Investigation of the role of typhoid toxin in acute typhoid fever in a human challenge model

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Salmonella Typhi is a human host-restricted pathogen that is responsible for typhoid fever in approximately 10.9 million people annually. The typhoid toxin is postulated to have a central role in disease pathogenesis, the establishment of chronic infection and human host restriction. However, its precise role in typhoid disease in humans is not fully defined. We studied the role of typhoid toxin in acute infection using a randomized, double-blind S. Typhi human challenge model. Forty healthy volunteers were randomized (1:1) to oral challenge with 10^6 colony-forming units of wild-type or an isogenic typhoid toxin deletion mutant (TN) of S. Typhi. We observed no significant difference in the rate of typhoid infection (fever ≥ 38 °C for ≥ 12 h and/or S. Typhi bacteremia) between participants challenged with wild-type or TN S. Typhi (15 out of 21 (71%) versus 15 out of 19 (79%); P = 0.58). The duration of bacteremia was significantly longer in participants challenged with the TN strain compared with wild-type (47.6 hours (28.9–97.0) versus 30.3(3.6–49.4); P ≤ 0.001). The clinical syndrome was otherwise indistinguishable between wild-type and TN groups. These data suggest that the typhoid toxin is not required for infection and the development of early typhoid fever symptoms within the context of a human challenge model. Further clinical data are required to assess the role of typhoid toxin in severe disease or the establishment of bacterial carriage.

Several preclinical studies have described the structure and function of the typhoid toxin in vitro and in small-animal models. Systemic administration of typhoid toxin to C57BL/6 mice results in the reproduction of many characteristic symptoms of typhoid fever; other studies have suggested that typhoid toxin may contribute to the establishment of chronic infection. It remains unclear how the surrogate end points of illness in mice—such as lethargy, weight loss, behavioral and motor changes—are representative of acute typhoid fever in humans. The toxin is also encoded by >40 clade B non-typhoidal Salmonella (NTS) serovars that display a broad host range and a distinct clinical phenotype to S. Typhi and Paratyphi, although some typhoid toxin-expressing NTS serovars appear to cause an enteric fever-like syndrome. Importantly, no previous studies have characterized the role of typhoid toxin in a human model of disease.

We aimed to characterize the role of typhoid toxin in human infection and pathogenesis using an S. Typhi human challenge model. This model has previously been used to test novel live-attenuated (MO1ZH09) and Vi-conjugate (Typbar-TCV) typhoid vaccines. We manufactured two challenge strains of S. Typhi to good manufacturing practice (GMP) standards. We used the wild-type S. Typhi Quailes strain (genotype 3.0.1) as the parent strain to generate an isogenic typhoid toxin-deficient knock-out strain (TN), as described previously. Whole-genome sequencing confirmed the absence of the typhoid toxin pathogenicity islet in the TN strain (Supplementary Information). The wild-type and TN strains harbored no other differences in relation to known key virulence factors (Supplementary Information). In particular, there were no differences identified in Salmonella pathogenicity island 7, a region encoding genes required for expression of the Vi-capsule—a key virulence factor in the pathogenesis of S. Typhi. Differences between strains were confined to highly variable regions encoding phage proteins, which were not known to impact on bacterial survival in the environment or persistence in the human host. However, deletion of the entire typhoid toxin pathogenicity island was associated with increased bacterial burden in a mouse model of S. Typhi infection, compared with a strain expressing a catalytic mutant of typhoid toxin (cdtB^H160Q pltB^S35A pltA^H133S; Fig. 1). Otherwise, the wild-type and TN challenge strain variants displayed comparable phenotypic properties with regards to Vi-capsule expression, cellular invasion, in vitro growth characteristics, antibiotic susceptibilidady and survival in environmental water and soil samples (data not shown). Cell cycle arrest in vitro was observed with the wild-type but not the TN strain (Fig. 1).

We enrolled a total of 41 healthy adults (aged 18–60 years) into a randomized, double-blind, human challenge study between 10 April and 1 August 2017. One volunteer withdrew prior to challenge,
and 40 completed the challenge protocol (Extended Data Fig. 1). The study was undertaken in a cohort of healthy adult volunteers in a setting non-endemic for typhoid fever (Oxford 14; see the Life Sciences Reporting Summary). Groups were well matched at baseline (Extended Data Fig. 2 and Supplementary Data). Participants fasted for 90 min before oral challenge with $1–5 \times 10^4$ colony-forming units (CFUs) of either wild-type or TN strains administered 2 min after sodium bicarbonate pretreatment (Supplementary Information). Study visits were scheduled for 12 h after challenge, and then daily for 14 d, when daily blood cultures were collected (Fig. 1) (ref. 7). Antibiotic treatment (ciprofloxacin 500 mg twice daily) was initiated at typhoid diagnosis or at day 14 for those without illness.

Using the primary composite diagnostic end point of fever $\geq 38 °C$ for $\geq 12$ h and/or $S$. Typhi bacteremia, we observed no significant difference in the rate of typhoid disease between participants challenged with wild-type or TN strains (15 out of 21 (71%) versus 15 out of 19 (79%); relative risk 1.11 (95% confidence interval (CI) 0.8–1.6); $P=0.58$; Fig. 2 and Supplementary Data). The attack rate ($n$ diagnosed/$n$ challenged) in the wild-type group met the target range of 60–75% and was consistent with earlier studies$^{7,13,14}$. There was no significant difference in the attack rate when we applied alternative diagnostic criteria (Supplementary Data). The challenge dose administered did not impact the outcome of the challenge (Fig. 2).

To determine if absence of the typhoid toxin was associated with an altered disease phenotype, we compared the clinical profiles between challenge groups (Fig. 2). Five participants met the prespecified criteria for severe typhoid fever; of these, one participant was randomized to wild-type (1 out of 15; 7%) and four (4 out of 15; 27%; $P=0.3$) were randomized to TN (Supplementary Data). Two serious
adverse events were reported, neither of which was assessed as being related to S. Typhi challenge (Supplementary Information). The most common symptoms reported by participants who developed typhoid were headache (30 out of 30; 100%), malaise (30 out of 30; 100%), anorexia (26 out of 30; 87%) and abdominal pain (23 out of 30; 77%; Fig. 2 and Supplementary Information).

**Fig. 2 | Clinical response to challenge with wild-type and TN S. Typhi.**

**a.** Time to diagnosis after challenge. Cumulative proportion of participants meeting the composite diagnostic end point defined as S. Typhi bacteremia and/or fever ≥38°C persisting ≥12 h. Participants not meeting the diagnostic criteria for typhoid diagnosis were censored at day 14. log-rank test.

**b.** Challenge dose administered between wild-type and TN challenge groups. Two-sided Mann–Whitney U-test, n_{TN}=19, n_{Wild-type}=21. c. Challenge dose administered according to outcome and challenge strain. Mann–Whitney U-test.

**d.** Time to first fever >38°C.

**e.** Fever clearance time categorized according to study group. Kaplan–Meier survival curve showing the cumulative proportion of participants with any fever >38°C by challenge group. Participants with no recorded fever were censored at day 14. log-rank test.

**f.** Cumulative symptom severity scores in all participants challenged; n_{TN}=19, n_{Wild-type}=21. Two-sided Mann–Whitney U-test.

