2020 to 2025, illustrating annual research productivity and evolving scientific interest in mucosal vaccination and *Shigella* research.

Table 1. Current *Shigella* vaccine strategies.

Platform (Type)	Antigens	Adjuvant	Route	Trial phase	Key outcomes	References
Oral inactivated whole-cell *Shigella* (trivalent), combined with ETEC (ETVAX)	*Shigella sonnei* + S. *flexneri* 2a, 3a LPS + outer-membrane/ surface proteins	dmLT (evaluated vs no adjuvant)	Oral	Preclinical (mouse model)	• Safe and well tolerated • No immune interference observed • Induced systemic IgG and intestinal IgA • dmLT enhanced LPS- and Ipa-specific responses	[26]
O-specific polysaccharide-protein conjugate	*Shigella flexneri* 3a O-polysaccharide–rTTHc conjugate	Alum	IM (Intramuscular)	Preclinical (guinea pig Sereny model)	• Strong OSP- and LPS-specific IgG/IgA responses • Functional bactericidal antibodies induced	[27]
Multi-epitope recombinant subunit vaccine	IpaA/B/C/D + OmpC/F + VirG epitopes	Freund's adjuvants	Subcutaneous	Preclinical (mouse model)	• Strong antigen-specific IgG responses • Neutralizing antibodies generated • 80%–100% protection against lethal challenge	[28]
Flexyn2a (bioconjugate vaccine)	*Shigella flexneri* 2a O-polysaccharide-EPA conjugate	None	IM	Phase 2b CHIM	• Well tolerated • Induced anti-LPS IgG • Partial protective efficacy demonstrated • Anti-LPS IgG identified as a correlate of protection	[29]
altSonflex1-2-3 (GMMA)	*Shigella sonnei* + *S. flexneri* 1b, 2a, 3a LPS + OM proteins	None	IM	Early clinical development	• Multivalent GMMA platform • Induced functional anti-*Shigella* IgG responses	[30]
Flexyn2a (bioconjugate vaccine)	*Shigella flexneri* 2a O-polysaccharide-EPA conjugate	Alum	IM	Phase I (single-blind, randomized)	• Safe and immunogenic • Induced LPS-specific IgG/ IgA • Functional SBA responses observed • Alum provided limited enhancement	[31]
Recombinant IpaB protein subunit	*Shigella* invasion protein invasion plasmid antigen B	Protein intrinsic adjuvanticity	Intranasal (immunization); Oral (challenge)	Preclinical (mouse model)	• Induced IgG and sIgA responses • Balanced Th1/Th2 immunity • Reduced intestinal colonization and colitis • 80%–90% protection against oral challenge	[32,33]
Protein subunit (multiepitope, conserved antigen)	VirG53–353 (conserved multiepitope region of VirG/IcsA)	Alum (Adju-Phos)	IM	Preclinical (mouse model)	• Strong anti-VirG IgG responses • Broad cross-protection against multiple serotypes • Anti-VirG IgG identified as a correlate of protection	[34]
Bacterial enzymatic combinatorial chemistry-engineered live-attenuated *Shigella*	*Shigella sonnei* + *S. flexneri* 2a whole cells (LPS/O-antigen; BECC-modified lipid A)	live-attenuated, BECC lipid A modification	Intranasal / Oral (mouse studies)	Preclinical (mouse)	• Reduced endotoxicity • Retained invasion capacity • Strong anti-LPS IgG/IgA responses • Improved safety profile for live-attenuated vaccines	[35]

immunity in indigenous populations and is included in a multicenter, phase Ia/b study in Europe and Africa (Table 1) [36].

