



Functional antibodies as immunological endpoints to evaluate protective immunity against *Shigella*

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ABSTRACT

The development, clinical advancement and licensure of vaccines, and monitoring of vaccine effectiveness could be expedited and simplified by the ability to measure immunological endpoints that can predict a favorable clinical outcome. Antigen-specific and functional antibodies have been described in the context of naturally acquired immunity and vaccination against *Shigella*, and their presence in serum has been associated with reduced risk of disease in human subjects. The relevance of these antibodies as correlates of protective immunity, their mechanistic contribution to protection (e.g. target antigens, interference with pathogenesis, and participation in microbial clearance), and factors that influence their magnitude and makeup (e.g. host age, health condition, and environment) are important considerations that need to be explored. In addition to facilitating vaccine evaluation, immunological correlates of protection could be useful for identifying groups at risk and advancing immune therapies. Herein we discuss the precedent and value of functional antibodies as immunological endpoints to predict vaccine efficacy and the relevance of functional antibody activity to evaluate protective immunity against shigellosis.

ARTICLE HISTORY

Received 22 November 2018
Revised 2 June 2019
Accepted 26 June 2019

KEYWORDS

Functional antibodies; correlates of protection; threshold of protection; *Shigella*; protective immunity; trials for developing countries; vaccinology

Shigella disease burden and the need for a vaccine

Diarrheal diseases are the second leading cause of death worldwide, resulting in the loss of about 500,000 children's lives under the age of 5 per year, primarily in the developing world.^{1,2} The contribution of *Shigella* to the global burden of diarrheal morbidity and mortality in this age group has been emphasized by estimates from the Global Enteric Multicenter Study (GEMS),³ the Malnutrition and Enteric Disease (MAL-ED) birth cohort study,⁴ and the Global Burden of Disease, Injuries, and Risk Factors Study (GBD 2016).⁵ Frequent and repeated episodes of diarrhea in children living in these resource-poor areas have been associated not only with increased mortality but also with severe and lifelong health impairment, which compromises growth and cognitive ability.^{6,7} Current prophylaxis to treat acute diarrheal episodes includes oral rehydration and antibiotics. While improved sanitation and access to clean water are certainly effective primary control measures, vaccination might be a more rapid and efficient preventive intervention. The success of this approach is exemplified by the rotavirus vaccine introduction, which resulted in substantial reduction in diarrhea-related hospital visits, admissions^{8–10} and death^{9,11–13} in children less than 5 years of age globally. *Shigella* has also been associated with diarrhea across all adult age groups, with increased incidence in the elderly.⁵ *Shigella* and Enterotoxigenic *Escherichia coli* (ETEC) are the main causes of diarrhea in travelers and military personnel. The development and clinical advancement of a *Shigella* vaccine are seen as an equitable, cost-effective prevention tool that could be deployed with existing vaccination programs for effective disease control.¹⁴

Naturally acquired immunity against shigellosis and the relevance of antibodies

It is known from observational epidemiological and human challenge studies that subjects repeatedly infected with *Shigella* acquire a natural immunity that prevents or reduces illness following subsequent infection.^{15–17} In endemic regions, disease incidence diminishes in older children and adults, and pathogen-specific host defenses, lacking in early infancy, progressively increase with age.^{17–19} This naturally acquired protective immunity tends to be serotype-specific and directed to the O-polysaccharide (OPS).^{15,16} Early studies involving Israeli soldiers reported associations between LPS-specific serum IgG and reduced illness during *Shigella* outbreaks.^{15,16,20} On the basis of this premise, OPS is the main antigen targeted by current vaccine approaches. Serum antibodies against plasmid-encoded antigens have been detected in individuals living in *Shigella*-endemic regions; these antibodies increase during convalescence and have been implicated in protective immunity.^{18,19,21} The magnitude of serum antibodies specific for virulence factors such as the invasion plasmid antigen (Ipa) proteins A, B, and C, and VirG at the time of challenge was found to be associated with reduced incidence of disease in US adult volunteers in a controlled infection study.^{22,23} Similarly, the frequency of IpaB-specific memory B cells in these individuals was linked to lower risk of infection.²⁴ In addition to serum antibodies, antibody-secreting cells (ASC) and antibody in lymphocyte supernatants (ALS) measured in circulating peripheral blood mononuclear cells (PBMC) 7–10 days following exposure to *Shigella* are used as indicators of robust mucosal immunity.^{25,26}