**g.** Maximum symptom severity score (day 0–21) in participants diagnosed with typhoid fever according to study group. Percentage of participants reporting one or more events, graded as mild, moderate or severe; n_{TN}=19, n_{Wild-type}=21. Two-sided Mann–Whitney U-test.
Fig. 3 | Microbiological response to challenge with wild-type and TN S. Typhi. a, b. Pattern of stool shedding after TN (a) and wild-type (b) challenge. The rows correspond to individual participants. Gray squares, negative sample; brown squares, positive stool culture; white squares = no sample collected. Tx is the day of treatment initiation. c. Probability of stool shedding S. Typhi over time after challenge. Samples were classified as culture-positive or culture-negative for S. Typhi and combined in mixed effects logistic regression models, as described previously. sTtyphi samples were classified as culture-positive or culture-negative for S. Typhi over time after challenge. Samples were classified as culture-positive or culture-negative for S. Typhi and combined in mixed effects logistic regression models, as described previously. 

sTtyphi over time after challenge. Samples were classified as culture-positive or culture-negative for S. Typhi over time after challenge. Samples were classified as culture-positive or culture-negative for S. Typhi and combined in mixed effects logistic regression models, as described previously. 

Comparison of the cumulative percentage with S. Typhi bacteremia after challenge (g) and time to S. Typhi bacteremia (h). Participants not meeting the diagnostic criteria were censored at day 14. Cumulative proportion of participants with ongoing bacteremia were measured from time of treatment initiation to first persistently negative blood culture, according to challenge group. Log-rank test. The box plots display the median and IQR, the upper whiskers extending to the largest value ≤1.5 × IQR from the 75th percentile and the lower whiskers extending to the smallest values ≤1.5 × IQR from the 25th percentile. The overlaid violin plots illustrate the distribution of the data points and their probability density.
Fig. 4 | Host response to challenge with wild-type and TN S. Typhi. a, CdtB−, PltA− and PltB-specific IFN-γ-producing PBMCs at baseline, day 14 and 28 after challenge. n\(^{\text{WT}}\) = 21, n\(^{\text{TN}}\) = 19; Wilcoxon signed-rank test for within-group comparisons on paired samples. b, Magnitude of ASC response at typhoid diagnosis. n\(^{\text{WT}}\) = 11, n\(^{\text{TN}}\) = 10; two-sided Mann–Whitney U-test. The box plots display the median and IQR. c, Plasma cytokine profiles after challenge with wild-type and TN S. Typhi. Heatmap showing log2 fold change in MFI for each cytokine (rows) and participant (columns) at time of diagnosis relative to baseline. n\(^{\text{WT}}\) = 15, n\(^{\text{TN}}\) = 15. Rows are annotated by significance of cytokine up- or downregulation relative to baseline in each challenge group (white, significant after adjustment for multiple testing; light gray, significant before adjustment; dark gray, non-significant). Two-sided moderated t-test with Benjamin–Hochberg correction. Clustering by Euclidean distance. FDR, false discovery rate. d, Volcano plots illustrate plasma cytokine up/downregulation at typhoid diagnosis in wild-type and TN challenge groups, with adjustment for baseline. The size of each point reflects average abundance (log2MFI) in the plasma. n\(^{\text{WT}}\) = 15, blue; n\(^{\text{TN}}\) = 15, red. e, Principal component plot of log2 fold change in MFI relative to baseline for each participant. Ellipses are drawn with a 95% confidence level. n\(^{\text{WT}}\) = 15, n\(^{\text{TN}}\) = 15. FGFB, basic fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; M-CSF, macrophage colony-stimulating factor.
Fever clearance time was comparable between wild-type and TN groups (median [IQR] hours 53.15 (23.0–87.4) versus 44.92 (12–96.6) hours; \(P = 0.71\); Fig. 2). Laboratory abnormalities (elevated C-reactive protein, lymphopenia, neutropenia) were all consistent with the expected presentation of typhoid fever in the field (Extended Data Fig. 3). Overall, the clinical phenotype was comparable between groups.

We next assessed if absence of typhoid toxin was associated with altered microbiological end points (Fig. 3). At least one stool culture was positive for \(S.\) Typhi in 13 out of 21 (62%) participants challenged with the wild-type strain and 11 out of 19 (58%) challenged with the TN strain. The pattern of stool shedding was comparable between groups, peaking 24–48 h after challenge, followed by a second peak in week 2 (Fig. 3) (ref. \(^{19}\)). There was no difference in the probability of shedding over the entire challenge period following challenge with wild-type compared with wild-type strain\(^{19}\). With a typhoid toxin-deficient strain of \(S.\) Typhi was associated with an increased bacterial burden compared with the wild-type strain.\(^{19}\)

Consistent with this observation, the duration of bacteremia was significantly longer in participants challenged with the TN strain compared with the wild-type strain (47.6 h (28.9–97.0) versus 30.3 (3.6–49.4); \(P \leq 0.001\); Fig. 3), although circulating quantitative colony counts did not differ (0.2 CFU ml\(^{-1}\) (0–21) versus 0.55 CFU ml\(^{-1}\) (0–3); \(P = 0.44\); Fig. 3). We next performed a principal component analysis of disease severity, using all clinical, microbiological and laboratory measures collected during the course of the challenge study. When all participants were included in the analysis, participants diagnosed with typhoid fever clearly cluster separately from individuals who did not develop disease (Extended Data Fig. 4); however, there was no clustering of participants by challenge group, suggesting that challenge with a typhoid toxin-deficient strain of \(S.\) Typhi was associated with an indistinguishable clinical phenotype to that caused by wild-type \(S.\) Typhi (Extended Data Fig. 5).

To determine if absence of the typhoid toxin modulated host immune responses to infection, we measured T-cell and antibody-secreting cell (ASC) responses between challenge groups. Interferon-\(\gamma\) (IFN-\(\gamma\))-producing T-cell responses to peptide pools comprising the typhoid toxin subunits CdtB, PltA and PltB were detectable in participants challenged with the wild-type strain, but not the TN strain, and peaked at day 28 post-challenge (Fig. 4). We observed a significant increase in circulating ASCs specific to the \(S.\) Typhi surface antigen \(O9\):LPS and H\(d\) at the time of typhoid diagnosis in both challenge groups (Extended Data Fig. 6) (ref. \(^{13}\)). The magnitude of the \(O9\):LPS-antigen- and H\(d\)-antigen-specific ASC response at typhoid diagnosis was greater generally in participants challenged with the TN strain. In particular, \(S.\) Typhi \(O9\):LPS-specific immunoglobulin A ASC responses at the time of typhoid diagnosis were significantly increased in the TN group (Fig. 4).

We next aimed to determine if the presence or absence of typhoid toxin was associated with a distinct plasma cytokine profile, measured using a 62-plex bead-based cytokine platform (LumineX) at baseline and during acute typhoid disease (Fig. 4). At the time of typhoid diagnosis, the plasma cytokines 10kDa interferon-gamma-induced protein (IP-10), monokine induced by interferon-gamma (MIG) and interleukin-1 receptor antagonist protein (IL-1RA) were significantly increased relative to baseline in both groups (Fig. 4). Hierarchical clustering and principal component analysis showed no separation of challenge groups by cytokine profile during acute typhoid disease (Fig. 4). Following adjustment for multiple testing, linear modeling found no cytokines to be significantly different between groups, although interleukin 8 (IL-8) was marginally downregulated in the toxin-negative but not the wild-type group (Fig. 4).

These data suggest that the typhoid toxin is not essential for \(S.\) Typhi infection nor the early acute presentation of typhoid fever.

