# Supporting Information

## Gondwe et al. 10.1073/pnas.0910497107

### SI Materials and Methods

***Salmonellae*.** The two strains of nontyphoidal *Salmonella* used in the study were isolated from blood cultures taken from children admitted to Queen Elizabeth Central Hospital, Blantyre, Malawi, in 2004. *S*. Typhimurium D23580 was from a 26-month-old girl and has resistance to ampicillin, chloramphenicol, and co-trimoxazole. This strain undergoes a two- to three-log10 kill in serum from healthy adults over a 3-h time course (Fig. S2) (1). The strain is representative of more than 90% of recent *S*. Typhimurium strains studied from Malawi (1, 2) and has been sequenced by the Sanger Core Sequencing Facility at the Wellcome Trust Sanger Institute (ref. 2; see also http://www.sanger.ac.uk/Projects/Salmonella/).

*S*. Typhimurium D26104 was from an 11-month-old girl. It has resistance to ampicillin and co-trimoxazole, but not chloramphenicol, and multiplies in serum from healthy adults (Fig. S2). This strain was identified as serum-resistant by screening invasive NTS isolates from children admitted to Queen Elizabeth Central Hospital using the serum bactericidal assay with pooled serum from 10 healthy HIV-uninfected Malawian adults ($\log_{10}$ growth at 180 min in pooled serum of 0.81 compared with $\log_{10}$ kill of D23580 at 180 min of 2.30). The basis of serum resistance is not an absence of antibody to this strain (titer of anti-D26104 IgG in pooled serum of 687 U, compared with titer of anti-D23580 IgG of 352 U) and is the subject of ongoing investigation.

**Sera and Ig Preparations.** Antibody-deficient serum from Malawian children was as described previously (1). This serum lacked anti–*S*. Typhimurium D23580 IgG and IgM antibodies measured by flow cytometry (titer < 1.5 U) and was unable to kill *S*. Typhimurium D23580 in the serum bactericidal assay. Immune serum from Malawian adults effected normal killing of *S*. Typhimurium D23580 in the serum bactericidal assay (>1.2 $\log_{10}$ kill at 180 min) (1) and contained anti–*S*. Typhimurium D23580 IgG and IgM antibodies (titers > 1.5 U). C6-deficient serum from a patient with genetic C6 deficiency contained anti–*S*. Typhimurium D23580 antibodies. Heat-inactivated serum had been incubated at 56 °C for 30 min to remove functional complement activity.

***Salmonella* Antibody Assays and Complement Assays.** For anti–whole *Salmonella* antibody titers and complement deposition on *Salmonella*, 5 μL *Salmonellae* in log-growth phase was mixed with 45 μL 10% serum for antibody determination or undiluted serum for complement deposition (final *Salmonella* concentration, $2 \times 10^8$/mL). FITC-conjugated anti–IgG/IgM/C3 antibody were used for detection before analysis on a FACSCalibur flow cytometer (Becton Dickinson). Antibodies to *Salmonella* LPS were measured using a fluorescent-bead-based immunoassay. *S*. Typhimurium LPS was conjugated to fluorescent microbeads (Bio-Rad) via a poly-L-lysine linker. Beads were incubated with serum at a 1:100 dilution and PE-conjugated anti-human IgG (Southern Biotech) used as the secondary antibody. Antibody-binding to beads was analyzed using a Luminex flow-cytometric instrument (Bio-Rad). Total and alternative pathway hemolytic complement assays were performed by radial immunodiffusion assays (Binding Site).


1. MacLennan CA, et al. (2008) The neglected role of antibody in protection against bacteremia caused by nontyphoidal strains of Salmonella in African children. *J Clin Invest* 118:1553–1562.

2. Kingsley RA, et al. (2009) Epidemic multiple drug resistant Salmonella Typhimurium causing invasive disease in sub-Saharan Africa have a distinct genotype. *Genomes Res* 19:2279–2287.


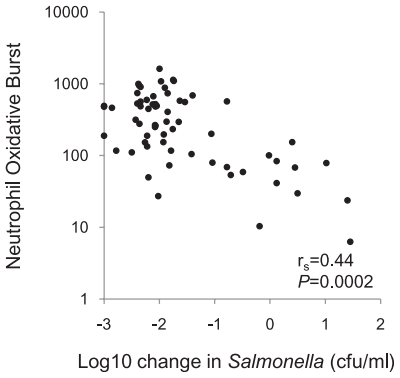


**Fig. S1.** Neutrophil oxidative burst activity in whole blood from Malawian children following stimulation with unopsonized *S*. Typhimurium D23580 (*y* axis) compared with in vitro serum killing of unopsonized *S*. Typhimurium D23580 (*x* axis). Each point corresponds with blood and serum from one child. Correlation coefficient is for respiratory burst and killing of *Salmonellae* (expressed as negative values on *x* axis), hence positive value given ($N$ = 64).