Vaccine-induced immunity and the relevance of antibodies

There is no approved vaccine to prevent shigellosis. A variety of *Shigella* vaccine concepts have been proposed and tested mainly for safety and immunogenicity in clinical studies (Reviewed in ref.²⁷). Modeling natural infection, oral *Shigella* vaccines are among the leading candidates tested in human clinical trials during the past decade. These include the whole cell inactivated *S. flexneri* 2a Sf2aWC,²⁸ and the live attenuated *S. flexneri* 2a CVD 1208S,²⁹ *S. flexneri* 2a SC602,³⁰ and *S. sonnei* WRSs2 and WRSs3.³¹ Orally delivered whole cell organisms generally elicit high levels of LPS-specific serum IgG and IgA as well as fecal IgA, and are potent inducers of LPS-specific IgA ASC. *Shigella* OPS-protein conjugate vaccines have gained interest and support in recent years due to the record of safety and ability to elicit potent systemic immunity. The concept of a *Shigella* conjugate vaccine was pioneered by John Robbins and colleagues at the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) in the early 1990s. A candidate vaccine produced by this group consisting of OPS from *S. sonnei* covalently bound to recombinant exoprotein A of *Pseudomonas aeruginosa* (rEPA) had an efficacy of about 70% in young adults in the Israeli military³² and in 3–4-year-old children in a Phase 3 clinical trial.³³ Protection was associated with the presence of high levels of serum IgG against the O-antigen. Unfortunately, the vaccine had no effect in 1–2-year olds, who would be primary targets for immunization. It remains unclear whether the protection seen in adults and in older children derived solely from vaccination or from boosting of pre-existing immunity acquired through natural exposure. A newer *Shigella* conjugate, Flexyn2a, was engineered based on the same principle, except this time using an elegant and simpler *in vivo* bioconjugation of the *S. flexneri* 2a OPS to rEPA. Flexyn2a elicited strong serum antibody and ALS responses in young adult volunteers, and up to 50% protection against severe shigellosis in an experimental human challenge.^{34,35} Another glycoconjugate vaccine candidate, SF2a-TT15, featuring repeating units of synthetic O-antigen from *S. flexneri* 2a conjugated to tetanus toxoid, has been shown to be safe and strongly immunogenic in a Phase 1 study.³⁶ An immunogenicity and efficacy study of SF2a-TT15 is ongoing at the University of Maryland in collaboration with Institut Pasteur, University of Tel Aviv, and The Walter Reed Army Institute of Research (WRAIR).

Shigella vaccine candidates that leverage the potential to offer broad protection have also advanced into clinical evaluation. One promising strategy is the Generalized Modules for Membrane Antigen (GMMA), which contain the O-antigen (and can be engineered to express O-antigen from multiple *Shigella* serotypes) plus outer membrane and periplasmic proteins. The *S. sonnei* prototype, GSK 1790GAHB, has been shown to be safe and immunogenic in clinical trials in naïve adult populations from France and the United Kingdom³⁷ and in adults living in Kenya, where *Shigella* is endemic.³⁸ The efficacy of this vaccine is under evaluation in a controlled human challenge study in adult volunteers sponsored by GlaxoSmithKline (NCT035227173). Another example is the

macromolecular complex Invaplex, which consists of the native lipopolysaccharide (LPS) and invasion plasmid antigen (Ipa) proteins B and C. The broad spectrum of this vaccine relies on immunity against the Ipa proteins, which are highly conserved among *Shigella* serotypes. Invaplex has been found to be safe and immunogenic in humans.³⁹ A Phase 1 dose-escalating study of detoxified *S. flexneri* 2a artificial complex (Invaplex AR-DETOX) administered intramuscularly to adult volunteers is being conducted at WRAIR (NCT03869333).

The inflow of new vaccine concepts and clinical studies, including refined, controlled human infection models (CHIM), and the strategic support of stakeholders have created unprecedented momentum, making this an exciting and hopeful time for a *Shigella* vaccine to finally materialize.^{40,41} The upcoming vaccine and challenge studies, in particular, offer an extraordinary opportunity to dissect host responses and mechanisms of protective immunity in the context of different immunization strategies. Specific ways in which antibodies deploy anti-microbial function to prevent *Shigella* infection as well as the potential of functional antibodies to predict protective immunity against shigellosis are discussed below.

