Antibody Responses to Salmonella Typhi Antigens among Typhoid Recovered Individuals in an Endemic Region

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Abstract

Purpose: This study focuses on evaluating the immune responses, specifically levels of IgA, IgG, and IgM antibodies against five distinct Salmonella Typhi antigens (Vi polysaccharide, H-protein, Lipopolysaccharide, Hemolysin E, and OMP) in the plasma samples collected from participants 3 months after typhoid. We also examined if the anti-Vi polysaccharide antibodies present in the typhoid-recovered participants' plasma had an activating effect on NK cells isolated from healthy individuals.

Methods: ELISA was used to measure the antibody responses to the five *S.* Typhi antigens in plasma samples from typhoid-recovered individuals and healthy participants. Vi polysaccharide specific plasma antibodies were tested for activation of the NK cells isolated from the healthy participants.

Results: Polysaccharide antigens mounted better IgA responses among typhoid recovered individuals as compared to healthy individuals. Anti-*S.* Typhi IgM was significantly elevated in the typhoid group for all five antigens ($p<0.05$). No significant differences were seen in IgG responses. Notably, healthy participants also exhibited considerable levels of antibodies to all the antigens, indicating background seroreactivity.

Conclusion: Polysaccharide antigens elicited significantly better IgA responses in recovered typhoid participants, possibly reflecting mucosal immune priming from the gut. Presence of IgM antibodies in recovered participants suggest a durable IgM response after Typhoid. Similar levels of IgG in both the groups may indicate waning IgG levels towards the baseline post-infection. These findings highlight the complexity of the immune response against *S.* Typhi in endemic settings.

Keywords

Salmonella Typhi, Typhoid, Vi polysaccharide, H antigen, Lipopolysaccharide, Hemolysin E, Outer Membrane Protein, Antibody-dependent NK cell activation (ADNKA)

1. Introduction

Typhoid and paratyphoid fevers, collectively known as enteric fevers, are caused by *Salmonella enterica* serovars Typhi and Paratyphi. The morbidity and mortality because of Typhoid fever is a major concern in developing countries, especially in those with poor healthcare systems. According to the World Health Organization (WHO), there are 9 million cases of typhoid annually, resulting in 1,10,000 deaths per year (CDC, 2025; WHO, 2019). *S.* Typhi is transmitted via the fecal–oral route, predominantly through ingestion of contaminated food and water (Crump & Mintz, 2010; Parry et al., 2002). Humans are the only natural reservoirs, and chronic carriers of the bacteria and play a critical role in sustaining transmission (Wain et al., 2005). Clinical manifestations of the disease range from sustained high fever, headache, and abdominal discomfort to hepatosplenomegaly, intestinal hemorrhage or perforation, and neuropsychiatric disturbances. Variations in disease presentation across endemic regions and the rise of multidrug resistant strains pose challenges for timely diagnosis and effective control (Parry et al., 2002; Wain et al., 2015)

Isolating *S.* Typhi using blood culture is the gold standard method of typhoid diagnosis but it has limited sensitivity and requires adequate laboratory infrastructure (Antillon et al., 2018; Bhan et al., 2005; P. Kumar & Kumar, 2017). Serological tests such as Widal agglutination assay and rapid immunoassays (e.g., Typhidot, Tubex) are used in resource limited areas and typically measure antibodies against O (lipopolysaccharide/LPS) and H (flagellin) antigens. These tests suffer from low sensitivity and specificity, where the obtained results are often unreliable and show cross-reactivity with other species of Salmonella (Gulati & Maheshwari, 2012; McKinnon & Abdool Karim, 2014; Moses et al., 2016; Tuuminen et al., 2013). There is no reliable method for the detection of chronic typhoid and serological tests may not distinguish between current and past infection (CDC, 2025).