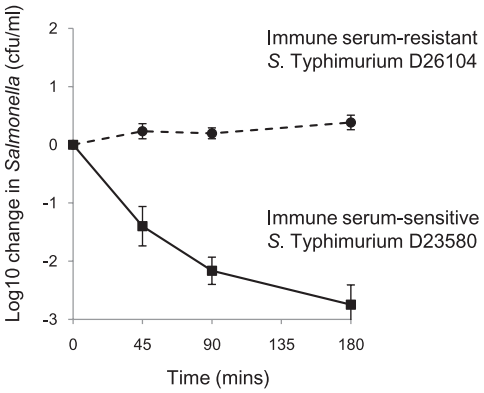


**Fig. S2.** In vitro killing of immune serum–sensitive *S*. Typhimurium D23580 (squares) and immune serum–resistant *S*. Typhimurium D26104 (circles) by serum from three healthy African adults at 45, 90, and 180 min. Negative values correspond with a decrease in viable *Salmonellae* compared with the initial concentration of $10^6$ *Salmonellae*/mL. Data are mean ±1 SD of three experiments.

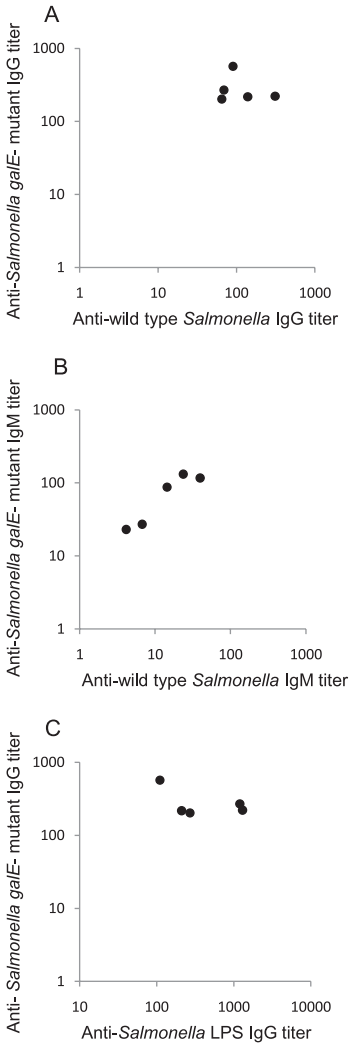


**Fig. S3.** Antibody to WT and rough *galE*⁻ mutants of *S*. Typhimurium and *S*. Typhimurium LPS in Malawian adult sera. Comparison of IgG (*A*) and IgM (*B*) antibody titers measured by flow cytometry versus WT *S*. Typhimurium D23580 and rough *galE*⁻⁻ mutant of *S*. Typhimurium D23580 that does not express O-antigen of LPS. (*C*) Comparison of anti–*S*. Typhimurium LPS IgG titer measured by fluorescent beads versus anti–*S*. Typhimurium D23580 *galE*⁻ IgG titer. Sera from five healthy HIV-uninfected Malawian adults. Scales are not comparable for *C* as different methods were used to assess the two antibody titers.

