Repertoire of naturally acquired maternal antibodies transferred to infants for protection against shigellosis

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Keywords: maternal antibodies, transplacental antibody transfer, *Shigella* antibodies, naturally acquired immunity, infant immunity

Abstract word count: 302

Text word count: 4859

Figures: 6

Tables: 3

Abstract

Shigella is the second leading cause of diarrheal diseases, accounting for >200,000 infections and >50,000 deaths in children under 5 years of age annually worldwide. The incidence of *Shigella*-induced diarrhea is relatively low during the first year of life and increases substantially, reaching its peak between 11 to 24 months of age. This epidemiological trend hints to an early protective immunity of maternal origin and an increase in disease incidence when maternally transferred immunity wanes. The magnitude, type, antigenic diversity and anti-microbial activity of maternal antibodies transferred via placenta that can prevent shigellosis during early infancy are not known. To address this knowledge gap, *Shigella*-specific antibodies directed against the lipopolysaccharide (LPS) and virulent factors (IpaB, IpaC, IpaD, IpaH, and VirG) and antibody mediated serum bactericidal (SBA) and opsonophagocytic killing antibody (OPKA) activity were measured in maternal and cord blood sera from a longitudinal cohort of mother-infant pairs living in rural Malawi. Protein-specific IgG (very high levels) and *Shigella* LPS were detected in maternal and cord blood sera; efficiency of placental transfer was 100% and 60%, respectively, and was associated with IgG subclass distribution (protein-specific IgG1 > LPS-specific IgG2). In contrast, SBA and OPKA activity in cord blood was substantially lower as compared to maternal serum and varied among *Shigella* serotypes. LPS was identified as a target of SBA and OPKA activity. Maternal sera had remarkably elevated *Shigella flexneri* 2a LPS IgM, indicative of recent exposure. Our study revealed a broad repertoire of maternally acquired antibodies in infants living in a *Shigella*-endemic region and highlights the abundance of protein-specific antibodies and their likely contribution to disease prevention during the first months of life. These results contribute new knowledge on maternal infant immunity and target antigens that can inform the development of vaccines or therapeutics that can extend protection after maternally transferred immunity wanes.

Introduction

Shigella spp. are major contributors of the global diarrheal disease burden, accounting for more than 250 million cases and 200,000 deaths annually (1, 2). The most affected are children under 5 years of age living in low- and middle-income countries (LMIC) (2, 3). Though usually self-limiting, repeated bouts of disease result in debilitating sequalae including malnutrition, stunted growth, and deficits in immune and cognitive development (3, 4). The preeminence of multidrug resistant *Shigella* strains globally makes the development of vaccines and therapeutics a compelling priority (5). Because the burden of disease disproportionately affects young children, a clear understanding of the elements and immune mechanisms that can protect this group is necessary to inform the development of efficacious vaccines or prophylaxes.

Most of what is known about *Shigella* immunity has been learned from infections in adults. Individuals living in endemic regions acquire natural immunity from repeated exposure (6-9). While there is no definitive immune correlate of protection against shigellosis, serum IgG against the *Shigella* surface-exposed lipopolysaccharide (LPS) has been associated with reduced risk of infection with serotype-matching strains in early field trials [reviewed in Ref (10)]. We have strong evidence that serum IgG specific for the *Shigella* invasion plasmid antigen (Ipa) B and the virulence protein VirG (IcsA) reduced risk of infection in a controlled human infection model (CHIM) study (11). In the same experimentally infected adult volunteers, complement-mediated serum bactericidal (SBA) and opsonophagocytic killing (OPKA) activity were identified as functional attributes of *Shigella*-specific antibodies associated with clinical protection (11).

Children living in endemic regions produce serum LPS- and Ipa-specific IgG in response to *Shigella* infection, and the magnitude of these responses increases progressively through adulthood (6, 9, 12, 13). Multiple surveillance studies have reported consistently that the rate of *Shigella* infection is relatively low during the first months of life, but gradually increases and reaches its peak during the second year of life (14, 15). The shielding of infants from *Shigella*-

induced diarrhea (although they may still suffer from other enteric infections such as rotavirus), hints at a putative pathogen-specific protection afforded by maternal immunity (antibodies transferred via placenta and the immune components of breast milk (16)). Studies of transplacental antibody transfer against other pathogens have shown that this process—termed placental "sieving" (17)—is regulated and selective, antigen-dependent (18, 19), and favors transfer of antibodies with specific biophysical features that make them most effective in the immature neonatal immune system (17). Information on antigen-specificity, magnitude, subclass distribution, and function of *Shigella* antibodies in mothers and infants and the process of placental transfer has been lacking. Here, we characterized the specificity and anti-microbial function of *Shigella*-specific antibodies in mothers and their infants at birth in a longitudinal cohort from rural Malawi. The magnitude of serum IgG (and IgG subclasses) specific for LPS against two *Shigella* serotypes, *S. flexneri* 2a and *S. sonnei*, and conserved *Shigella* proteins IpaB, IpaC, IpaD, IpaH, and VirG were determined. SBA and OPKA levels and the target antigen mediating complement- and phagocytic-effector functions were investigated. Finally, correlative analysis and comparison with protective thresholds were conducted to identify unique features and the potential anti-microbial activity in vivo of *Shigella* antibodies in mother-infant pairs.

Results

Cohort characteristics

This study utilized a mother-infant cohort from a malaria surveillance study in Malawi. Participants were enrolled from rural villages in Chikwawa in the southern region. Out of 108 mother and infant pairs enrolled, 63 pairs were analyzable (Figure 1). Cord blood was collected at birth. Maternal blood was obtained at recruitment within 3 months of delivery. Characteristics

of the cohort are summarized in Table 1. Mean age of the mothers was 26.8 years (17-43 years). Median maternal parity was 3 (0-8). Among the infants, 36 (57%) were female and 5 (8%) had a low birth weight (less than 2.5kg). As for the season of birth, 21 (33%) were born during the rainy season (November – April).

Shigella antigen-specific IgG in mothers and their newborns

Naturally acquired *Shigella*-specific antibodies were determined in paired maternal and cord blood sera. The antigenic repertoire analysis focused on *S. flexneri* 2a and *S. sonnei*; these species had been attributed the highest incidence of moderate-to-severe diarrhea (MSD) in <5-year-old children (37.8% and 13.5%, respectively) in Malawi-neighboring Mozambique by the Global Enteric Multicenter Study (GEMS) (20); precise information on *Shigella* prevalence in Malawi is not available. *S. flexneri* 2a and *S. sonnei* LPS-specific IgG titers in maternal sera were significantly higher as compared to those in cord blood (Figure 2A). The median cord-blood to maternal *S. flexneri* 2a and *S. sonnei* LPS IgG transfer ratios were 0.51 and 0.57 respectively (Figure 2B, Table 2), indicating low transplacental sieving efficiency of LPS IgG.

Maternal and cord IgG levels against five different *Shigella* virulence factors: IpaB, IpaC, IpaD, IpaH, and VirG and their placental transfer efficiency were also determined (Figure 2A and 2B). High levels of circulating IgG specific for all protein antigens were detected in both maternal and cord blood sera, which far surpassed the levels of IgG against LPS (Figure 2A). Likewise, placental transfer of protein-specific maternal IgG was more efficient than the transfer of LPS-specific IgG; median transfer ratios were: 1.06, 1.02, 0.93, 0.97, and 0.96 for anti-IpaB, -IpaC, -IpaD, -IpaH, and -VirG antibodies, respectively (Figure 2B and Table 2). Despite differences in transfer efficiency between LPS- and protein-specific antibody titers, there was a significant and positive linear correlation between maternal and cord blood IgG levels for all antigens, confirming the selective and distinct regulation of placental antibody transport (Figure 2C).

Geometric mean titers (GMT), median transfer ratios, and range of maternal and cord serum titers are summarized in Table 2.

Shigella **protein- and LPS-specific IgG subclass placental transfer**

Placental transport of maternal antibodies is primarily mediated through binding to the neonatal Fc receptor (FcRn) expressed in the syncytiotrophoblast (21, 22). Qualitative differences in the Fc structure, such as in the human IgG subclasses, can influence FcRn binding and placental transfer (23). We therefore explored IgG subclass distribution of *Shigella*-specific antibodies as a contributor to the observed differences in IgG transfer efficiency.