This study represents the first application of a typhoid human challenge model to prospectively study the role of a specific virulence factor in the pathogenesis of typhoid fever. Previous trials of live-attenuated \(S.\) Typhi vaccines have offered insights into the importance of other \(Salmonella\) genes to human disease (including \(aroC/\ aroD, htrA, phoP, phoQ, ssaV\) and \(cyA\))\(^{19}\). Overall, in this study, the clinical presentation was indistinguishable between the TN and wild-type groups. Counterintuitively, there was a trend toward a more severe disease phenotype in the TN group, including a shorter time to diagnosis, higher number of cases meeting the criteria for severe enteric fever, elevated ASC response and prolonged duration of bacteremia. These observations suggest that the typhoid toxin may have an important role in modifying host immune responses to infection. These data raise questions as to the utility of targeting typhoid toxin in the development of novel therapeutics or vaccine strategies. Notably, currently utilized typhoid vaccines, including Vi capsular polysaccharide/conjugate vaccines, are capable of inducing protection despite targeting a virulence factor that is not strictly necessary for the establishment of enteric fever\(^{19–22}\). Antibody and T-cell responses to typhoid toxin components have been detected in patients with typhoid fever\(^{23–25}\). Further studies are required to correlate host responses to typhoid toxin with protection against disease and to further characterize its function in the context of natural \(S.\) Typhi infection.

We acknowledge the limitations of our experimental approach. Due to ethical considerations, this model is not suited to assess the role of typhoid toxin in severe typhoid fever, including typhoid encephalopathy, which has been associated with typhoid toxin in animal models\(^{26–27}\). The primary diagnostic criteria minimizes risk to study participants by early treatment initiation\(^{28}\), but could mask differences between groups by treating self-limiting disease. The study population may not be generalizable to typhoid endemic countries, owing to differences in prior immune priming and/or baseline genetic differences\(^{22}\).

The absence of experiments showing reversion to virulence after complementation of the typhoid toxin genes in vitro is a limitation of this study. Additional studies with a strain expressing inactive components of typhoid toxin (for example, \(cdtB^{H160Q}, pltA^{E133A}, pltB^{E130A}\)) could address whether deletion of typhoid toxin genes is associated with an altered phenotype beyond loss of toxicity. Importantly, the study was underpowered to detect anything other than a large effect size (80% power to detect at 72% relative reduction). Additional in vitro studies and deeper analysis of challenge samples are ongoing to further characterize the potential immunobiological role of typhoid toxin in the pathogenesis of typhoid fever.

These data indicate that typhoid toxin is not essential for the development of early acute typhoid fever within the context of a controlled human infection model. These data highlight some of the benefits and challenges of studying bacterial virulence factors using controlled human infection models, in particular for the screening of potential vaccine and therapeutic targets.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at https://doi.org/10.1038/s41591-019-0505-4.

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Author contributions

J.G. and A.I.P conceived the project. M.M.G. and A.I.P designed the clinical study. M.M.G., C.J.M., D.C., E.J., S.C., C.J.B., C.Dold, C.Darlow, L.B., J.H., H.T.-B. and L.S.R. collected the data. M.M.G, J.M, C.Black and C.Jones co-ordinated study approvals and recruitment. The plasma cytokine assays were performed by Y.H.-R. and G.O.A. performed the cytokine analysis. M.I. and X. J. constructed the typhoid toxin mutant strain and carried out its in vitro characterization. G. S. conducted the mouse infection studies. E.H. and G.D. analyzed the sequencing data. U.G. provided the statistical oversight. B.A. and A.I.P provided clinical oversight. M.M.G. wrote the first draft of the manuscript and all authors reviewed and edited the manuscript and approved the final version.

Competing interests

A.I.P chairs the UK Department of Health’s (DHI) Joint Committee on Vaccination and Immunisation (JCVI) and the European Medicines Agency Scientific Advisory Group on Vaccines, and is a member of the World Health Organization’s (WHO) Strategic Advisory Group of Experts. A.I.P previously received grant funding from Okairos, which ended in 2016. The views expressed in this manuscript are those of the authors and do not necessarily reflect the views of the JCVI, DHI or WHO. All other authors declare no competing interests.

Additional information

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Methods

Study design and participants. The OVG2016/03 (TYGER) study was a randomized, double-blind, controlled human infection study comparing the response to challenge with wild-type S. Typhi with a typhoid toxin-deficient isogenic mutant strain (S. Typhi ΔpltB, ΔpltA and ΔcdtB). The study was designed as an outpatient challenge study, conducted in a cohort of healthy community adult volunteers in a setting non-endemic for typhoid fever (Oxford).

Challenge strains. To facilitate comparisons with earlier challenge studies and to minimize the risk to study participants, we used the Shigella strain (genotype 3.0.1 (ref. 1)) as the parent strain to generate the typhoid toxin-deficient knockout strain. Deletion of typhoid toxin subunit genes was carried as described previously.

Briefly, deletion of typhoid toxin subunit genes was carried out using the R6K-derived, suicide vector pSB890. The pSB890 plasmid cannot replicate in S. Typhi since it requires the bacteriophage λpir protein to replicate. The plasmid vector also encodes a counterselectable marker sacB, which encodes an enzyme that is lethal to bacteria when grown in the presence of sucrose. The pSB890 plasmid vector is maintained in a specially constructed strain of Escherichia coli, which encodes the bacteriophage λpir protein. This E. coli strain also carries a deletion mutation in the asd gene (aadD), which encodes the aspartate-semialdehyde dehydrogenase required for peptidoglycan synthesis—growth of this strain will only occur in media supplemented with lysine diaminoheptanodioate.

Due to the genomic organization of the typhoid toxin pathogenicity island, the pltB gene was deleted first, followed by simultaneous deletion of the pltA and cdtB genes (encoded immediately downstream of pltB) in the ΔcdtB strain. Chromosomal DNA fragments encoding sequences upstream and downstream of the target genes were expanded by PCR and cloned into the pSB890 plasmid, maintained in the E. coli λpir strain. The plasmid vector encoding the cloned sequences was then transferred to S. Typhi by conjugation, counterselecting the donor E. coli strain by plating the transconjugants in media lacking lysine diaminoheptanodioate (L−) and aminoglycoside (kanR). Transconjugants of S. Typhi possessing deletions of the toxin integrated into the chromosome were identified by plating in sucrose that counterselects for the plasmid vector. Colonies were screened by PCR to identified mutants carrying the specific deletions.

Challenge strains were manufactured to a GMP standard at the Walter Reed Army Institute of Research (Silver Spring) and stored as a frozen suspension in soyatrome medium containing 10% sucrose at −80 °C before use.

Strain characterization. Growth curves of wild-type and toxin-deficient strains of S. Typhi were performed in lysogeny broth (LB) using wild-type and toxin-deficient strains (S. Typhi ΔpltB, ΔpltA and ΔcdtB with S. Typhi Quailes cdtBΔkan, pltBΔ900 and pltAΔ900). Strains were cultured in an incubator overnight at 37 °C, 5% CO2. On the following day, colonies were washed and culture medium containing gentamicin (50 μg ml−1) was added and infection continued for 48 h. Cells were collected by centrifugation and the fixative decanted and 90% ethanol solution with continuous mixing. Cells were kept in fixative for 2 h on ice. The fixed cells were collected by centrifugation and the fixative decanted and 90% ethanol solution with continuous mixing. Cells were kept in fixative for 2 h on ice. The fixed cells were collected by centrifugation and the fixative decanted thoroughly. The pellets were washed once with 5 ml PBS and the cell pellet was resuspended in 1 ml of a solution containing 0.1% Triton X-100, DNA-free ribonuclease A (20 μg ml−1) and propidium iodide (20 μg ml−1: Molecular Probes) in PBS. The stained cells were analyzed by flow cytometry with a FACSCalibur flow cytometer (BD Biosciences). Intactocytes showed a larger proportion of cells in the G2/M phase of the cell cycle and thus exhibited a larger amount of DNA content.

