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

Esther Ndungo[1], Liana R. Andronescu[1], Andrea G Buchwald [1, #], Patricia Mawindo[2], Miriam K. Laufer[1], Marcela F. Pasetti[1*]

[1]Center for Vaccine Development and Global Health, University of Maryland School of Medicine, 685 W, Baltimore Street, Baltimore, MD, 21201, USA

[2] Blantyre Malaria Project, University of Malawi College of Medicine, Blantyre, Malawi.

#Present address: Department of Environmental and Occupational Health, University of Colorado, Anschutz, Aurora, USA.

[*]**Correspondence:**

Marcela F. Pasetti

e-mail: mpasetti@som.umaryland.edu

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

Abstract word count: 299

Text word count: 4899

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 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 maternal 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 maternal 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, growth stunting, 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 to date 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 reported a strong correlation between serum IgG specific for the *Shigella* invasion plasmid antigen (Ipa) B and the virulence protein, VirG (IcsA), and 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) 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 young infants from *Shigella*-induced diarrhea (while they still suffer from other enteric infections such as rotavirus), hints to 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 render them most functional in the context of 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 is 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 *S. flexneri* 2a LPS, *S. sonnei* LPS, 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 the rural villages in Chikwawa and in the southern region of Malawi. Out of 108 mother and infant pairs enrolled, 63 mother-infant 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 was focused on *S. flexneri* 2a and *S. sonnei*, as these strains 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 cord to maternal *S. flexneri* 2a and *S. sonnei* LPS IgG transfer ratio were 0.60 and 0.70 respectively (Figure 2B, Table 2), indicating low transplacental sieving efficiency of LPS IgG.

Maternal and cord IgG levels against five different *Shigella* virulent 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; mean transfer ratios were: 1.10, 1.53, 0.98, 0.99 and 1.01 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, implicating a distinct regulated transport (Figure 2C). A comparative analysis of maternal antibody specificity

(including all *Shigella* antigens tested) revealed positive associations on the basis of antigenic target (protein or LPS). Protein-specific antibody levels were generally highly and positively associated ($r > 0.5$) as were *S. flexneri* 2a and *S. sonnei* LPS IgG titers (Pearson's $r > 0.6$) (Figure 2D). Antibodies against *S. flexneri* 2a and *S. sonnei* LPS are not expected to be cross-reactive (7, 21, 22). Therefore, the observed association between the two suggests that most mothers had been exposed and responded similarly to both serotypes. Correlations between protein- and LPS-specific antibody titers were generally lower (Pearson's $r < 0.4$), except for the association between IpaC- and both *S. flexneri* 2a and *S. sonnei* LPS IgG (Figure 2D). These associations were mirrored in the antibody responses in the infant serum, confirming the selective and regulated principles of placental antibody transfer (Figure 2D). Geometric mean titers (GMT), mean transfer ratios, and standard deviations (SD) between 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 syncytiotrophoblast (23, 24). Qualitative differences in the Fc structure, such as in the human IgG subclasses, can influence FcRn binding and placental transfer (25). 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. 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). For protein antigens, IgG1 and IgG4 had the highest cord-blood:maternal mean transfer ratios (1.05-1.27 and 1.29-1.68, respectively, Figure 3B and C).

147 For LPS antigens, IgG1 also exhibited the highest mean transfer ratios: 1.3 and 1.58 for *S. flexneri*
148 2a and *S. sonnei,* respectively (Figure 3B). Mean transfer ratios for LPS IgG2 were lower,
149 regardless of the serotype. The predominance of maternal LPS-IgG2 and the lower transfer
150 efficiency of IgG2 explains the low levels of LPS IgG in the cord blood despite their abundance in
151 maternal circulation. The mean transfer ratios for antigen-specific IgG compared to IgG
152 subclasses are represented in a heatmap (Figure 3C).

153

154 **Functional capacity of placentally transferred *Shigella*-specific antibodies**

155 In addition to antibody specificity through direct binding, we examined the functional capacity of
156 maternal and placentally-acquired antibodies to render complement-dependent bactericidal and
157 opsonophagocytic activity. SBA and OPKA activity was detected in both maternal and newborn
158 sera. Maternal SBA and OPKA titers against *S. flexneri* 2a were significantly higher as compared
159 to those against *S. sonnei* (GMT 26,944 compared to 306, respectively). Maternal SBA and OPKA
160 titers specific for *S. flexneri* 2a were also significantly higher in maternal sera as compared to
161 those of cord blood (Figure 4A and 4B); the mean transfer ratios were 0.16 and 0.18, respectively
162 (Figure 4A and B, and Table 3). In contrast, SBA and OPKA titers against *S. sonnei* in maternal
163 serum and in infant serum were comparable (Figure 4A); the mean transfer ratios were 1.29 and
164 0.82 (Figure 4B and Table 3). It was noticed that while maternal and cord blood functional antibody
165 titers against *S. sonnei* were strongly correlated, those against *S. flexneri* 2a were not (Figure 4C
166 and D). The discrepancy in functional antibody titer against *S. flexneri* 2a between mothers and
167 infants prompted us to investigate the specificity and type of antibodies involved in bactericidal
168 and opsonophagocytic killing.