4. INDUCTION OF MUCOSAL ANTIBODY RESPONSES AND CELLULAR IMMUNITY

Mucosal surfaces represent the major portals of entry for many pathogens, including *Shigella*, and protection of these sites is critical for infection control. SIgA provides the first line of mucosal immune defense by inhibiting microbial adherence and invasion [37]. At mucosal surfaces, SIgA operates by "immune exclusion"; by binding antigens with very high avidity, SIgA prevents microbes from adhering to and invading the mucosa. This mechanism has been documented for respiratory viruses; intranasal SIgA neutralizes influenza and SARS-CoV-2 more effectively than serum IgG by trapping virions in the mucus layer [38]. In addition to blocking, SIgA agglutinates bacteria and entrains them in mucus for clearance. Unlike IgG, polymeric SIgA does not fix complement via the classical pathway, but it can activate the lectin/complement cascade and engage Fcα receptors on phagocytes. Thus, SIgA not only prevents infection but can also recruit innate effector functions in the gut and lung [39]. Apart from neutralization, SIgA modulates the mucosal microbiota through selective coating of commensal and pathogenic bacteria, thereby influencing microbial composition and maintaining intestinal homeostasis [40]. It also promotes retro-transport of antigens with dendritic cells (DCs) in gut-associated lymphoid tissue, thereby priming adaptive immune responses [41]. The activity of SIgA extends to downregulating pro-inflammatory responses that maintain tolerance to non-pathogenic antigens and prevent excessive inflammation that might disrupt the integrity of the mucosal barrier [39].

Mucosal vaccines, especially the mucosal route such as oral and intranasal administration, are specifically engineered to induce antigen-specific SIgA responses at mucosal surfaces. This tends to prevent entry through this portal, as well as induce systemic immunity [39]. After the vaccination antigen is taken up there, the vaccine antigen is sampled at specialized inductive sites, such as Peyer's patches in the gut and nasopharynx-associated lymphoid tissue, where antigen presentation results in activation and differentiation of SIgA-producing B cells [42]. Furthermore, intranasal immunization has been demonstrated to promote a strong SIgA response in the upper respiratory epithelium and to establish tissue-resident memory T cells in the respiratory epithelium as a benefit for long-term immune surveillance [43]. The improvements in adjuvants and delivery systems have further bolstered the performance of mucosal vaccines. Of particular interest are subunit vaccines formulated with innate immune receptor agonists and mucosal permeation enhancers, which boost antigen uptake and add to the local immune activation generated by the vaccine, without incurring excessive safety risks [44]. Because mucosal vaccines cannot induce immunity in the absence of strong