As with total IgG titers, protein-specific IgG subclasses had common features, which differed from those against LPS. In both the mothers and their infants, IgG1 was the most abundant subclass against all protein antigens, followed by IgG2 and IgG3 (Figure 3A and Supplementary table 1). In contrast, IgG2 was the most abundant subclass against both *S. flexneri* 2a and *S. sonnei* LPS. IgG4 titers were generally the lowest for all antigens tested (Figure 3A and Supplementary table 1). For protein antigens, IgG1 and IgG4 had the highest cord-blood:maternal median transfer ratios: 0.97-1.14 and 1.25-1.72, respectively (Figure 3B and Supplementary table 1).

For LPS antigens, IgG1 also exhibited the highest median transfer ratios: 1.13 and 1.28 for *S. flexneri* 2a and *S. sonnei,* respectively (Figure 3B and Supplementary table 1). Median transfer ratios for LPS IgG2 were lower, the lowest being for IgG2 against *S. flexneri* 2a. The predominance of maternal LPS-IgG2 and the lower transfer efficiency of IgG2 explains the low levels of LPS IgG in the cord blood despite their abundance in maternal circulation. The median transfer ratios for antigen-specific IgG compared to IgG subclasses are represented in a heatmap (Figure 3B).

Functional capacity of placentally transferred *Shigella*-specific antibodies

In addition to antibody specificity through direct binding, we examined the functional capacity of maternal and placentally-acquired antibodies to render complement-dependent bactericidal and opsonophagocytic activity. SBA and OPKA activity was detected in both maternal and newborn sera. Maternal SBA and OPKA titers against *S. flexneri* 2a were significantly higher as compared to those against *S. sonnei* (GMT 26,944 compared to 306, respectively). Maternal SBA and OPKA titers specific for *S. flexneri* 2a were also significantly higher in maternal sera as compared to those of cord blood (Figure 4A and 4B); the median transfer ratios were 0.02 and 0.03, respectively (Figure 4A and B, and Table 3). In contrast, SBA and OPKA titers against *S. sonnei* in maternal and infant sera were comparable (Figure 4A); the median transfer ratios were 0.91 and 0.75 (Figure 4B and Table 3). It was noticed that while maternal and cord blood functional antibody titers against *S. sonnei* were strongly correlated, those against *S. flexneri* 2a were not (Figure 4C and D). The discrepancy in functional antibody titer against *S. flexneri* 2a between mothers and infants prompted us to investigate the specificity and type of antibodies involved in bactericidal and opsonophagocytic killing.

Specificity of maternally acquired functional antibodies

Mouse monoclonal antibodies specific for *Shigella* LPS were reported to have bactericidal activity (24). Several studies have reported increases in SBA titers in response to *Shigella* polysaccharide-based vaccine candidates in adult volunteers (25-27). LPS is therefore presumed to be the main antigenic target of antibody-mediated shigellacidal activity. We probed the antigen-specificity of the SBA activity in our cohort by evaluating complement-dependent *Shigella* killing in sera that had been pre-incubated with increasing amounts of IpaB or LPS to

sequester specific antibodies; efficiency of antibody removal was demonstrated by a decrease of ELISA binding signal (Figure 5A left). Depletion of *S. flexneri* 2a and *S. sonnei* LPS antibodies from both maternal and cord blood serum resulted in a proportional (dose-responsive) reduction of bactericidal activity that was serotype-specific (Figure 5A). Depletion of IpaB-specific antibodies had no effect on *S. flexneri* 2a killing. These results confirm the LPS-specificity of the maternal and infant SBA antibodies.

Having identified LPS as the molecular target of antibody function, we compared LPS IgG and SBA titers in both maternal and infant serum. A positive and significant correlation was observed between maternal *S. sonnei* LPS IgG and SBA (Pearson's r = 0.686) but the same was not true for *S. flexneri* 2a (Pearson's r = 0.281) (Figure 5B). However, in the infants, LPS IgG and SBA titers were positively and significantly correlated for both *S. flexneri* 2a (Pearson's r = 0.614) and *S. sonnei* (Pearson's r = 0.707) (Figure 5F). These results hinted that another component, other than IgG, was contributing to maternal *S. flexneri* 2a SBA but was not sieved through the placenta.

IgM is a strong activator of complement that could account for the excess maternal *S. flexneri* 2a SBA and OPKA observed. LPS-specific IgM titers against both *S. flexneri* 2a and *S. sonnei* LPS were detected in maternal serum and in a handful of cord blood samples by ELISA (Figure 5C). While similar in the infants, there was substantially higher IgM against *S. flexneri* 2a as compared to *S. sonnei* LPS (mean of 263 EU/mL compared to 99 EU/mL) in maternal blood (Figure 5C). Depletion of maternal IgM greatly diminished *S. flexneri* 2a SBA (Figure 5D). *S. flexneri* 2a SBA titers measured in the IgM-depleted maternal sera were strongly associated with maternal LPS-specific IgG (Pearson's r = 0.64); this was in contrast to the limited correlation observed when SBA was measured in intact (IgM- and IgG-containing) sera (Figure 5E). These results attribute complement dependent *Shigella* killing activity to both LPS-specific circulating IgG and IgM. In the correlation analyses, maternal *S. flexneri* 2a SBA was more

closely associated with LPS IgM (likely due to its abundance in sera) while *S. sonnei* SBA was mostly associated with LPS IgG (Figure 5F). Associations were also calculated for SBA and LPS-specific IgG, IgG1-4, and IgM for both strains in maternal and cord blood. IgG2 (the predominant LPS-specific antibody) was the subclass most associated with *S. sonnei* and *S. flexneri* 2a SBA in cord blood serum (Figure 5F). IgG1 against *S. sonnei* LPS was equally associated with SBA in the infants.

Comparisons with protective titers

Finally, to place the mother-infant antigen-specific and functional antibody titers determined in this study in the context of protective immunity, we compared serological outcomes in the dyad with those measured in individuals who remained healthy or had only mild disease when challenged with wild type *S. flexneri* 2a in a CHIM study (11). IpaB and *S. flexneri* 2a LPS-IgG titers in maternal serum and cord blood were significantly higher than those found in adult American volunteers that remained healthy post experimental oral challenge (Figure 6A). Likewise, maternal *S. flexneri* SBA and OPKA titers were similar or higher than those of the same protected individuals. In contrast, the functional SBA and OPKA antibodies in the infants were significantly lower than those of volunteers clinically protected against experimental *Shigella* infection (Figure 6B).

Discussion

Through lifelong exposure, adults living in endemic regions develop natural immunity against *Shigella*, which has been attributed to antibodies against serotype specific LPS (10). The incidence of moderate to severe diarrhea attributable to *Shigella* progressively increases after the first year of life, reaching its peak in young children 24 to 59 months of age. Although they experience other diarrheal diseases, young infants are shielded from *Shigella* dysentery

presumably through maternal immunity acquired via placenta or breast milk (16). The exact elements that prevent infection in these children, the contribution of antibodies specific for LPS or for other bacterial antigens, and the antimicrobial mechanisms involved are not known. An understanding of the particular components of this shielding immunity is important as new candidate vaccines targeted for the susceptible infant and toddler groups continue to advance in the clinical pathway. To identify maternally derived humoral immune components that may contribute to protection of young infants during the first months of life, we characterized the repertoire of *Shigella*-specific antibodies in a cohort of Malawian mothers and their infants at the time of birth.

Serum IgG specific for both *S. flexneri* 2a LPS and *S. sonnei* LPS were detected in maternal and in cord blood sera, although their placental transfer efficiency was moderate, 0.51 and 0.57, respectively. Two previous studies of transplacental transfer of antibodies against *S. flexneri* 2a and *S. sonnei* LPS in Israel (28) and against *S. sonnei* LPS in Vietnam (29), also found high levels of IgG specific for *Shigella* LPS in maternal sera. Our data suggest that the mothers in our cohort had been exposed to both *S. flexneri* 2a and *S. sonnei*, which is consistent with the reported serotype prevalence in countries neighboring Malawi (20, 30), and other endemic regions (28, 31, 32). The study in Vietnam reported a much higher median transfer ratio (1.33) for *S. sonnei* LPS IgG (29) than was observed in our cohort, which may reflect regional differences in seroprevalence due to *Shigella* circulation (and possibly increased proportions of LPS-specific IgG1).