There is a gap in our understanding about *S.* Typhi infection and its pathogenesis, where the human-restricted nature of this pathogen has limited our ability to model host-pathogen interactions effectively. Adaptive immunity against *S.* Typhi includes both cellular and humoral arms (Sztein et al., 2014). Humoral responses involve antibodies to diverse antigens such as surface polysaccharides (LPS [O antigen], Vi capsule) and bacterial proteins (e.g., flagellin [H

antigen], HlyE toxin, outer membrane proteins). The role of antibodies in protection against *S.* Typhi is not fully understood, however anti-Vi-polysaccharide responses correlate with vaccine-induced immunity (Dahora et al., 2019; Sztein et al., 2014). Recent studies have identified IgA against HlyE and LPS as strong markers of acute typhoid infection (S. Kumar et al., 2020). However, little is known about how long these antibodies persist post-infection or how they differ among individuals of endemic-area without clinical illness.

Key virulence determinants of *S.* Typhi include the Vi capsular polysaccharide, Type III secretion systems (T3SS-1 and T3SS-2), and typhoid toxin, which facilitate intestinal invasion, intracellular survival within macrophages, and systemic dissemination (Haghjoo & Galán, 2004; Song et al., 2013). Protective immunity against typhoid fever involves antibodies directed against the Vi antigen, robust Th1 responses characterized by IL-12 and IFN-γ production, and cytotoxic T cell lymphocyte activity that promotes intracellular killing of bacteria (Hornick, 1970; Sztein, 2007). A detailed understanding of these correlates of protection underpins current vaccine strategies (Patel et al., 2021).

S. Typhi capsule consists of Vi polysaccharide and is encoded by the genes of Salmonella Pathogenicity Islands (SPI) (Marcus et al., 2000; Wain et al., 2005). It has been noted in a study from India, that about 10% of the fresh isolates lack Vi-polysaccharide antigens (Maurya et al., 2010). However, strains of *S.* Typhi with Vi polysaccharide are more infectious and virulent than Vi-negative strains (Parry et al., 2002). H-protein, a flagellar antigen recognized by Toll-like receptor 5 (TLR5), triggers strong antibody responses, while the Vi capsular polysaccharide antigen acts as a virulence factor by masking O antigens and protecting the bacteria from complement-mediated lysis and phagocytosis (Chaban et al., 2015; Dufresne et al., 2018; Dunstan et al., 2005). LPS, composed of lipid A, core oligosaccharide, and O-specific polysaccharide chains, is recognized by TLR4 and induces robust inflammatory responses (Chessa et al., 2014; Hoare et al., 2006). Interactions among these antigens are complex; for instance, Vi expression can suppress immune recognition of LPS, altering host-pathogen interactions (De Jong et al., 2012; Wilson et al., 2008). In addition to these antigens, Outer Membrane Proteins (OMPs) represent significant immunogenic component involved with host-pathogen interactions during *S.* Typhi infection. These proteins play essential roles in the pathogen's adaptation to environmental conditions, motility, adherence, and host cell

colonization. *S.* typhi OMPs have demonstrated potent immunogenic properties, eliciting long-lasting and protective immunity, which makes them a potential vaccine candidate, and diagnostic antigen (Isibasi et al., 1988).

Interactions among these antigens are complex; for instance, Vi expression can suppress immune recognition of LPS (De Jong et al., 2012; Wilson et al., 2008). In endemic regions, where typhoid fever is widespread, immune responses to *S.* Typhi are complicated by frequent exposure to the pathogen. How different antigenic components contribute to long-term immunity in endemic settings, where individuals may experience repeated exposure and subclinical infections, remains poorly understood.

This study focuses on evaluating the antibody responses- IgA, IgG, and IgM; against five critical *S.* Typhi antigens: Vi polysaccharide, H-protein, LPS, Hemolysin E and Outer Membrane Protein to understand the long-term immune landscape shaped by *S.* Typhi exposure in endemic regions. Plasma samples were collected three months after typhoid to assess the dynamics of the humoral immunity, with a focus on identifying immunological patterns that could inform vaccine response studies. To further improve our understanding about cellular innate immunity, we tested whether Vi-polysaccharide specific antibodies can activate the NK cells, owing to the role of the NK cells in eliminating cells infected by an intracellular pathogen. The findings of this study reveal heterogeneous humoral responses among typhoid-recovered individuals against these antigens. Notably, the substantial immune responses observed in healthy participants also highlight the *S.* Typhi exposure in this region, emphasizing the challenges posed by the pathogen endemicity.

