Supporting Information

Lee et al. 10.1073/pnas.1111413109

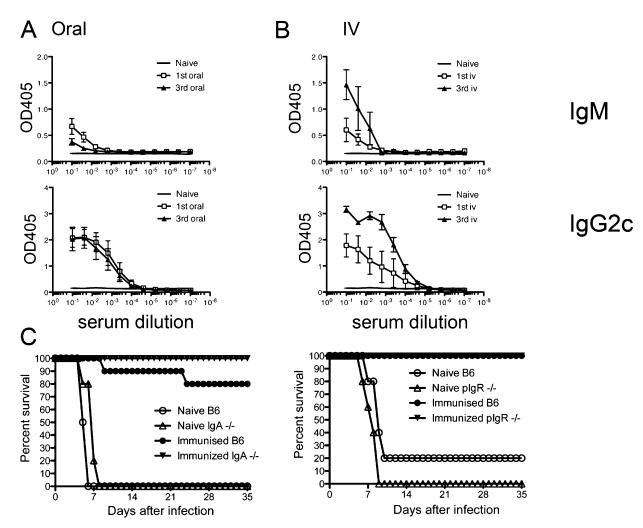


Fig. S1. Vaccination with attenuated *Salmonella* induces a systemic antibody response. C57BL/6 mice were infected (*A*) orally with 5×10^9 or (*B*) i.v. with 5×10^5 BRD509. Some mice received a single immunization, and other mice were immunized three times with 1 mo between doses. Blood was obtained from the retro-orbital plexus 55 d after immunization, serum was prepared, and *Salmonella*-specific IgM (*Upper*) and IgG2c (*Lower*) were measured by ELISA. Line graphs show mean absorbance \pm SEM of four mice per group. (C) C57BL/6 mice and (*Left*) IgA-deficient (IgA^{-/-}) or (*Right*) polymeric IgReceptor-deficient (pIgR^{-/-}) mice were immunized orally with 5×10^9 BRD509, and 42 d later naive or immunized mice were infected orally with 5×10^7 virulent *Salmonella* SL1344. Line graphs represent the percentage of survival of the infected mice and at least six to eight mice per group.

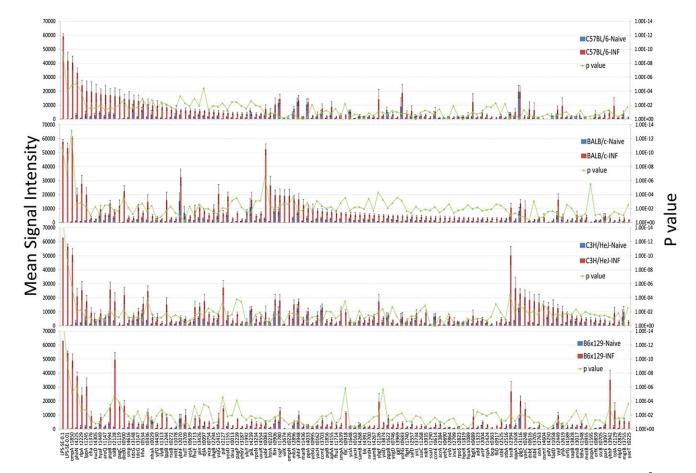


Fig. S2. Strain-specific antigens detected in *Salmonella*-infected mice. C57BL/6, BALB/c, C3H/HeJ, and B6 \times 129F1 mice were infected i.v. with 5 \times 10⁵ BRD509 and boosted 1 and 2 mo later. Blood was collected from naive mice, and 55 d after the last immunization serum was tested for the ability to bind to *Salmonella* proteomic arrays. Bound IgG was detected using biotinylated anti-mouse IgG and SA-Surelight. Slides were scanned using a ProScanArray HT microarray scanner, and the signal intensity of each spot was quantified and normalized. Histograms show the average signal intensity \pm SEM for all antigens identified for each mouse strain. The strain-specific antigens have been ordered by mean signal intensity, and common antigens that are detected by two or more strains are displayed only once. *P* values are overlaid for each antigen examined in each mouse strain.