Cellular infection assay. Henle–407 intestinal epithelial cells were infected with wild-type S. Typhi Quailes or the toxin-deficient SB6000 derivative for 2 h at three multiplicity of infection (MOI) levels (50, 100 and 500). Cells were washed and gentamycin (50 μg ml−1) was added to the culture medium. After 2 h, cells were washed again, lysed and colony counts of both strains were determined by plating dilutions of the cell lysates. The invasive ability was expressed as the percentage of the bacterial inoculum that survived gentamicin treatment.

Comparison of the bacterial loads of S. Typhi Quailes ΔpltB, ΔpltA and ΔcdtB with S. Typhi Quailes cdtBΔkan, pltBΔ900 and pltAΔ900 were derived from S. Typhi Quailes and were constructed by standard recombinant DNA techniques as described previously. CmH1–S1 mice, which are susceptible to S. Typhi infection, were intraperitoneally infected with equal numbers (1010 CFU) of S. Typhi Quailes and the derivative mutant strains carrying either deletions in the pltB, pltA and pltB genes (S. Typhi Quailes ΔpltB, ΔpltA and ΔcdtB) or expressing an inactivated version of typhoid toxin by virtue of catalytic mutations in its active subunits PTA and CdtB and a mutation in the receptor-binding site of PltB (S. Typhi Quailes cdtBΔkan, pltBΔ900 and pltAΔ900). The strains were alternatively marked by a chloramphenicol (cmlR) or kanamycin (kanR) resistance genes, as indicated, inserted within the STY6407 gene, which previous studies have shown not to affect virulence. All animal experiments were conducted in accordance with protocols approved by Yale University’s Institutional Animal Care and Use Committee. Seven-to-ten week old, CmH1–S1 mice were infected intraperitoneally with ∼108 CFU of each of the two strains. The inoculum was plated to confirm the equivalent ratio of the bacterial strains. Mice were killed at day 5 post-infection and the CFUs of each strain in the spleens of infected animals were determined by plating on LB plates containing chloramphenicol (30 μg ml−1) or kanamycin (50 μg ml−1).

Phenotypic characterization of the wild-type and TN strains comprised growth characterization in liquid culture, aggregation, cellular adhesion and cell intoxication assays. Whole-genome sequencing using both the MiSeq (Illumina) and PacBio (Pacific Biosciences) platforms was performed by the Wellcome Sanger Institute (Hinxton). DNA for MiSeq sequencing was extracted using the Wizard Genomic DNA purification kit according to the manufacturer’s instructions. Sequence reads were assembled using HCAP v.3 of the SMRT analysis software v2.2.3 (Supplementary Information).

Establishment of challenge dose. Challenge agents were prepared in batches for a maximum of six participants at any one time. All work was performed in the containment level 3 facility at the Centre for Clinical Vaccinology and Tropical Medicine (Oxford) in a class II biological safety hood dedicated for challenge agent preparation.

Two GMP master stock vials of typhoid toxin-negative S. Typhi TN strain (BPR–1218–00, lot 1977; cell concentration 1×1010) or wild-type S. Typhi Quailes strain (BPR–1218–00, lot 1977; cell concentration 9.8×1010) were selected at random from stocks stored in a −80 °C freezer. Vials were thawed at room temperature for approximately 30 min and mixed by vortexing. The volume of two GMP master stock vials were transferred to a master stock tube and mixed for 6–10s by vortexing. A 1:10 dilution in sodium bicarbonate was performed by transferring 1,600 μl from the tube labeled ‘master stock’ to a fresh 50 ml falcon labeled as ‘master stock 1.10’. To create the challenge inoculum of the toxin-negative strain, 1.74 μl from the ‘master stock 1:10 dilution’ was transferred to a sterile sodium bicarbonate containing 4.2 g sodium bicarbonate dissolved in 240 ml bottled mineral water (challenge flask). The challenge inoculum for the wild-type strain was generated by transferring 1.85 μl from the 1:10 dilution into an equivalent challenge flask. The challenge agents were then prepared by transferring 30 ml from the challenge flask to prelabelled 50 ml falcon tubes, sealed and stored on ice.

The challenge dose was confirmed by pipetting 200 μl from the challenge dose onto six TrypTone Soya Agar plates (code no. PO0163A; Oxoid). The bacterial suspension was spread over the surface of the agar using an L-shaped spreader and cultured in an incubator overnight at 37 °C, 5% CO2. On the following day, colonies were manually counted and checked by a second operator. The CFUs of the challenge inoculum were calculated by multiplying the area of the CFU counts for the plates by the dilution factor of the volume plated (×150 for a total challenge inoculum of 30 ml for a plating of 0.2 ml (30/0.2××150)).

Sodium bicarbonate was prepared by dissolving 2.1 g sodium bicarbonate in 120 ml bottled mineral water.

Participant characteristics. Healthy adults aged 18–60 years, without prior residency in an enteric fever endemic country for ≥6 months, were considered eligible for enrollment. Key exclusion criteria included significant medical, surgical or psychiatric history and gallbladder disease. A full description of the inclusion and exclusion criteria is provided in the Nature Research Reporting Summary.

Randomization and masking. Participants were randomized 1:1 to challenge with either wild-type strain S. Typhi or toxin-negative strain S. Typhi (TN) in varying block sizes. Anti-Vi IgG was measured at screening using a commercial ELISA kit (VacZyme; The Binding Site Ltd) according to the manufacturer’s instructions.
Randomization was stratified by anti-Vi IgG measured (low (<7.4 EU ml⁻¹) or high (≥7.4 EU ml⁻¹)). The exception was a sentinel group of two participants who were randomized 1:1 to receive the wild-type strain or TN knockout strain using a block size of two. Randomization was performed at the prechallenge visit, one week before challenge. We generated a randomization list in STATA v.14.2 (StataCorp), which was implemented in the computerized randomization software Sortition (Nuffield Department of Primary Care, Clinical Trials Unit, University of Oxford), which matched a masked allocation group to each participant. The software generated a randomization number, corresponding to the challenge allocation group. A locked, challenge agent randomization allocation list was maintained by the study statistician and unblinded laboratory team responsible for challenge agent preparation.

The study was conducted double-blind from the time of randomization until participant unblinding, such that participants, and clinical or laboratory staff undertaking follow-up procedures, were unaware of challenge agent allocation. Both wild-type and TN strains were prepared suspended in sodium bicarbonate and had an indistinguishable appearance (transparent, colorless liquid).

**Procedures.** Participants fasted for 90 min before challenge. Two minutes before challenge, participants drank a sodium bicarbonate solution (2.1 g 120 ml⁻¹) to neutralize stomach acid. The oral challenge inoculum was administered suspended in sodium bicarbonate (0.53 g 30 ml⁻¹) and was kept on ice before administration within 3 h of preparation. Participants were observed for 90 min post-challenge. The challenge dose administered was 1–5×10⁶ CFUs calculated as described previously. Participants attended the clinical site 12 h after challenge and then daily for 14 d, as described previously. Daily visits comprised comprised continued consent check, oral temperature measurement, heart rate and blood pressure measurements and sample collection, as outlined in the study protocol. Solicited symptoms and twice-daily temperature measurements were recorded in an electronic diary for 21 d after challenge. Symptoms were categorized as not present, mild, moderate or severe (Supplementary Information).