Functional antibodies as immunological correlates of protection

Understanding the mechanisms harnessed by the host immune system to prevent infection and avert pathogenicity is essential to identify correlates of protective immunity.⁴² Establishing the antimicrobial capacity of vaccine-induced antibodies is important to confirm that these antibodies can operate in such a manner *in vivo*. Impairment of antibody function (e.g. due to age, immune adaptation, underlying health conditions, or vaccine failure) cannot be discerned by traditional serological methods (such as ELISA) that rely only on antibody binding to an immobilized antigen. In contrast, functional assays appraise not only the magnitude of an antibody response but also the operative anti-microbial activity. A functional outcome may reflect the contribution of multiple antibody specificities as opposed to antibody titers directed to a single, independent antigen, and therefore may be more informative of the breadth of host immunity, and potentially a more robust tool to predict vaccine-induced protection. The production of functional antibodies requires, and therefore indirectly reflects, strong underlying T-cell immunity, which is more difficult to determine and is an impractical primary/secondary clinical trial endpoint.

There are multiple examples for the use of functional antibodies as immune correlates of protection against mucosal bacterial pathogens. Based on evidence that clearance of *Streptococcus pneumoniae* in the lungs is primarily achieved by opsonophagocytosis,⁴³ opsonophagocytic antibodies have been the primary immunogenicity readout of pneumococcal polysaccharide vaccines.^{44,45} Bactericidal antibody activity has been associated with protection against meningococcal infection.^{46,47} Vibriocidal antibodies are indicators of protective immunity against *Vibrio cholerae*.^{48,49} These correlates have helped advance vaccine candidates through the regulatory pathway. The assessment of functional antibody responses post-vaccination, when assays are available, is recommended by the World Health

Organization (WHO) Expert Committee on Biological Standardization.⁵⁰ The following sections discuss the relevance of functional antibody to *Shigella* immunity.

Antibody function relevant to *Shigella* immunity

Classical studies demonstrated the capacity of serum antibodies from patients with shigellosis to activate complement and promote bactericidal activity.⁵¹⁻⁵³ Immune sera from convalescent individuals were reported to mediate phagocytosis by K-lymphocytes, monocytes, and granulocytes present in circulation.⁵³⁻⁵⁵ Bactericidal antibody activity against endemic *Shigella* strains has been demonstrated in the context of natural infection in adults living in Israel³² and Bangladesh.^{53,56} Our group reported associations between elevated serum bactericidal antibody (SBA) and opsonophagocytic killing antibody (OPKA) titers at the time of challenge and protection from moderate to severe disease in a challenge study of North American volunteers.²² *Shigella* SBA activity has been attributed primarily to antibodies specific for the OPS,⁵⁷ although this may not be the only target of antibody and complement-mediated killing.²² That may also be the case for antibodies mediating opsonophagocytic killing, since both SBA and OPKA tend to follow the same response pattern.²² Adult volunteers immunized with the conjugate vaccine candidates SF2a-TT15 and Flexyn2a had robust SBA titers^{34,36} and, incidentally, Flexyn2a had >50% efficacy in a subsequent challenge study.³⁵ Detailed analysis of antibody function and clinical outcomes emerging from these and other well-controlled CHIM studies will provide critical insights into the value of functional serological readouts as predictors of vaccine efficacy.

Given that *Shigella* is an enteric pathogen, protective immune effectors likely operate within the gut. Antibodies, as primary humoral effector molecules, may be produced locally or transported via circulation. We reported the presence of bactericidal and opsonophagocytic killing antibody activity in ALS from human volunteers immunized orally with whole cell inactivated and live attenuated *Shigella* vaccines.⁵⁸ ASC are considered robust markers of *Shigella*-specific B cells (plasmablasts) that originate in and migrate back to the gut,⁵⁹ and ALS represent the antibodies produced *in vitro* by transiently circulating ASC.²⁶ It is therefore plausible that antibody-mediated, complement-dependent killing may represent a local (gut mucosal) mechanism of bacterial clearance. Such function would have to be mediated by IgG (or IgM), since IgA does not fix complement. An OPS IgG-mediated local bactericidal and phagocytic killing could explain the protective capacity of the *Shigella* conjugate vaccine approach. In the early 1990s, John Robbins proposed that IgG could transudate into the intestinal lumen and kill *Shigella* through complement-mediated lysis at the epithelial cell surface.^{60,61} We would argue that a combination of vaccine-induced IgG as well as IgM produced shortly after infection (both potent opsonin and complement fixing antibodies) are likely responsible for antibody-mediated protection against *Shigella in vivo*.