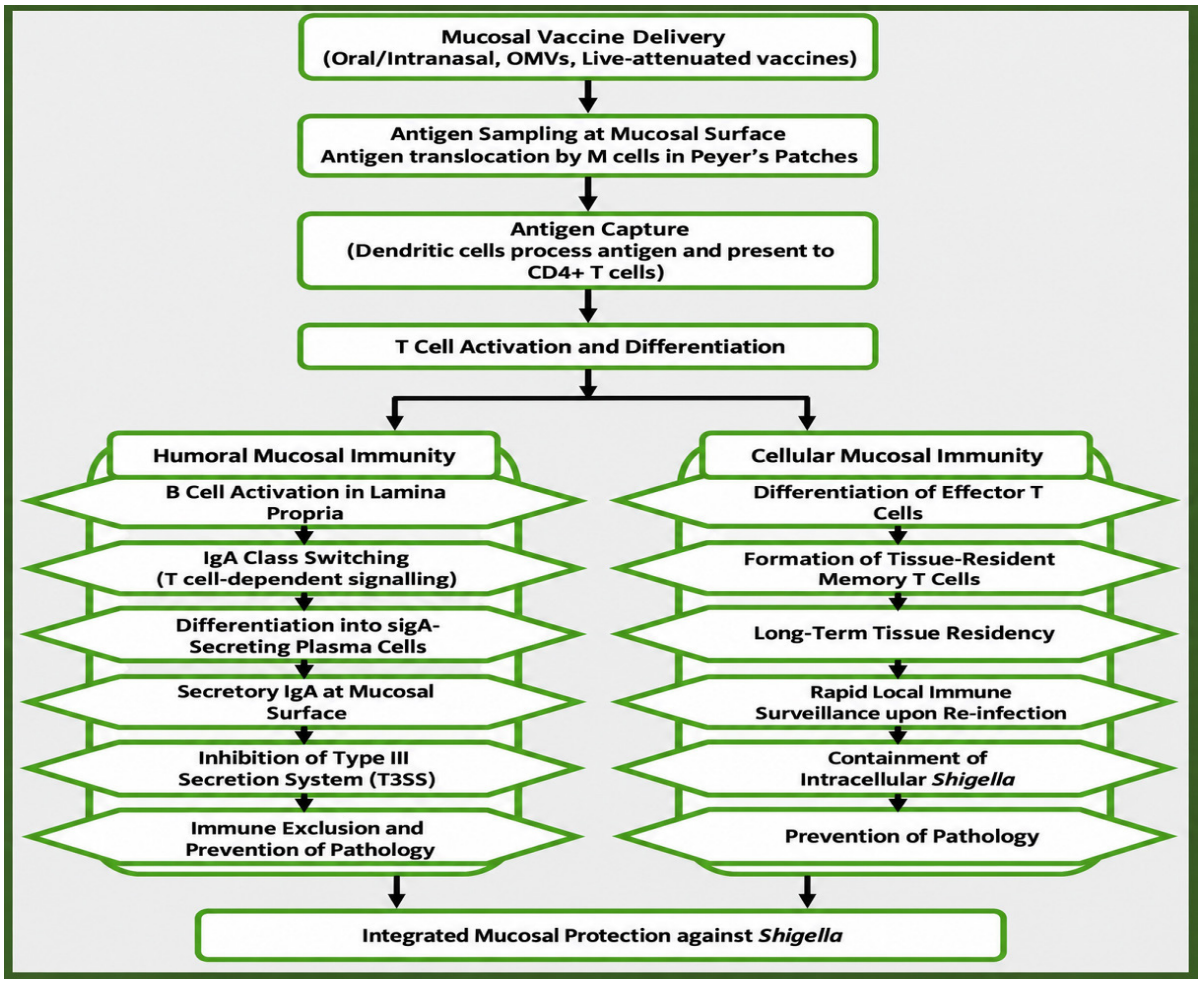


Figure 3. Mucosal immune mechanisms induced by *Shigella* mucosal vaccines.

adjuvants, it is necessary to incorporate strong adjuvants in mucosal vaccines. dmLT promotes dendritic-cell activation and the polarization of Th17 lymphocytes [45]. In early clinical trials, co-administration of dmLT has been shown to enhance systemic and mucosal antibody responses to the *Shigella* antigens, indicating its potential usefulness for the continued early clinical trial phase I InvaplexAR-Detox with Ipa proteins + LPS [36]. dmLT continuously expands IL-17-producing T cells, leading to elevations of SIgA at the site of exposure, and has an acceptable safety profile at tested intramuscular doses [45,46,8]. TLR agonists (CpG: TLR9, poly(I: C): TLR3, imidazoquinolines: TLR7/8, or flagellin: TLR5) are other emerging mucosal adjuvants, as are also the STING agonists, such as c-di-GMP. CpG DNA skews the Th response to Th1 and induces strong levels of IgG2a, and poly(I) induces type I interferon and cross-priming of CD8+ T cells [47]. These agents have been demonstrated to have promise in preclinical mucosal vaccines [47]. Chitosan is a cationic polysaccharide that is mucoadhesive and able to increase tight junction uptake and SIgA production in addition to being an adjuvant/carrier [48]. Biomimetic nanoparticles, such as virus-like particles and liposomes, can provide PAMP-like signals and improve APC uptake [49].