Antibiotic treatment was initiated on fulfilment of composite diagnostic criteria or at day 14 for those without illness. First-line treatment was oral ciprofloxacin 500 mg twice daily for 14 d.

**Outcomes.** The primary objective of this study was to compare the proportion of participants meeting the composite diagnostic end point for typhoid fever (attack rate) following oral challenge with (1–5)×10⁶ CFUs wild-type S. Typhi Quailes strain, compared to challenge with (1–5)×10⁶ CFUs of a typhoid toxin-deficient isogenic mutant of S. Typhi Quailes strain SB6000 (TN). The composite diagnostic end point for typhoid fever was defined as a temperature ≥38°C persisting for ≥12 h and/or S. Typhi bacteremia collected ≥72 h after oral challenge.

Secondary end points were: mode of diagnosis; time to typhoid diagnosis; time to first temperature ≥38°C; fever clearance time; time to bacteremia; duration of bacteremia; and quantitative blood culture (for definitions, see the Life Sciences Reporting Summary). Descriptive end points included: severe adverse events; solicited symptom profiles; proportion of participants meeting the criteria for severe enteric fever; pathologiocal and biochemical measures; plasma cytokine profiles; pattern of bacteremia; and pattern of stool shedding (see Life Sciences Reporting Summary).

Stool samples for culture, blood samples for culture (10 ml), and hematological and biochemical testing were processed by the local hospital's accredited pathology laboratory as described previously.

**Criteria for severe enteric fever.** Severe enteric fever was defined as participants meeting any of the following criteria: oral temperature >40°C; systolic blood pressure <85 mmHg; significant lethargy or confusion; gastrointestinal bleeding; gastrointestinal perforation; or any grade 4 laboratory abnormality.

**Ex vivo ASC enzyme-linked immune absorbent spot (ELISPOT).** Ex vivo IgG₃, IgA- and IgM-producing ASC responses against O- and H-antigen were measured at baseline and 24–48 h after typhoid diagnosis in those meeting the diagnostic criteria as described previously. Multiscroll filtration ELISPOT plates (catalog no. MAHAS5450; Merck Millipore) were coated with S. Typhi O9.PFS, S. Typhi Hd antigen (University of Maryland) and Panaglobulin (catalog no. H17090; Invitrogen) each at a final concentration of 10 μg ml⁻¹ in carbonate-bicarbonate buffer and incubated overnight at 4°C. Plates were blocked with 200 μl per well of R10 medium for 1 h before use at 37°C, 5% CO₂. Peripheral blood mononuclear cells (PBMCs) were separated using ACCUSPIN tubes (Sigma-Aldrich), counted and resuspended in R10 media. PBMCs at a concentration of 2.5×10⁶ were added in duplicate to the ELISPOT plate (100 μl per well) and incubated overnight at 37°C, 5% CO₂. Plates were washed four times with PBS-0.25% Tween, once with PBS and soaked with PBS for 5 min. Goat anti-human IgG, IgA and IgM secondary antibodies conjugated to alkaline phosphatase (catalog nos. 401442, 401132 and 401092, respectively; Sigma-Aldrich) were diluted to 1:500 in PBS and incubated for 4 h at room temperature. After incubation, the plates were washed five times with PBS-0.25% Tween and four times with diH₂O. Alkaline phosphatase substrate (catalog no. 170-6432; Bio-Rad) was added at 50 μl per well, allowed to develop over approximately 10 min and stopped with diH₂O as spots began to develop.

ELISPOT plates were read using an automated ELISPOT reader (ELR30/ ESR30-TV; Autoimmun Diagnostika) and the AID ELISPOT software v.5.0. Study- and antigen-specific count settings for spot intensity, size and gradient were applied to the plate counts and manually verified to remove artifacts. Raw counts (spots per 2.5×10⁶ PBMCs) were averaged across duplicate wells and multiplied by four to give the number of spot-forming units (SFUs) per 10⁶ PBMCs.

**Fluorospots.** Measurements were taken from frozen PBMCs collected at baseline, and on day 14 and 28 post-challenge. Precocated plates (catalog no. FSP-010308-10; Mabtech) were blocked before adding 50 μl per well toxin peptide pools consisting of 15-mer sequences with 11-amino acid overlaps and covering the sequence of proteins CD1b, PRA and PHB (think peptides). The peptides were dissolved in 100% DMSO (Sigma-Aldrich) and arranged in three pools. Concentration was adjusted to 0.6 mg ml⁻¹ and used in the fluorospot assay at a final concentration of 3 μg ml⁻¹ of each peptide. DMSO and concanavalin A (Sigma-Aldrich) were used as negative and positive controls, respectively. After defrosting and resting for 1 h, 50 μl per well of PBMCs were added to the peptide wells at a concentration of 4×10⁶ cells ml⁻¹ in triplicate and incubated overnight at 37°C in 5% CO₂, 95% humidity. Detection of spots was carried out according to the manufacturer's instructions (Mabtech) and analyzed with the iSpot ELISPOT reader (Autoimmun Diagnostika).

**Plasma cytokine analysis.** Plasma was isolated from heparinized blood by centrifugation. Protease inhibitor was added in a 1:40 dilution before storage at −80°C. Immunofluorimetric assay was carried out in a blinded manner by research participants by the Human Immuno Monitoring Center at Stanford University using a 62-plex Luminex system (brain-derived neurotrophic factor, beta-nerve growth factor, CD40 ligand, epidermal growth factor, ENA-78 (CXCL5), eotaxin, fibroblast growth factor 2, granulocyte-colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, GRO-α (CXCL1), hepatocyte growth factor, IFN-α, IFN-β, IFN-γ, IL-1α, IL-1β, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17A, IL-17F, IL-18, IL-1αR, IL-2, IL-21, IL-23, IL-27, IL-31, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IP-10, leukenia inhibitory factor, macrophage colony-stimulating factor 1, monocoy chemotactic protein 1 (MCP-1), MCP-3, MIG, macrophage inflammatory protein 1-α (MIP-1-α), MIP-1-β, plasminogen activator inhibitor 1, platelet-derived growth factor subunit B, RANTES, resistin, stem cell factor, stromal cell-derived factor 1, soluble Fas ligand, soluble intercellular adhesion molecule 1, soluble vascular cell adhesion protein 1, transforming growth factor-α (TGF-α), TGF-β, tumor necrosis factor-α (TNF-α), TNF-β, tumor necrosis factor ligand superfamily member 10, vascular endothelial growth factor A (VEGF-A) and VEGF-D). Samples were run in duplicate and the mean fluorescence intensity (MFI) of duplicates was used for analysis. To minimize plate-to-plate variation, samples across time points for each individual were run on the same plates and each plate contained an equal mix of individuals allocated to wild-type or toxin-negative challenge. Control beads (CHEX 1–4) and control sera were used per plate.

**Clinical scoring.** The MFI of all samples were examined by principal component analysis to confirm consistency between duplicates and identify outliers. One participant was excluded on this basis. Duplicates were then averaged and the MFI quantile normalized. Significance testing was performed using linear modeling in limma v3.43.9 (ref. 35), incorporating plate, dose and sex as covariates. P values were corrected for multiple testing using the Benjamini–Hochberg correction. Hierarchical clustering was carried out based on Euclidean distance.