An important and often overlooked study conducted by Carol Tacket in the early 1990s showed that oral administration of bovine colostrum concentrate containing high levels of *Shigella* LPS-specific immunoglobulin protected adult

volunteers against shigellosis, thus providing direct *in vivo* evidence of IgG functional activity in the gut.⁶² We have detected LPS-IgG and antibody-mediated complement-dependent *Shigella*-cidal activity in maternal milk from mothers living in Malawi.⁶³ Together, these observations support a protective role of IgG (and possibly IgM) in mucosal secretions.

What then would be the role for IgA, and is it dispensable in *Shigella* immunity? A combination of protective effects has been attributed to LPS-specific agglutinating secretory IgA (SIgA), including reduced bacterial attachment to intestinal epithelial cell monolayers, maintenance of tight junction and cell morphology, and inhibition of pro-inflammatory pathways – all in the absence of other immune components.⁶⁴ Intestinal IgA produced by oral vaccines⁶⁵ may be an adjunct mode of bacterial immobilization and exclusion that helps preserve barrier integrity.

IgA against *Shigella* LPS and Ipa proteins have been found in breast milk.⁶⁶⁻⁶⁸ The formation of immune complexes could, likewise, be an effective mechanism of protection in the gut of the breastfeeding infant. The interaction between antibodies and cells in breast milk is an important area that deserves investigation. One study reported that leukocytes isolated from colostrum of healthy mothers and used as effector cells in phagocytic/bactericidal assays were able to kill *Shigella* in the presence of antibodies.⁶⁹

Antibodies targeting *Shigella* antigens other than LPS likely deploy additional effector functions. Antibodies directed to the Ipa proteins would be expected to inhibit TTSS protein translocation⁷⁰ and prevent invasion of epithelial cells.⁷¹ We have shown that combined murine IpaB and IpaD antibodies did not exhibit complement-mediated killing activity, but they inhibited *Shigella*-induced macrophage toxicity.⁷² Antibodies against VirG (IcsA) could be important for inhibition of bacterial adhesion⁷³ or cell-to-cell spread.⁷⁴ Blocking *Shigella* invasion in this way conceivably averts the release of pro-inflammatory cytokines and the recruitment of neutrophils, which exacerbates the damage of the intestinal epithelium. The exact operative mechanisms of antibodies against the multiple *Shigella* antigens (antibody type, interacting cells, and other contributing molecules) and their contribution to protection *in vivo* remain to be elucidated.

Functional antibodies as immunological correlates of protection against shigellosis and protective thresholds

From the perspective of vaccine development, evaluation and licensure, it would be important and advantageous to identify an immunological endpoint or immune signature that correlates with clinical protection against shigellosis. These immune effectors might be mediators of protection themselves or indirectly reflect other components or determinants of protective immunity.

An immune correlate of protection requires a well-defined cutoff value, or threshold, that could be used to reliably infer vaccine effectiveness, alleviating the need for human challenge or efficacy studies.⁷⁵ Ideally, a correlate of protection should be established as early as possible in the process of vaccine development. Such a threshold would allow differentiating

individuals with adequate immunity from those at risk of contracting disease. It may be an absolute number above which protection is denoted, or relative, meaning that protection increases with higher threshold values.⁷⁶ As an example, to evaluate group B meningococcal vaccines with an SBA assay that employs baby rabbit complement, a titer $\geq 1:8$ was established as a protective threshold.⁴⁶ In the case of *S. pneumoniae*, establishment of an opsonophagocytic threshold titer of $\geq 1:8$ allows the use of non-inferiority trials to assess subsequent vaccines, and was the basis for the licensure of the 13-valent pneumococcal conjugate vaccine (PCV-13).⁷⁷

Important aspects in the definition of a protective threshold include: differences in populations, age dependency, and vaccine compositions.