While secretory IgA mediates immune exclusion at mucosal surfaces, effective protection against invasive pathogens such as *Shigella* also requires rapid cellular immune containment once epithelial invasion has occurred. This function is primarily mediated by tissue-resident memory T cells (T-RM), which constitute a central arm of mucosal cellular immunity [50]. Unlike circulatory memory T cells, tissue-resident memory T cells reside indefinitely in tissue under the epithelial layer and are locally clustered to deploy a swift response to re-encounter. T_RM subsets, $CD4^+$ and $CD8^+$, can provide protection against infection by quickly discerning and eliminating transformed cells and orchestrating local immune activation [50,51]. These cells exhibit special transcriptional and functional programs, which play a role in the preservation of long-term tissue residency and the synthesis of effective and rapid responses to intracellular pathogens to control infection and limit the dissemination of pathogens. Colonic tissue-resident memory T (T_RM) cells have been found within the human gut and are characterized by their continued expression of retention markers (CD69 and CD103), absence of lymphoid-homing receptors, and their non-circulating effector phenotype that is optimized for supporting localized mucosal immunity [15]. From a functional standpoint, T-RM cells have polyfunctional effector profiles that generate cytokines, attract innate immune cells, and promote local inflammatory responses necessary for containment of bacterial dissemination [50]. Importantly, it was strongly indicated that depletion of circulating T cells does not abrogate mucosal protection when T-RM cells are not depleted: These cells are sufficient in mediating durable mucosal immunity (Fig. 3) [52].

5. INNOVATIVE ADJUVANTS AND DELIVERY SYSTEMS ENHANCING MUCOSAL IMMUNITY

Shigella invades and replicates in the intestinal mucosa, so effective vaccines must induce strong gut mucosal immunity. Novel adjuvants and delivery systems are significantly improving mucosal immunity against pathogens such as *Shigella* by enhancing antigen-specific responses and integrating both adaptive and trained innate immunity (Table 2). Toxin-derived mucosal adjuvants have long been studied for enteric vaccines. The dmLT from *Escherichia coli coli* (LT_R192G/L211A) retains immunostimulatory activity while being non-toxic. dmLT binds GM1 on epithelial cells and triggers IL-8 and G-CSF release, recruiting and activating DCs [43]. Activated DCs upregulate costimulatory molecules (CD80/86) and secrete IL-1, IL-23, and IL-6, promoting strong Th17 and T follicular helper responses [8]. As a result, dmLT dramatically enhances both mucosal SIgA and systemic IgG production against co-delivered antigens [49]. In *Shigella* models, dmLT-adjuvanted subunit vaccines (e.g., IpaB/D antigens) delivered intranasally induced strong Th17/Th1 responses and cross-protection against multiple *Shigella* serotypes [53]. Clinical data also support dmLT's promise; it was safe and immunogenic in phase I trials of enterotoxigenic *E. coli* and is now being tested with a *Shigella* Invaplex subunit vaccine in a phase Ia/b trial [36]. Another toxin-derived adjuvant is CTA1-DD, a non-toxic fusion of cholera toxin A1 with an IgG-binding domain (DD) from *Streptococcus pyogenes*. CTA1-DD targets B cells and follicular DCs, thereby amplifying germinal-center formation and promoting the production of secretory IgA without causing inflammation or neurologic side effects [54]. In preclinical studies (mostly influenza and bacterial vaccines), CTA1-DD given intranasally markedly enhanced mucosal and systemic IgA responses, suggesting potential applicability for *Shigella* antigens. Both dmLT and CTA1-DD exemplify how detoxified ADP-ribosylating toxins can be repurposed to focus adaptive immunity at mucosal surfaces.