169

170 **Specificity of maternally acquired functional antibodies**

Mouse monoclonal antibodies specific for *Shigella* LPS were reported to have bactericidal activity (21). Several studies have reported increases in SBA titers in response to *Shigella* polysaccharide-based vaccine candidates in adult volunteers (26-28). 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 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, different from 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 around 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, between 0.6 and 0.7. Two previous studies of transplacental transfer of antibodies against *S. flexneri* 2a and *S. sonnei* LPS in Israel (29) and against *S. sonnei* LPS in Vietnam (30), also found high levels of IgG specific for *Shigella* LPS in maternal sera. The mothers in our cohort had clearly been exposed to both *S. flexneri* 2a and *S. sonnei*, which is consistent with the reported serotype prevalence in countries neighboring Malawi (20), and other endemic regions (29, 31, 32). The study in Vietnam reported a much higher median transfer ratio (1.33) for *S. sonnei* LPS IgG (30) than was observed in our cohort, which may reflect regional differences in seroprevalence due to *Shigella* circulation (and possibly increased proportion of LPS-specific IgG1).

An abundance of serum IgG against *Shigella* type 3 secretion system proteins IpaB, IpaC, IpaD, IpaH, and the virulence factor, VirG were observed in maternal and infant circulation, with levels being substantially higher than those of antibodies specific for LPS. 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 LPS compared to protein 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 of 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 the bulk of LPS-IgG, is a poor complement activator. Notwithstanding, 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. pneumonia* (39), which is consistent with OPKA activity observed in our study.

An 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 dissimilar functional activity between maternal and infant human sera, also due to maternal IgM, had 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 countries 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 very young infants living in endemic regions is the epidemiological evidence of the lowest risk of infection in this group. 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

295 challenge with wild-type *S. flexneri* 2a organisms (11). The functional antibody activity in the
296 infants was noticeably below the threshold of clinical protected adults. In contrast, serum IgG
297 against protective target antigens IpaB and VirG in mother and infant sera were well above those
298 detected in the clinically protected challenged volunteers (11). The low levels of LPS-specific IgG
299 and their limited functional capacity (SBA and OPKA) in cord blood, along with high levels of
300 maternal protein-specific IgG and its efficient transfer, argue in favor of a more prominent role of
301 antibodies against *Shigella* virulence antigens in preventing *Shigella* infection than originally
302 appreciated. Similarly, others observed that naturally acquired maternal antibodies against
303 pneumococcal proteins, unlike anti-polysaccharide antibodies, were associated with protection
304 against nasal carriage in infants during the first 3 months of life (45). Further studies are warranted
305 to dissect the mechanisms by which such antibodies block microbial infection (not captured by
306 our traditional assays) and to corroborate their disease protective capacity in humans.

307 A *Shigella* vaccine that is efficacious in children under 3 years of age, the most vulnerable target
308 group, would make a major public health impact. The limited efficacy of a clinically-advanced O-
309 polysaccharide based vaccine candidate in young children (46, 47) has been linked with young
310 children's hypo-responsiveness to *Shigella* LPS (and likely impaired bactericidal/phagocytic
311 antibody activity). Our results showing the abundance of protein-specific antibodies in groups
312 naturally immune (low-risk group) to *Shigella* and the ease with which children respond to protein-
313 based immunization highlight the prospect of a *Shigella* protein-based vaccine approach. Purified
314 IpaB and IpaD (48-50) or a formulation containing IpaB and IpaC (Invaplex, (51)) have been
315 shown to prevent *Shigella* infection in preclinical studies. A vaccine combining multiple conserved
316 *Shigella* proteins would not only be likely broadly protective, but also effective, targeting multiple
317 mechanisms important for *Shigella* invasion and virulence.

318 One limitation in our study was that maternal serum was obtained within 3 months of birth. While
319 some antibody features may change after birth, we did not find significant differences in maternal

320 titers determined in sera collected prior to or after delivery. The limited sample size may have also
321 precluded more extensive and complex statistical analyses. It would be important to conduct
322 similar analyses of the antibody repertoire beyond birth and through the first 3 years of life to
323 better understand trends of disease and immune acquisition, and to investigate age-specific
324 antibody mediated antimicrobial functions using age-relevant immune cells to recreate elements
325 that would operate in vivo (17).

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

335

336 **Acknowledgments**

337 We are grateful to the women who volunteered to participate and to the nurse midwives of the
338 Mfera Health Centre maternity ward and antenatal clinic who supported this study. We thank Dr.
339 Neeraj Kapoor and Dr. Jeff Fairman at Vaxcyte Inc. for providing IpaH for our ELISA studies. We
340 thank Syze Gama and Dr. Avital Shimanovich for providing technical and logistic expertise during
341 the sample collection process.

