# *Shigella*-Specific Immune Profiles Induced after Parenteral Immunization or Oral Challenge with Either *Shigella flexneri* 2a or *Shigella sonnei*

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**ABSTRACT** *Shigella* spp. are a leading cause of diarrhea-associated global morbidity and mortality. Development and widespread implementation of an efficacious vaccine remain the best option to reduce *Shigella*-specific morbidity. Unfortunately, the lack of a well-defined correlate of protection for shigellosis continues to hinder vaccine development efforts. *Shigella* controlled human infection models (CHIM) are often used in the early stages of vaccine development to provide preliminary estimates of vaccine efficacy; however, CHIMs also provide the opportunity to conduct in-depth immune response characterizations pre- and postvaccination or pre- and postinfection. In the current study, principal-component analyses were used to examine immune response data from two recent *Shigella* CHIMs in order to characterize immune response profiles associated with parenteral immunization, oral challenge with *Shigella flexneri* 2a, or oral challenge with *Shigella sonnei*. Although parenteral immunization induced an immune profile characterized by robust systemic antibody responses, it also included mucosal responses. Interestingly, oral challenge with *S. flexneri* 2a induced a distinctively different profile compared to *S. sonnei*, characterized by a relatively balanced systemic and mucosal response. In contrast, *S. sonnei* induced robust increases in mucosal antibodies with no differences in systemic responses across shigellosis outcomes postchallenge. Furthermore, *S. flexneri* 2a challenge induced significantly higher levels of intestinal inflammation compared to *S. sonnei*, suggesting that both serotypes may also differ in how they trigger induction and activation of innate immunity. These findings could have important implications for *Shigella* vaccine development as protective immune mechanisms may differ across *Shigella* serotypes.

**IMPORTANCE** Although immune correlates of protection have yet to be defined for shigellosis, prior studies have demonstrated that *Shigella* infection provides protection against reinfection in a serotype-specific manner. Therefore, it is likely that subjects with moderate to severe disease post-oral challenge would be protected from a homologous rechallenge, and investigating immune responses in these subjects may help identify immune markers associated with the development of protective immunity. This is the first study to describe distinct innate and adaptive immune profiles post-oral challenge with two different *Shigella* serotypes. Analyses conducted here provide essential insights into the potential of different immune mechanisms required to elicit protective immunity, depending on the *Shigella* serotype. Such differences could have significant impacts on vaccine

design and development within the *Shigella* field and should be further investigated across multiple *Shigella* serotypes.

*Shigella* spp. are a major cause of diarrheal disease-associated morbidity and mortality, with approximately 75 million annual episodes of shigellosis and the greatest burden observed in children younger than 5 years of age living in low- and middle-income nations (1–4). *Shigella* also remains an important cause of travelers' diarrhea. *Shigella* morbidity extends beyond acute gastroenteritis, and infection is associated with multiple postinfectious sequalae, including reactive arthritis, irritable bowel syndrome (5, 6), and physical and cognitive stunting in children (7, 8). Furthermore, children with repeated enteric infections are also at a higher risk of mortality due to other infectious diseases (9). Although antibiotics are generally effective in treating shigellosis, increasing antibiotic resistance has served to greatly diminish their effectiveness. Consequently, efforts to accelerate the development of protective interventions, such as vaccines, have been expanded (10, 11). While several *Shigella* vaccine candidates are under clinical investigation (12, 13), there is currently no widely available licensed vaccine.

A hindrance to *Shigella* vaccine development has been the lack of a well-defined immune correlate of protection (CoP). When defining CoPs, one must consider that they can be mechanistic (mCoP) or nonmechanistic (nCoP), with mCoPs causally associated with protective efficacy and often functioning at the site of infection. While nCoPs are not the causal means of protective immunity, they can predict protection and serve as surrogate measures for mCoPs (14). A CoP can guide the rational design and evaluation of candidate vaccines and can also be used to accelerate licensure, especially where alterations or additions to pathogen serotypes are required (15).

While there is not a defined CoP for shigellosis, *Shigella* challenge/rechallenge studies have shown that prior infection protects from subsequent infection in a serotype-specific manner for at least 6 months to a year (16, 17). Results from these studies demonstrate the importance of the O antigen in *Shigella* lipopolysaccharide (LPS) as a key protective antigen, leading to the clinical evaluation of several conjugate vaccine candidates (18–20). These studies have indicated that LPS-specific serum IgG may serve as a CoP for *Shigella* infection (21–23). However, it is unclear if LPS-specific serum IgG is an mCoP via IgG transudate into the intestinal lumen or an nCoP or surrogate measure for a yet to be defined immunological protective mechanism (21, 24). Additional CoPs have also been suggested, including LPS-specific IgA-secreting B cells (ASCs) (25), LPS-specific IgA-secreting memory B cells (26), and even a combination of LPS-specific serum IgG and IgA ASCs (27, 28). The importance of LPS-specific serum IgA has also been discussed in the context of its association with a reduced risk of disease post-oral challenge with *S. sonnei* (29). Additional *Shigella* antigens, including the surface-exposed invasion plasmid antigen (Ipa) proteins, may also be important vaccine targets given that oral challenge or immunization with live attenuated vaccine candidates can induce robust immune responses to the Ipa proteins (30). Additionally, IpaB-specific memory B cell (26) and serum IgG (unpublished data) responses have been associated with resistance to infection or progression to disease in a controlled human infection model (CHIM) setting.

Investigation of CoPs and developing efficacious vaccines is often complicated by multiple host and environmental factors, such as population genetics or epigenetics, the target population versus the investigational population, and host nutritional or immune status, as well as infection history, preexisting immunity, and/or concurrent infections (14, 31–36). Furthermore, pathogen strain or serotype, route of infection, and dose/bacterial load can influence not only pathogen virulence but also the ensuing host immune responses (37–41). Consequently, attempts to define a single

immune correlate may not adequately consider these complexities impacting host immune responses or host-pathogen interactions.

Recent omics-based research has provided helpful insights into host responses to vaccination and experimental challenge (31, 42–46) and has demonstrated that protective immunity often requires an integrated and networked immune response profile (47). Immune response profiles postvaccination or postchallenge have been described or proposed for several different pathogens (45, 48–52). Recently, *Salmonella enterica* serovar Typhi-specific immune response profiles have been described after parenteral immunization with two different vaccine constructs (capsular polysaccharide alone or conjugated to tetanus toxoid) followed by oral challenge with live *S.* Typhi (52). Interestingly, while both vaccine constructs provided similar levels of efficacy postchallenge, the polysaccharide alone protected from challenge through a serum IgA-dominated response, while the tetanus toxoid polysaccharide conjugate protected subjects through increased serum IgA responses in conjunction with high-avidity serum IgG1 antibodies (52). Different protective immune profiles have also been described for different vaccine constructs and/or immunization routes in the case of cholera (53) and polio infection (54, 55).

As *Shigella* spp. are enteric pathogens with a complex life cycle and over 50 different serotypes, there may be multiple host immune mechanisms that could impact infection, intestinal invasion, and the severity or duration of disease. Given these complexities associated with *Shigella* infection, protection from shigellosis may be better described as an immune profile rather than a single immune correlate. In order to investigate *Shigella*-specific immune profiles, immune response data previously generated from two *Shigella* CHIMs (29, 56–58) were compiled for use in principal-component analyses (PCA). The use of such multidimensional analyses aids in examining the combined contribution of several immune response parameters to the generation of a protective immune profile and has been recently used to gain greater insights into correlates of protection associated with typhoid (52). In the current study, PCA were used to investigate similarities and/or differences in immune profiles across the following three exposures: (i) parenteral immunization with a *S. flexneri* 2a bioconjugate vaccine, (ii) oral challenge with *S. flexneri* 2a 2457T, and (iii) oral challenge with *S. sonnei* 53G. PCA results revealed potential differences in immune response profiles, not only across the route of exposure (parenteral versus oral), but also across the *Shigella* challenge strain (*S. flexneri* 2a versus *S. sonnei*). Such comparative analyses may help broaden our understanding of protective immune mechanisms associated with different routes of antigenic delivery and could inform *Shigella* vaccine development efforts.

## RESULTS

**Comparison of immune profiles after parenteral immunization versus oral challenge. (i) PCA results and loading plot.** Populations 1 (Fig. 1A) and 2 (Fig. 1B) were used to compare protective immune profiles associated with parenteral immunization or oral challenge with *S. flexneri* 2a 2457T. The following immune response variables had a Pearson's $r$ of ≥0.3 with at least one other immune response variable (data not shown) and were therefore included in the PCA: *S. flexneri* 2a LPS-specific serum IgG, IgA, IgM, IgG1, IgG2, IgG3, and IgG4, memory B cell antibody in lymphocyte secretion (ALS) IgG and IgA, $\alpha 4 \beta 7^+$ and $\alpha 4 \beta 7^-$ ALS IgG and IgA, and *S. flexneri* 2a 2457T-specific serum bactericidal activity (SBA). Peak responses postvaccination (population 1) or postchallenge (population 2) were used for all immune response variables. Data sets were considered adequately sized for use in PCA, with a Kaiser-Meyer-Olkin (KMO) value of 0.761 and a significant result ($P < 0.0001$) in the Bartlett test for sphericity (data not shown). Principal components 1 to 4 (C1 to C4) had eigenvalues of ≥1 and together explained 75.7% of the variability in immune responses across populations 1 and 2. The first two components were used for population comparisons, with C1 and C2 accounting for 45.5% and 12.2% of the total variability, respectively (Fig. 2). All immune response variables were positively correlated with C1, indicating that this component represents the presence, or magnitude, of an immune response (Fig. 2A).
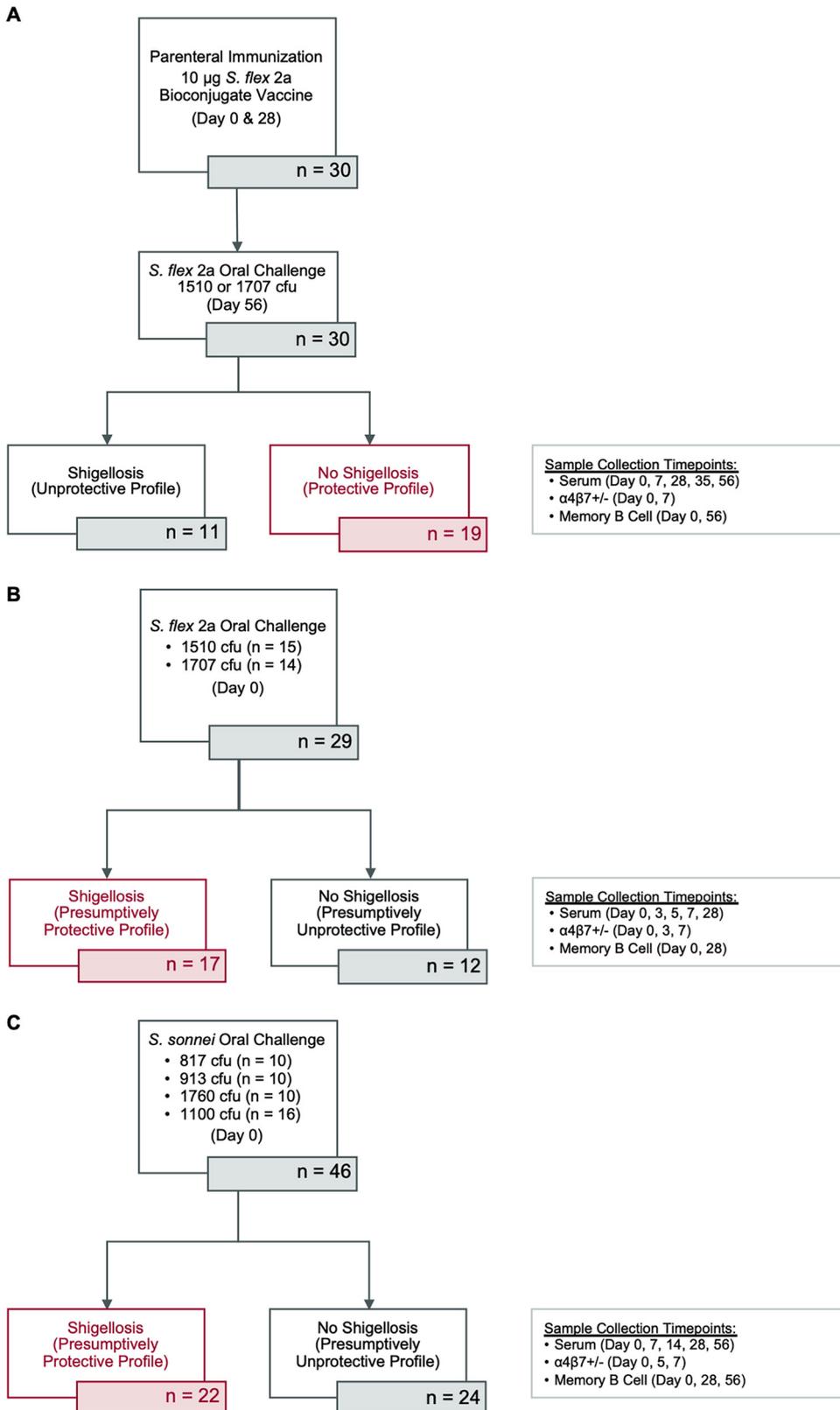
**A**