pattern recognition receptor (PRR) agonist adjuvants have emerged as versatile enhancers of mucosal vaccines. TLR ligands, such as CpG oligodeoxynucleotides (TLR9), monophosphoryl lipid A (MPLA, a TLR4 agonist), Pam3CSK4 (TLR2), and flagellin (TLR5), activate innate signaling pathways that link to adaptive immunity [55]. These adjuvants engage APCs via MyD88 or TRIF adaptors, driving DC maturation, cytokine release, and germinal center formation [55]. In mice, a newly developed TLR4 agonist (BECC438, a detoxified lipid A analog) admixed with *Shigella* T3SS fusion antigens conferred high protection when given intranasally [53]. The BECC438 formulation induced strong IFN-γ and IL-17 responses from mucosal lymphocytes, correlating with survival after *Shigella* challenge [53]. Of note, combinations of TLR agonists and STING agonists can synergistically shape mucosal T-cell memory. STING pathway agonists (e.g., cyclic di-AMP, cyclic di-GMP) are potent mucosal adjuvants that induce type I interferons and robust Th1/CD8+ responses [56,57]. For example, intranasal liposomes co-delivering SARS-CoV-2 spike protein and the STING agonist 2′3′-cGAMP elicited broad mucosal immunity in preclinical models [43]. More recent work shows that pairing a TLR agonist with c-di-GMP intranasally can markedly induce $IL\text{-}17^+$ tissue-resident memory T cells (T_RM) in the respiratory tract [58]. By analogy, such strategies could generate long-lived Th17/T_RM cells in the gut to rapidly counter *Shigella* invasion.

Beyond adaptive responses, "trained" innate immunity is an emerging concept for mucosal vaccines. Vaccination or priming with specific microbial components can epigenetically reprogram innate cells (such as macrophages and neutrophils) for enhanced reactivity upon re-exposure [1,2]. *Shigella* itself can induce trained innate responses: zebrafish exposed to a sublethal *Shigella* dose developed neutrophils with an altered epigenetic landscape (active H3K4me3 on innate genes) and greater bacterial killing capacity [59]. Such *Shigella*-trained neutrophils exhibited improved clearance and accelerated infection resolution upon rechallenge [18]. These findings imply that mucosal vaccines could be designed to harness trained immunity, for example, by including microbial motifs or adjuvants that prime innate cells (e.g., with β-glucans, TLR2 ligands, or even live-attenuated vectors). Exploiting trained innate pathways might enhance first-line defense in vaccines, complementing the adaptive SIgA/T_RM responses.

Engineered delivery systems are crucial for optimal antigen and adjuvant presentation to the mucosal immune system. *Shigella* antigens and immunostimulatory molecules can be encapsulated in biodegradable nanoparticles, usually composed of polymers such

Table 2. Major mucosal adjuvants and delivery platforms investigated for *Shigella* mucosal vaccine development, highlighting their mechanisms of action, advantages, limitations, vaccine targets, and developmental status.