Differences in population and pre-existing immunity

Shigella vaccine efficacy traditionally studied in industrialized nations cannot be extrapolated to other geographical settings. Individuals living in endemic regions and repeatedly infected with *Shigella* acquire natural immunity that includes high levels of antibodies (Figure 1(a)) as well as mucosal and systemic T and B cell-mediated immunity. An initial infection prompts an IgM and modest IgG response, while subsequent infections trigger recall immunity with rapid increases in serum IgG in repeating

cycles, as illustrated in Figure 1(a). An SBA response originally mediated by IgM is later supplemented and eventually replaced by an overwhelming IgG-mediated bactericidal activity, as has been shown for *V. cholerae*.⁷⁸ In agreement with this model, an increase in the length of exposure to *Shigella* has been shown to improve protection in field settings.⁷⁹

In contrast, populations living in industrialized regions (North America or Europe) are immunologically naïve and highly susceptible to infection (Figure 1(b)).^{38,80} These individuals typically respond vigorously to vaccine candidates. Ideally, a safe and effective vaccine given to naïve individuals should induce a strong and effective IgG immune response resulting in serum antibody levels above a protective threshold and comparable to, or better than, those found in naturally immune individuals with few required boosters (Figure 1(a,b)). Unfortunately, this was not achieved with previous oral candidates for which immunogenicity and efficacy in an industrialized region could not be reproduced in endemic areas.^{30,81} The microbiome or other pathogens within the gut microenvironment in these settings might have played a role in diminishing the immune response. The gut microbiome and enteric host factors that may directly affect oral immunization are not expected to influence immune responses induced by the new parenteral OPS conjugate formulations.

Another important consideration in the evaluation of *Shigella* vaccines, inherent to specific populations, is the blunting of