342 **Funding**

343 This project was supported in part by federal funds from the U.S. National Institutes of Health,
344 under grant numbers R01AI117734 and R01AI125841 to M.F.P, a Research Supplement to

345 Promote Diversity in Health-Related Research Program, 3R01AI117734-04S1, and
346 T32DK067872 to E.N.

347

348

349 Methods

350 Study population and sample collection

351 Our study population consisted of mothers and infants recruited between January and November
352 2016 at Mfera Health Clinic, on the outskirts of Blantyre in Malawi. Healthy pregnant women who
353 were HIV seronegative were enrolled either before birth (screening) or during delivery. Infants
354 were enrolled at birth. Baseline information on mother and the infant's health was obtained during
355 the first hours post-delivery. Other information such as village, baseline health info, and current
356 physical complaints was also collected during the visit. Umbilical cord blood was collected at
357 delivery at the Mfera Health Clinic. Venous maternal blood was obtained at enrollment, either
358 during screening before birth, at a well-child visit (week 1, 6 or 10) or at 3 months. Samples were
359 frozen and shipped to the University of Maryland School of Medicine in Baltimore for analysis.
360 This study was approved by the Institutional Review Board of University of Maryland School of
361 Medicine, and the College of Medicine Research and Ethics Committee (COMREC) at the College
362 of Medicine in Malawi. All participating mothers provided written informed consent for themselves
363 and their infants.

364 Antigen-specific antibody analysis

365 *Shigella* antigens IpaB, IpaC, IpaD, *S. flexneri* 2a LPS and *S. sonnei* LPS were obtained from
366 Walter Reade Army Institute of Research (WRAIR). The N-terminal domain of VirG was
367 expressed and purified inhouse in an *E. coli* expression system (Chitra STS et al, unpublished).
368 The C-terminal domain of IpaH was expressed and purified using a cell-free expression system
369 (Neeraj Kapoor, Vaxcyte, Inc. personal communication). Antigen-specific serum IgG titers were

370 measured by ELISA as previously described (52). Briefly, Immulon 2HB plates (Thermo Scientific,
371 Waltham MA) were coated with IpaB, IpaC, IpaD and IpaH at 0.1µg/mL in PBS, and VirG, *S.*
372 *flexneri* 2a LPS and *S. sonnei* LPS at 5µg/mL in carbonate buffer, pH 9.6. Plates were incubated
373 for 3h at 37°C and blocked at 4°C overnight in PBS containing 10% w/v non-fat dry milk (NFDM).
374 Sera diluted in PBS containing 10% NFDM and 0.05% Tween-20 (PBS-T) were added, and the
375 plates incubated at 37°C for 1h. Plates were incubated with HRP-labeled goat IgG specific for
376 human IgG (Jackson Immuno Research, West Grove, PA) for another 1h at 37°C. Plates were
377 washed 6 times with PBS-T following every incubation step. Tetramethylbenzidine (TMB; KPL,
378 Gaithersburg, MD) was added as substrate for 15 min in the dark with shaking, and the reaction
379 was stopped by adding 1M phosphoric acid (Millipore Sigma, Burlington, MA). Endpoint titers
380 were calculated as the inverse serum dilution that resulted in an absorbance value at 450 nm of
381 0.2 above background and were reported as the ELISA units/mL.
382 Antigen-specific IgG subclasses were measured using *Shigella* multiplex assay using the
383 MesoScale Diagnostic platform (MSD, Rockville, MD). Assays are run in the same way as the
384 antigen-specific ELISAs, with a few exceptions: 1. There is no antigen-coating step as the
385 antigens are pre-printed on MSD plates. 2. After the serum incubation step, the plates were
386 incubated with biotinylated anti-IgG subclass antibodies (SouthernBiotech, Birmingham, AL) plus
387 the SULFOTag-STREP (MSD) for another 1h at 37°C. 3. Binding was then detected using MSD
388 GOLD Read buffer. The plates were read using an MSD sector imager, model 2400 and data
389 analyzed by the MSD workbench software provided by the manufacturer. The ECL signal (minus
390 background from blank) for each sample (diluted at 1:100 in PBS containing 10% NFDM) was
391 reported.
392 **Functional assays**
393 SBA. The SBA assay was performed as previously described (53). SBA titers were determined
394 by Opsotiter (53) as the reciprocal of the serum dilution that produced 50% bacterial killing as
395 determined by Reed-Muench regression analysis. The provisional reference serum sample,