An abundance of serum IgG against *Shigella* type 3 secretion system (TTSS) proteins IpaB, IpaC, IpaD, IpaH, and the virulence factor VirG were observed in maternal and infant circulation. Such a detailed serological interrogation of protein specific antibodies had been hindered by the difficulty in obtaining *Shigella* antigens of high quality and in sufficient yield; classical literature that report Ipa antibody analyses in endemic regions typically relied on crude and undefined

protein extracts (9, 12). Different from antibodies to LPS, protein-specific antibodies were efficiently transferred to the newborns; cord blood IgG titers were similar or even higher than those in maternal sera for all the proteins examined. Consistent with our findings, a reduced placental transfer of polysaccharide- compared to protein-specific antibodies has been reported for other pathogens, such as *Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumoniae* (33-35). Despite the differences in magnitude, we observed that maternal and infant antibody levels were correlated for all antigens, which confirms the regulated and selective nature of transplacental transfer in a process that is antigen/antibody dependent. *Shigella*-specific placental antibody sieving was distinctly linked to IgG subclass. While protein-specific antibodies were primarily IgG1 (followed by IgG2, IgG3, and IgG4), *S. flexneri* 2a and *S. sonnei* LPS-specific IgG contained mainly IgG2 (followed by IgG1, IgG3, and IgG4). The superior levels of protein-specific IgG1 in infant blood as compared to LPS-specific IgG2 is consistent with the hierarchy of receptor-mediated IgG subclass transport based on affinity to FcRn and other placental Fc receptors (34-36). Given their distinct functional attributes, the IgG subclass profile available to the infants will determine the anti-microbial capacity of humoral immunity early in life (1, 23, 43).

SBA and OPKA titers have been correlated with clinical protection in adults experimentally infected with virulent *S. flexneri* 2a (11). We confirmed *Shigella* LPS as a target antigen for *S. flexneri* 2a SBA activity in both maternal and infant sera. Consistently, LPS IgG (and particularly IgG2) was strongly correlated with SBA activity for both *S. flexneri* 2a and *S. sonnei*. Interestingly, IgG2, which makes up the bulk of LPS-IgG, is a poor complement activator. However, IgG2 is known to mediate complement-dependent killing of *Haemophilus influenzae* type b, albeit not as efficiently as IgG1 (37). On the other hand, LPS IgG2 may activate an alternate pathway when epitope densities are high (38), which is likely the case for a surface-exposed target like LPS. IgG2 can act in a complement-independent manner as has been

shown with opsonophagocytic activity against *S. pneumoniae* (39), which is consistent with OPKA activity observed in our study.

Unexpectedly enhanced SBA and OPKA activity against *S. flexneri* 2a was observed in the mothers (but not in the infants) from our cohort that was linked to high levels of IgM (a potent activator of complement that is not placentally transferred). A similar reduction in functional activity in infant compared to maternal sera, also due to maternal IgM, has been shown against *E. coli* and *Salmonella* (40, 41). Though mothers in our cohort had comparable levels of LPS IgG against both serotypes, maternal LPS-specific IgM against *S. flexneri* 2a was markedly higher resulting in heightened SBA activity. Differences in IgM levels likely reflect frequency of exposures and strain circulation, suggesting, in our study, a higher prevalence of *S. flexneri* 2a as compared to *S. sonnei* in the Blantyre, Malawi, region. A handful of infant samples had detectable IgM against LPS from both serotypes. IgM against environmental and vaccine antigens has been reported in cord blood from infants in LMIC but not in those from industrialized nations. The origin of this IgM is unclear and presumed to reflect environmental factors, such as intrauterine infections that affect placenta integrity (42, 43), and non-specific natural antibodies (42, 44). Maternal SBA and OPKA against both serotypes were strongly associated implying shared antibody contribution to microbial killing.

The underlying premise for dissecting the humoral immune profile against *Shigella* in mothers and infants living in endemic regions is the epidemiological evidence of the lowest risk of infection in these groups. Maternal *S. flexneri* 2a LPS-IgG, SBA, and OPKA titers were comparable or higher than those observed in North American volunteers who remained healthy following challenge with wild-type *S. flexneri* 2a organisms (11). The functional antibody activity in the infants was noticeably below the threshold of clinically protected adults. In contrast, serum IgG against protective target antigens IpaB and VirG in mother and infant sera were well above those detected in the clinically protected challenged volunteers (11). The low levels of LPS-

specific IgG and their limited functional capacity (SBA and OPKA) in cord blood, along with high levels of maternal protein-specific IgG and its efficient transfer, argue in favor of a more prominent role of antibodies against *Shigella* virulence antigens in preventing *Shigella* infection than originally thought. Similarly, others observed that naturally acquired maternal antibodies against pneumococcal proteins, unlike anti-polysaccharide antibodies, were associated with protection against nasal carriage in infants during the first 3 months of life (45). Further studies are warranted to dissect the mechanisms, not captured by our traditional assays, by which these protein-specific antibodies block microbial infection and to corroborate their disease protective capacity in humans.

A *Shigella* vaccine that is efficacious in children under 3 years of age, the most vulnerable target group, would make a major public health impact. The limited efficacy of a clinically advanced O-polysaccharide-based vaccine candidate for young children (46, 47) has been linked with young children's hypo-responsiveness to *Shigella* LPS (and likely impaired bactericidal/phagocytic antibody activity). Our results showing the abundance of protein-specific antibodies in groups naturally immune (low-risk group) to *Shigella* and the ease with which children respond to protein-based immunization highlight the prospect of a *Shigella* protein-based vaccine approach. Purified IpaB and IpaD (48-50) or a formulation containing IpaB and IpaC (Invaplex, (51)) have been shown to prevent *Shigella* infection in preclinical studies. While the exact operative mechanisms are yet to be determined, antibodies targeting *Shigella* proteins are expected to block *Shigella* attachment and TTSS protein translocation (anti-IpaB, -C, and -D), prevent bacterial spread and replication (anti-VirG and anti-IpaH) and prevent inflammation (52). A vaccine combining multiple conserved *Shigella* proteins would not only be likely broadly protective, but also effective, targeting multiple mechanisms important for *Shigella* invasion and virulence.

One limitation in our study was that maternal serum was obtained within 3 months of birth. While some antibody features may change after birth, we did not find significant differences in maternal titers determined in sera collected prior to or after delivery. The limited sample size may have also precluded more extensive and complex statistical analyses. It would be important to conduct similar analyses of the antibody repertoire beyond birth and through the first 3 years of life to better understand trends of disease and immune acquisition, and to investigate age-specific antibody mediated antimicrobial functions using age-relevant immune cells to recreate elements that would operate in vivo (17).

In summary, we have demonstrated, for the first time, the efficient placental transfer of maternal antibodies against *Shigella* protein antigens and their availability at high levels to the infant at birth along with the less efficient transfer of LPS-specific IgG with bactericidal and opsonophagocytic killing activity. Our results define the maternally acquired protective antibody repertoire available to infants at birth and suggest a larger role for protein-specific immunity than has previously been appreciated. Exploring the concept of a protein-based vaccine that would target these antigens either alone or in conjunction with *Shigella* LPS is warranted. Finally, these findings emphasize the need to better understand strain-specific immunity in young children to inform preventive strategies.

Acknowledgments

We are grateful to the women who volunteered to participate and to the nurse midwives of the Mfera Health Centre maternity ward and antenatal clinic who supported this study. We thank Syze Gama and Dr. Avital Shimanovich for providing technical and logistic expertise during the sample collection process.

Funding

This project was supported in part by federal funds from the U.S. National Institutes of Health, under grant numbers R01AI117734, R01AI125841 and Research Supplement to Promote Diversity in Health-Related Research Program, 3R01AI117734-04S1. EN is supported by T32DK067872.

Methods

Study population and sample collection

Our study population consisted of mothers and infants recruited between January and November 2016 at Mfera Health Clinic in Chikwawa, Malawi. Healthy pregnant women who were HIV seronegative were enrolled either at the antenatal clinic or during delivery. Infants were enrolled at birth. Baseline information on mother and the infant's health was obtained at enrollment. Other information such as village, baseline health info, and current physical complaints was also collected during the visit. Umbilical cord blood was collected at delivery at the Mfera Health Clinic. Venous maternal blood was obtained at enrollment, either during screening before birth, at a well-child visit (week 1, 6, or 10) or at 3 months. Samples were frozen and shipped to the University of Maryland School of Medicine in Baltimore for analysis. This study was approved by the Institutional Review Board of University of Maryland School of Medicine, and the College of Medicine Research and Ethics Committee (COMREC) at the College of Medicine in Malawi. All participating mothers provided written informed consent for themselves and their infants. Serum samples from North American individuals were obtained from a previous clinical study performed at the Center for Vaccine Development (University of Maryland, Baltimore) under approved IRB protocols. Serum samples tested were obtained at day -1, prior to challenge with 1 X 10^3 CFU of the wild-type *S. flexneri* 2a strain 2457T as described previously (53); some of these volunteers had been previously vaccinated and had

varying degrees of immunity. Specimens were selected from volunteers who remained healthy or who experienced mild disease, as previously described (11).