**B**



**C**



**FIG 1** Flow chart of populations 1 to 3 used for analyses, including treatments, sample collections, and timelines. (A) Subjects parenterally (i.m.) immunized with a *S. flexneri* 2a bioconjugate vaccine and subsequently orally challenged with *S. flexneri* 2a 2457T, (B) subjects orally challenged with *S. flexneri* 2a 2457T without any prior intervention, and (C) subjects orally challenged with *S. sonnei* 53G without any prior intervention.
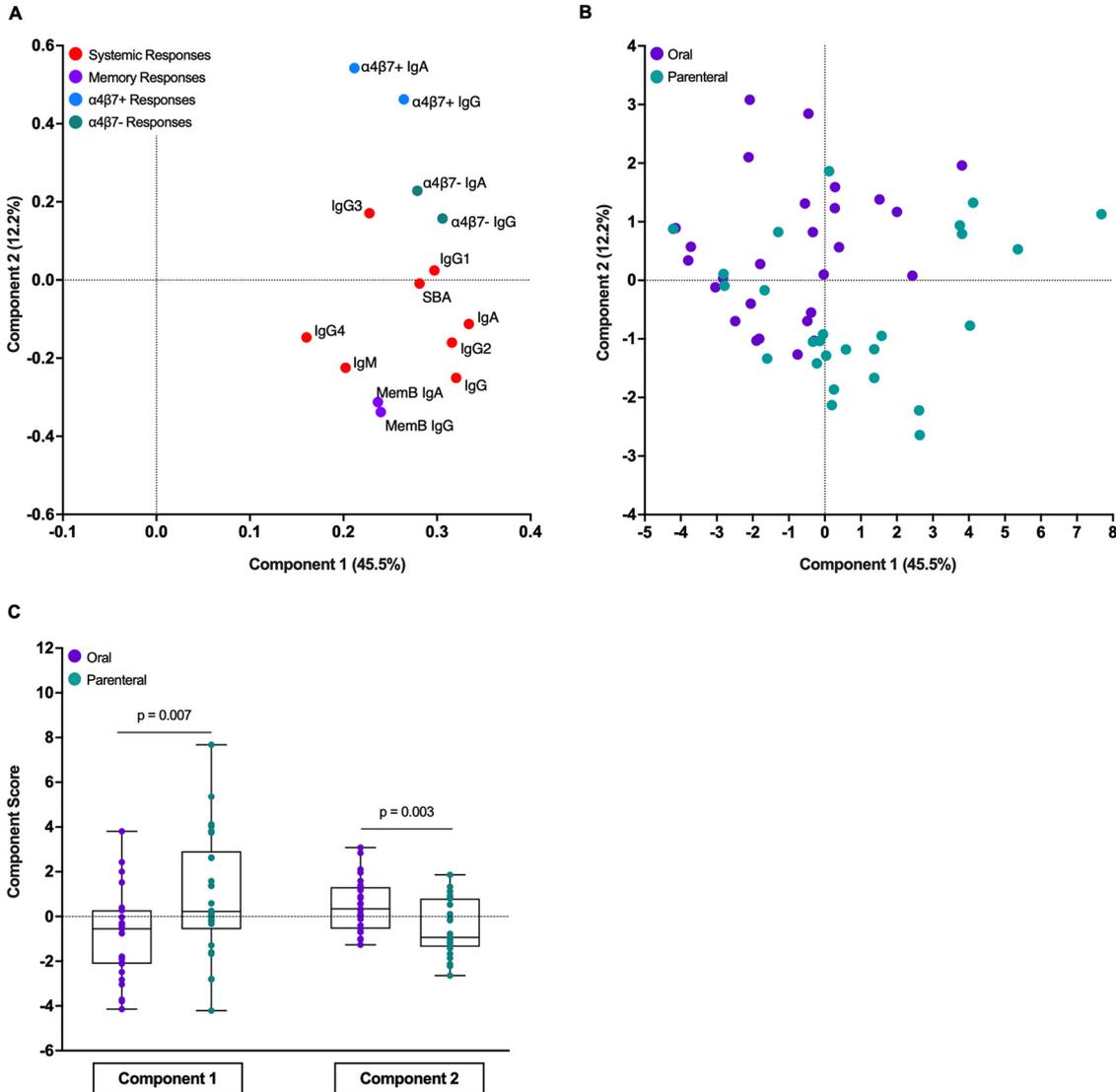
**FIG 2** PCA results comparing components 1 and 2 in subjects either parenterally immunized with a bioconjugate vaccine or orally challenged with *S. flexneri* 2a. (A) PCA loading plot for all systemic, memory, and mucosal immune response variables included in analysis, (B) component 1 and 2 scores for all subjects, grouped by exposure route, and (C) box and whisker (minimum to maximum) plots of component 1 and 2 scores across subjects either parenterally immunized or orally challenged. *P* values were determined by Welch's *t* test.

Interestingly, variables were spread across C2, generally grouping into anti-*S. flexneri* 2a LPS immune response variables that define a mucosal ($\alpha 4\beta 7^+$ and $\alpha 4\beta 7^-$ ALS IgG and IgA) or systemic (serum IgG, IgA, IgM, IgG1, IgG2, IgG3, and IgG4 and SBA) and memory B cell immune response (IgA and IgG memory).

**(ii) PCA scores grouped by exposure route.** Immune response profiles varied across exposure route (Fig. 2B). Parenterally immunized subjects were largely divided across C1, with 62% of subject scores positively correlated with C1 (see Table S2 in the supplemental material). In contrast, orally challenged subjects were divided across C2, with 67% of subjects having scores positively correlated with C2 (Table S2). C1 scores in parenterally immunized subjects were higher (*P* = 0.007) (Fig. 2C) than scores from orally challenged subjects, demonstrating the robust immune response induced by parenteral immunization. However, orally challenged subjects showed a stronger positive correlation with C2 than parenterally immunized subjects (*P* = 0.003) (Fig. 2C), indicating a stronger mucosal antibody response after oral challenge compared with
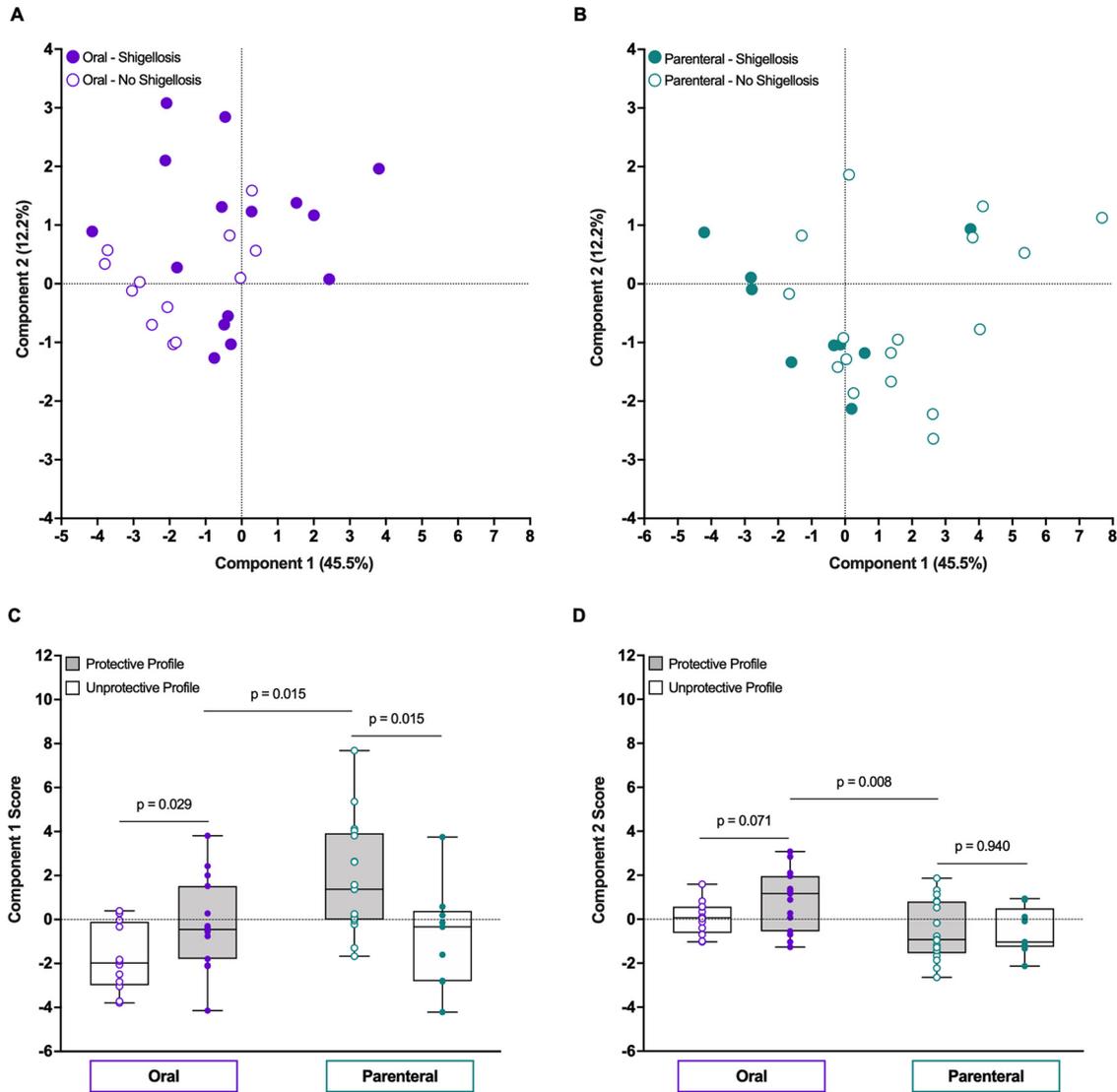
FIG 3 Comparisons of component 1 and 2 scores in subjects either (A) orally challenged with *S. flexneri* 2a or (B) parenterally immunized with a *S. flexneri* 2a bioconjugate vaccine, grouped by subjects with (closed circles) or without (open circles) shigellosis. Box and whisker (minimum to maximum) plots of (C) component 1 scores and (D) component 2 scores grouped by shigellosis outcome. *P* values were determined by Welch's *t* test.

parenteral immunization, which correlated more with serum and memory B cell antibody responses (Fig. 2).