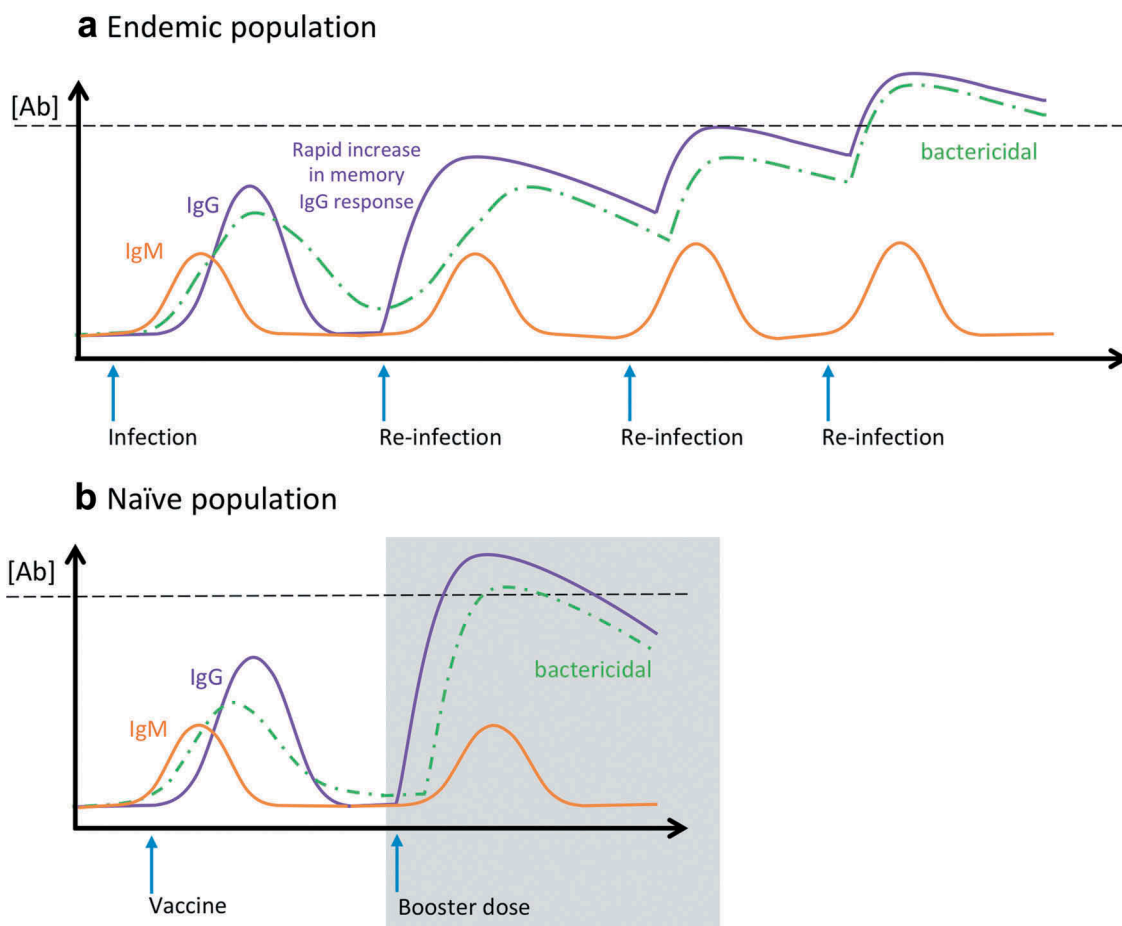


Figure 1. Protective immunity and threshold of protection. Schematic representation of IgM, IgG, and serum bactericidal antibody responses measured following (a) infection and multiple re-infections in an endemic population, and (b) initial vaccination and booster dose administered to a naïve population. Black dashed line represents threshold of protection.

responses by preexisting immunity. This is a complex phenomenon that affects not only responses to *Shigella* but to other vaccines as well (i.e. measles, pertussis). A variety of possible mechanisms (antibody level, type of antigen administered, B cell receptor interaction, etc.) have been proposed.⁸² How exactly and why pre-existing immunity precludes further *Shigella* vaccine responses is not well understood. In recent clinical studies, volunteers have been screened prior to enrollment to exclude those with high levels of serum LPS-IgG, the rationale being that this scenario better represents non-immune vaccine target groups (i.e. young children). Interestingly, immune blunting does not appear to affect in the same way (or to the same extent) individuals from endemic areas who experience multiple cycles of infection that gradually boost immunity (Figure 1(a)). One possibility is the higher stringency of infection compared to vaccination, and the fact that (a more potent) infection may override effectors or responses that prevent vaccine-induced immune activation. For potent vaccines, this may not be an issue. The type of vaccine and route of administration may also influence the outcome. Understanding the influence of pre-existing immunity in immunological priming against *Shigella* will be critical for the success of any vaccine. The effects of prior immunological exposure on vaccine take (when immune blunting occurs, why it occurs, and who is affected) is therefore a fundamental topic that warrants further investigation.

Differences in age groups

Host susceptibility, immune development, and competence are different for children and adults. Therefore, antibody effector functions and even threshold levels that determine protective immunity in adults may not be the same as those required in young children. Infants and toddlers up to about 2 years of age are more susceptible to *Shigella* than older children and adults¹⁷ and their responses to vaccines are also lower than that of adults.³³

Back-tracking to early infancy, *Shigella*-specific antibodies seem to be efficiently transferred from mothers to infants, as shown by the levels detected in cord blood at birth.⁸³ However, the functionality of these antibodies remains to be investigated. In a study of maternal-infant transfer of functional antibodies against *E. coli* and *Salmonella*, Gitlin and colleagues found that unlike the paired maternal sera, most of the cord blood antibodies lacked bactericidal antibody activity – this functionality was contained primarily in the maternal IgM fraction and therefore not transferred through the placenta.⁸⁴ For newborns who did exhibit serum bactericidal activity, this was found to be IgG-specific and correlated with IgG-mediated functional antibody activity in maternal blood.⁸⁴ Systemic placentally acquired antibodies wane at about 4 months of age at which time infants rely on passive protection through immune components in maternal milk. Vaccination within this time frame would have the highest impact in preventing disease. The efficacy of this approach would have to be answered by studies in the field.

Because young children lack other potentially enhancing mechanisms such as robust T and B cells, innate immunity, and cross-reactive adaptive immunity, more stringent requirements than those defined for adults may be in order. This has

been proposed for seasonal influenza: while a hemagglutination inhibition (HAI) titer of >40 appears sufficient to protect adults against seasonal influenza, young (unprimed) children may require an HAI titer of >330.^{85,86} Establishing what constitutes protective immunity against shigellosis in young children and identifying thresholds of protection for vaccine target age groups are challenges for the immediate future.

Vaccine composition

Because of the differences in vaccine platforms, antigenic composition, and/or mode of delivery, an immune correlate of protection identified for one vaccine may not be applicable to another. For example, SBA and OPKA may be appropriate to assess OPS-based vaccine efficacy but would not be suitable for protein-based subunits. Mucosal responses induced by oral vaccines (ASC, ALS, fecal antibodies) may not be relevant to conjugate vaccines given parenterally. Different functionality, antibody specificity or even type of immunological readout may be necessary to properly assess some of the new vaccine concepts. A combination of end-points reflecting the contribution of multiple antigens may be necessary to accommodate multivalent (e.g. broadly protective) formulations.