Antigen-specific antibody analysis

Shigella antigens IpaB, IpaC, IpaD, *S. flexneri* 2a LPS and *S. sonnei* LPS were obtained from Walter Reed Army Institute of Research (WRAIR). The N-terminal domain of VirG was expressed and purified inhouse in an *E. coli* expression system (Chitra STS et al., unpublished). IpaH was produced by Vaxcyte (N. Kapoor et al., manuscript in preparation). Antigen-specific serum IgG titers were measured by ELISA as previously described (54). Briefly, Immulon 2HB plates (Thermo Scientific, Waltham MA) were coated with IpaB, IpaC, IpaD, and IpaH at 0.1µg/mL in PBS, and VirG, *S. flexneri* 2a LPS and *S. sonnei* LPS at 5µg/mL in carbonate buffer, pH 9.6. Plates were incubated for 3h at 37°C and blocked at 4°C overnight in PBS containing 10% w/v non-fat dry milk (NFDM). Sera diluted in PBS containing 10% NFDM and 0.05% Tween-20 (PBS-T) were added, and the plates incubated at 37°C for 1h. Plates were incubated with HRP-labeled goat IgG specific for human IgG (Jackson Immuno Research, West Grove, PA) for another 1h at 37°C. Plates were washed 6 times with PBS-T following every incubation step. Tetramethylbenzidine (TMB; KPL, Gaithersburg, MD) was added as substrate for 15 min in the dark with shaking, and the reaction was stopped by adding 1M phosphoric acid (Millipore Sigma, Burlington, MA). Endpoint titers were calculated as the inverse serum dilution that resulted in an absorbance value at 450 nm of 0.2 above background and were reported as the ELISA units/mL.

Antigen-specific IgG subclasses were measured using *Shigella* multiplex assay using the MesoScale Diagnostic platform (MSD, Rockville, MD). Assays were run in the same way as the antigen-specific ELISAs, with a few exceptions: 1. There was no antigen-coating step as the antigens were pre-printed on MSD plates. 2. After the serum incubation step, the plates were incubated with biotinylated anti-IgG subclass antibodies (SouthernBiotech, Birmingham, AL) plus the SULFOTag-STREP (MSD) for another 1h at 37°C. 3. Binding was then detected using

MSD GOLD Read buffer. The plates were read using an MSD sector imager, model 2400 and data analyzed by the MSD workbench software provided by the manufacturer. The ECL signal (minus background from blank) for each sample (diluted at 1:100 in PBS containing 10% NFDM) was reported.

Functional assays

SBA. The SBA assay was performed as previously described (55). SBA titers were determined by Opsotiter (55) as the reciprocal of the serum dilution that produced 50% bacterial killing as determined by Reed-Muench regression analysis. The provisional reference serum sample, Korean QC19, was run with each assay to normalize and reduce variability between assays. Korean QC19 was assigned a titer = 28000 for *S. flexneri* 2a and 1100 for *S sonnei* (55). The lowest dilution tested was 1:200 so that the lowest titer is 100.

OPKA. OPKA was also performed as previously described (11) with some modifications. Briefly, 10 µL of target bacteria (~10^4 CFU/mL) was opsonized by mixing with 20µL of a heat-inactivated, serially diluted test sample in a well of a round-bottom microtiter plate and incubated for 15 min at 37°C in room air, with shaking. As with the SBA, control wells had bacteria, baby rabbit complement (BRC), and buffer only; no test sample was added to these wells. 10µL of baby rabbit complement (10% final concentration), mixed with 60µL of 10^5 dimethylformamide; (DMF)-differentiated HL-60 cells (ATCC CCL-240) were then added to the reaction mixture (for a 100µL total volume). Following a 45 min incubation at 37°C, 5% CO_2, 10 µL from each well was spotted on LB agar. The agar plates were incubated overnight at 29°C for *S. flexneri* 2a and 26°C for *S. sonnei*. The percentage of bacteria that were phagocytosed and killed per well was determined by measuring the colony counts and determining the titer, as was done for the SBA (above). Standardization of the *Shigella* OPKA has not yet been done, but the Korean QC19 was run with each assay and was found to have an average titer of 15574 for *S. flexneri* 2a and 288 for *S sonnei*. The lowest dilution tested was 1:200 so that the lowest titer is 100.

Antibody depletion. (i) LPS and IpaB-specific antibodies were sequestered by incubating serum samples into ELISA plates coated with serial dilutions of antigen (0 - 50µg/mL) as described above for 3h at 37°C with shaking. Antibody depletion was confirmed by ELISA. The depleted serum sample was then tested for SBA activity as described above. (ii) IgM was removed by incubating serum samples with 50mM of beta-mercaptoethanol for 1h at 37°C. The IgM-depleted serum was further diluted and used in the SBA reaction as described above.

Statistical analysis

Geometric mean titers (GMT) were calculated for antigen-specific IgG in maternal and cord blood sera. Comparisons of titers between maternal and cord samples, or between antibody levels against different antigens, were measured by paired t-test or one-way analysis of variance (ANOVA) with Tukey's post-test correction. Placental transfer ratios were assessed as a ratio of cord blood divided by maternal antibody titers. Associations between maternal and cord blood titers were calculated using Pearson's correlation. A two-way ANOVA with Tukey's post-test correction was used to compare transfer ratios between IgG subclasses. All statistical analysis was conducted using GraphPad Prism 9**.**

References

1. GBD 2017 Causes of Death Collaborators. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2018;392:1736-88.
2. Khalil IA, Troeger C, Blacker BF, Rao PC, Brown A, Atherly DE, et al. Morbidity and mortality due to *Shigella* and enterotoxigenic *Escherichia coli* diarrhoea: the Global Burden of Disease Study 1990-2016. Lancet Infect Dis. 2018;18(11):1229-40.
3. Khalil I, Troeger CE, Blacker BF, Reiner RC, Jr. Capturing the true burden of Shigella and ETEC: The way forward. Vaccine. 2019;37(34):4784-6.
4. Guerrant RL, DeBoer MD, Moore SR, Scharf RJ, Lima AA. The impoverished gut--a triple burden of diarrhoea, stunting and chronic disease. Nat Rev Gastroenterol Hepatol. 2013;10(4):220-9.
5. WHO. WHO publishes list of bacteria for which new antibiotics are urgently needed 2017 [Available from: https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed.
6. Oaks EV, Hale TL, Formal SB. Serum immune response to *Shigella* protein antigens in rhesus monkeys and humans infected with *Shigella spp*. Infect Immun. 1986;53(1):57-63.
7. Cohen D, Green MS, Block C, Rouach T, Ofek I. Serum antibodies to lipopolysaccharide and natural immunity to shigellosis in an Israeli military population. J Infect Dis. 1988;157(5):1068-71.
8. Robin G, Cohen D, Orr N, Markus I, Slepon R, Ashkenazi S, et al. Characterization and quantitative analysis of serum IgG class and subclass response to Shigella sonnei and Shigella flexneri 2a lipopolysaccharide following natural Shigella infection. J Infect Dis. 1997;175(5):1128-33.
9. Oberhelman RA, Kopecko DJ, Salazar-Lindo E, Gotuzzo E, Buysse JM, Venkatesan MM, et al. Prospective study of systemic and mucosal immune responses in dysenteric patients to specific *Shigella* invasion plasmid antigens and lipopolysaccharides. InfectImmun. 1991;59(7):2341-50.
10. Cohen D, Meron-Sudai S, Bialik A, Asato V, Goren S, Ariel-Cohen O, et al. Serum IgG antibodies to Shigella lipopolysaccharide antigens - a correlate of protection against shigellosis. Hum Vaccin Immunother. 2019;15(6):1401-8.
11. Shimanovich AA, Buskirk AD, Heine SJ, Blackwelder WC, Wahid R, Kotloff KL, et al. Functional and Antigen-Specific Serum Antibody Levels as Correlates of Protection against Shigellosis in a Controlled Human Challenge Study. Clin Vaccine Immunol. 2017;24(2).
12. Van de Verg LL, Herrington DA, Boslego J, Lindberg AA, Levine MM. Age-specific prevalence of serum antibodies to the invasion plasmid and lipopolysaccharide antigens of *Shigella* species in Chilean and North American populations. JInfectDis. 1992;166(1):158-61.
13. Raqib R, Qadri F, SarkEr P, Mia SM, Sansonnetti PJ, Albert MJ, et al. Delayed and reduced adaptive humoral immune responses in children with shigellosis compared with in adults. Scand J Immunol. 2002;55(4):414-23.
14. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet. 2013;382(9888):209-22.
15. Ferreccio C, Prado V, Ojeda A, Cayyazo M, Abrego P, Guers L, et al. Epidemiologic patterns of acute diarrhea and endemic Shigella infections in children in a poor periurban setting in Santiago, Chile. Am J Epidemiol. 1991;134(6):614-27.