**(iii) PCA scores grouped by shigellosis outcome.** The majority of scores from orally challenged subjects without shigellosis were negatively correlated with C1 (75%) and positively correlated with C2 (58%) (Fig. 3A; Table S2). In contrast, component scores from the majority of subjects with shigellosis (67%) were positively correlated with C2 and negatively correlated with C1 (Fig. 3A; Table S2). Subjects with shigellosis postchallenge showed a greater magnitude of response compared to subjects without shigellosis, represented by higher C1 scores (*P* = 0.029) (Fig. 3C). Although not significant (*P* = 0.071) (Fig. 3D), a similar trend is observed across C2 scores, with subjects developing shigellosis having higher mucosal responses compared to subjects without shigellosis (Fig. 3A, C, and D).

Parenterally immunized subjects developing shigellosis demonstrate a similar immune profile to orally challenged subjects not developing shigellosis (Fig. 3). In contrast, parenterally immunized subjects not developing shigellosis had robust systemic immune responses (see Fig. S1 in the supplemental material), with 77% having positive

C1 scores ($P = 0.015$) (Fig. 3C; Table S2). As expected, there were protected vaccinees clustered in the lower right quadrant of the score plot (Fig. 3B), highlighting the strong positive correlation with C1 and negative correlation with C2, indicative of the association of robust systemic responses with protection from shigellosis after parenteral immunization. Interestingly, 35% of protected vaccinees had scores positively correlated with C2 (Table S2), indicating that parenteral immunization with the bioconjugate vaccine is capable of inducing a protective immune response driven by mucosal antibodies (Fig. 3B; Fig. S1 and Table S2). Overall, protective immune response profiles comparing parenteral and oral exposure differed, with strong C1 (systemic/memory responses) correlations ($P = 0.015$) (Fig. 3C) in protected vaccinees, while orally challenged subjects progressing to shigellosis show a stronger correlation with C2 (mucosal immune responses) ($P = 0.008$) (Fig. 3D).

**Comparison of immune profiles after oral challenge with *S. flexneri* 2a or *S. sonnei*. (i) PCA results and loading plot.** Populations 2 (Fig. 1B) and 3 (Fig. 1C) were used to characterize presumptively protective immune profiles associated with *S. flexneri* 2a 2457T or *S. sonnei* 53G infection. The following immune response variables had a Pearson's $r$ of $\geq 0.3$ with at least one other immune response variable (data not shown) and were included in the PCA: *S. flexneri* 2a or *S. sonnei* LPS-specific serum IgG, IgA, IgM, IgG1, IgG2, and IgG3, memory B cell ALS IgG and IgA, $\alpha 4 \beta 7^+$ and $\alpha 4 \beta 7^-$ ALS IgG and IgA, and SBA directed to either *S. flexneri* 2a strain 2457T or *S. sonnei* strain Moseley. Postchallenge immune responses on day 7 ($\alpha 4 \beta 7^{+/-}$ ALS) or day 28 were used in analyses. Data sets were considered adequately sized for use in PCA, with a KMO value of 0.742 and a significant result ($P < 0.0001$) in the Bartlett test for sphericity (data not shown). Principal components 1 to 3 had eigenvalues of $\geq 1$ and together explained 68.3% of the variability in immune responses across populations 2 and 3. The first two components accounted for 43.1% and 15.9% of the total variability, respectively (Fig. 4). All immune response variables were positively correlated with C1, indicative of the magnitude of immune response (Fig. 4A). Immune response variables showed a high degree of clustering across C2, with a similar differentiation across this component of mucosal ($\alpha 4 \beta 7^+$ and $\alpha 4 \beta 7^-$ ALS IgG and IgA) or systemic (serum IgG, IgA, IgM, IgG1, and IgG2 and SBA) and memory (IgA and IgG memory) responses. Interestingly, an exception to this clustering pattern was observed with serum IgG3, which clustered with the $\alpha 4 \beta 7$ responses. The association of serum IgG3 and mucosal responses is interesting and deserves further investigation as serum IgG3 has been associated with protective immune responses across other mucosal pathogens, including HIV and influenza virus (59).

**(ii) PCA scores grouped by challenge strain.** No differences in C1 scores were observed across challenge strains ($P = 0.505$) (Fig. 4C). Additionally, a wide range in C1 scores was observed across both strains (Fig. 4B; see Table S3 in the supplemental material), indicating a large amount of variability in the magnitude of immune responses induced after challenge with either *Shigella* serotype. Importantly, component scores were largely divided across C2, with 59% of *S. sonnei*-challenged subjects having positively correlated C2 scores (Table S3) and an overall higher magnitude ($P < 0.0001$) (Fig. 4C) compared to subjects challenged with *S. flexneri* 2a. Moreover, the majority (78%) of subject scores after oral challenge with *S. flexneri* 2a were negatively correlated with C2 (Fig. 4B and C; Table S3), indicating a predominantly memory/systemic immune response profile compared to mucosal alone.

**(iii) PCA scores by shigellosis outcome.** Subjects receiving *S. sonnei* showed no differences in C1 scores among those with or without shigellosis ($P = 0.260$) (Fig. 5A and C). In contrast, subjects with shigellosis following *S. sonnei* challenge had significantly higher C2 scores ($P < 0.0001$) (Fig. 5A and D) compared to subjects without shigellosis, with 77% of subjects with shigellosis (Table S3) having positively correlated C2 values. The immune response profile driven by mucosal antibodies is consistent with the magnitude of $\alpha 4 \beta 7^+$ ALS IgG and IgA responses in *S. sonnei*-challenged subjects, comparing those with and without shigellosis (see Fig. S2 in the supplemental
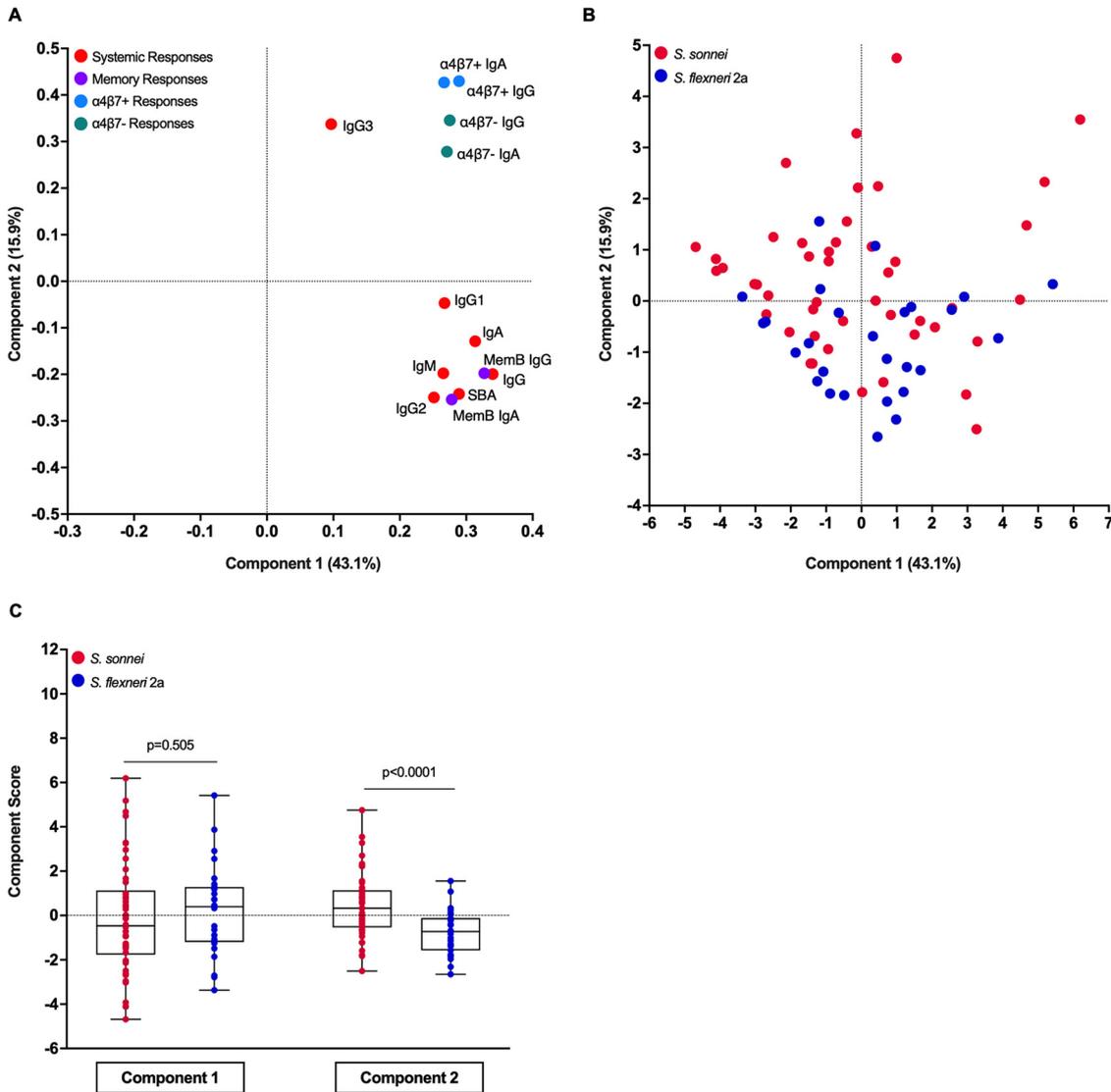
FIG 4 PCA results comparing components 1 and 2 in subjects orally challenged with either *S. flexneri* 2a or *S. sonnei*. (A) PCA loading plot for all systemic, memory and mucosal immune response variables included in analysis, (B) component 1 and 2 scores for all subjects, grouped by challenge strain, and (C) box and whisker (minimum to maximum) plots of component 1 and 2 scores across subjects orally challenged with either *S. flexneri* 2a or *S. sonnei*. P values were determined by Welch's *t* test.

material). Similar to previous reports (29, 56), no differences were observed in the systemic or memory responses across shigellosis outcome.

In contrast, approximately 80% of subjects progressing to shigellosis following *S. flexneri* 2a oral challenge (Table S3) had positively correlated C1 scores that were greater in magnitude ($P = 0.043$) (Fig. 5B and C) than those of subjects without shigellosis. Furthermore, no differences were observed in C2 scores across shigellosis outcome in *S. flexneri* 2a-challenged subjects ($P = 0.102$) (Fig. 5B and D). Minimal to undetectable immune responses were observed in *S. flexneri* 2a-challenged subjects not developing shigellosis, while in contrast, subjects developing shigellosis had a range of immune response magnitudes across multiple different immune parameters (Fig. 5B to D; Fig. S2). Overall, protective immune response profiles comparing oral challenge with either *S. sonnei* or *S. flexneri* 2a did not differ across C1 (systemic/memory responses) ($P = 0.440$) (Fig. 5C) but showed a strong segregation across C2 (mucosal immune responses) ($P = 0.0002$) (Fig. 5D).