Adjuvant / Platform	Mechanism of action	Advantages	Limitations	Vaccine Candidate / Target	Development stage	References
Double-mutant labile toxin (dmLT) / multiple-mutant CT (mmCT)	• Activates APCs through cAMP signaling • Promotes Th1/Th17 and SIgA responses	• Strong mucosal and systemic immunity • Promotes T_RM formation	• Residual enterotoxicity risk • Complex manufacturing	• InvaplexAR-Detox • ETVAX • ShigETEC	Phase I–II	[14,25,60]
GMMA (generalized modules for membrane antigens)	• OMV-based self-adjuvanted platform • Preserves native O-antigen and membrane proteins	• Scalable and low-cost • Strong immunogenicity • Suitable for multivalent vaccines	• Partial mucosal imprinting after parenteral delivery • Variable O-antigen expression	• altSonflex1-2-3	Phase II	[4,61,62]
Live attenuated platforms	• Mimics natural infection • Stimulates SIgA and T-cell immunity	• Broad antigen exposure • Strong cellular responses	• Reactogenicity concerns • Risk of over-attenuation	• WRSs2 • SC602 • CVD1208S	Phase I/II	[63,64]
Bioconjugate / Polysaccharide platform	• Converts T-cell-independent O-polysaccharide responses into T-cell-dependent immunity	• Strong SBA responses • High serotype specificity	• Multiple-dose requirement • Carrier suppression risk	• Flexyn2a • S4V	Phase I/II	[65,66]
Invaplex (*Shigella* Invasin Complexes)	• Delivers Ipa proteins and O-antigen • Enhances epithelial uptake and T3SS signaling	• Conserved antigens • Induces mucosal and systemic immunity	• Requires potent co-adjuvants in endemic regions	• InvaplexAR-Detox	Phase Ia/b	[67]
Chitosan nanoparticles	• Mucoadhesive carrier system • Enhances epithelial transport and antigen stability	• Biodegradable • Enhances mucosal IgA and systemic IgG	• Weak T-cell stimulation alone • pH-sensitive stability	• MxiH antigen systems	Preclinical	[68]
TLR agonists (MPL, CpG, flagellin)	• Activates PRR signaling pathways • Promotes Th1/Th17 immunity	• Strong mucosal immune activation • Enhances IgA responses	• Excessive inflammatory responses possible	• *Shigella* T3SS antigens	Preclinical / Clinical	[16, 69]
STING agonists (cyclic dinucleotides)	• Activates cGAMP-mediated innate immune signaling	• Strong Th1/Th17 and SIgA responses	• Early-stage development • Potential reactogenicity	• Experimental mucosal vaccines	Preclinical	[57,70]
IpaC surface-modified nanoparticles	• Enhances APC targeting and antigen uptake	• Improved targeted antigen delivery	• Limited clinical data available	• *Shigella* subunit vaccines	Preclinical	[71]

as poly(lactic-co-glycolic acid) or chitosan, which prevent them from being degraded by enzymes in the mucosal environment [68]. Moreover, these nanosystems can also be targeted toward microfold (M) cells and DCs in order to improve the delivery of antigen and effective induction of mucosal immune responses [72]. For example, *Shigella* proteins have been intranasally delivered by alginate NPs, which improves the controlled release and antigen presentation [73]. Additionally, liposomes and immunostimulating complexes are used for delivery as carriers: liposomal complexes can fuse with cell membranes and can deliver antigens (and TLR/STING agonists) efficiently to mucosal APCs. Liposomes that contain a STING agonist in combination with a second compound were found to provide a potent intranasal immunization in animal models in one example [74]. Beyond conventional formulations, "alginate-derived micro- and nanosystems offer a mild, aqueous approach for incorporating structurally sensitive protein antigens," while poly(anhydride)-based nanoparticles provide an additional particulate vaccine-delivery strategy. In experimental "*Shigella*" vaccination studies, outer-membrane vesicles formulated within poly(anhydride) nanoparticles enhanced protection following mucosal administration, with particularly favorable performance at reduced intranasal antigen quantities [75]. Viral vectors (e.g., replication-deficient adenovirus, modified vaccinia Ankara) are explored for viral diseases; these mimic infection, strongly activate innate sensors, and can be delivered mucosally to induce high systemic and mucosal T-cell and antibody responses [76]. To date, candidates for *Shigella* vaccine that have advanced toward the clinical development stage are dead whole-cell, live-attenuated, or protein/bioconjugate formulations; viral-vectored candidates, if any, are still relatively underrepresented in the clinical pipeline [77].