Korean QC19, was ran 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* (53). 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 15min 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 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 3 h at 37°C with shaking. 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 1 h at 37°C. The IgM-depleted serum was the 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.
21. 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.
22. Cohen D, Green MS, Block C, Slepon R, Ofek I. Prospective study of the association between serum antibodies to lipopolysaccharide O antigen and the attack rate of shigellosis. JClinMicrobiol. 1991;29(2):386-9.
23. Simister NE. Placental transport of immunoglobulin G. Vaccine. 2003;21(24):3365-9.
24. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. Front Immunol. 2014;5:520.
25. 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.
26. 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.
27. 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.
28. 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.
29. 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.
30. 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.
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. 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.
53. 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 titer (EU/mL) GMT (range)	Maternal serum titer (EU/mL) GMT (range)	Transfer ratio Mean (range)
S. flexneri 2a LPS	1,752 (120-26,789)	3,491 (153-123,844)	0.60 (0.05-2.20)
S. sonnei LPS	1,264 (45-19,002)	2,109 (135-32,727)	0.70 (0.23-3.38)
IpaB	77,134 (7,274-419,898)	73,737 (6,818-324,773)	1.10 (0.27-2.00)
IpaC	21,856 (279-125,268)	21,732 (292-127,040)	1.06 (0.34-2.02)
IpaD	9,761 (84-92,804)	10,653 (81-112,576)	0.98 (0.26-2.08)
IpaH	19,890 (1,224-122,206)	21,156 (1,067-145,110)	1.00 (0.21-1.98)
VirG	14,840 (3,038-63,877)	15,726 (2,003-75,273)	1.01 (0.29-3.71)

588

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

Antigen	Cord blood GMT (range)	Maternal serum GMT (range)	Transfer ratio Mean (range)
S. flexneri 2a SBA	1,227 (200-113,628)	44,076 (6,889-1,278,483)	0.16 (0.00-2.78)
S. flexneri 2a OPKA	895 (100-178,424)	26,944 (2,770-596,279)	0.18 (0.00-1.77)
S. sonnei SBA	832 (200-13,536)	1,017 (200-17,916)	1.29 (0.19-13.61)
S. sonnei OPKA	245 (100-2,468)	357 (100-2,460)	0.78 (0.18-3.03)

589

590 ## Figure Legends

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

592 **Figure 2. *Shigella*-specific maternal antibody repertoire and placental transfer efficiency**.
593 (A) IgG against *Shigella* LPS and protein antigens in maternal (M) and cord blood (CB) sera.
594 Symbols represent individual titers. Asterisks indicate statistically significant differences between
595 M and CB titers as determined by paired t test. * $P < 0.05$, **** $P < 0.0001$. (B) Placental transfer
596 ratios (CB titer/M titer) for antibodies against each antigen. Whiskers show minimum and
597 maximum values. Line is at ratio = 1. (C) Associations between maternal and cord blood titers for
598 each antigen. Pearson's r and *P* values are shown within each graph. (D) Heatmaps showing
599 association between the antibody titers against each antigen in maternal serum (left) and in cord
600 blood serum (right). Pearson correlation, r, is shown in each box.

601 **Figure 3. IgG subclass distribution of maternal and infant placentally acquired antibodies**.
602 (A) ECL signal for IgG1, IgG2, IgG3 and IgG4 against *Shigella* antigens measured in serum from
603 mothers (M) and cord blood (CB) diluted at 1:100. Symbols represent individual titers. (B) Mean
604 placental transfer ratios (CB titer/M titer) for subclass. Dashed line is at ratio = 1. (C) Heatmap
605 representing transfer ratios of total IgG and IgG subclasses against *Shigella* antigens. Asterisks
606 indicate statistically significant differences as determined by Paired t test. *, $P < 0.05$, **, $P < 0.01$,

607 ***, $P < 0.001$, ****, $P < 0.0001$. For the bar graphs, significance was evaluated using a two-way
608 ANOVA with a Tukey's post-test correction.

609 **Figure 4. Maternal and infant placentally acquired functional antibodies against *Shigella*.**
610 (A) Serum bactericidal antibody (SBA) and (B) opsonophagocytic killing antibody (OPKA) titers
611 measured in serum from mothers (M) and cord blood (CB) (left graphs). Data represent individual
612 titers. Placental transfer ratios (CB titer/M titer) are shown on the graphs on the right. Data
613 represent individual titers. Asterisks indicate statistically significant differences between M and
614 CB titers as determined by one-way ANOVA with a Tukey's post-test correction (** P<0.01, ***
615 P<0.001 , **** P<0.0001). Whiskers indicate minimum and maximum values. Line is at ratio = 1.
616 (C) and (D) Associations between maternal and cord blood SBA and OPKA titers, respectively.
617 Pearson's r and P values are shown within each graph.