**(iv) Fecal inflammatory marker responses.** Inflammatory marker concentrations were compared across subjects challenged with either *S. flexneri* 2a or *S. sonnei*. Prior
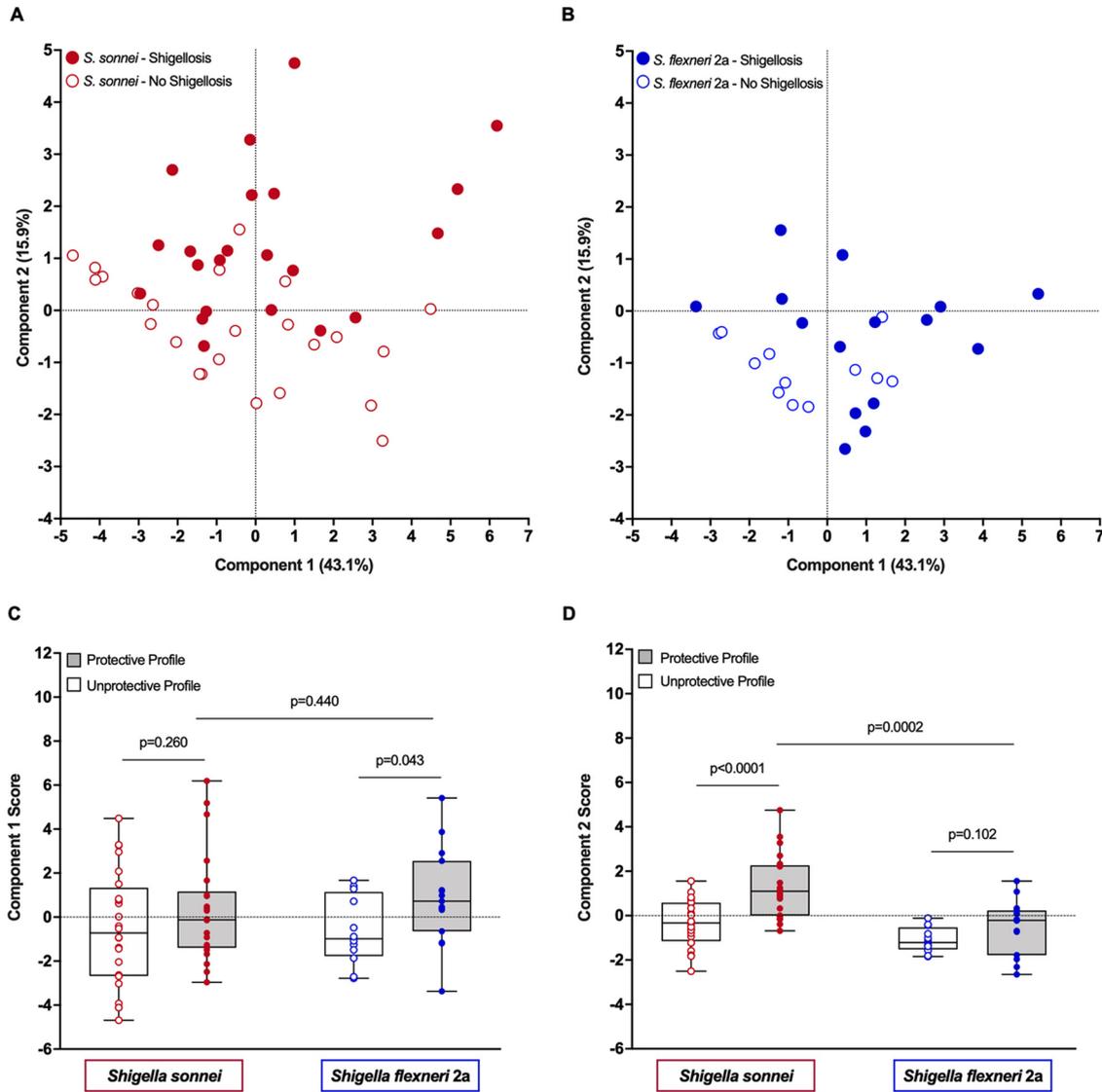
**FIG 5** Comparisons of component 1 and 2 scores in subjects orally challenged with either (A) *S. sonnei* or (B) *S. flexneri* 2a, grouped by subjects with (closed circles) or without (open circles) shigellosis. (C and D) Box and whisker (minimum to maximum) plots of (C) component 1 scores and (D) component 2 scores grouped by shigellosis outcome. *P* values were determined by Welch's *t* test.

to challenge, mean calprotectin and myeloperoxidase concentrations were within previously reported ranges considered normal for healthy North American adults (calprotectin, $\leq 60$ $\mu$g/g; myeloperoxidase, $\leq 10$ $\mu$g/g) (60), and peak inflammatory marker concentrations were observed approximately 3 to 4 days postchallenge with either serotype. Both myeloperoxidase ($P = 0.003$, *t* test) (Fig. 6) and calprotectin ($P = 0.006$, *t* test) (Fig. 6) levels 3 days postchallenge were significantly increased after *S. flexneri* 2a challenge compared to those of *S. sonnei*, indicating that, in a CHIM setting at the bacterial doses tested, *S. flexneri* 2a induces a higher degree of inflammation during infection, which suggests that these two challenge strains may also differ in how they trigger the innate immune response.

**Comparison of peak fold rise and percentage of responders across populations 1 to 3.** Peak fold rise in LPS-specific immune responses and percentage of responders (defined as a peak fold rise of $\geq 4$) were compared across the three populations, grouped by shigellosis outcome (Table 1). Subjects immunized parenterally with the *S. flexneri* 2a bioconjugate and protected from oral challenge had robust increases in serum IgG as well as moderate increases in serum IgG1, IgG2, and $\alpha 4\beta 7^{+/-}$ ALS IgG (all
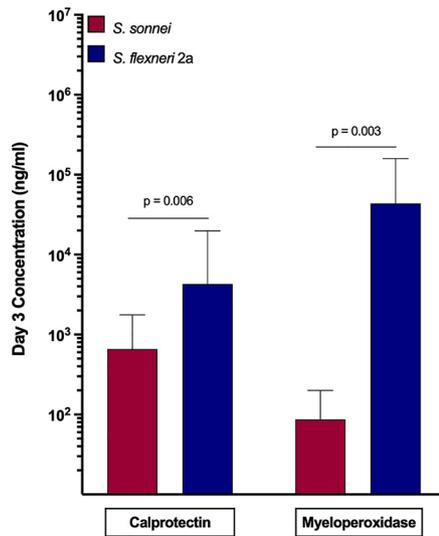
**FIG 6** Inflammatory marker concentrations (mean with standard deviation) 3 days postchallenge with either *S. sonnei* or *S. flexneri* 2a. *P* values were determined by *t* test of log-transformed concentrations.

$P \leq 0.01$, Mann-Whitney U) (Table 1). In contrast, unvaccinated subjects progressing to shigellosis after oral challenge with *S. flexneri* 2a had higher $\alpha 4\beta 7^+$ ALS IgG responses ($P = 0.018$, Mann-Whitney U) (Table 1) compared to parenterally immunized subjects protected from shigellosis, with the addition of significant increases in $\alpha 4\beta 7^+$ ALS IgA ($P = 0.033$, Mann-Whitney U) (Table 1). However, oral challenge with *S. flexneri* 2a also induced moderate increases across a range of systemic immune responses, including serum IgG and IgA and SBA (all $P \leq 0.05$, Mann-Whitney U) (Table 1).

Interestingly, a rather different response profile was observed after oral challenge with *S. sonnei*. Moderate increases in several systemic and functional antibody responses were observed after *S. sonnei* challenge; however, there were no differences in the magnitude of the increase across shigellosis outcome groups (Table 1). The differences resided in the $\alpha 4\beta 7^+$ ALS IgG and IgA responses after *S. sonnei* challenge, with the magnitude of fold rise and frequency of responders significantly increased ($P = 0.0001$, Mann-Whitney U) (Table 1) in subjects progressing to shigellosis. These findings indicate that subjects with shigellosis after oral challenge with *S. sonnei* develop a predominantly mucosal immune response, whereas oral challenge with *S. flexneri* 2a induces a more balanced systemic and mucosal response.

## DISCUSSION

Given that a CoP for *Shigella* infection has yet to be defined, immune response characterizations are key to furthering our understanding of *Shigella*-specific protective immune mechanisms. As previous *Shigella* infection protects against subsequent infections with the same serotype (16, 17, 61), investigation of immune responses among subjects developing disease postchallenge provides an opportunity to identify immune markers associated with recovery from infection and development of protective immunity. However, while investigating immune correlates or surrogates of protection is important, it is also essential to examine how multiple immune parameters work in concert to induce a protective immune profile, which may differ, depending on the initial route of antigenic exposure or bacterial serotype. In this regard, data presented here on the immune profiles induced after oral challenge with either *S. flexneri* 2a or *S. sonnei* suggest that one may have to consider the possibility of distinct protective profiles associated with these two *Shigella* serotypes.

*S. sonnei* is a unique serotype among *Shigella* spp. for several reasons, with one of the more recent findings revealing that *S. sonnei* harbors a type 6 secretion system

**TABLE 1** Comparisons of percentages of responders and fold rise responses across shigellosis outcome after parenteral immunization or oral challenge with either *S. flexneri* 2a or *S. sonnei*

| | Fold rise in immune response (% of responders)[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Parenteral immunization with *S. flexneri* 2a bioconjugate vaccine | | | Oral challenge | | | | | |
| | | | | *S. flexneri* 2a | | | *S. sonnei* | | |
| Response | No shigellosis | Shigellosis | P value | No shigellosis | Shigellosis | P value | No shigellosis | Shigellosis | P value |
| Serum | | | | | | | | | |
| IgG | 72 (94.7) | 6 (54.5) | 0.004 | 3 (33.3) | 7 (70.6) | 0.020 | 46 (66.7) | 17 (100.0) | 0.264 |
| IgA | 37 (100.0) | 41 (54.5) | 0.177 | 6 (50.0) | 20 (88.2) | 0.017 | 34 (66.7) | 39 (100.0) | 0.135 |
| IgM | 4 (31.6) | 2 (9.1) | 0.109 | 2 (16.7) | 4 (35.3) | 0.229 | 6 (41.7) | 8 (77.3) | 0.042 |
| IgG1 | 9 (57.9) | 2 (9.1) | 0.022 | 1 (0.0) | 1 (5.9) | 0.220 | 7 (37.5) | 4 (22.7) | 0.332 |
| IgG2 | 24 (84.2) | 4 (45.5) | 0.013 | 3 (25.0) | 5 (52.9) | 0.070 | 3 (25.0) | 2 (22.7) | 0.578 |
| IgG3 | 1 (5.3) | 1 (0.0) | 0.844 | 1 (0.0) | 1 (5.9) | 0.107 | 2 (16.7) | 2 (13.6) | 0.721 |
| IgG4 | 1 (10.5) | 1 (0.0) | 0.122 | 1 (0.0) | 1 (0.0) | | 1 (0.0) | 1 (0.0) | |
| | | | | | | | | | |
| Bactericidal activity | 33 (94.7) | 7 (63.6) | 0.056 | 5 (25.0) | 17 (70.6) | 0.026 | 398 (83.3) | 220 (90.9) | 0.629 |
| | | | | | | | | | |
| Memory B cell ALS | | | | | | | | | |
| IgG | 21 (31.6)[b] | 4 (27.3)[b] | 0.120 | 3 (16.7) | 19 (58.8) | 0.221 | 10 (54.2) | 10 (81.8) | 0.347 |
| IgA | 5 (26.3)[b] | 4 (27.3)[b] | 0.706 | 3 (25.0) | 35 (58.8) | 0.055 | 6 (58.3) | 14 (77.3) | 0.121 |
| | | | | | | | | | |
| $\alpha4\beta7^{+/-}$ ALS | | | | | | | | | |
| $\alpha4\beta7^-$ IgG | 89 (73.7) | 5 (27.3) | 0.014 | 2 (16.7) | 6 (47.1) | 0.045 | 2 (12.5) | 13 (36.4) | 0.030 |
| $\alpha4\beta7^-$ IgA | 11 (31.6) | 1 (9.1) | 0.165 | 1 (0.0) | 6 (23.5) | 0.107 | 1 (4.2) | 10 (18.2) | 0.093 |
| $\alpha4\beta7^+$ IgG | 50 (52.6) | 5 (9.1) | 0.002 | 11 (33.3) | 93 (82.4) | 0.018 | 66 (54.2) | 1,544 (90.9) | 0.0001 |
| $\alpha4\beta7^+$ IgA | 15 (42.1) | 5 (27.3) | 0.133 | 24 (33.3) | 197 (64.7) | 0.033 | 114 (66.7) | 991 (95.5) | 0.0001 |

[a]The values shown are the mean peak fold rise in LPS-specific immune response within each exposure and shigellosis outcome group, with percentages of responders in parentheses. The percentage of responders was calculated as (no. of subjects with peak fold rise of $\geq4$/total no. of subjects within that exposure and shigellosis outcome group) $\times$ 100. P values show significance comparing peak fold rise responses across shigellosis outcome within a given exposure group. P values were determined by Mann-Whitney test.
[b]The percentage of responders was determined only in subjects with available baseline memory B cell response data ($n = 8$ with shigellosis and 9 without shigellosis).