6. HARNESSING TRAINED INNATE IMMUNITY IN MUCOSAL VACCINE DESIGN

Immunity provided by trained innate immunity is important for the concept of *Shigella* vaccine design because the disease outcome is decided during the first hours of infection when epithelial invasion, macrophage death, and recruitment of neutrophils are occurring, before the onset of adaptive immunity [67]. Through long-term epigenetic and metabolic changes, innate immune training, not antigen specificity, confers enhanced responsiveness and magnitude of mucosal myeloid cells to *Shigella* [11]. Experimental evidence directly connects *Shigella* exposure to innate immune training: exposure to sublethal doses of *Shigella* leads to long-term epigenetic changes in neutrophils, including alterations in chromatin accessibility and increased bactericidal activity after re-exposure, leading to increased survival of the host and faster clearance of bacteria [59]. This sets the stage for the possibility of imprinting trained innate states and the development of a vaccine strategy to exploit this pathway to immunity. Clinically, trained immunity during *Shigella* infection starts with strong activation of PRRs, including the toll-like receptor 4 (TLR-4) and the nucleotide-binding oligomerization domain pathways [69]. These receptors are stimulated by the components of *Shigella*, including LPS, lipid A, and type III secretion system-associated ligands [80]. The activation of these events results in an increase in activating histone modifications at the promoter sites of genes that regulate inflammatory and antimicrobial responses [78,79]. In combination, these modifications permit the transcription response to be even faster and more pronounced if a cell is subsequently stimulated [68]. Functionally, this reprogramming leads to more robust IL-1β-dependent signaling, enhanced phagocytic activity, and improved killing of bacteria in the endosome, all of which directly oppose *Shigella*'s ability to gain a foothold within the intestine by overwhelming initial innate immune responses at the mucosal surface [20]. There are several vaccine platforms for *Shigella* that naturally target pathways linked to trained innate immunity [81]. GMMA and outer membrane vesicle-based vaccines present native membrane PAMPs in particulate form, which allow for prolonged and multivalent PRR stimulation that resembles natural infection [82]. The construction of live-attenuated strains of *E. coli* via the bacterial endotoxin conditioning center is another example of rational design of lipid A to retain its innate immunostimulatory properties while decreasing its endotoxicity. This balance is crucial because it promotes beneficial innate training outcomes and not immune tolerance or pathological inflammation [35]. It remains to be determined whether GMMA vaccination induces epigenetic changes in humans, but the extended innate immune activation and gut-homing immune cell profiles seen after vaccination with GMMA are consistent with the presence of partial innate immune imprinting [83].

7. CONCLUSION

The emergence of mucosal vaccines for *Shigella* represents a paradigm shift in rational vaccine design, moving from traditional approaches aimed at systemic immunity to more specific ones that directly target the biological pathways of mucosal immunity. To achieve long-lasting protection, a comprehensive, localized protective response is required because *Shigella* infection begins with rapid penetration of the colonic epithelium. Next-generation vaccine formulations do so by coordinating a three-member immune triumvirate: induction of antigen-specific frontline secretory IgA to exclude pathogens from the mucus layer; establishment of tissue-resident memory T cells to mediate local cellular containment independent of blood-borne lymphocyte trafficking; and harnessing of trained innate immunity through epigenetic and metabolic reprogramming. The clinical relevance of these highly complex mechanisms depends critically on the use of novel mucosal delivery systems, such as nanoparticles, liposomes, and general modules for membrane antigens, as well as potent and targeted adjuvants, such as dmLT and CTA1-DD. These platforms are highly effective at stimulating both local and systemic immune responses and at maintaining effective control of excess inflammation when used orally or via nasal delivery. In conclusion, the ongoing refinement of these complex strategies has the potential to reduce the heavy burden of shigellosis experienced by populations in endemic areas around the world, and provides a roadmap for the design of next-generation vaccines to address a wide range of difficult-to-vaccinate, mucosal pathogens.

8. AUTHOR'S CONTRIBUTIONS

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; participated in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be authors as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

9. CONFLICTS OF INTEREST

The authors report no financial or other conflicts of interest in this work.

10. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

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12. DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting this review are available within the article.

13. INFORMED CONSENT STATEMENT

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14. PUBLISHER'S NOTE

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15. USE OF ARTIFICIAL INTELLIGENCE-ASSISTED TOOLS

Artificial intelligence (AI)-based language tools were used solely to improve the readability, clarity, and English language of the manuscript. These tools were not used to generate scientific content, analyze or interpret data, formulate conclusions, or create references, figures, or images. The authors carefully reviewed and edited all AI-assisted text and take full responsibility for the accuracy, integrity, and originality of the manuscript.

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