Distinct glycosylation and functional profile of typhoid vaccine-induced antibodies in a UK challenge study and Nepalese children. Stockdale *et al. doi: 10.3389/frans.2022.1005558*

Supplementary Methods

## Assays to measure antibody function

Antibody dependent monocyte, and neutrophil phagocytosis (ADCP, ADNP), NK cell activation (ADNKA), complement deposition (ADCD), and serum bactericidal activity analysis were performed based upon previously described methods and carried out on UK samples at all timepoints studied here and are reported by Jin et al (1). ADNP was investigated using the same method for Nepalese samples but the assay carried out in a different laboratory and data are reported by Johnson et al (2). Methods for ADCD and ADCP are described by Barouche *et al*  (3), and ADNP and ADNOB by Ackerman *et al* (4). Mean Fluorescence Intensities (MFIs) were reported after background subtraction, and results were calculated from the mean result of sample replicates across two assay runs. Data were acquired on an S1000EXi cytometer (Stratedigm) and analyzed using FlowJo v10.6 software. Data from the UK cohort have been previously reported by Jin et al (1). Luminescent-Serum Bactericidal Activity Assay (SBA) was carried out as per previously described method and the data from the UK cohort have been described in Jones *et al* (5) .

## ADCD (antibody dependent complement deposition)

Serum samples were heat-inactivated, diluted 1:10 and incubated at 37°C for 2 h with red fluorescent NeutrAvidin-coated micro-spheres (Thermo Fisher Scientific) which had been previously coated with biotinylated Vi-antigen (provided by Dr. Sjoerd Rijpkema, NIBSC, Potters Bar,UK) in 96-well round-bottom plates. Guinea pig complement (Cedarlane), reconstituted in veronal buffer with 0.1% gelatin, was added and incubated at 37°C for a further 20 min. Beads were washed twice with 15 mM EDTA in PBS. FITC-conjugated goat anti–guinea pig complement C3b antibody (MP Biomedical) was added and incubated in the dark for 15 min at room temperature. Beads were washed, resuspended in PBS, and analysed using flow cytometry. FITC MFIs were measured for each sample.

## ADCP (antibody dependent cellular phagocytosis)

ADCP was assessed by quantifying phagocytosis of antibody-opsonized Vi-coated fluorescent microspheres using monocyte cell line THP-1 (IB-202; American Type Culture Collection). Serum samples were diluted 1:250 and incubated with NeutrAvidin-coated yellow-green 1-μm fluorescent microspheres coupled with biotinylated Vi-antigen. 2.5 × 104 THP-1 cells (American Type Culture Collection) were added to each well and incubated overnight at 37°C. Cells were pelleted, fixed with 4% paraformaldehyde (PFA), and analyzed using flow cytometry. A phagocytic score was calculated using the mean MFI for each sample and the following formula: [(% microsphere-positive cells) × (MFI of microsphere-positive cells)]/10,000.

## ADNP (antibody dependent neutrophil phagocytosis)

ADNP was assessed by measuring the uptake of antibody-opsonized Vi-coated fluorescent microspheres using freshly isolated neutrophils. Serum samples, diluted 1:100, were incubated with NeutrAvidin-coated yellow-green 1-μm fluorescent microspheres coupled with biotinylated Vi-antigen. Leukocytes were isolated from acid citrate dextrose–treated fresh blood from healthy anonymized donors using ammonium-chloride-potassium lysis buffer. 5 × 104leukocytes were added to each well containing antibody-opsonized beads and incubated for 1 hat 37°C. Cells were pelleted, the supernatant was removed, and cells were stained with APC-Cy7 anti-CD14 (clone MψP9; BDBiosciences) and Pacific Blue anti-CD66b (clone G10F5; BioLegend). Cells were washed, fixed with 4% PFA, and analyzed using flow cytometry. The phagocytic score was calculated from gated neutrophils (CD14−, CD66b+).