(T6SS) (62). T6SSs not only serve to directly kill other bacterial species, therefore playing a role in microbial competition, but T6SSs have also been associated with increased severity of host disease and prolonged survival during infection with other enteric pathogens (63–67). *S. sonnei* uses its T6SS during infection to kill nearby microbial host commensals, potentially having substantial effects on the immune responses induced by *S. sonnei*. Intestinal microbiota play an integral role in host immune induction and regulation, with especially important roles in the large intestine. Not only can commensals in this region contribute to the fitness and tolerance of regulatory T cells (68), but they can also control the production of pro-interleukin-1$\beta$ (pro-IL-1$\beta$) in order to condition resident macrophages to quickly respond and produce the mature, active form of IL-1$\beta$ (68). Furthermore, some resident commensals have the ability to induce inflammasome-mediated secretion of IL-1$\beta$ and IL-18 on their own during an active enteric infection (68). While the release of proinflammatory cytokines does increase intestinal inflammation, it also aids in the recruitment of additional innate cells, such as neutrophils, to the site of infection (69, 70). The killing of commensal microbiota via the T6SS of *S. sonnei* may reduce the recruitment of innate immune cells, subsequently resulting in a lowered inflammatory environment. This reduction in intestinal inflammation could contribute to the reduced levels of fecal inflammatory markers observed postchallenge with *S. sonnei* compared to *S. flexneri* 2a and indicate that *S. sonnei* and *S. flexneri* 2a may differ in how they activate the innate immune response to infection.

Another unique feature of *S. sonnei* is the presence of an O-antigen group 4 capsule (G4C) (70, 71), which has been associated with increased virulence and environmental persistence (72, 73), potentially translating to an increased fitness of *S. sonnei* compared to *S. flexneri* 2a. *S. sonnei* expresses O antigen on both core-linked LPS and G4C, creating a thick layer of O polysaccharide (OPS) on its surface (69–71). This thick layer of OPS on the surface of *S. sonnei* reduces type 3 secretion system (T3SS) accessibility,

thereby reducing T3SS-dependent uptake into macrophages and subsequent vacuole escape of *S. sonnei* (69, 70). While the T3SS and associated virulence proteins are essential during macrophage invasion and vacuole escape for *S. flexneri* species, *S. sonnei* has demonstrated T3SS-independent uptake into macrophages (69), providing *S. sonnei* with the choice of utilizing either T3SS-dependent or -independent uptake. Given that T3SS-dependent and -independent uptake induce different host cell signaling pathways, a reduction in the T3SS-dependent cellular uptake could ultimately work to further lower the inflammatory response during *S. sonnei* infection through the reduced activation of caspase-1 inflammasome-induced pyroptosis. Lowered caspase-1 activation may lead to reduced macrophage cell death and result in less release of proinflammatory cytokines and subsequent neutrophil recruitment (69, 70).

Although neutrophils can effectively control *Shigella* infection, they also contribute to damaging the epithelial cell layer during the early stages of infection. *Shigella* spp. make use of this damaged epithelium and inflammatory environment by manipulating the epithelial cell layer and passing across the barrier without the use of M cells (74). Although this would imply that an inflammatory environment is beneficial during *Shigella* infection, recent evidence suggests that *S. sonnei* has adapted to an extracellular lifestyle (69). Therefore, the ability of *S. sonnei* to utilize its unique virulence factors to reduce the inflammatory environment during infection would lead to a reduction in neutrophil recruitment and would ultimately contribute to prolonged survival of extracellular *S. sonnei*. Additionally, extended extracellular survival would result in prolonged exposure of *S. sonnei* to the intestinal mucosa and may explain the robust mucosal immune responses associated with disease after challenge with *S. sonnei*. In contrast, assuming *S. flexneri* 2a follows the dogmatic life cycle associated with facultative intracellular pathogens, a large portion of the *S. flexneri* 2a life cycle would be spent in an intracellular phase, thereby inducing a different immune response profile, possibly presenting as a more balanced systemic and mucosal response compared to *S. sonnei*.

Non-pathogen-specific factors, including study design and conduct, may also explain some of the observed differences in immune profiles across serotypes. The current analysis uses populations from two separate studies conducted at different times and geographical locations, and although there are no differences in study demographics across exposure groups, the influence of these population-level characteristics on results has not been investigated in detail. Nonetheless, both studies used similar inclusion and exclusion criteria, and while the studies do not control for genetics, epigenetics, or microbiome differences, all subjects were screened for both preexisting health conditions as well as *Shigella*-specific preexisting immunity (56, 58). The use of similar screening procedures likely minimized potential population differences and reduced the possibility that preexisting *Shigella*-specific immunity is responsible for the observed differences in immune response profiles.

Antigenic dose can also impact immune responses postchallenge (75, 76), and it is important to consider that subjects challenged with *S. flexneri* 2a were administered an approximate dose of 1,600 CFU, while subjects challenged with *S. sonnei* were administered a range of doses, averaging 1,147 CFU across cohorts. Although the broad range of doses used for the *S. sonnei* challenge should be considered, it is relevant to note that the number of subjects progressing to shigellosis in each cohort (817 CFU = 60%, 913 CFU = 30%, 1,100 CFU = 44%, and 1,760 CFU = 60%) was not dose dependent (56). Furthermore, no differences in immune responses postchallenge were observed across cohorts (29, 56), implying that the dose of *S. sonnei* delivered had minimal impacts on the analysis of immune response profiles. In addition to the differences in challenge dose, the *S. sonnei* challenge inoculum was prepared from a lyophilized strain, whereas a frozen strain was used for the *S. flexneri* 2a inoculum (56, 58, 77). Although this is an important consideration, the lyophilized *S. sonnei* strain underwent extensive characterization to ensure ≥80% retention of the virulence plasmid after lyophilization and overall comparability to frozen *S. sonnei* strains (56, 77). Comparison testing between

the lyophilized and frozen *S. sonnei* strains showed no differences in cellular invasion, plaque formation, or virulence/disease outcomes (as measured in the guinea pig keratoconjunctivitis model) (A. E. Suvarnapunya, unpublished data). Furthermore, protective immune profiles were compared across individuals progressing to the same definition of shigellosis, thereby creating a similar population of subjects with disease postchallenge, regardless of the challenge strain used, and reducing the potential bias introduced by using a lyophilized *S. sonnei* strain.

Antigenic content and route of delivery also impact the immune response postexposure (52, 75, 76, 78), as observed during comparisons of parenteral versus oral exposure routes. Unsurprisingly, parenteral immunization induced a protective immune profile characterized by systemic and memory B cell antibodies, while oral challenge with *S. flexneri* 2a induced a more balanced profile characterized by systemic and mucosal immune responses. An altogether different profile was observed postchallenge with *S. sonnei*, with the profile in subjects progressing to shigellosis after *S. sonnei* challenge dominated by mucosal antibodies.

When comparing immune responses across challenge strain in subjects not progressing to shigellosis, the majority of subjects challenged with *S. flexneri* 2a presented with a small magnitude of systemic and mucosal immune responses, while subjects without shigellosis post-challenge with *S. sonnei* had a wide range in magnitude of systemic responses, while mucosal responses remained low to undetectable. These contrasting immune response magnitudes in subjects without shigellosis provide further support to the potential of different protective immune mechanisms across the challenge strain, further warranting additional investigations.

Finally, while the analyses conducted consist of a robust set of immune response characterizations postexposure, the number of immune parameters used in analyses is still limited. Including a select number of parameters, primarily representing humoral immune responses, could have biased the results. The addition of other immune parameters such a T cell responses or cytokine profiles could provide a more complete understanding of protective immune profiles. Alternatively, a systems biology approach of investigating immune response profiles would likely provide a more refined picture of the potential differences in *Shigella*-specific protective immune profiles.

Nonetheless, the data reported here may have important implications for vaccine development. While challenge with *S. flexneri* 2a induced a balanced immune response with both systemic and mucosal antibodies among individuals developing shigellosis, subjects progressing to shigellosis following *S. sonnei* challenge had a response driven by robust mucosal antibodies. It is also possible that the robust intestinal inflammatory responses postinfection with *S. flexneri* 2a impacted the kinetics of the mucosal immune response, with the peak response post-*S. flexneri* 2a infection missed due to the study sample collection schedule. However, while the $\alpha 4 \beta 7^+$ ALS responses 7 days postinfection were used for analyses, samples were also collected and analyzed 3 days postinfection with *S. flexneri* 2a, and no significant increases in $\alpha 4 \beta 7^+$ ALS IgG or IgA were observed at this earlier time point (data not shown).

Many vaccine candidates currently in clinical development are *Shigella* LPS-conjugate vaccines delivered parenterally (19, 20, 79–81), aligning with the concept of LPS-specific serum IgG serving as a CoP (21–23). The *S. flexneri* 2a bioconjugate vaccine provides an excellent example of a parenterally delivered *Shigella* vaccine being capable of inducing protective responses correlating with LPS-specific serum IgG levels (58); however, it remains to be seen if comparable response profiles achieved with *S. sonnei* conjugate vaccines, which have been previously reported to be efficacious in field trials (21), would protect against *S. sonnei* challenge in a CHIM setting. The *S. flexneri* 2a bioconjugate vaccine also demonstrated the ability to induce mucosal antibody responses associated with protection, providing further support for the combined effect of systemic and mucosal responses (28). However, the benefit of these combined responses was only evaluated in the context of *S. flexneri* 2a efficacy, and therefore, additional investigations are required to determine if a greater magnitude of mucosal

response than what has been reported with parenterally delivered *Shigella* vaccines would be needed to protect against *S. sonnei* infection. As mentioned earlier, reports of efficacy after parenteral immunization with a *S. sonnei* conjugate vaccine are available (21, 79, 80); however, efficacy in these studies was age related, with only adults and children ≥3 years old protected from *S. sonnei* infection. Although the efficacy trials also included cohorts immunized with an *S. flexneri* 2a conjugate vaccine, the *S. flexneri* 2a attack rate was too low to evaluate vaccine efficacy, precluding an assessment of potential differences in efficacy provided by the conjugate across different *Shigella* serotypes (79, 80).

It is also important to consider that the aforementioned *S. sonnei* conjugate vaccine efficacy studies were conducted in populations where *Shigella* is endemic, and as the majority of *Shigella* infections occur prior to the age of 4 in such settings (3, 82), one cannot discount that the efficacy reported in these trials may have resulted from the conjugate vaccine acting as a booster in mucosally primed individuals. Prior *Shigella* infection would provide some level of preexisting immunity, potentially contributing to the observed pattern of age-related efficacy, in that as age increased, so did the number of previously exposed individuals, resulting in a larger proportion of mucosally primed subjects within the older age groups, thus leading to the increased vaccine efficacy after immunization with the conjugate. The concept of mucosal priming has also been studied in preclinical models, which demonstrated that a priming immunization with a live attenuated *Shigella* vaccine candidate was required in order for the conjugate vaccine to be efficacious (83). The low clinical efficacy observed in the population under age 3 could therefore be explained by a lack of exposure to *Shigella* prior to immunization, resulting in a truly naive population that had not been mucosally primed and therefore was not protected after vaccination alone. While the analyses described here were conducted on adult populations, subjects were recruited from settings where shigellosis is not endemic and were also screened for preexisting *Shigella*-specific immunity, potentially resulting in a population more closely aligned with a *Shigella*-naive under-3 population. Although the vaccine-induced immune responses may not have been sufficiently potent in the younger age groups, it is possible that the addition of an adjuvant or the use of different vaccine manufacturing strategies may be helpful in inducing protective immune responses.