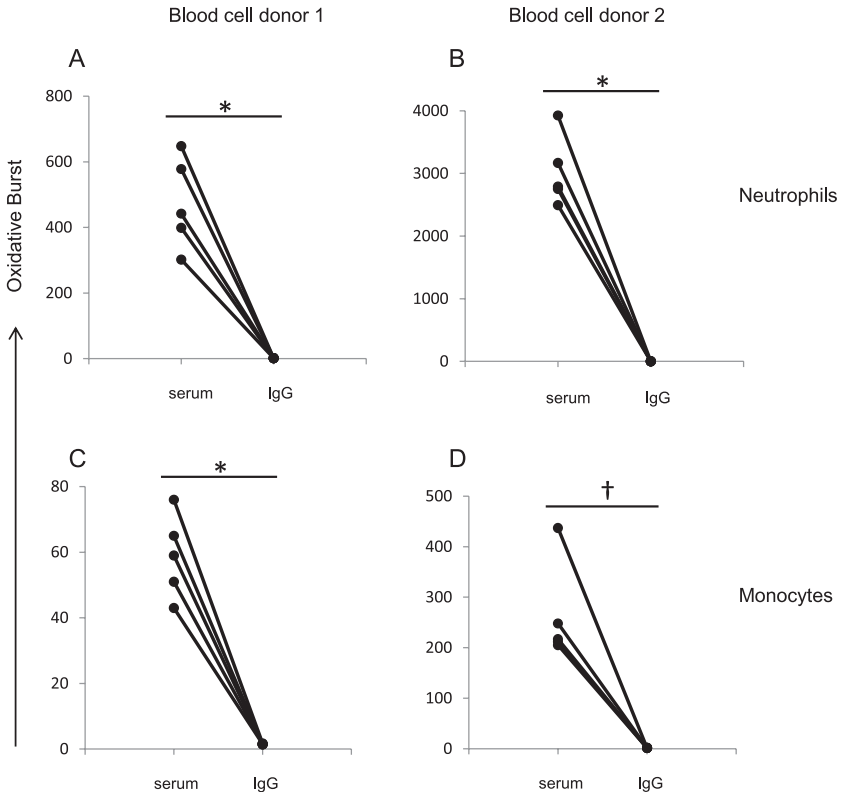


**Fig. S4.** Oxidative burst activity in washed peripheral blood neutrophils (*A* and *B*) and monocytes (*C* and *D*) from two healthy HIV-uninfected adults in response to stimulation by *S*. Typhimurium D23580 opsonized with whole serum (*serum*) or purified IgG (*IgG*) from five healthy Malawian adults. Each point represents oxidative burst activity in response to stimulation with *Salmonella* opsonized with a different serum or IgG preparation. A linear scale has been used to help distinguish data points for serum-opsonized *Salmonella*. Data points for stimulations with IgG-opsonized *Salmonellae* are superimposed on each other. Data points corresponding to *Salmonella* opsonized with serum and IgG from the same individual are joined by a solid line. $^{*}P < 0.0001$, $^{\dagger}P = 0.0003$.

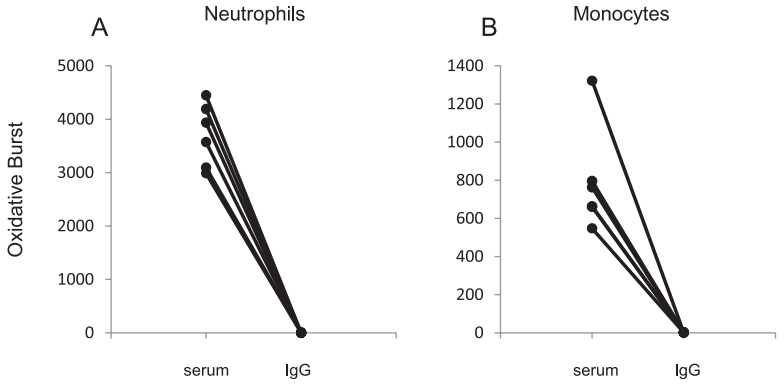


**Fig. S5.** Oxidative burst activity in peripheral blood cells in response to stimulation by laboratory *S.* Typhimurium strain SL1344 opsonized with serum and IgG. Oxidative burst activity in washed neutrophils (*A*) and monocytes (*B*) from a healthy HIV-uninfected adult in response to stimulation by *S*. Typhimurium SL1344 opsonized with whole serum (*serum*) or purified IgG (*IgG*) from five healthy Malawian adults. Each point represents oxidative burst activity in response to stimulation with *Salmonella* opsonized with a different serum or IgG preparation. Data points corresponding to *Salmonella* opsonized with serum and IgG from the same individual are joined by a solid line.