16. Ogra PL. Immunology of Human Milk and Lactation: Historical Overview. Nestle Nutr Inst Workshop Ser. 2020;94:11-26.
17. Jennewein MF, Goldfarb I, Dolatshahi S, Cosgrove C, Noelette FJ, Krykbaeva M, et al. Fc Glycan-Mediated Regulation of Placental Antibody Transfer. Cell. 2019;178(1):202-15 e14.
18. Fu C, Lu L, Wu H, Shaman J, Cao Y, Fang F, et al. Placental antibody transfer efficiency and maternal levels: specific for measles, coxsackievirus A16, enterovirus 71, poliomyelitis I-III and HIV-1 antibodies. Sci Rep. 2016;6:38874.
19. Clements T, Rice TF, Vamvakas G, Barnett S, Barnes M, Donaldson B, et al. Update on Transplacental Transfer of IgG Subclasses: Impact of Maternal and Fetal Factors. Front Immunol. 2020;11:1920.
20. Livio S, Strockbine NA, Panchalingam S, Tennant SM, Barry EM, Marohn ME, et al. Shigella Isolates From the Global Enteric Multicenter Study Inform Vaccine Development. ClinInfectDis. 2014;59(7):933-41.
21. Simister NE. Placental transport of immunoglobulin G. Vaccine. 2003;21(24):3365-9.
22. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. Front Immunol. 2014;5:520.
23. Wilcox CR, Holder B, Jones CE. Factors Affecting the FcRn-Mediated Transplacental Transfer of Antibodies and Implications for Vaccination in Pregnancy. Front Immunol. 2017;8:1294.
24. Lin J, Smith MA, Benjamin WH, Jr., Kaminski RW, Wenzel H, Nahm MH. Monoclonal Antibodies to Shigella Lipopolysaccharide Are Useful for Vaccine Production. Clin Vaccine Immunol. 2016;23(8):681-8.
25. Riddle MS, Kaminski RW, Di Paolo C, Porter CK, Gutierrez RL, Clarkson KA, et al. Safety and Immunogenicity of a Candidate Bioconjugate Vaccine against *Shigella flexneri* 2a Administered to Healthy Adults: a Single-Blind, Randomized Phase I Study. Clin Vaccine Immunol. 2016;23(12):908-17.
26. Cohen D, Atsmon J, Artaud C, Meron-Sudai S, Gougeon ML, Bialik A, et al. Safety and immunogenicity of a synthetic carbohydrate conjugate vaccine against Shigella flexneri 2a in healthy adult volunteers: a phase 1, dose-escalating, single-blind, randomised, placebo-controlled study. Lancet Infect Dis. 2020.
27. Clarkson KA, Talaat KR, Alaimo C, Martin P, Bourgeois AL, Dreyer A, et al. Immune response characterization in a human challenge study with a Shigella flexneri 2a bioconjugate vaccine. EBioMedicine. 2021;66:103308.
28. Passwell JH, Freier S, Shor R, Farzam N, Block C, Lison M, et al. Shigella lipopolysaccharide antibodies in pediatric populations. PediatrInfectDisJ. 1995;14(10):859-65.
29. Thompson CN, Le TP, Anders KL, Nguyen TH, Lu LV, Nguyen VV, et al. The transfer and decay of maternal antibody against *Shigella sonnei* in a longitudinal cohort of Vietnamese infants. Vaccine. 2016;34(6):783-90.
30. Chisenga CC, Bosomprah S, Simuyandi M, Mwila-Kazimbaya K, Chilyabanyama ON, Laban NM, et al. Shigella-specific antibodies in the first year of life among Zambian infants: A longitudinal cohort study. PLoS One. 2021;16(5):e0252222.
31. Islam D, Wretlind B, Ryd M, Lindberg AA, Christensson B. Immunoglobulin subclass distribution and dynamics of Shigella-specific antibody responses in serum and stool samples in shigellosis. Infect Immun. 1995;63(5):2054-61.
32. Sayem MA, Ahmad SM, Rekha RS, Sarker P, Agerberth B, Talukder KA, et al. Differential host immune responses to epidemic and endemic strains of Shigella dysenteriae type I. J Health Popul Nutr. 2011;29(5):429-37.

33. Palmeira P, Quinello C, Silveira-Lessa AL, Zago CA, Carneiro-Sampaio M. IgG Placental Transfer in Healthy and Pathological Pregnancies. Clinical and Developmental Immunology. 2012;2012:985646.
34. Stach SC, Brizot ML, Liao AW, Palmeira P, Francisco RP, Carneiro-Sampaio MM, et al. Placental transfer of IgG antibodies specific to Klebsiella and Pseudomonas LPS and to group B Streptococcus in twin pregnancies. Scand J Immunol. 2015;81(2):135-41.
35. van den Berg JP, Westerbeek EA, Berbers GA, van Gageldonk PG, van der Klis FR, van Elburg RM. Transplacental transport of IgG antibodies specific for pertussis, diphtheria, tetanus, haemophilus influenzae type b, and Neisseria meningitidis serogroup C is lower in preterm compared with term infants. Pediatr Infect Dis J. 2010;29(9):801-5.
36. Einhorn MS, Granoff DM, Nahm MH, Quinn A, Shackelford PG. Concentrations of antibodies in paired maternal and infant sera: relationship to IgG subclass. J Pediatr. 1987;111(5):783-8.
37. Amir J, Scott MG, Nahm MH, Granoff DM. Bactericidal and opsonic activity of IgG1 and IgG2 anticapsular antibodies to Haemophilus influenzae type b. J Infect Dis. 1990;162(1):163-71.
38. Aase A, Michaelsen TE. Opsonophagocytic activity induced by chimeric antibodies of the four human IgG subclasses with or without help from complement. Scand J Immunol. 1994;39(6):581-7.
39. Lortan JE, Kaniuk AS, Monteil MA. Relationship of in vitro phagocytosis of serotype 14 Streptococcus pneumoniae to specific class and IgG subclass antibody levels in healthy adults. Clin Exp Immunol. 1993;91(1):54-7.
40. Gitlin D, Rosen FS, Michael JG. Transient 19S gammaglobulin deficiency in the newborn infant, and its significance. Pediatrics. 1963;31:197-208.
41. de Alwis R, Tu LTP, Quynh NLT, Thompson CN, Anders KL, Van Thuy NT, et al. The Role of Maternally Acquired Antibody in Providing Protective Immunity Against Nontyphoidal Salmonella in Urban Vietnamese Infants: A Birth Cohort Study. J Infect Dis. 2019;219(2):295-304.
42. Deorari AK, Broor S, Maitreyi RS, Agarwal D, Kumar H, Paul VK, et al. Incidence, clinical spectrum, and outcome of intrauterine infections in neonates. J Trop Pediatr. 2000;46(3):155-9.
43. King CL, Malhotra I, Wamachi A, Kioko J, Mungai P, Wahab SA, et al. Acquired immune responses to Plasmodium falciparum merozoite surface protein-1 in the human fetus. J Immunol. 2002;168(1):356-64.
44. Xia L, Gildersleeve JC. Anti-glycan IgM repertoires in newborn human cord blood. PLoS One. 2019;14(7):e0218575.
45. Ojal J, Goldblatt D, Tigoi C, Scott JAG. Effect of Maternally Derived Anti-protein and Anticapsular IgG Antibodies on the Rate of Acquisition of Nasopharyngeal Carriage of Pneumococcus in Newborns. Clin Infect Dis. 2018;66(1):121-30.
46. Passwell JH, Ashkenazi S, Harlev E, Miron D, Ramon R, Farzam N, et al. Safety and immunogenicity of Shigella sonnei-CRM9 and Shigella flexneri type 2a-rEPAsucc conjugate vaccines in one- to four-year-old children. Pediatr Infect Dis J. 2003;22(8):701-6.
47. Passwell JH, Ashkenzi S, Banet-Levi Y, Ramon-Saraf R, Farzam N, Lerner-Geva L, et al. Age-related efficacy of *Shigella* O-specific polysaccharide conjugates in 1-4-year-old Israeli children. Vaccine. 2010;28(10):2231-5.
48. Heine SJ, Diaz-McNair J, Martinez-Becerra FJ, Choudhari SP, Clements JD, Picking WL, et al. Evaluation of immunogenicity and protective efficacy of orally delivered Shigella type III secretion system proteins IpaB and IpaD. Vaccine. 2013;31(28):2919-29.
49. Heine SJ, Diaz-McNair J, Andar AU, Drachenberg CB, Van D, V, Walker R, et al. Intradermal delivery of Shigella IpaB and IpaD type III secretion proteins: kinetics of cell

recruitment and antigen uptake, mucosal and systemic immunity, and protection across serotypes. JImmunol. 2014;192(4):1630-40.