The current analyses provide important insights into potential immunological response differences associated with infection across different *Shigella* serotypes. The differences in immune responses postinfection may be understandable when considering the number of molecular and pathogenic responses reported across the studied serotypes. Nonetheless, additional investigations should be conducted with parenterally administered *S. sonnei* vaccine candidates and with data from live attenuated *Shigella* vaccine candidate studies. Additionally, immune responses induced postinfection in subjects living in settings where shigellosis is endemic may provide a different immune response profile altogether and should be investigated as thoroughly as possible. Rigorous characterization of immune responses and immune profiles is essential to understanding the mechanisms underlying *Shigella* serotype-specific protective immunity associated with either oral challenge or parenteral vaccination and could guide future *Shigella* vaccine development efforts.

## MATERIALS AND METHODS

**Source studies.** Data from two *Shigella* CHIMs recently conducted in North American adults were used for the current investigations. The first study was a *S. flexneri* 2a CHIM conducted in Baltimore, MD (Clinicaltrials.gov registration no. NCT02646371), which included two treatment groups: (i) vaccinated subjects who later underwent oral challenge with *S. flexneri* 2a 2457T to evaluate vaccine efficacy and (ii) subjects that underwent oral challenge with *S. flexneri* 2a 2457T without any prior intervention (58). The second study was a *S. sonnei* CHIM conducted in Cincinnati, OH (ClinicalTrials.gov registration no. NCT02816346), and only included subjects orally challenged with different doses of *S. sonnei* 53G without any prior interventions (56). Subjects from each CHIM were grouped into three populations (as described below) to evaluate and compare *Shigella*-specific immune response profiles associated with different antigenic exposures. The population demographics are summarized in Table S1 in the supplemental material but have also been described in detail elsewhere, along with study inclusion/exclusion

criteria and study outcomes specific to each CHIM (56, 58). Both studies aimed to enroll *Shigella*-naive subjects using the following exclusion criteria: recent travel to a region where *Shigella* is endemic, history of confirmed *Shigella* infection, or serologic evidence of prior exposure to *S. flexneri* 2a or *S. sonnei* as determined by LPS-specific serum IgG enzyme-linked immunosorbent assay (ELISA) (56, 58).

**Protection of human subjects.** The investigators have adhered to the policies for protection of human subjects as prescribed in AR 70-25.

**Immune response data.** In-depth immune response characterizations pre- and post-antigenic exposure were conducted in each CHIM, with results fully detailed elsewhere (29, 57). All immunoassays were conducted in the same laboratory using the same procedures as previously described (29, 57). The following LPS-specific systemic, memory, and mucosal immunologic parameters were used in analyses: serum antibody responses, SBA, memory B cell ALS responses, and ALS responses from B cell populations that were either positive or negative for the intestinal homing marker $\alpha4\beta7$. Mucosal responses were defined as antibody responses from $\alpha4\beta7^+$ B cell populations.

**Fecal inflammatory markers.** Intestinal inflammatory responses post-oral challenge with *S. flexneri* 2a or *S. sonnei* were investigated by quantifying the levels of myeloperoxidase and calprotectin in stool samples pre- and postchallenge. Stool samples collected prior to challenge and 3 days postchallenge were processed for inflammatory marker analyses and assayed by ELISA per the manufacturer's instructions (Epitope Diagnostics). Inflammatory marker concentrations were interpolated from a standard curve, with values below the assay limit of detection (LOD) being assigned a value of half the lowest concentration in the standard curve (1/2 LOD).

**Disease outcomes and definitions.** Results are presented by clinical outcome postchallenge using the consensus shigellosis CHIM endpoint definition (84). A protective immune profile in population 1 (see below) is defined as vaccinated subjects not developing shigellosis after oral challenge. Protective immune profiles in populations 2 and 3 (see below) are presumptive and are defined as naive subjects progressing to shigellosis after oral challenge. This presumption of protection is based on the prior documentation that previous *Shigella* infection protects from subsequent illness with the same serotype (16, 17, 61). Given that prior infection can induce protective immunity, it is reasonable to assume that subjects in populations 2 and 3 (see below) with moderate to severe disease postchallenge would be expected to be protected from subsequent challenge with the same serotype. However, there are examples of this not being the case with other enteric pathogens, and protection cannot be verified without subjects undergoing a homologous rechallenge.

**Populations used in analyses. (i) Population 1.** The first population ($n = 30$) (Fig. 1A) was compiled of subjects from the *S. flexneri* 2a CHIM and was used to analyze immune profiles associated with parenteral immunization with a candidate *S. flexneri* 2a bioconjugate vaccine (57, 58). Subjects in this population were intramuscularly (i.m.) immunized twice, 28 days apart, and orally challenged 28 days after their second immunization with either 1,510 CFU ($n = 15$) or 1,707 CFU ($n = 15$) of virulent *S. flexneri* 2a 2457T. After challenge, subjects without shigellosis ($n = 19$) were used to characterize protective immune profiles associated with parenteral immunization. Immune responses induced after vaccination but prior to challenge were used for analyses.

**(ii) Population 2.** The second population ($n = 29$) (Fig. 1B) was also compiled of subjects from the *S. flexneri* 2a CHIM and was used to investigate immune profiles associated with oral challenge with *S. flexneri* 2a 2457T (57, 58). Subjects in this population were orally challenged with either 1,510 CFU ($n = 15$) or 1,707 CFU ($n = 14$) of virulent *S. flexneri* 2a 2457T without any prior interventions. Postchallenge immune responses in subjects developing shigellosis ($n = 17$) were used to characterize presumptively protective immune profiles associated with *S. flexneri* 2a infection.

**(iii) Population 3.** The third population ($n = 46$) (Fig. 1C) was compiled of subjects from the *S. sonnei* CHIM (29, 56). This study contained a total of 5 cohorts orally challenged with various doses (567, 817, 913, and 1,760 CFU at $n = 10$/dose group or 1,100 CFU at $n = 16$) (Fig. 1C) of virulent *S. sonnei* 53G. The lowest inoculum dose cohort (567 CFU, $n = 10$) was excluded from this analysis due to the absence of shigellosis cases. Postchallenge immune responses in subjects progressing to shigellosis ($n = 22$) were used to characterize presumptively protective immune profiles associated with *S. sonnei* infection.

**Principal-component analysis.** PCA was used to reduce immune response data dimensionality and redundancy, and principal components were generated for each analysis of the three populations detailed above. Since PCA results are based on linear correlations between variables, all immune response data were log transformed prior to analyses to ensure a Gaussian distribution. Inclusion of immune response variables in PCA was determined by examining variable colinearity or redundancy using a Pearson correlation matrix. Any immune response variable not demonstrating colinearity or redundancy with any other variable (defined as a Pearson's $r$ of $<0.3$) was excluded from analyses (85). PCA sampling adequacy was assessed using the KMO measure and Bartlett's test of sphericity, with a KMO value of $\geq0.6$ and a significant ($P \leq 0.05$) Bartlett's test required in order for sampling adequacy to be considered acceptable (85). Principal-component scores were assigned to all subjects for any principal component with an eigenvalue of $\geq1$ (85). Component scores for principal components 1 and 2 were used for comparisons across populations and shigellosis outcome. PCA component score distribution was assessed for normality via distribution plots and Shapiro-Wilk's normality test, with $P$ values of $\leq0.05$ indicating that component scores within a population did not follow a Gaussian distribution. Nonnormally distributed component score data were log transformed prior to analysis. All statistical tests were interpreted in a two-tailed fashion ($\alpha = 0.05$), with $P$ values of $\leq0.05$ considered statistically significant in Stata (version 14 for MAC).

**Immune response heat maps.** The peak fold rise over baseline (preexposure) across each immune response variable was graphed using heat maps to display the magnitude of immune responses across

each population. Peak fold rise was calculated by dividing the maximum titer postexposure (population 1, postvaccination/prechallenge; populations 2 and 3, postchallenge) by the preexposure titer. Fold rise in memory B cell IgG and IgA responses could not be calculated for 10 subjects in population 1 as baseline memory B cell responses were not measured in these subjects (57). All heat maps were generated using Prism (version 9 for MAC).

## SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

**FIG S1**, TIF file, 0.5 MB.
**FIG S2**, TIF file, 0.7 MB.
**TABLE S1**, PDF file, 0.1 MB.
**TABLE S2**, PDF file, 0.1 MB.
**TABLE S3**, PDF file, 0.1 MB.

## REFERENCES

1. GBD Diarrhoeal Disease Collaborators. 2018. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect Dis 18:1211–1228. https://doi.org/10.1016/S1473-3099(18)30362-1.

2. Khalil IA, Troeger C, Blacker BF, Rao PC, Brown A, Atherly DE, Brewer TG, Engmann CM, Houpt ER, Kang G, Kotloff KL, Levine MM, Luby SP, MacLennan CA, Pan WK, Pavlinac PB, Platts-Mills JA, Qadri F, Riddle MS, Ryan ET, Shoultz DA, Steele AD, Walson JL, Sanders JW, Mokdad AH, Murray CJL, Hay SI, Reiner RC, Jr. 2018. Morbidity and mortality due to shigella and enterotoxigenic Escherichia coli diarrhoea: the Global Burden of Disease Study 1990–2016. Lancet Infect Dis 18:1229–1240. https://doi.org/10.1016/S1473-3099(18)30475-4.

3. Kotloff KL, Nasrin D, Blackwelder WC, Wu Y, Farag T, Panchalingham S, Sow SO, Sur D, Zaidi AKM, Faruque ASG, Saha D, Alonso PL, Tamboura B, Sanogo D, Onwuchekwa U, Manna B, Ramamurthy T, Kanungo S, Ahmed S, Qureshi S, Quadri F, Hossain A, Das SK, Antonio M, Hossain MJ, Mandomando I, Acacio S, Biswas K, Tennant SM, Verweij JJ, Sommerfelt H, Nataro JP, Robins-Browne RM, Levine MM. 2019. The incidence, aetiology, and adverse clinical consequences of less severe diarrhoeal episodes among infants and children residing in low-income and middle-income countries: a 12-month case-control study as a follow-on to the Global Enteric Multicenter Study (GEMS). Lancet Glob Health 7:e568–e584. https://doi.org/10.1016/S2214-109X(19)30076-2.

4. Lamberti LM, Bourgeois AL, Fischer Walker CL, Black RE, Sack D. 2014. Estimating diarrheal illness and deaths attributable to shigellae and enterotoxigenic Escherichia coli among older children, adolescents, and adults in South Asia and Africa. PLoS Negl Trop Dis 8:e2705. https://doi.org/10.1371/journal.pntd.0002705.

5. Porter CK, Choi D, Cash B, Pimentel M, Murray J, May L, Riddle MS. 2013. Pathogen-specific risk of chronic gastrointestinal disorders following bacterial causes of foodborne illness. BMC Gastroenterol 13:46. https://doi.org/10.1186/1471-230X-13-46.

6. Porter CK, Choi D, Riddle MS. 2013. Pathogen-specific risk of reactive arthritis from bacterial causes of foodborne illness. J Rheumatol 40:712–714. https://doi.org/10.3899/jrheum.121254.

7. Guerrant RL, Leite AM, Pinkerton R, Medeiros PH, Cavalcante PA, DeBoer M, Kosek M, Duggan C, Gewirtz A, Kagan JC, Gauthier AE, Swann J, Mayneris-Perxachs J, Bolick DT, Maier EA, Guedes MM, Moore SR, Petri WA, Havt A, Lima IF, Prata MM, Michaleckyj JC, Scharf RJ, Sturgeon C, Fasano A, Lima AA. 2016. Biomarkers of environmental enteropathy, inflammation, stunting, and impaired growth in children in northeast Brazil. PLoS One 11:e0158772. https://doi.org/10.1371/journal.pone.0158772.

8. Kosek M, Haque R, Lima A, Babji S, Shrestha S, Qureshi S, Amidou S, Mduma E, Lee G, Yori PP, Guerrant RL, Bhutta Z, Mason C, Kang G, Kabir M, Amour C, Bessong P, Turab A, Seidman J, Olortegui MP, Quetz J, Lang D, Gratz J, Miller M, Gottlieb M. 2013. Fecal markers of intestinal inflammation and permeability associated with the subsequent acquisition of

linear growth deficits in infants. Am J Trop Med Hyg 88:390–396. https://doi.org/10.4269/ajtmh.2012.12-0549.