Composite antibody measurements and immune signatures

Instead of a single readout, establishing an immune correlate comprised of an aggregate (or composite) of antibody measurements would be a more robust approach to define protective status. Such composite could include serum antibodies (levels, function, and avidity) and/or mucosal antibodies (ALS levels, function, and avidity). A recent microarray analysis identified the *Shigella* conserved proteins IpaA, IpaB, and IpaC as an immune signature associated with protection from severe disease.²³ Mathematical models⁸⁷ and machine learning⁸⁸ tools have the potential to probe complex datasets consisting of multiple parameters across multiple studies to predict measurements that correlate with protective immunity. However, the value of these models is dependent on the breadth and quality of data available. The input from upcoming prospective CHIM studies can break new ground in the field. Systems serology, a new technology that combines the analysis of biophysical features of antibodies and their broad functional capacity using bioinformatic analyses, would be useful to investigate and establish antibody features and novel cell-associated activities relevant for protection against shigellosis. This method was used to interrogate and compare Fc-mediated immune responses from multiple vaccine trials; it allowed successful identification of an “Fc fingerprint” associated with protective immunity against HIV,⁸⁹ and discrimination of antibody function in latent versus active TB.⁹⁰

Going forward

What constitutes an effective (protective) response against shigellosis through a “serological lens” remains to be defined. A careful serological analysis of sick versus healthy individuals during case-control and/or household contact studies could yield threshold

values to distinguish immune from at-risk individuals. Thresholds of protective immunity can also be established through CHIM. This method, however, traditionally involves a different (non-target) population and an artificial setting (dose, interval between vaccination and challenge, administration of antibiotics post-challenge). Mechanistic analyses are necessary to delineate the contribution of antibodies in promoting bacterial clearance and preventing *Shigella* infection in the gut mucosa. It will be important to know the antigenic targets and type of antibodies (i.e. IgG subclasses) involved, the intrinsic properties of functional antibodies (e.g. structure, glycan profile), and their interactions with innate immune cells and other host cells. Investigating the capacity of antibodies to interfere with different steps of *Shigella* pathogenesis and methods that can reliably quantify such antibodies will expand the assay repertoire to facilitate vaccine development and evaluation. Selection, optimization, and harmonization of assays will enable comparative analysis of results derived from multiple studies. The identification and utilization of calibrated reagents (i.e. antigens, standard sera) would be necessary to define and implement a reliable protective threshold or serological cutoff.⁹¹ High throughput technologies, including automation, multiplexing,⁴⁴ and luminescence-based assays⁹² will facilitate and simplify the implementation of functional assays. A recently reported *S. flexneri* 2a, *S. flexneri* 3a, and *S. sonnei* SBA qualification and demonstration of interlaboratory agreement is a step towards wider adoption of functional assays.⁹³ A consortium sponsored by Bill and Melinda Gates Foundation and coordinated by the National Institute for Biological Standards and Control (NIBSC), with the participation of WRAIR, University of Maryland, University of Tel Aviv, GSK Vaccines Institute for Global Health (GVGH), and Johns Hopkins University is working toward a harmonized LPS IgG assay and the creation of a standard control. Similar efforts would facilitate rigorous and standardized measurement of functional antibodies.

Concluding remarks

There is precedent for the use of functional antibodies as correlates of protection and promise for their use as predictors of protective immunity to *Shigella* infection. The induction of high levels of antibodies with a defined anti-bacterial effect is indicative of more complex and robust responses that involve T cell activation and B cell expansion in response to infection and vaccination. Functional antibody assays can be performed rigorously in laboratories that conduct low complexity tests and are simple and reliable tools to evaluate vaccine-induced and natural immunity. Upcoming CHIM and field efficacy studies will offer the opportunity to carefully examine markers and thresholds of protection in different settings, including endemic populations. The ultimate goal would be to identify a robust serological correlate of protection in young children, who would benefit the most from a *Shigella* vaccine.

Acknowledgments

We are grateful to Dr. Ed Oaks for critical reading of this manuscript and Dr. Robert Kaminski for insights and discussions on these topics.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by NIH awards R01AI117734 and R01AI125841 to M.F.P. and a Research Supplement to Promote Diversity in Health-Related Research Program, 3R01AI117734-04S1, to E.N.

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