**Sample size.** The sample size was dictated primarily by the number of participants that could be feasibly enrolled within the time frame and budget of the study; therefore, it represents a convenience sample. Assuming typhoid toxin is central to the clinical presentation of acute typhoid fever, it was anticipated that the attack rate following challenge with the TN strain would be reduced compared with the wild-type strain, although the effect size was unknown. Assuming an attack rate of 65% following wild-type challenge (as observed in previous studies) and 50% attack rate following TN challenge, and accounting for a 10% dropout, 20 participants in each group had 95% CIs for attack rate of 41–85% in the wild-type group and 27–73% in the TN group. Twenty participants were enrolled to have 80% power to detect an absolute reduction in attack rate of 55% (65% with the wild-type strain versus 10% with the TN strain, corresponding to an 85% relative risk reduction) and 80% power to detect an absolute reduction in attack rate of 47% (65% with the S. Typhi wild-type strain versus 18% with the S. Typhi toxin-negative strain, corresponding to a 72% relative risk reduction) based on Fisher's exact test with 5% alpha.

**Statistical considerations.** Attack rates and 95% CIs were calculated for each challenge group for the per-protocol population (that is, participants who completed the 14-d challenge period) as the primary end point. All participants were included in the analyses if they were successfully challenged on day 0 and had at least one post-challenge assessment. The difference in attack rate (and other categorical variables) between naive and rechallenge groups was tested using Fisher's exact test. Time-to-event data were summarized using the Kaplan–Meier method.
method, with participants censored at day 14. Group comparisons were performed using a log-rank test. Continuous variables were compared using the Mann–Whitney U-test for unpaired samples and the Wilcoxon signed-rank test for paired samples. All statistical tests were two-sided.

Paired samples across time points were compared using the Wilcoxon signed-rank test. Comparisons between groups were performed using the Mann–Whitney U-test. ELISpot/FluoroSpot data were log_{10}-transformed to approximate a normal distribution; wells with no spots were assigned an arbitrary value of 0.5, corresponding to half the lower limit of detection. Raw counts were averaged across replicate wells. The number of background spots detected in blank wells were subtracted from the test samples to give the final cell count per sample.

Clinical data were recorded on a web-based database (OpenClinica Enterprise v3.13). Symptom and ELISpot were extracted using Microsoft Excel. Data analysis was performed using R v.3.4.4. Variables were normalized by z-score before inclusion in the principal component analysis, which was performed using the FactoMineR package v1.41 (ref. 36).

Approvals. The OVG2016/03 study was sponsored by the University of Oxford (Clinical Trials & Research Governance). Ethical approvals for the primary protocol, and any study amendments, were obtained from the South Central-Oxford A Research Ethics Committee (16/SC/0338). In the UK, legislation governing the deliberate release of genetically modified organisms is currently provided by the Environmental Protection Act 1990, sections 111 and 112 (ref. 37), and the Genetically Modified Organisms (Deliberate Release) Regulations 2002 (ref. 38). Approvals for deliberate release of the genetically modified strain of S. Typhi were obtained from the United Kingdom Department for Environment, Food & Rural Affairs (16/R48/01) (ref. 39). The study was registered with clinicaltrials.gov (NCT03067961) and was performed according to the provisions of the Declaration of Helsinki (2013) and Good Clinical Practice guidelines. This work is licensed under the Creative Commons Attribution 4.0 International License.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
The datasets generated and/or analyzed during the current study are attached. Any additional data are available from the corresponding author. No participant identifiable information will be disclosed. The raw sequence reads for the wild-type and TN strains used in the challenge are available under accession nos. ERS3381923 and ERS3381927.

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Extended Data Fig. 1 | Trial profile. One participant randomized to wild-type S. Typhi withdrew before challenge and was excluded from all further analyses, leaving 40 participants in the per-protocol analysis.
Extended Data Fig. 2 | Baseline anti-Vi IgG. S. Typhi Quailes strain (n = 21). Two-sided Mann–Whitney U-test. The box plots represent the median and IQR. The overlaid violin plots illustrate the distribution of the data points and their probability density. Samples below the lower limit of detection of the ELISA (7.4 EU ml⁻¹) were assigned a value equating to half the lower limit of detection (3.7 EU ml⁻¹)14. The box plots display the median and IQR, with the upper whiskers extending to the largest value ≤1.5 × IQR from the 75th percentile and the lower whiskers extending to the smallest values ≤1.5 × IQR from the 25th percentile. The overlaid violin plots illustrate the distribution of the data points and their probability density31. TN S. Typhi (n = 19).
Extended Data Fig. 3 | Hematological and biochemical laboratory measures after challenge. Participants challenged with TN (red) and wild-type (blue) S. Typhi and diagnosed with typhoid fever, n^TN^ = 15, n^WT^ = 15. Data are presented relative to the time of typhoid diagnosis (time point 0 = day of diagnosis). The dot plots correspond to individual values colored according to challenge group (TN, red; wild-type, blue). The solid, colored lines connect the median values at each time point. The dashed lines represent the upper and lower reference limits for the individual parameters measured. The box plots display the median and IQR, with the upper whiskers extending to the largest value ≤1.5 × IQR from the 75th percentile and the lower whiskers extending to the smallest values ≤1.5 × IQR from the 25th percentile, and are colored according to challenge group.
Extended Data Fig. 4 | Principal component analysis (PCA) biplot of disease severity by outcome of challenge. Participants challenged with TN (n = 19) or wild-type (n = 21) S. Typhi based on the typhoid severity score. The data points are colored by outcome of challenge (yellow, typhoid diagnosis; blue, no typhoid diagnosis). The ellipses represent the 95% confidence levels for diagnosis status. The biplot arrows represent the contributions of individual variables to a given principal component, scaled according to their relative contribution.
Extended Data Fig. 5 | PCA biplot of disease severity by challenge agent. PCA biplot of participants challenged with typhoid fever following challenge with TN (n = 19) or wild-type (n = 21) S. Typhi based on all clinical, microbiological and laboratory measures. The data points are colored by challenge agent allocation. The ellipses represent the 95% confidence levels for challenge agent. The biplot arrows represent the contributions of individual variables to a given principal component, scaled according to their relative contribution.36
Extended Data Fig. 6 | O9:LPS- and Hd-specific IgG, IgA and IgM ex vivo ASC responses. a, O9:LPS. b, Hd. Participants diagnosed with typhoid, illustrated as log_{10} SFUs per 10^6 PBMCs. D0, baseline; TD, time of diagnosis (samples processed 24–48 h after initiation of treatment). Two-sided matched-pairs Wilcoxon signed-rank test. n^{O9LPS tox}=11, n^{TN} = 10. The box plots display the median and IQR, with the upper whiskers extending to the largest value \leq 1.5 \times IQR from the 75th percentile and the lower whiskers extending to the smallest values \leq 1.5 \times IQR from the 25th percentile.
Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Software and code

Policy information about availability of computer code

Data collection

Clinical data were recorded on a web-based database (OpenClinica Enterprise). Symptom and ELISPOT/Flurospot were extracted using Microsoft Excel. ELISPOT plates were read using an automated ELISPOT reader (AID ELR03/ELR030408215) and AID ELISPOT software V5.0.
Data analysis was performed using R version 3.4.4. Plots were generated using the ggplot2 package. The principal component analysis was performed using the FactoMineR package 37 with R version 3.4.4. The randomisation list was generated in STATA version 14.2. Randomisation was implemented using Sortition® (Oxford University Innovation Ltd & Nuffield Department of Primary Care, Clinical Trials Unit, University of Oxford).