9. Anderson JD, Bagamian KH, Muhib F, Amaya MP, Laytner LA, Wierzba T, Rheingans R. 2019. Burden of enterotoxigenic Escherichia coli and Shigella non-fatal diarrhoeal infections in 79 low-income and lower middle-income countries: a modelling analysis. Lancet Glob Health 7:e321–e330. https://doi.org/10.1016/S2214-109X(18)30483-2.

10. Bhattacharya D, Bhattacharya H, Sayi DS, Bharadwaj AP, Singhania M, Sugunan AP, Roy S. 2015. Changing patterns and widening of antibiotic resistance in Shigella spp. over a decade (2000-2011), Andaman Islands, India. Epidemiol Infect 143:470–477. https://doi.org/10.1017/S0950268814000958.

11. World Health Organization. 2019. The 6th Annual Meeting of the WHO Product Development for Vaccines Advisory Committee (PDVAC): executive summary of meeting and recommendations. https://www.who.int/immunization/research/meetings_workshops/pdvac_june19/en/.

12. Mani S, Wierzba T, Walker RI. 2016. Status of vaccine research and development for Shigella. Vaccine 34:2887–2894. https://doi.org/10.1016/j.vaccine.2016.02.075.

13. Walker RI. 2015. An assessment of enterotoxigenic Escherichia coli and Shigella vaccine candidates for infants and children. Vaccine 33:954–965. https://doi.org/10.1016/j.vaccine.2014.11.049.

14. Plotkin SA, Gilbert PB. 2012. Nomenclature for immune correlates of protection after vaccination. Clin Infect Dis 54:1615–1617. https://doi.org/10.1093/cid/cis238.

15. Qin L, Gilbert PB, Corey L, McElrath MJ, Self SG. 2007. A framework for assessing immunological correlates of protection in vaccine trials. J Infect Dis 196:1304–1312. https://doi.org/10.1086/522428.

16. DuPont HL, Hornick RB, Snyder MJ, Libonati JP, Formal SB, Gangarosa EJ. 1972. Immunity in shigellosis. II. Protection induced by oral live vaccine or primary infection. J Infect Dis 125:12–16. https://doi.org/10.1093/infdis/125.1.12.

17. Formal SB, Oaks EV, Olsen RE, Wingfield-Eggleston M, Snoy PJ, Cogan JP. 1991. Effect of prior infection with virulent Shigella flexneri 2a on the resistance of monkeys to subsequent infection with Shigella sonnei. J Infect Dis 164:533–537. https://doi.org/10.1093/infdis/164.3.533.

18. Cohen D, Ashkenazi S, Green M, Lerman Y, Slepon R, Robin G, Orr N, Taylor DN, Sadoff JC, Chu C, Shiloach J, Schneerson R, Robbins JB. 1996. Safety and immunogenicity of investigational Shigella conjugate vaccines in Israeli volunteers. Infect Immun 64:4074–4077. https://doi.org/10.1128/iai.64.10.4074-4077.1996.

19. Riddle MS, Kaminski RW, Di Paolo C, Porter CK, Gutierrez RL, Clarkson KA, Weerts HE, Duplessis C, Castellano A, Alaimo C, Paolino K, Gormley R, Gambillara Fonck V. 2016. 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 23:908–917. https://doi.org/10.1128/CVI.00224-16.

20. Phalipon A, Tanguy M, Grandjean C, Guerreiro C, Belot F, Cohen D, Sansonetti PJ, Mulard LA. 2009. A synthetic carbohydrate-protein conjugate vaccine candidate against Shigella flexneri 2a infection. J Immunol 182:2241–2247. https://doi.org/10.4049/jimmunol.0803141.

21. Cohen D, Meron-Sudai S, Bialik A, Asato V, Goren S, Ariel-Cohen O, Reizis A, Hochberg A, Ashkenazi S. 2019. Serum IgG antibodies to Shigella lipopolysaccharide antigens—a correlate of protection against shigellosis. Hum Vaccin Immunother 15:1401–1408. https://doi.org/10.1080/21645515.2019.1606971.

22. Cohen D, Green MS, Block C, Slepon R, Ofek I. 1991. Prospective study of the association between serum antibodies to lipopolysaccharide O antigen and the attack rate of shigellosis. J Clin Microbiol 29:386–389. https://doi.org/10.1128/jcm.29.2.386-389.1991.

23. Robbins JB, Chu C, Schneerson R. 1992. Hypothesis for vaccine development: protective immunity to enteric diseases caused by nontyphoidal salmonellae and shigellae may be conferred by serum IgG antibodies to the O-specific polysaccharide of their lipopolysaccharides. Clin Infect Dis 15:346–361. https://doi.org/10.1093/clinids/15.2.346.

24. Passwell JH, Harlev E, Ashkenazi S, Chu C, Miron D, Ramon R, Farzan N, Shiloach J, Bryla DA, Majadly F, Roberson R, Robbins JB, Schneerson R. 2001. Safety and immunogenicity of improved Shigella O-specific polysaccharide-protein conjugate vaccines in adults in Israel. Infect Immun 69:1351–1357. https://doi.org/10.1128/IAI.69.3.1351-1357.2001.

25. Coster TS, Hoge CW, VanDeVerg LL, Hartman AB, Oaks EV, Venkatesan MM, Cohen D, Robin G, Fontaine-Thompson A, Sansonetti PJ, Hale TL. 1999. Vaccination against shigellosis with attenuated Shigella flexneri 2a strain SC602. Infect Immun 67:3437–3443. https://doi.org/10.1128/IAI.67.7.3437-3443.1999.

26. Wahid R, Simon JK, Picking WL, Kotloff KL, Levine MM, Sztein MB. 2013. Shigella antigen-specific B memory cells are associated with decreased disease severity in subjects challenged with wild-type Shigella flexneri 2a. Clin Immunol 148:35–43. https://doi.org/10.1016/j.clim.2013.03.009.

27. Davis CL, Wahid R, Toapanta FR, Simon JK, Sztein MB, Levy D. 2013. Applying mathematical tools to accelerate vaccine development: modeling Shigella immune dynamics. PLoS One 8:e59465. https://doi.org/10.1371/journal.pone.0059465.

28. Arevalillo JM, Sztein MB, Kotloff KL, Levine MM, Simon JK. 2017. Identification of immune correlates of protection in Shigella infection by application of machine learning. J Biomed Inform 74:1–9. https://doi.org/10.1016/j.jbi.2017.08.005.

29. Clarkson KA, Frenck RW, Jr, Dickey M, Suvarnapunya AE, Chandrasekaran L, Weerts HP, Heaney CD, McNeal M, Detizio K, Parker S, Hoeper A, Bourgeois AL, Porter CK, Venkatesan MM, Kaminski RW. 2020. Immune response characterization after controlled infection with lyophilized Shigella sonnei 53G. mSphere 5:e00988-19. https://doi.org/10.1128/mSphere.00988-19.

30. Ndungo E, Randall A, Hazen TH, Kania DA, Trappl-Kimmons K, Liang X, Barry EM, Kotloff KL, Chakraborty S, Mani S, Rasko DA, Pasetti MF. 2018. A novel Shigella proteome microarray discriminates targets of human antibody reactivity following oral vaccination and experimental challenge. mSphere 3:e00260-18. https://doi.org/10.1128/mSphere.00260-18.

31. Trautmann L, Sekaly RP. 2011. Solving vaccine mysteries: a systems biology perspective. Nat Immunol 12:729–731. https://doi.org/10.1038/ni.2078.

32. Katona P, Katona-Apte J. 2008. The interaction between nutrition and infection. Clin Infect Dis 46:1582–1588. https://doi.org/10.1086/587658.

33. Mondal D, Minak J, Alam M, Liu Y, Dai J, Korpe P, Liu L, Haque R, Petri WA, Jr. 2012. Contribution of enteric infection, altered intestinal barrier function, and maternal malnutrition to infant malnutrition in Bangladesh. Clin Infect Dis 54:185–192. https://doi.org/10.1093/cid/cir807.

34. Flores J, Okhuysen PC. 2009. Genetics of susceptibility to infection with enteric pathogens. Curr Opin Infect Dis 22:471–476. https://doi.org/10.1097/QCO.0b013e3283304eb6.

35. Ducarmon QR, Zwittink RD, Hornung BVH, van Schaik W, Young VB, Kuijper EJ. 2019. Gut microbiota and colonization resistance against bacterial enteric infection. Microbiol Mol Biol Rev 83:e00007-19. https://doi.org/10.1128/MMBR.00007-19.

36. Zimmermann P, Curtis N. 2019. Factors that influence the immune response to vaccination. Clin Microbiol Rev 32:e00084-18. https://doi.org/10.1128/CMR.00084-18.

37. Day T. 2001. Parasite transmission modes and the evolution of virulence. Evolution 55:2389–2400. https://doi.org/10.1111/j.0014-3820.2001.tb00754.x.

38. Sela U, Euler CW, Correa da Rosa J, Fischetti VA. 2018. Strains of bacterial species induce a greatly varied acute adaptive immune response: the contribution of the accessory genome. PLoS Pathog 14:e1006726. https://doi.org/10.1371/journal.ppat.1006726.

39. Martins NE, Faria VG, Teixeira L, Magalhaes S, Sucena E. 2013. Host adaptation is contingent upon the infection route taken by pathogens. PLoS Pathog 9:e1003601. https://doi.org/10.1371/journal.ppat.1003601.

40. Sperandio V. 2018. Pathogens' adaptation to the human host. Proc Natl Acad Sci U S A 115:9342–9343. https://doi.org/10.1073/pnas.1813379115.

41. Relman DA, Hamburg MA, Choffnes ER, Mack A. 2009. Microbial evolution and co-adaptation: a tribute to the life and scientific legacies of Joshua Lederberg: workshop summary. National Academies Press, Washington, DC.

42. Pulendran B, Li S, Nakaya HI. 2010. Systems vaccinology. Immunity 33:516–529. https://doi.org/10.1016/j.immuni.2010.10.006.

43. Li S, Nakaya HI, Kazmin DA, Oh JZ, Pulendran B. 2013. Systems biological approaches to measure and understand vaccine immunity in humans. Semin Immunol 25:209–218. https://doi.org/10.1016/j.smim.2013.05.003.

44. Nakaya HI, Pulendran B. 2015. Vaccinology in the era of high-throughput biology. Philos Trans R Soc Lond B Biol Sci 370:20140146. https://doi.org/10.1098/rstb.2014.0146.

45. Querec TD, Akondy RS, Lee EK, Cao W, Nakaya HI, Teuwen D, Pirani A, Gernert K, Deng J, Marzolf B, Kennedy K, Wu H, Bennouna S, Oluoch H, Miller J, Vencio RZ, Mulligan M, Aderem A, Ahmed R, Pulendran B. 2009. Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. Nat Immunol 10:116–125. https://doi.org/10.1038/ni.1688.

46. Hasin Y, Seldin M, Lusis A. 2017. Multi-omics approaches to disease. Genome Biol 18:83. https://doi.org/10.1186/s13059-017-1215-1.

47. Haks MC, Bottazzi B, Cecchinato V, De Gregorio C, Del Giudice G, Kaufmann SHE, Lanzavecchia A, Lewis DJM, Maertzdorf J, Mantovani A, Sallusto F, Sironi M, Uguccioni M, Ottenhoff THM. 2017. Molecular signatures of immunity and immunogenicity in infection and vaccination. Front Immunol 8:1563. https://doi.org/10.3389/fimmu.2017.01563.

48. Valletta JJ, Recker M. 2017. Identification of immune signatures predictive of clinical protection from malaria. PLoS Comput Biol 13:e1005812. https://doi.org/10.1371/journal.pcbi.1005812.