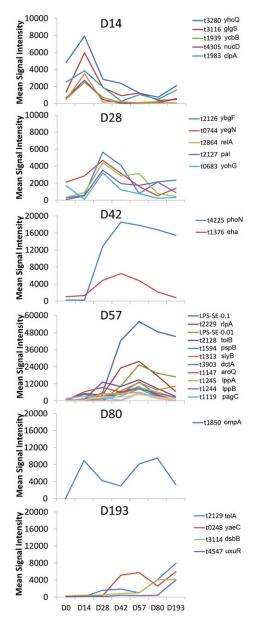


Fig. S3. Examination of *Salmonella* antigenic targets at different time points during the primary response. C57BL/6 mice were immunized i.v. with 5×10^5 BRD509, and blood was collected 14, 28, 42, 57, 80, and 193 d later. Sera from naive and immunized mice were used to probe the *Salmonella* protein array, and bound IgG was detected as described above. Mean reactivity of no DNA controls ± 2.5 SDs was used as a cutoff for determining whether antigens were included or not. Data show the mean response to each target antigen at each time point indicated. Antigens have been grouped together according to when the peak IgG response to each protein was first detected, as indicated at the top of each line graph.

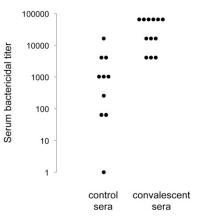


Fig. S4. Serum bactericidal titers against the invasive Malawian serovar Typhimurium strain D23580 in control and convalescent Malawian child sera following invasive *Salmonella* disease. *Salmonella* was added to serum from control or convalescent individuals, and complement-mediated bacterial killing was measured as described in *Materials and Methods*. Titer is the maximum dilution that can effect a 0.5 log 10 kill at 180 min in the serum bactericidal assay.

Table S1. Salmonella proteins recognized in three of four infected mouse strains
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Antigen no.	Symbol	Product name	Predicted location	Comments		
1850 ompA		Outer membrane protein A	Outer membrane	Target antigen in mice and humans (1–3)		
4225	phoN	Nonspecific acid phosphatase precursor	Periplasmic	Target antigen in human typhoid (1)		
2229	rlpA	Rare lipoprotein A	Unknown			
1594	pspB	Phage shock protein B	Cytoplasmic			
2128	tolB	Translocation protein B	Periplasmic			
0500	dedD	DedD protein	Unknown			
0336	yfgL	Outer membrane protein assembly complex subunit	Unknown			
1244	lppB	Major outer membrane lipoprotein	Unknown			
3426	pin	Putative regulatory protein	Unknown			
0097	djlA	DNA-J like membrane chaperone protein	Cytoplasmic membrane			
0754	wza	Putative polysaccharide export protein	Outer membrane			
2127	pal	Peptidoglycan-associated outer membrane lipoprotein	Outer membrane			
2239	pagP	Palmitoyl transferase for lipid A	Outer membrane			
3234	rpoN	Hypothetical protein	Unknown			
1245	lppA	Major outer membrane lipoprotein	Unknown			
1119	pagC	Outer membrane invasion protein	Outer membrane	Identified as a target antigen in human typhoid (1, 4)		
4616	smp	Putative membrane protein	Unknown			
0029	nhaA	Putative exported protein	Unknown			
2294	ykgB	Hypothetical protein	Cytoplasmic			
1155	yeaD	Hypothetical protein	Unknown			
0013	dnaJ	Chaperone protein	Cytoplasmic			
4239	pilL	Hypothetical protein	Unknown			
4554	uxuR	Hypothetical protein	Unknown			
0906	fliH	Flagellar assembly protein H	Unknown			
1780	csgF	Curli assembly protein F	Unknown	Identified as a target antigen in human typhoid (4)		
4155	aphA	Acid phosphatase/phosphotransferase	Unknown			
0918	fliC	Flagellin	Extracellular	Identified as a target antigen in mice and humans (5, 6)		
2126	ybgF	Hypothetical protein	Unknown			
3874	yiaD	Putative outer membrane lipoprotein	Outer membrane			

1. Charles RC, et al. (2010) Characterization of anti-Salmonella enterica serotype Typhi antibody responses in bacteremic Bangladeshi patients by an immunoaffinity proteomics-based technology. *Clin Vaccine Immunol* 17:1188–1195.