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

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

638

639

Figure 1

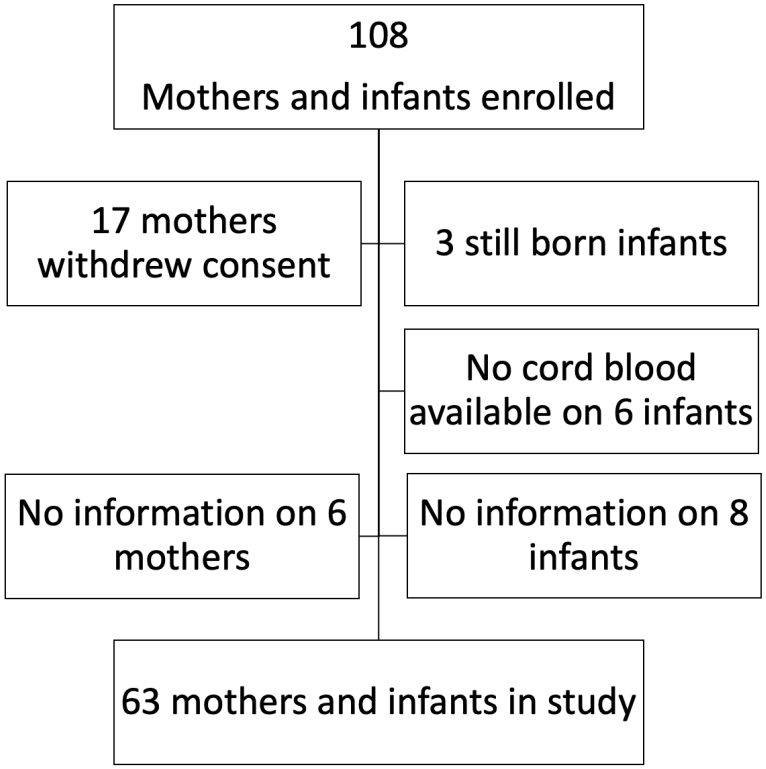

Figure 1. Flowchart showing selection of paired maternal-infant samples

Figure 2

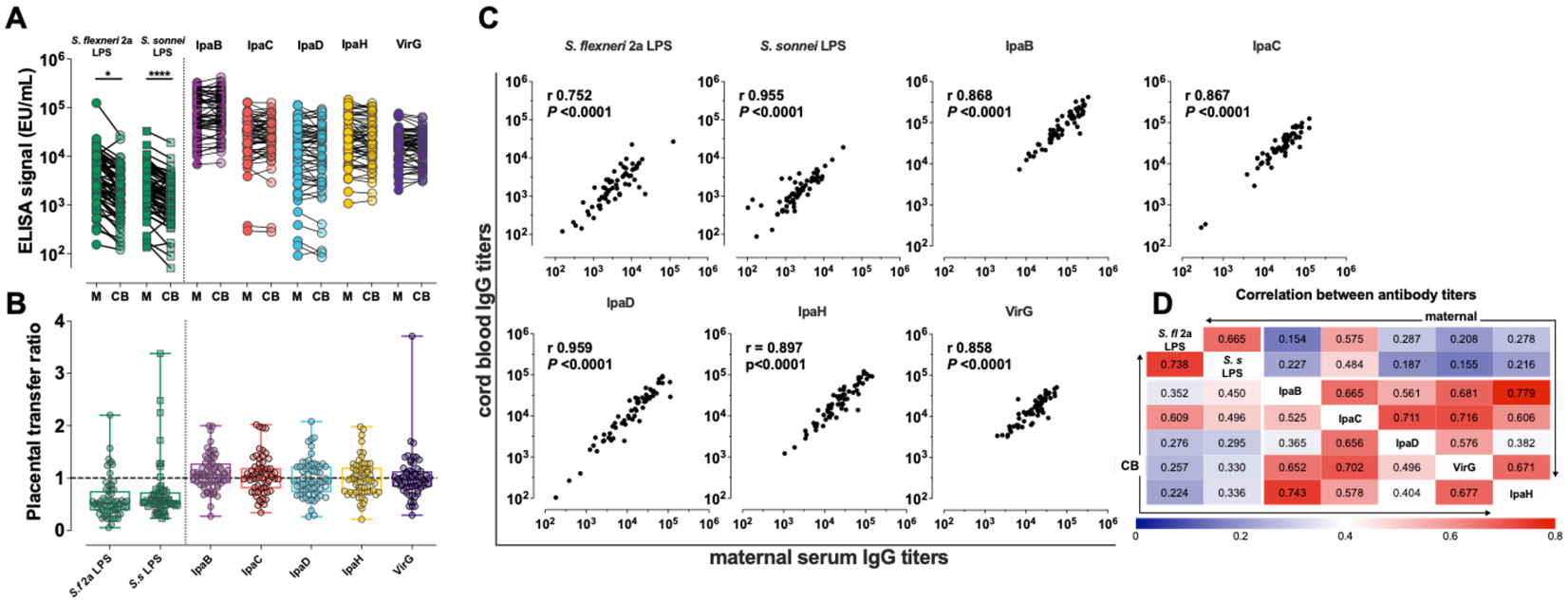

Figure 2. *Shigella*-specific maternal antibody repertoire and placental transfer efficiency. (A) IgG against *Shigella* LPS and protein antigens in maternal (M) and cord blood (CB) sera. Symbols represent individual titers. Asterisks indicate statistically significant differences between M and CB titers as determined by paired t test. * $P < 0.05$, **** $P < 0.0001$. (B) Placental transfer ratios (CB titer/M 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. (D) Heatmaps showing association between the antibody titers against each antigen in maternal serum (left) and in cord blood serum (right). Pearson correlation, r, is shown in each box.