49. Gaucher D, Therrien R, Kettaf N, Angermann BR, Boucher G, Filali-Mouhim A, Moser JM, Mehta RS, Drake DR, III, Castro E, Akondy R, Rinfret A, Yassine-Diab B, Said EA, Chouikh Y, Cameron MJ, Clum R, Kelvin D, Somogyi R, Greller LD, Balderas RS, Wilkinson P, Pantaleo G, Tartaglia J, Haddad EK, Sekaly RP. 2008. Yellow fever vaccine induces integrated multilineage and polyfunctional immune responses. J Exp Med 205:3119–3131. https://doi.org/10.1084/jem.20082292.

50. Alter G, Barouch D. 2018. Immune correlate-guided HIV vaccine design. Cell Host Microbe 24:25–33. https://doi.org/10.1016/j.chom.2018.06.012.

51. Tomaras GD, Plotkin SA. 2017. Complex immune correlates of protection in HIV-1 vaccine efficacy trials. Immunol Rev 275:245–261. https://doi.org/10.1111/imr.12514.

52. Dahora LC, Jin C, Spreng RL, Feely F, Mathura R, Seaton KE, Zhang L, Hill J, Jones E, Alam SM, Dennison SM, Pollard AJ, Tomaras GD. 2019. IgA and IgG1 specific to Vi polysaccharide of Salmonella Typhi correlate with protection status in a typhoid fever controlled human infection model. Front Immunol 10:2582. https://doi.org/10.3389/fimmu.2019.02582.

53. Ryan ET, Calderwood SB. 2000. Cholera vaccines. Clin Infect Dis 31:561–565. https://doi.org/10.1086/313951.

54. Plotkin SA. 2001. Immunologic correlates of protection induced by vaccination. Pediatr Infect Dis J 20:63–75. https://doi.org/10.1097/00006454-200101000-00013.

55. Plotkin SA. 2010. Correlates of protection induced by vaccination. Clin Vaccine Immunol 17:1055–1065. https://doi.org/10.1128/CVI.00131-10.

56. Frenck RW, Jr, Dickey M, Suvarnapunya AE, Chandrasekaran L, Kaminski RW, Clarkson KA, McNeal M, Lynen A, Parker S, Hoeper A, Mani S, Fix A, Maier N, Venkatesan MM, Porter CK. 2020. Establishment of a controlled human infection model with a lyophilized strain of Shigella sonnei 53G. mSphere 5:e00416-20. https://doi.org/10.1128/mSphere.00416-20.

57. Clarkson KA, Talaat KR, Alaimo C, Martin P, Bourgeois AL, Dreyer A, Porter CK, Chakraborty S, Brubaker J, Elwood D, Frolich R, DeNearing B, Weerts HP, Feijoo B, Halpern J, Sack D, Riddle MS, Fonck VG, Kaminski RW. 2021. Immune response characterization in a human challenge study with a Shigella flexneri 2a bioconjugate vaccine. EBioMedicine 66:103308. https://doi.org/10.1016/j.ebiom.2021.103308.

58. Talaat KR, Alaimo C, Martin P, Bourgeois AL, Dreyer AM, Kaminski RW, Porter CK, Chakraborty S, Clarkson KA, Brubaker J, Elwood D, Frolich R, DeNearing B, Weerts H, Feijoo BL, Halpern J, Sack D, Riddle MS, Fonck VG. 2021. Human challenge study with a Shigella bioconjugate vaccine: analyses of clinical efficacy and correlate of protection. EBioMedicine 66:103310. https://doi.org/10.1016/j.ebiom.2021.103310.

59. Chu TH, Patz EF, Jr, Ackerman ME. 2021. Coming together at the hinges: therapeutic prospects of IgG3. MAbs 13:1882028. https://doi.org/10.1080/19420862.2021.1882028.

60. Wagner M, Peterson CG, Ridefelt P, Sangfelt P, Carlson M. 2008. Fecal markers of inflammation used as surrogate markers for treatment outcome in relapsing inflammatory bowel disease. World J Gastroenterol 14:5584–5589. https://doi.org/10.3748/wjg.14.5584.

61. Herrington DA, Van de Verg L, Formal SB, Hale TL, Tall BD, Cryz SJ, Tramont EC, Levine MM. 1990. Studies in volunteers to evaluate candidate Shigella vaccines: further experience with a bivalent Salmonella typhi-Shigella sonnei vaccine and protection conferred by previous Shigella sonnei disease. Vaccine 8:353–357. https://doi.org/10.1016/0264-410x(90)90094-3.

62. Anderson MC, Vonaesch P, Saffarian A, Marteyn BS, Sansonetti PJ. 2017. Shigella sonnei encodes a functional T6SS used for interbacterial competition and niche occupancy. Cell Host Microbe 21:769–776.e3. https://doi.org/10.1016/j.chom.2017.05.004.

63. Ma AT, Mekalanos JJ. 2010. In vivo actin cross-linking induced by Vibrio cholerae type VI secretion system is associated with intestinal inflammation. Proc Natl Acad Sci U S A 107:4365–4370. https://doi.org/10.1073/pnas.0915156107.

64. Ma J, Bao Y, Sun M, Dong W, Pan Z, Zhang W, Lu C, Yao H. 2014. Two functional type VI secretion systems in avian pathogenic Escherichia coli are involved in different pathogenic pathways. Infect Immun 82:3867–3879. https://doi.org/10.1128/IAI.01769-14.

65. Troxell B. 2018. A type 6 secretion system (T6SS) encoded gene within Salmonella enterica serovar Enteritidis contributes to virulence. Virulence 9:585–587. https://doi.org/10.1080/21505594.2017.1421829.

66. Sana TG, Flaugnatti N, Lugo KA, Lam LH, Jacobson A, Baylot V, Durand E, Journet L, Cascales E, Monack DM. 2016. Salmonella Typhimurium utilizes a T6SS-mediated antibacterial weapon to establish in the host gut. Proc Natl Acad Sci U S A 113:E5044–E5051. https://doi.org/10.1073/pnas.1608858113.

67. Cheng AT, Ottemann KM, Yildiz FH. 2015. Vibrio cholerae response regulator VxrB controls colonization and regulates the type VI secretion system. PLoS Pathog 11:e1004933. https://doi.org/10.1371/journal.ppat.1004933.

68. Belkaid Y, Hand TW. 2014. Role of the microbiota in immunity and inflammation. Cell 157:121–141. https://doi.org/10.1016/j.cell.2014.03.011.

69. Watson JL, Sanchez-Garrido J, Goddard PJ, Torraca V, Mostowy S, Shenoy AR, Clements A. 2019. Shigella sonnei O-antigen inhibits internalization, vacuole escape, and inflammasome activation. mBio 10:e02654-19. https://doi.org/10.1128/mBio.02654-19.

70. Caboni M, Pedron T, Rossi O, Goulding D, Pickard D, Citiulo F, MacLennan CA, Dougan G, Thomson NR, Saul A, Sansonetti PJ, Gerke C. 2015. An O antigen capsule modulates bacterial pathogenesis in Shigella sonnei. PLoS Pathog 11:e1004749. https://doi.org/10.1371/journal.ppat.1004749.

71. Xu DQ, Cisar JO, Ambulos N, Jr, Burr DH, Kopecko DJ. 2002. Molecular cloning and characterization of genes for Shigella sonnei form I O polysaccharide: proposed biosynthetic pathway and stable expression in a live Salmonella vaccine vector. Infect Immun 70:4414–4423. https://doi.org/10.1128/IAI.70.8.4414-4423.2002.

72. Shifrin Y, Peleg A, Ilan O, Nadler C, Kobi S, Baruch K, Yerushalmi G, Berdichevsky T, Altuvia S, Elgrably-Weiss M, Abe C, Knutton S, Sasakawa C, Ritchie JM, Waldor MK, Rosenshine I. 2008. Transient shielding of intimin and the type III secretion system of enterohemorrhagic and enteropathogenic Escherichia coli by a group 4 capsule. J Bacteriol 190:5063–5074. https://doi.org/10.1128/JB.00440-08.

73. Gibson DL, White AP, Snyder SD, Martin S, Heiss C, Azadi P, Surette M, Kay WW. 2006. Salmonella produces an O-antigen capsule regulated by AgfD and important for environmental persistence. J Bacteriol 188:7722–7730. https://doi.org/10.1128/JB.00809-06.

74. Schroeder GN, Hilbi H. 2008. Molecular pathogenesis of Shigella spp.: controlling host cell signaling, invasion, and death by type III secretion. Clin Microbiol Rev 21:134–156. https://doi.org/10.1128/CMR.00032-07.

75. Fehr T, Ochsenbein AF. 2004. Outcome of the antibody response: a question of antigen dose and distribution. Trends Immunol 25:165–168. https://doi.org/10.1016/j.it.2004.02.003.

76. Tongren JE, Drakeley CJ, McDonald SL, Reyburn HG, Manjurano A, Nkya WM, Lemnge MM, Gowda CD, Todd JE, Corran PH, Riley EM. 2006. Target antigen, age, and duration of antigen exposure independently regulate immunoglobulin G subclass switching in malaria. Infect Immun 74:257–264. https://doi.org/10.1128/IAI.74.1.257-264.2006.

77. Talaat KR, Bourgeois AL, Frenck RW, Chen WH, MacLennan CA, Riddle MS, Suvarnapunya AE, Brubaker JL, Kotloff KL, Porter CK. 2019. Consensus report on Shigella controlled human infection model: conduct of studies. Clin Infect Dis 69:S580–S590. https://doi.org/10.1093/cid/ciz892.

78. Hjelholt A, Christiansen G, Sorensen US, Birkelund S. 2013. IgG subclass profiles in normal human sera of antibodies specific to five kinds of microbial antigens. Pathog Dis 67:206–213. https://doi.org/10.1111/2049-632X.12034.

79. Cohen D, Ashkenazi S, Green MS, Gdalevich M, Robin G, Slepon R, Yavzori M, Orr N, Block C, Ashkenazi I, Shemer J, Taylor DN, Hale TL, Sadoff JC, Pavliakova D, Schneerson R, Robbins JB. 1997. Double-blind vaccine-controlled randomised efficacy trial of an investigational Shigella sonnei conjugate vaccine in young adults. Lancet 349:155–159. https://doi.org/10.1016/S0140-6736(96)06255-1.

80. Passwell JH, Ashkenazi S, Banet-Levi Y, Ramon-Saraf R, Farzam N, Lerner-Geva L, Even-Nir H, Yerushalmi B, Chu C, Shiloach J, Robbins JB, Schneerson R, Israeli Shigella Study Group. 2010. Age-related efficacy of Shigella O-specific polysaccharide conjugates in 1–4-year-old Israeli children. Vaccine 28:2231–2235. https://doi.org/10.1016/j.vaccine.2009.12.050.

81. Barel LA, Mulard LA. 2019. Classical and novel strategies to develop a Shigella glycoconjugate vaccine: from concept to efficacy in human. Hum Vaccin Immunother 15:1338–1356. https://doi.org/10.1080/21645515.2019.1606972.

82. Kotloff KL, Riddle MS, Platts-Mills JA, Pavlinac P, Zaidi AKM. 2018. Shigellosis. Lancet 391:801–812. https://doi.org/10.1016/S0140-6736(17)33296-8.

83. Hartman AB, Van de Verg LL, Collins HH, Jr, Tang DB, Bendiuk NO, Taylor DN, Powell CJ. 1994. Local immune response and protection in the guinea pig keratoconjunctivitis model following immunization with Shigella vaccines. Infect Immun 62:412–420. https://doi.org/10.1128/iai.62.2.412-420.1994.

84. MacLennan CA, Riddle MS, Chen WH, Talaat KR, Jain V, Bourgeois AL, Frenck R, Kotloff K, Porter CK. 2019. Consensus report on Shigella controlled human infection model: clinical endpoints. Clin Infect Dis 69:S591–S595. https://doi.org/10.1093/cid/ciz891.

85. Keho Y. 2012. The basics of linear principal components analysis, p 181–206. *In* Sanguansat P (ed), Principal component analysis. IntechOpen, London, United Kingdom.