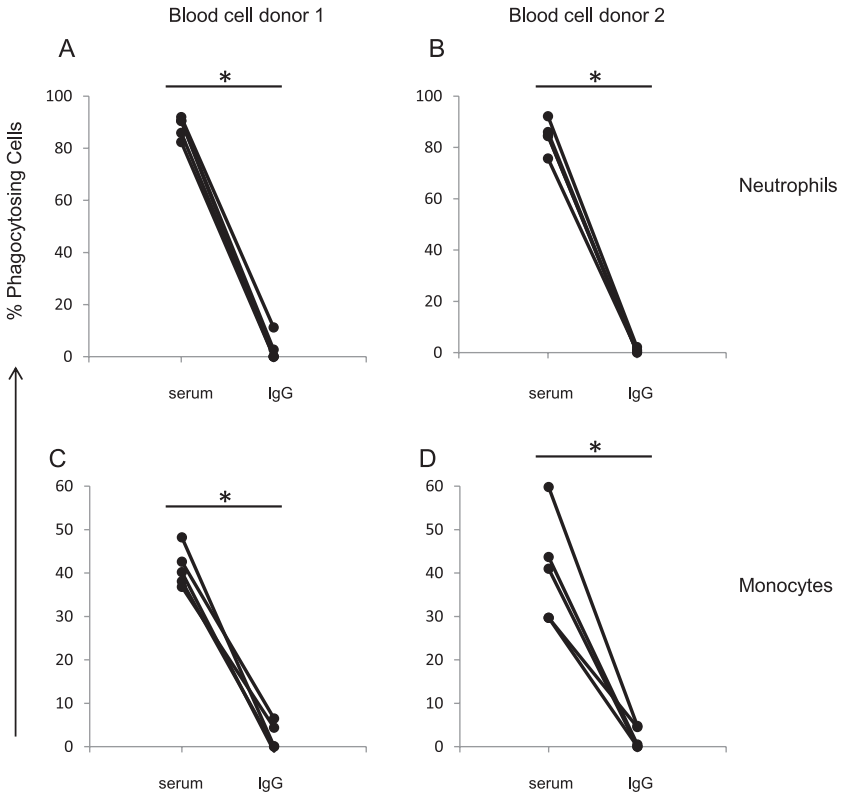


**Fig. S6.** Phagocytosis of *Salmonella* opsonized with serum and IgG by peripheral blood cells. Percentage of washed neutrophils (*A* and *B*) and monocytes (*C* and *D*) from two healthy HIV-uninfected adults phagocytosing *S*. Typhimurium D23580 opsonized with whole serum (*serum*) or purified IgG (*IgG*) from five healthy Malawian adults. Each point represents the percentage of phagocytosing blood cells when incubated with *Salmonella* opsonized with a different serum or IgG preparation. Some data points are superimposed on each other. Data points corresponding to *Salmonella* opsonized with serum and IgG from the same individual are joined by a solid line. $*P < 0.0001$.

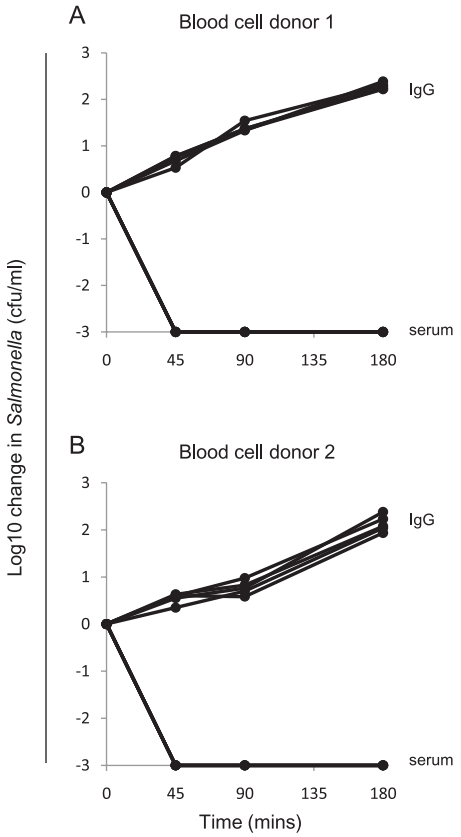


**Fig. S7.** Killing of *Salmonella* opsonized with serum and IgG by peripheral blood cells. In vitro killing of *S*. Typhimurium D23580 opsonized with whole serum (*serum*) or purified IgG (*IgG*) from five healthy Malawian adults by washed peripheral blood cell cells from two healthy adults (*A* and *B*) at 45, 90, and 180 min. Negative values correspond with a decrease in viable *Salmonellae* compared with the initial concentration of $10^5$ *Salmonellae*/mL. All *Salmonellae* opsonized with sera underwent a three-log10 kill, the limit of detection of the assay, within 45 min, and lines for killing of serum-opsonized *Salmonellae* are superimposed.