50. Heine SJ, Franco-Mahecha OL, Chen X, Choudhari S, Blackwelder WC, van Roosmalen ML, et al. *Shigella* IpaB and IpaD displayed on *L. lactis* bacterium-like particles induce protective immunity in adult and infant mice. ImmunolCell Biol. 2015;93(7):641-52.
51. Turbyfill KR, Clarkson KA, Vortherms AR, Oaks EV, Kaminski RW. Assembly, Biochemical Characterization, Immunogenicity, Adjuvanticity, and Efficacy of Shigella Artificial Invaplex. mSphere. 2018;3(2).
52. Ndungo E, Pasetti MF. Functional antibodies as immunological endpoints to evaluate protective immunity against Shigella. Hum Vaccin Immunother. 2020;16(1):197-205.
53. Kotloff KL, Losonsky GA, Nataro JP, Wasserman SS, Hale TL, Taylor DN, et al. Evaluation of the safety, immunogenicity, and efficacy in healthy adults of four doses of live oral hybrid *Escherichia coli- Shigella flexneri* 2a vaccine strain EcSf2a-2. Vaccine. 1995;13(5):495-502.
54. Kotloff KL, Pasetti MF, Barry EM, Nataro JP, Wasserman SS, Sztein MB, et al. Deletion in the *Shigella* enterotoxin genes further attenuates *Shigella flexneri* 2a bearing guanine auxotrophy in a phase 1 trial of CVD 1204 and CVD 1208. JInfectDis. 2004;190(10):1745-54.
55. Nahm MH, Yu J, Weerts HP, Wenzel H, Tamilselvi CS, Chandrasekaran L, et al. Development, Interlaboratory Evaluations, and Application of a Simple, High-Throughput Shigella Serum Bactericidal Assay. mSphere. 2018;3(3).

Tables

Table 1. Baseline Characteristics of 63 Participating Mother-Infant Pairs

Characteristic	Value
Maternal characteristics	
Age, y, median (range)	26 (17-43)
Parity, median (range)	3 (0-8)
Infant characteristics	
Female sex (%)	36 (57%)
Birth weight, kg, median (range)	3.1 (1.5-4.3)
< 2.5 kg (low birth weight), n (%)	5 (8%)
Twin births, n (%)	3(4.8%)
Born during rainy season (November- April), n (%)	21 (33%)

Table 2. *Shigella* antigen-specific titers

Antigen	Cord blood GMT EU/mL (range)	Maternal serum GMT EU/mL (range)	Transfer ratio Median (range)
S. flexneri 2a LPS	1,752 (120-26,789)	3,491 (153-123,844)	0.51 (0.05-2.20)
S. sonnei LPS	1,264 (45-19,002)	2,109 (135-32,727)	0.57 (0.23-3.38)
IpaB	77,134 (7,274-419,898)	73,737 (6,818-324,773)	1.06 (0.27-2.00)
IpaC	21,856 (279-125,268)	21,732 (292-127,040)	1.02 (0.34-2.02)
IpaD	9,761 (84-92,804)	10,653 (81-112,576)	0.93 (0.26-2.08)
IpaH	19,890 (1,224-122,206)	21,156 (1,067-145,110)	0.97 (0.21-1.98)
VirG	14,840 (3,038-63,877)	15,726 (2,003-75,273)	0.96 (0.29-3.71)

Table 3. *Shigella* functional antibody (SBA and OPKA) titers

Antigen	Cord blood GMT (range)	Maternal serum GMT (range)	Transfer ratio Median (range)
S. flexneri 2a SBA	1,227 (200-113,628)	44,076 (6,889-1,278,483)	0.02 (0.00-2.78)
S. flexneri 2a OPKA	895 (100-178,424)	26,944 (2,770-596,279)	0.03 (0.00-1.77)
S. sonnei SBA	832 (200-13,536)	1,017 (200-17,916)	0.91 (0.19-13.61)
S. sonnei OPKA	245 (100-2,468)	357 (100-2,460)	0.75 (0.18-3.03)

Figure Legends

Figure 1. Flowchart showing selection of paired maternal-infant samples

Figure 2. *Shigella*-specific maternal antibody repertoire and placental transfer efficiency. (A) IgG against *Shigella* LPS and protein antigens in maternal (M) and cord blood (CB) sera. Symbols represent individual titers. Asterisks indicate statistically significant differences determined by paired t-test. * $P < 0.05$, **** $P < 0.0001$. (B) Placental transfer ratios (cord blood titer/maternal titer) for antibodies against each antigen. Whiskers show minimum and maximum values. Line is at ratio = 1. (C) Associations between maternal and cord blood titers for each antigen. Pearson's r and *P* values are shown within each graph.

Figure 3. IgG subclass distribution of maternal and infant placentally acquired antibodies. (A) Radar plots depict the ECL signal for IgG1, IgG2, IgG3, and IgG4 against *Shigella* antigens measured in maternal and cord blood sera diluted at 1:100. Symbols represent the geographic mean signal. (B) Heatmap representing median placental transfer ratios (cord blood titer/maternal titer) for *Shigella* antigen-specific IgG and IgG subclasses. Statistically significant differences between the transfer ratio of each IgG subclass compared to the total IgG transfer ratio were evaluated by one-way ANOVA with Dunnett's post-test correction following ROUT analysis to exclude outliers. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Figure 4. Maternal and infant placentally acquired functional antibodies against *Shigella*. (A) Serum bactericidal antibody (SBA) and (B) opsonophagocytic killing antibody (OPKA) titers measured in maternal (M) and cord blood (CB) sera (left graphs). Data represent individual titers. Placental transfer ratios (cord blood titer/maternal titer) are shown on the graphs on the right. Data represent individual titers. Asterisks indicate statistically significant differences between M and CB titers as determined by one-way ANOVA with a Tukey's post-test correction

(** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). Whiskers indicate minimum and maximum values. Line is at ratio = 1. (C) and (D) Associations between maternal and cord blood SBA and OPKA titers, respectively. Pearson's r and *P* values are shown within each graph.

Figure 5. *Shigella* LPS-specific antibodies exhibit bactericidal activity. (A) Depletion of IpaB- or LPS-specific antibodies shown by decrease in percent ELISA signal in sera that had been incubated with increasing antigen concentration on ELISA plate (left panel) and percent killing of *S. flexneri* 2a in a bactericidal assay using antibody-depleted sera (right panel). Darker-shaded symbols represent maternal sera and lighter-shaded symbols represent infant sera. (B) Correlations between maternal LPS IgG and SBA against *S. flexneri* 2a (left panel) or *S. sonnei* (right panel). (C) Mean (bars) and individual (symbols) IgM titers against *S. flexneri* 2a and *S. sonnei* LPS in maternal (M) and cord blood (CB) sera. Maternal and cord blood titers were compared by one-way ANOVA with a Tukey's post-test correction (**** $P < 0.0001$). (D) Mean (bars) and individual (symbols) SBA titers in maternal serum before (Total) or after IgM depletion (IgM depleted). SBA titers in the two groups were compared by paired t test (** $P < 0.01$). (E) Associations between *S. flexneri* 2a LPS IgG and SBA titers before (filled circle) and after IgM depletion (open circles). Pearson's r values are shown within the graph. (F) Heatmap showing associations (Pearson's r) between SBA and Total IgG, IgG subclasses, and IgM against LPS.