Figure 3

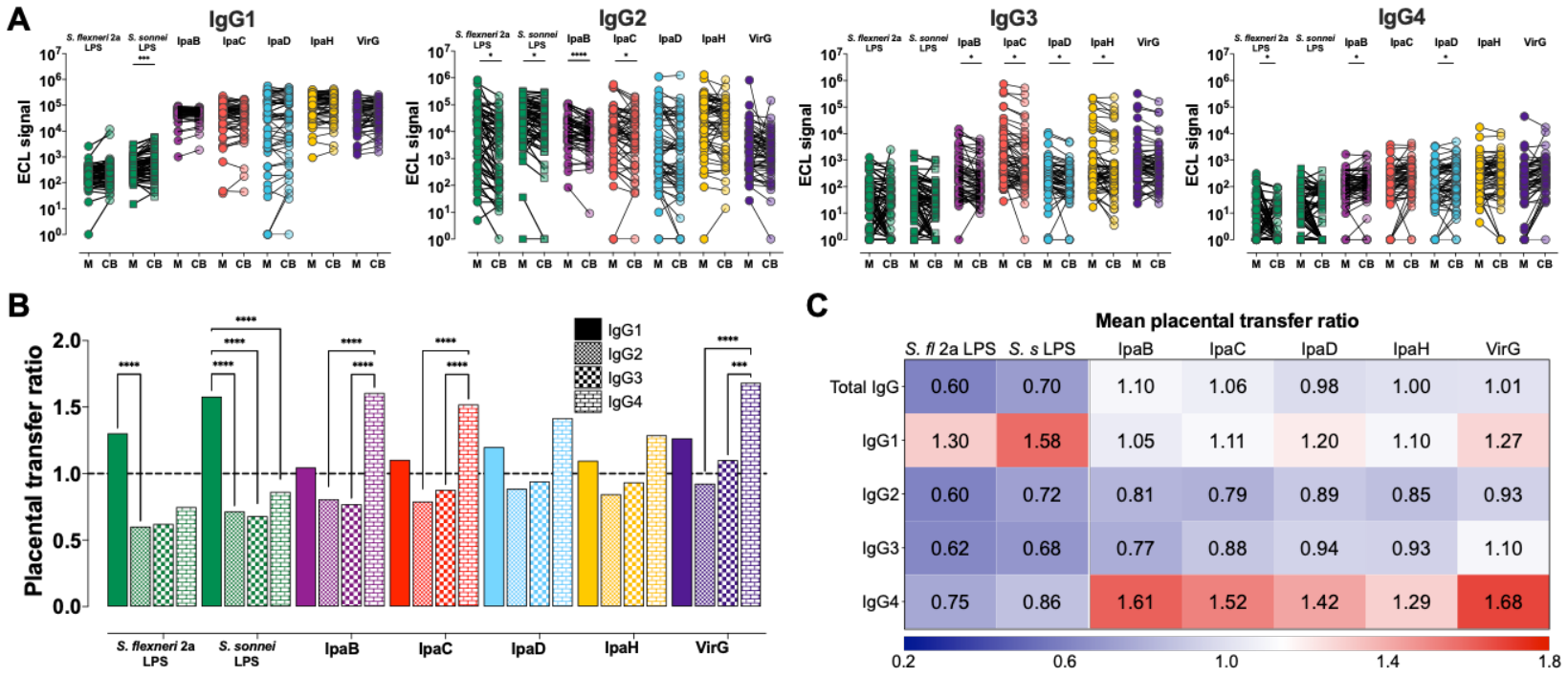

Figure 3. IgG subclass distribution of maternal and infant placentally acquired antibodies. (A) ECL signal for IgG1, IgG2, IgG3 and IgG4 against *Shigella* antigens measured in serum from mothers (M) and cord blood (CB) diluted at 1:100. Symbols represent individual titers. (B) Mean placental transfer ratios (CB titer/M titer) for subclass. Dashed line is at ratio = 1. (C) Heatmap representing transfer ratios of total IgG and IgG subclasses against *Shigella* antigens. Asterisks indicate statistically significant differences as determined by Paired t test. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, ****, $P < 0.0001$. For the bar graphs, significance was evaluated using a two-way ANOVA with a Tukey's post-test correction.