Sequencing:
Sequence reads were assembled using HGAP v3 (PMID: 23644548) of the SMRT analysis software v2.3.0 (Available from: https://github.com/PacificBiosciences/SMRT-Analysis). The assembly was circularized using Circulator v1.1.3 (PMID: 26714481). The circularized assembly was polished using the PacBio RS_Resequencing protocol and Quiver v1 of the SMRT analysis software v2.3.0 (Available from: https://github.com/PacificBiosciences/SMRT-Analysis). Automated annotation, as well as annotation steps on manual assemblies, was performed using PROKKA v1.11 [(PubMed PMID: 24642063) and a genus specific databases from RefSeq (PubMed PMID: 22121212)](https://github.com/PacificBiosciences/SMRT-Analysis).

SNP/Indel calling
The in-house script uses SMALT v0.7.4 (Available from: https://sourceforge.net/projects/smalt/) to map reads against a selected reference including randomly mapping the repeats and using the GATK indel alignment option. Variation detection was performed using samttools mpileup v0.1.19 and bcftools v0.1.19 to produce a BCF file of all sites and all variant sites.

The datasets generated during and/or analysed during the current study are available from the corresponding author. No participant identifiable information will be disclosed.

The raw sequence reads are available under accessions ERS3381923 (sample 1 Oxford w/t), ERS3381924 (sample 2 w/t Pre-GMT), ERS3381925 (sample 3 w/t Post-GMT), ERS3381926 (sample 4 k/o Pre-GMT), and ERS3381927 (sample 5 k/o Post-GMT). Manually refined hybrid assemblies as described above are given for the wild type strain (sample 2 w/t Pre-GMT) under accession GCA_901457615 and for the knock-out strain (sample 5 k/o Post-GMT) under accession GCA_901457625.
Anti-Vi IgG was measured at screening using a commercial ELISA kit (VaccZyme, The Binding Site Ltd, Birmingham, UK) according to the manufacturer’s instructions. Randomization was stratified by anti-Vi IgG (low <7.4 EU/ML or high >=7.4 EU/ml). The exception was a sentinel group of two participants who were randomized 1:1 to receive the WT strain or TN knock-out strain, using a block size of two.

Randomization was performed at the pre-challenge visit (Day -7), one week prior to challenge. We generated a randomisation list in STATA version 14.2, which was implemented in the computerised randomisation software Sortition (Nuffield Department of Primary Care, Clinical Trials Unit, University of Oxford), which matched a masked allocation group to each participant.

Blinding

The study was conducted in a double-blind from the time of randomisation until participant unblinding, such that the participants, and clinical or laboratory staff undertaking follow-up procedures, were unaware of challenge agent allocation. Both WT and TN strains were prepared suspended in sodium bicarbonate and had an indistinguishable appearance (transparent, colourless liquid).

Reporting for specific materials, systems and methods
We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☒  | Antibodies            |
| ☒  | Eukaryotic cell lines |
| ☒  | Palaeontology         |
| ☒  | Animals and other organisms |
| ☒  | Human research participants |
| ☒  | Clinical data         |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☒  | ChIP-seq              |
| ☒  | Flow cytometry        |
| ☒  | MRI-based neuroimaging |

Antibodies

Antibodies used

Antibodies:
Manufacturer: Merck Millipore
Catalog/lot number: Goat anti-human IgG γ-Chain specific conjugated to alkaline phosphatase 401442-1ml/2689825; Goat anti-human IgA alpha-Chain specific conjugated to alkaline phosphatase 401132/D00165538.
Used at a 1:5000 dilution.

Fluorospot detection antibodies, using Mabtech kit FSP-010308-10 (batch 3).
Manufacturer: Mabtech
Anti IFNg monoclonal antibody, clone 7-B6-1-BAM, batch 1, 1/200 dilution
Anti IL17A monoclonal antibody biotinylated, clone MTS04, batch 7, 1/250 dilution
Fluorophore conjugates:
anti BAM-490, batch 3, 1/200 dilution; SA-550, batch 7, 1/200 dilution; anti-WASP-641, batch 7, 1/200 dilution

Validation

Immunoaffinity purified goat polyclonal anti-human IgA and anti-human IgG antibody conjugated to alkaline phosphatase, validated by manufacturer Merck Millipore.
(Manufacturers information available at http://www.merckmillipore.com/GB/en/product/Goat-Anti-Human-IgG-Chain-Specific-Alkaline-Phosphatase-Conjugate,EMD_BIO-401442#anchor_PDS)

Antibodies used in fluorospot assay validated by manufacturer Mabtech.
(Manufacturers information available at https://www.mabtech.com/products/anti-human-ifn-gamma-antibody-7-b6-1-biotinylated-3420-6)

Antibodies used in ex-vivo ELISPOT assays as outlined in https://www.ncbi.nlm.nih.gov/pubmed/18032593 and https://www.ncbi.nlm.nih.gov/pubmed/27533046

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Henle-407 intestinal epithelial cells from the Roy Curtiss laboratory collection.

Authentication

The cells were frequently checked for their morphological features, growth speed and functionalities, but were not
Authentication authenticated by short tandem repeat (STR) profiling.

Mycoplasma contamination All cell lines tested negative for mycoplasma contamination.

Commonly misidentified lines (See ICLAC register) No commonly misidentified cell lines were used.

### Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | CmaH -/- bloc3 -/- mice (Figure 1).
| Age: 7 - 10 week old
| Sex: male and females, randomly assigned to the different groups |
| Wild animals | The study did not involve wild animals. |
| Field-collected samples | The study did not involve field collected specimens. |
| Ethics oversight | All animal experiments were conducted according to protocols approved by Yale University’s Institutional Animal Care and Use Committee. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

### Human research participants

Policy information about studies involving human research participants

| Population characteristics | Healthy adults aged 18-60 years |

Key exclusion criteria included significant medical, surgical or psychiatric history, gallbladder disease and high-risk occupations as defined by Public Health England guidelines. A full description of inclusion and exclusion criteria is provided in the attached study protocol and below.

Study eligibility
Male or female participants aged 18-60 years inclusive who were in good health (as determined by a study doctor, medical investigation and review of medical history provided by their General Practitioner) and who were able to provide written informed consent were eligible for inclusion in this study.

Inclusion Criteria
Participants must satisfy all of the following criteria to be considered eligible for the study:
- Agree to give informed consent for participation in the study.
- Aged between 18 and 60 years inclusive at time of challenge.
- In good health as determined by medical history, physical examination and clinical judgment of the study team.
- Agree (in the study team’s opinion) to comply with all study requirements, including capacity to adhere to good personal hygiene and infection control precautions.
- Agree to allow his or her General Practitioner (and/or Consultant if appropriate), to be notified of participation in the study.
- Agree to allow study staff to contact his or her GP to access the participant’s vaccination records and summary of medical history.
- Agree to allow Public Health England to be informed of their participation in the study.
- Agree to give his or her close contacts written information informing them of the participant’s involvement in the study and offer them voluntary screening for S. Typhi carriage.
- Agree to have 24-hour contact with study staff during the four weeks post challenge and to be able to ensure that they are contactable by mobile phone for the duration of the challenge period until antibiotic completion.
- Agree to allow the study team to hold the name and 24-hour contact number of a close friend, relative or housemate who will be kept informed of the study participant’s whereabouts for the duration of the challenge period (from the time of challenge until completion of antibiotic course). This person will be contacted if study staff are unable to contact the participant.
- Have internet access to allow completion of the e-diary and real-time safety monitoring.
- Agree to avoid antipyretic/anti-inflammatory treatment from the time of challenge (Day 0) until advised by a study doctor or until 14 days after challenge.
- Agree to refrain from donating blood for the duration of the study.
- Agree to provide their National Insurance/Passport number for the purposes of TOPS registration and bank account details for payment of reimbursement expenses.