Figure 6. Comparative analysis of *Shigella* antigen-specific and functional antibody titers in the mother-infant dyad in relation to clinical protection. *Shigella* antigen-specific (A) and *S. flexneri* 2a functional (B) antibody titers measured in pre-challenge serum from North American individuals (black squares, protected) that were either healthy or had mild disease after wild-type *Shigella* challenge and in maternal (M) or cord blood (CB) sera from the Malawi cohort. Data represent individual titers. Differences between groups were determined by paired t-test (ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

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Figure 1

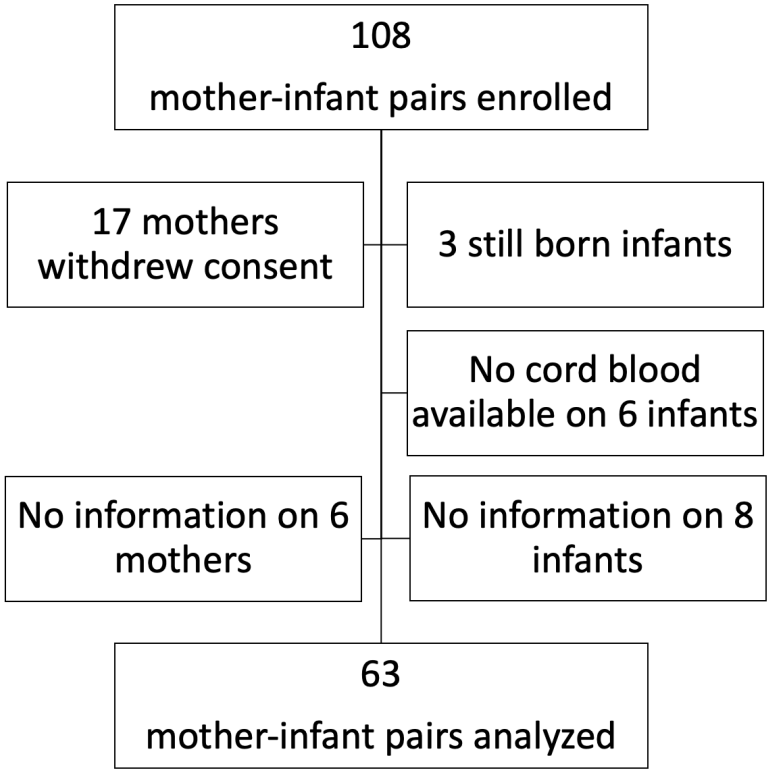

Figure 1. Flowchart showing selection of paired maternal-infant samples

Figure 2

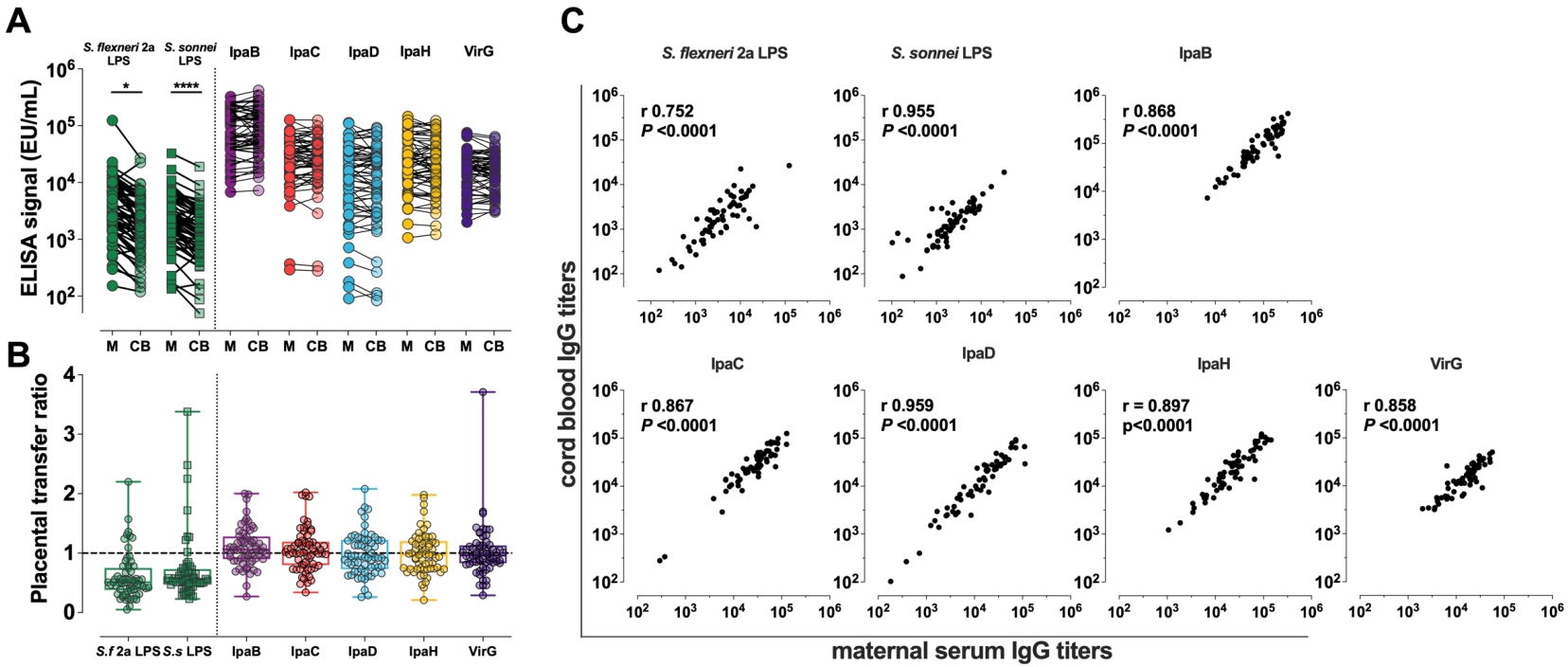

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Figure 3

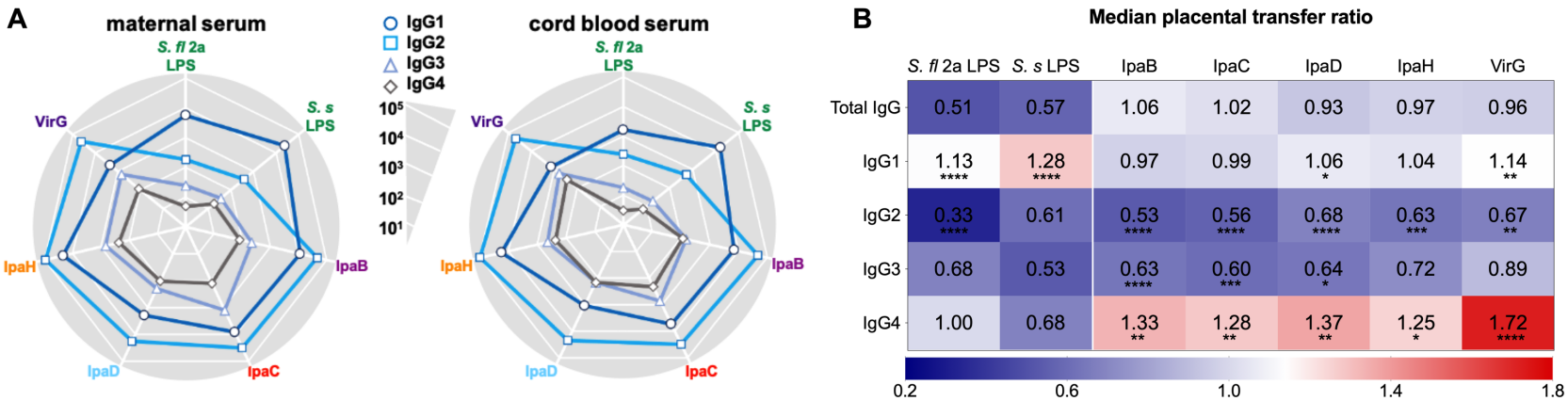

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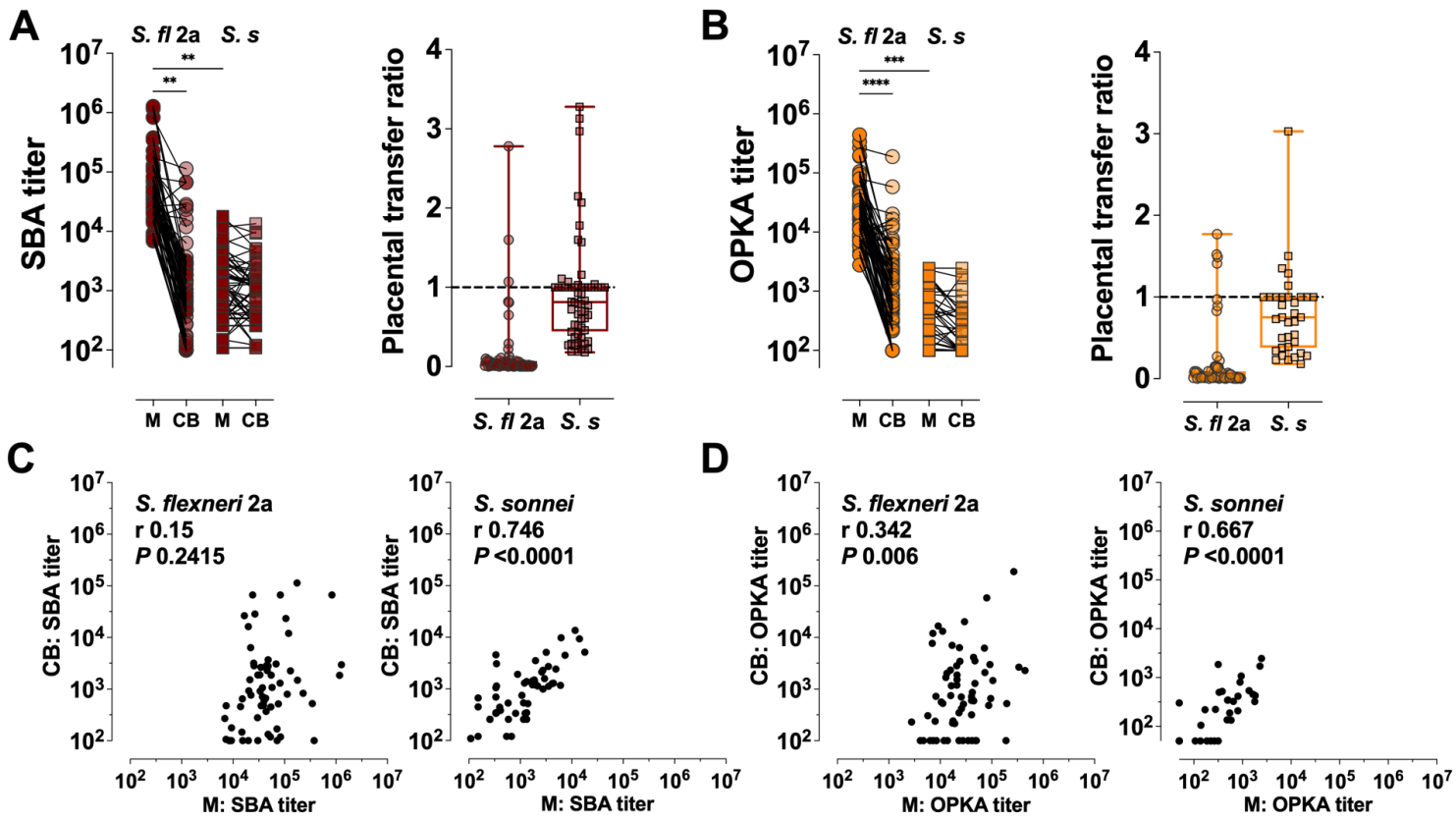
Figure 4



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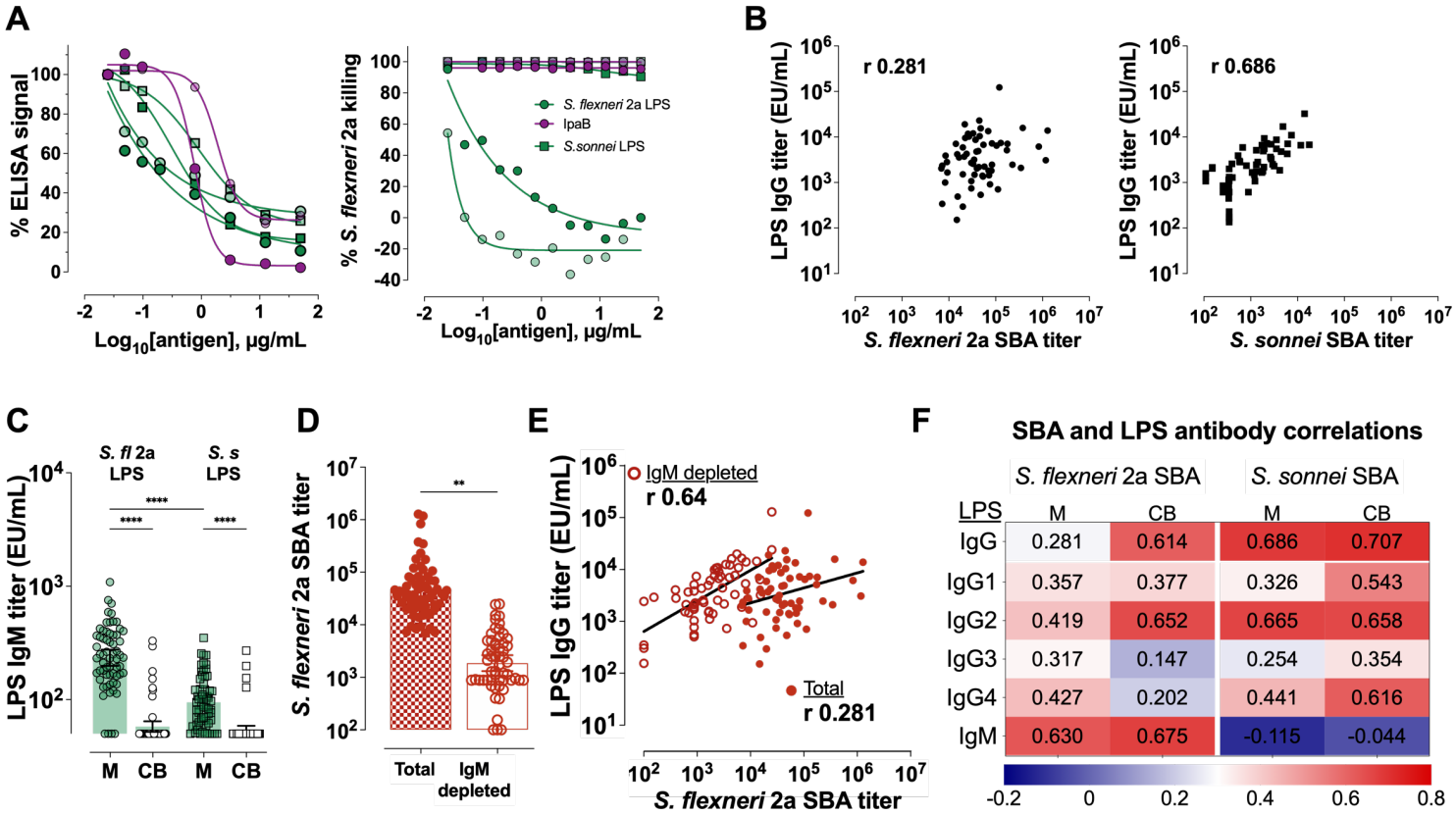

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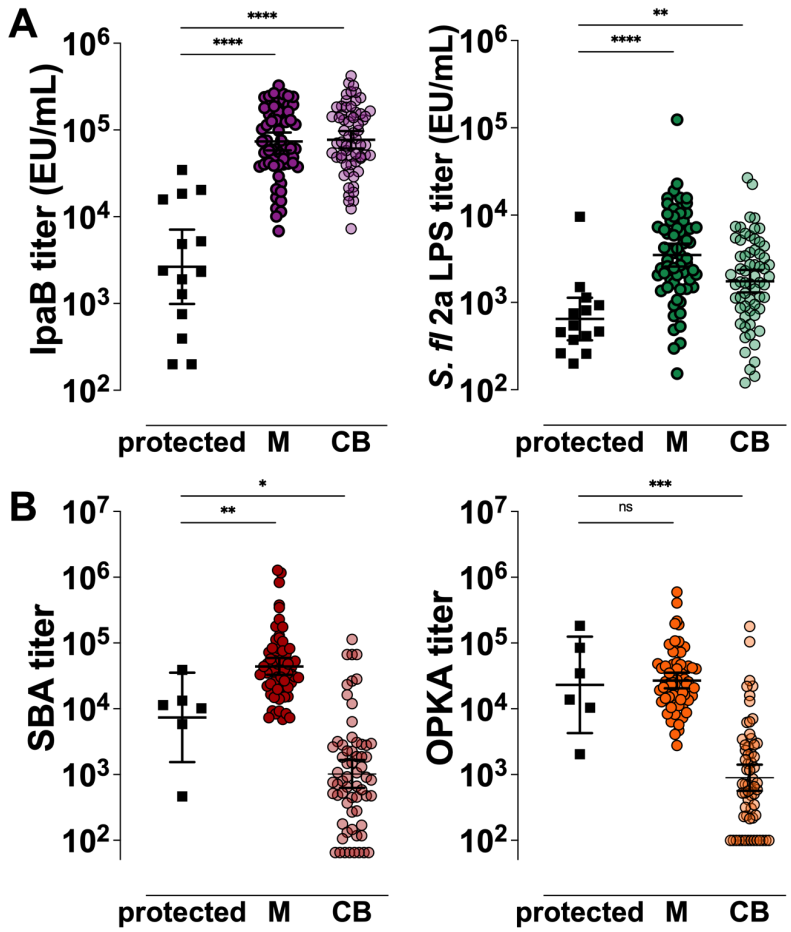
Figure 6



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