Figure 4

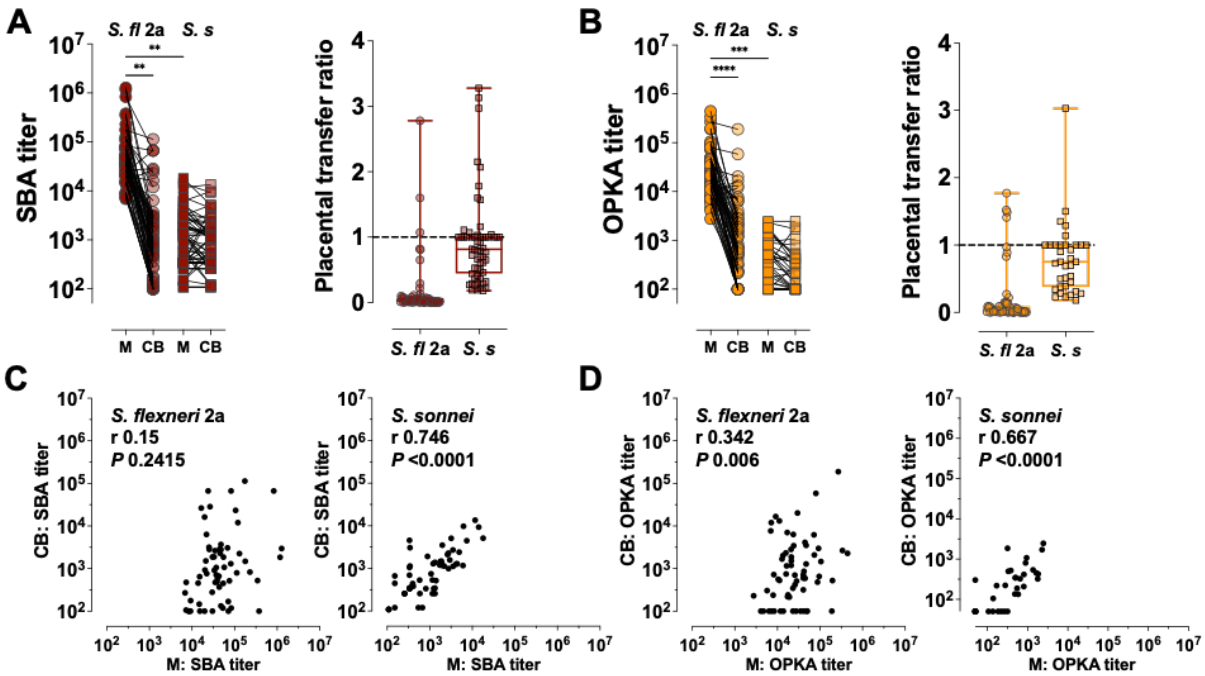

Figure 4. Maternal and infant placentally acquired functional antibodies against *Shigella*. (A) Serum bactericidal antibody (SBA) and (B) opsonophagocytic killing antibody (OPKA) titers measured in serum from mothers (M) and cord blood (CB) (left graphs). Data represent individual titers. Placental transfer ratios (CB titer/M 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