Exclusion Criteria
Participants were not enrolled if any of the following applied:
- History of significant organ/system disease that could interfere with trial conduct or completion. Including, for example, but not restricted to:
  - Cardiovascular disease
  - Respiratory disease
  - Haematological disease
  - Endocrine disorders
  - Renal or bladder disease, including history of renal calculi
Recruitment

Several strategies were employed to recruit participants, including:

- NHS database: Potential study participants will be identified via databases such as the National Health Applications and Infrastructure Services (NHAIS) who hold the central NHS patient database (Open Exeter) or their equivalent.

- Poster advertising: Display of posters advertising the study throughout local hospitals and doctor’s surgeries, tertiary education institutions and other public places with the permission of the owner/proprietor.

- Direct mail-out

- E-mail communication: We will contact representatives of local tertiary education establishments and local employers and ask them to circulate posters and information, and to circulate a link to study information on the OVG website by email.
- Oxford Vaccine Centre (OVC) database for healthy volunteers
- Media advertising: Local media, newspaper, radio, website and social media advertisement placed in locations relevant for the target age group with brief details of the study and contact details for further information
- Website advertising: Description of the study and copy of information booklet on the Oxford Vaccine Group website.

Exhibitions: Advertising material and/or persons providing information relating to the study will exhibit using stalls or stands at exhibitions and/or fairs, such as University Fairs

Royal Mail Leaflet: Royal Mail door-to-door service with delivery of invitation letters in OVG envelopes to every household within certain postcode areas.

Potential participants who were interested in study participation contacted the study site by telephone, email, by out website online registration with self-screening questions or paper reply slip for further information. Once an expression of interest was received, an information booklet was be sent via mail or email to the potential participants to read at their leisure. Participants were also be directed to the Oxford Vaccine Group website, where the information booklet was available. If participants were willing to proceed they were invited for a screening and consent visit, where a member of the clinical research team at the Oxford Vaccine Group assessed their eligibility. We also took consent for clinical staff to access electronic patient records (EPR) to assess eligibility.

We acknowledge the potential self-selection bias in such human-challenge studies. We contend that any potential biases are mitigated by standardized procedures and consistent inclusion/exclusion criteria. These limitations are discussed within the manuscript.

**Ethics oversight**

The OVG2016/03 study was sponsored by the University of Oxford (Clinical Trials & Research Governance). Ethical approvals for the primary protocol, and any study amendments, were obtained from the South-Central Oxford A research ethics committee (16/SC/0358). In the UK, legislation governing the deliberate release of genetically modified organisms is currently provided by the Environmental Protection Act 1990 section 111 and 11242, and the Genetically Modified Organisms (Deliberate Release) Regulations 200243. Approvals for deliberate release of the genetically modified strain of S. Typhi were obtained from the United Kingdom Department for Environment, Food & Rural Affairs (16/R48/01)44

Note that full information on the approval of the study protocol must also be provided in the manuscript.

**Clinical data**

Policy information about clinical studies. All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

| Clinical trial registration | Clinicaltrials.gov NCT03067961 |
|----------------------------|----------------------------------|
| Study protocol             | Submitted alongside manuscript   |
| Data collection            | Centre for Clinical Vaccinology & Tropical Medicine, The Churchill Hospital, Oxford, United Kingdom OX3 7LE 10th April 2017 and 1st August 2017 |

**Outcomes**

Primary Outcome Measure

The primary outcome was the attack rate post challenge, defined as the proportion of participants meeting the composite diagnostic endpoint for typhoid fever during the challenge period. The composite diagnostic endpoint for typhoid fever was defined as a temperature of 38°C persisting for >12hrs and/or S. Typhi bacteraemia collected >72hours after oral challenge

Secondary clinical and microbiological outcome variables are listed by below

- Time to diagnosis - Time from date/time of challenge to date/time of first temperature >38C that subsequently lasted for >12hours OR the date/time of first positive blood culture collection (whichever occurs earliest).
- Time to first blood culture positive for S. Typhi - Time from date/time of challenge to date/time of blood culture collection.
- Time to clinical diagnosis (fever >38C lasting >12 hours) - Time from date/time of challenge to date/time of first recorded temperature >38C which subsequently lasted 12 hours.
- Mode of diagnosis - The proportion of participants diagnosed with typhoid/paratyphoid fever based upon either clinical criteria (persistent fever >38C for >12 hours) OR microbiological criteria (blood culture positive for S. Typhi collected >72 hours from diagnosis)

Detailed mode of diagnosis - Proportion of participants diagnosed with typhoid/paratyphoid fever based upon either of the following specific diagnostic criteria:

- Temperature >38C preceding positive blood culture;
- o Temperature >38C without positive blood culture;
- o S. Typhi bacteraemia preceding temperature >38C;
- o S. Typhi bacteraemia without temperature >38C.
- Time to first fever - Time from date/time of challenge to date/time of first recorded temperature >38C.
- Fever clearance time - Time from initiation of antibiotics or start of fever (whichever was later) to first recorded temperature <38C persisting for at least 48hours. Only diagnosed participants with fever were included in the analysis.
Symptom severity - Severity of symptoms in each challenge group were assessed by:

- The proportion of participants with maximum symptom severity score graded as mild, moderate or severe following challenge.
- The proportion of participants meeting the criteria for severe enteric fever.
- Individual enteric fever severity scores calculated by summing numerical values assigned to the severity of individual solicited symptoms, clinical observations (heart rate, systolic blood pressure, diastolic blood pressure and temperature) and laboratory measurements between Day 0 to Day 21 (0=not present; 1=mild; 2=moderate; 3=severe; 4 = Hospitalisation).
- Duration of bacteraemia - Time (Hours/Days) from collection of first positive blood culture until date/time of the last positive blood culture.
- Bacteraemia clearance time - Time (Hours/Days) from collection of first positive blood culture until date/time of the first negative blood culture remaining persistently negative. Participants with missing data (e.g. no negative blood cultures after commencing antibiotics) were censored in the analysis at the time point of the last culture taken.
- Stool shedding - Daily stool culture(s) positive for S. Typhi for 14 days post-challenge.

Quantitative blood culture - Concentration of bacteria in 10ml blood taken at the time of diagnosis using the Wampole™ Isostat® Isolator system (Colony forming units/ml). For values below the lower limit of detection (0.1 CFU/ml), a value of 0.05 CFU/ml was assigned.

Haematological and biochemical end-points - The following haematological parameters were measured from time of challenge to Day 28 and/or Day 90.

- Total Haemoglobin (g/L)
- Haemoglobin change from baseline (Hb g/l D0 – Hb g/l D14)
- Total White Cell Count (x10⁹/l)
- Neutrophil count (x10⁹/l)
- Lymphocyte count (x10⁹/l)
- Monocyte count (x10⁹/l)
- Eosinophil count (x10⁹/l)
- Monocyte/Lymphocyte ratio
- Urea & Electrolytes (Na, K+, Urea, Creatinine –mmol/l)
- C-reactive protein (mg/l)
- Liver function tests (Bilirubin [umol/l], aspartate transaminase (AST IU/l), alkaline phosphatase (ALP IU/l), alanine transaminase (ALT IU/l), Albumin (g/L)

Safety outcome measures - Adverse events, adverse events of special interest, SAE’s and SUSARs according to each study group.