## ADNOB (antibody dependent neutrophil oxidative burst)

Neutrophil oxidative burst was measured by addition of dihydrorhodamine 123 to freshly isolated neutrophils with antibody-opsonized red fluorescent NeutrAvidin-coated microspheres. Heat inactivated serum samples were diluted 1:50 before incubation with Vi-coated red fluorescent microspheres. Leukocytes were isolated from EDTA-treated blood from healthy anonymized donors using ammonium-chloride-potassium lysis buffer. 5 × 104 leukocytes were added to each well of antibody-opsonized beads, followed by 10 µl per well of dihydrorhodamine 123 (50 µM), and incubated for 1 h at 37°C. Cells were pelleted and stained as above with APC-Cy7 anti-CD14 (clone MφP9; BD Biosciences), Alexa Fluor 700 anti-CD3 (clone UCHT1; BD Biosciences), and Pacific Blue anti-CD66b (clone G10F5; BioLegend). Cells were washed, fixed with 4% PFA, and analyzed immediately using flow cytometry. The oxidative burst score was calculated from gated neutrophils (CD14+,CD3−,CD66b+).

## ADNKA (antibody-dependent NK cell activation)

96-well ELISA plates were coated with streptavidin (5 μg/ml) at37°C overnight. The following day, streptavidin solution was removed, and biotinylated Vi (3 μg/ml) was added to plates before incubation at 37°C for 3 h. Plates were washed with PBS, and serum samples diluted 1:10 were added to Vi-coated wells before incubation for 2 h at 37°C. After incubation, plates were washed with PBS before addition of NK cells. NK cells were isolated from buffy coats using a RosetteSep NK cell enrichment kit (StemCell Technologies), and a cocktail of PE-Cy5 anti-CD107a (cloneH4A3), brefeldin A (Sigma-Aldrich), and GolgiStop (BD Biosciences) was added immediately before addition of NK cells to plate wells at 5 × 104 cells/well. Plates were then incubated at 37°C for 5 h. Following incubation, cells were transferred to a 96-well U-bottom plate containing an antibody surface stain cocktail of PE-Cy7 anti-CD56 (clone B159), APC-Cy7 anti-CD16 (clone 3G8), and A700 anti-CD3 (clone UCHT1) and incubated for 15 min. Cells were then washed before permeabilization with PermA (Life Technologies); intracellular staining for 15 min with MIP-1β-PE (clone D21-1351), IFNγ-APC (clone B27), and PermB (Life Technologies); and finally washing with PBS. Cells were analyzed using flow cytometry. NK IFNγ, CD107a, and MIP-1β production is represented as the proportion of NK cells (CD3+, CD56+, and CD16+) for the expression of these activation markers. All antibodies were sourced from BD Biosciences.

## Luminescent-Serum Bactericidal Activity Assay (L-SBA)

The L-SBA assay methods are based on the publication by Necchi et al. [20]. Briefly, participant serum samples were heat-inactivated by incubating in a water bath at 56◦C for30 min before making a dilution series starting at 1:1.5 in PBS. *S*. typhi bacteria (Quailes strain, a wild-type, Vi+ strain isolated from a chronic carrier) were grown to log phase and diluted 1:60 in LB broth (Sigma, St. Louis, MO, USA) before adding to diluted test sera in the presence of 10% rabbit complement (CedarLane, Burlington, Canada) (10μL diluted bacterial suspension, 10μL test serum, 10μL rabbit complement, 70μL LB) in a 96 well plate (VWR Ltd., Radnor, PA, USA). Plates were incubated for 3 h at 37◦C, with shaking at220 rpm before centrifugation at 3220×g for 10 min. The supernatant was discarded and the pelleted bacteria re-suspended in PBS. The resultant bacterial suspension was transferred to a white flat-bottomed 96 well plate (VWR International Ltd., Radnor, PA, USA) and mixed in a 1:1 ratio with Promega BacTiter-Glo for quantification of luminescence (relative light units, RLU) using the LUMIstar OMEGA (BMG Labtech, Ortenberg, Germany), RLU output is directly proportional to the number of whole bacteria in the final suspension. SBA titres were calculated by normalising the luminescence measured for each sample dilution by that of the active complement only control (no serum), before fitting a 4-parametersigmoidal curve to each dilution series and determining the serum dilution at which 50%killing of S.Typhi occurred. Data were included only if the R2 of the sample dilution curve was greater than 0.7, and the sample had been sufficiently diluted so the final RLU was comparable to the RLU of the complement only control. Samples were re-run if the titre of the positive control sample (international Vi standard 16/138, NIBSC UK) run on the same plate fell out of range (average±1 standard deviation). The limit of detection was defined as an SBA titre of 39.

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