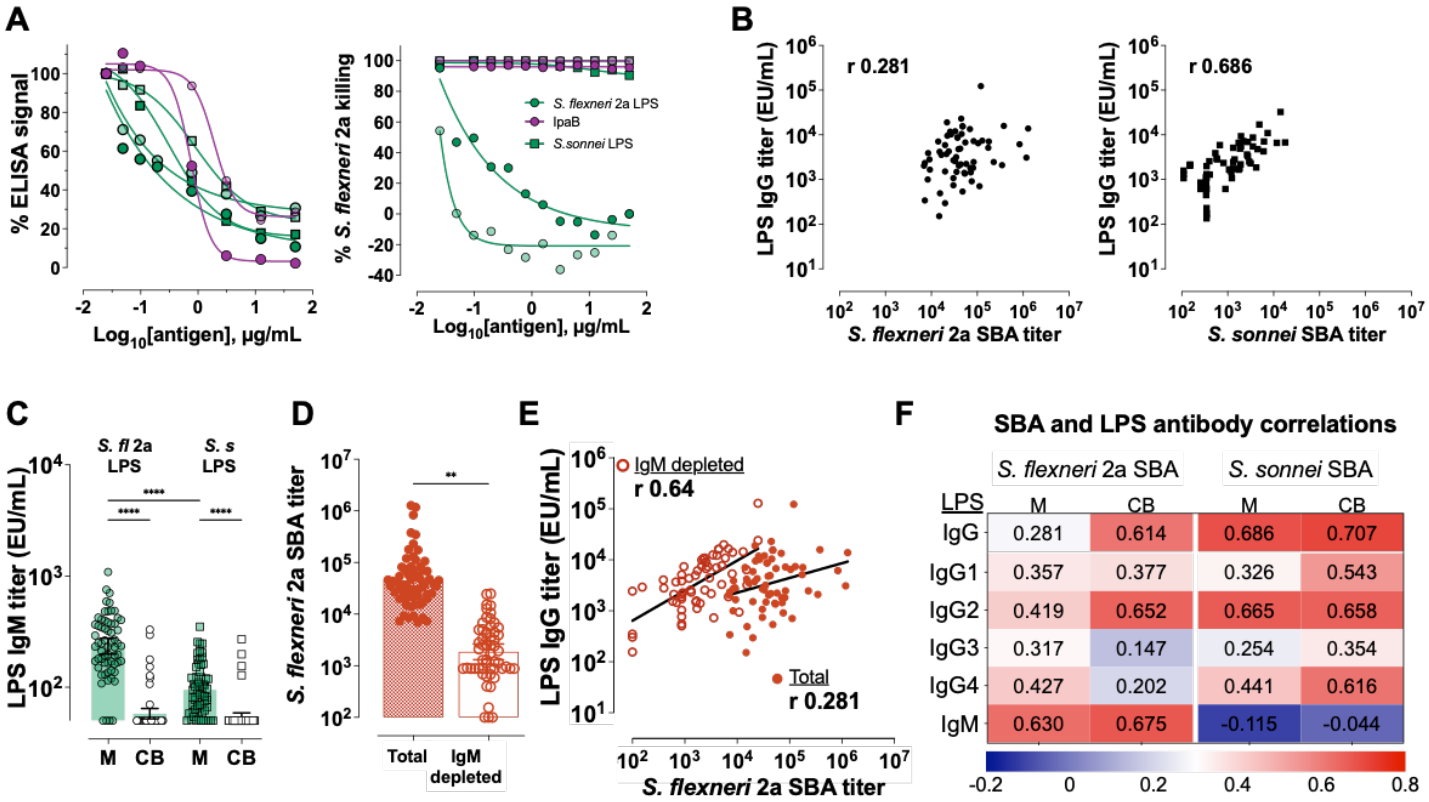

Figure 5. *Shigella* LPS-specific antibodies exhibit bactericidal activity. (A) Depletion of IpaB- or LPS-specific antibodies (left panel) and percent killing of *S. flexneri* 2a in a bactericidal assay using antibody-depleted sera (right panel). Darker-shaded symbols represent maternal sera and lighter-shaded symbols represent infant sera. (B) Correlations between maternal LPS IgG and SBA for *S. flexneri* 2a (left panel) or *S. sonnei* (right panel). (C) Mean IgM titers (bars) against *S. flexneri* 2a and *S. sonnei* LPS in maternal (M) and cord blood (CB) sera. Symbols represent individual titers. Maternal and cord blood titers were compared by one-way ANOVA with a Tukey's post-test correction (****, $P < 0.0001$). (D) Mean SBA titers (bars) in maternal serum before (Total) or after IgM depletion (IgM depleted). SBA titers in the two groups were compared by paired t test (**, $P < 0.01$). Symbols represent individual titers. (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

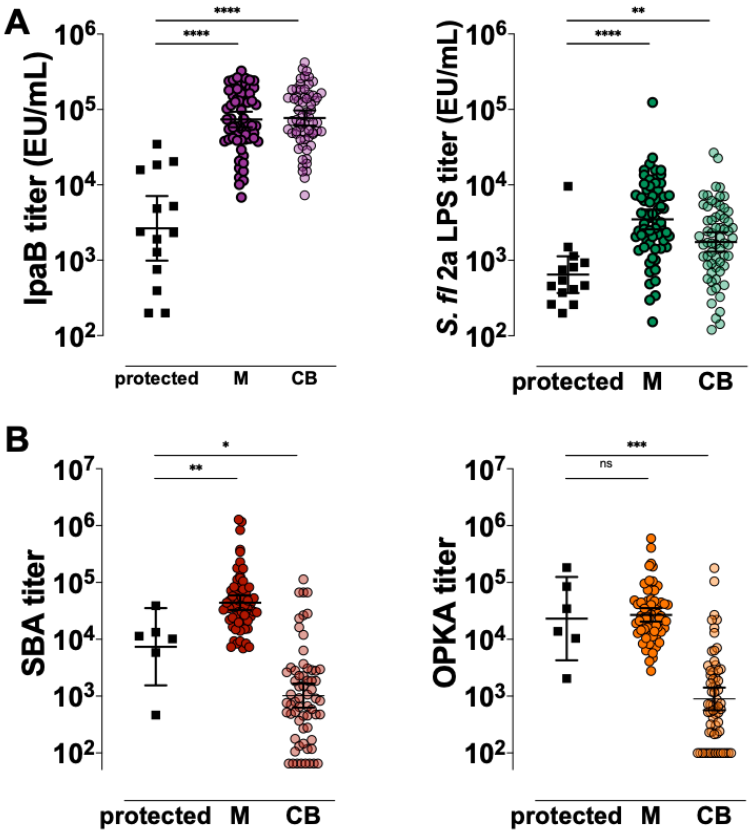

Figure 6. Comparative analysis of *Shigella* antigen-specific and functional antibody titers in the mother-infant dyad in relation to clinical protection. *Shigella* antigen-specific (A) and *S. flexneri* 2a functional (B) antibody titers measured side-by-side in pre-challenge serum from North American 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 was determined by paired t-test (ns $P>0.05$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$).