2. Ortiz V, Isibasi A, García-Ortigoza E, Kumate J (1989) Immunoblot detection of class-specific humoral immune response to outer membrane proteins isolated from Salmonella typhi in humans with typhoid fever. J Clin Microbiol 27:1640–1645.

3. Brown A, Hormaeche CE (1989) The antibody response to Salmonellae in mice and humans studied by immunoblots and ELISA. Microb Pathog 6:445-454.

4. Harris JB, et al. (2006) Identification of in vivo-induced bacterial protein antigens during human infection with Salmonella enterica serovar Typhi. Infect Immun 74:5161–5168.

5. Sadallah F, et al. (1990) Production of specific monoclonal antibodies to Salmonella typhi flagellin and possible application to immunodiagnosis of typhoid fever. J Infect Dis 161:59-64.

6. Calderón I, et al. (1986) Antibodies to porin antigens of Salmonella typhi induced during typhoid infection in humans. Infect Immun 52:209–212.

Table S2. Clinical features of Malawian children with Salmonella bacteremia

Patient no	Age (mo)	Sex	Serovar	Source	MDR	Clinical presentation	HIV	Other diagnoses and comorbidities
1	2	F	Typhimurium	CSF		Fever, convulsions, vomiting	+	Meningitis
2	4	М	Typhimurium	Blood	\checkmark	Fever, cough and shortness of breath, diarrhea, and vomiting		Severe pneumonia, malnutrition
3	6	Μ	Typhimurium	Blood		Fever, cough		
4	10	F	Typhimurium	Blood		Fever, cough		
5	13	F	Typhimurium	Blood		Fever, shortness of breath, diarrhea, and vomiting		Severe malarial anemia
6	15	Μ	Typhimurium	Blood		Fever, diarrhea, and vomiting	+	
7	16	Μ	Typhimurium	Blood		Fever, cough, diarrhea, and vomiting	+	Malnutrition
8	20	F	Typhimurium	Blood		Fever, cough, vomiting		
9	43	М	Typhimurium	Blood		Fever	+	
10	72	F	Typhimurium	Blood		Fever		Moderate anemia
11	90	F	Typhi	Blood		Fever, cough		
12	151	М	Typhi	Blood		Fever, cough, and shortness of breath		Moderate anemia

M, male; F, female; MDR, multidrug resistant (resistant to cotrimoxazole, ampicillin, and chloramphenicol).

Table S3. List of 14 antigens differentially reactive in human NTS patients vs. contro	Table S3.	s differentially reactive in human NTS patients vs. controls
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Human NTS antigens	C57BL/6	BALB/c	C3H/HeJ	B6x129	Gene	Product name (from NCBI)
4225	+	+	+	+	phoN	Nonspecific acid phosphatase precursor
1850	+	+	+	+	ompA	Outer membrane protein A
1449	+		+		hyaF	Putative exported protein
2070	+				ybhF	HlyD-family secretion protein
2126		+	+	+	ybgF	Hypothetical protein
4155		+	+	+	aphA	Acid phosphatase/phosphotransferase
2864		+	+		relA	Hypothetical protein
4189			+		nrfG	Formate-dependent nitrite reductase complex subunit
3119						Putative exported protein
2941						Conserved hypothetical protein
1459						Putative secreted hydrolase
1266					sseB	Pathogenicity island effector protein
0510					hisJ	Histidine-binding periplasmic protein
3708					hslT	Putative lipoprotein

NTS, non-Typhi Salmonella; NCBI, National Center for Biotechnology Information.

Other Supporting Information Files

Dataset S1 (XLS) Dataset S2 (XLS)

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