2. Materials and Methods

2.1 Ethics statement

This study was conducted at the BRIC-Translational Health Science and Technology Institute, Faridabad, India. Participant enrolment and sample collection were done at ESIC Medical College and Hospital, Faridabad. Written informed assent/consent was obtained from each of the study participants. This study was approved by the Institutional Ethics Committees from both

institutes [*ESIC Hospital and Medical College, Faridabad File no. 134 X/11/13/2022- IEC/110 and THSTI Faridabad Ref No: THS 1.8.1/ (152)*].

2.2 Participant details

Blood samples were collected from a total of 80 participants **(Table 1)**. Their age, gender, infection date (in the case of typhoid-recovered patients) and blood collection date were recorded. Among the 80 participants we recruited for the study, 33 had recovered from typhoid fever at least for 3 months, while 47 were healthy participants and had no recorded history of the disease. A positive Widal test was not considered as a confirmatory test; a positive Typhidot IgM/IgG or blood culture tests were used instead.

2.3 Blood collection and sample processing

Eight ml of blood was collected from each participant in the Greiner Bio-One tubes (Cat. No. 22-040-134) coated with sodium heparin. Blood samples were processed immediately to isolate plasma and peripheral blood mononuclear cells (PBMCs) using Lymphoprep density gradient centrifugation method. Plasma was stored at -80 °C and PBMCs were resuspended in fetal bovine serum (FBS) supplemented with 10% dimethyl sulfoxide (DMSO) and then stored in liquid nitrogen (Crotty et al., 2004).

2.4 ELISA

2.4.1 Polysaccharide antigens

Anti- *S.* Typhi IgA, IgG and IgM antibody responses are measured against Vi polysaccharide and Lipopolysaccharide after the three-months from infection of the typhoid patients by using indirect ELISA. Nunc Maxisorp 96-well plate was coated with 100 µL of poly-l-lysine (2 µg/ml) and incubated at 37°C for 2 hours. The poly-l-lysine coating was not done in case of the H antigen. All antigens were diluted in sterile PBS (2 µg/ml for Vi polysaccharide; 1 µg/ml for Lipopolysaccharide and H-protein). Antigens were coated and incubated overnight (37°C for Vi polysaccharide and lipopolysaccharide; 4°C for H-protein). Coated plates were blocked with a 200 µl block buffer (1% BSA in PBS) and incubated for 1 hour at 37°C. Plasma samples were diluted 1:100 in a diluent (1% BSA, 0.05% Tween-20 in PBS) and 100 µl/well was added and

incubated for one hour at 37°C. Then, 1/10000 secondary antibody was diluted in a buffer containing 1% BSA, and 0.05% Tween-20 in PBS and 100 µl was added to each well and incubated. After an hour 100 µl pNPP (p-Nitrophenyl Phosphate) substrate is added to each well which is diluted in Tris-$MgCl_2$ and incubated for an hour at room temperature in dark conditions. At last, 50 µl 3M NaOH is added to stop the reaction. After every step of ELISA, 4 to 5 washing is given with 0.05% Tween-20 in PBS except after the addition of substrate. Then, optical density is measured using a plate reader at 405 nm and 490 nm. The readings at 490 nm were subtracted from those at 405 nm to correct for optical imperfections.

2.4.2 Protein Antigens

For the protein antigens (H-protein, Hemolysin E and OMP), similar ELISA was performed with some modifications. HRP-TMB was used as the conjugate-substrate combination instead of alkaline phosphatase-pNPP; because it was found to give better and consistent results during the optimization experiments. Hence, the stop solution was changed to 2N H_2SO_4.

2.5 Avidity assay

The 96-well ELISA plate (Maxisorp, ThermoFisher) was coated with 100 µL of Poly-L-Lysine (10 mg/mL) in each well and was incubated at 37 □ for 2 hours. After incubation, the plates were washed with a wash buffer (0.85% NaCl + 0.1% Brij 35 in PBS) 5 times. After washing, the antigen was coated by adding 100 µL Vi antigen (0.5 µg/mL) and LPS of *S.* Typhi (0.5 µg/mL) in each well and was kept for overnight incubation at 37□. Washing was repeated 5 times with a wash buffer post-incubation. Blocking of the plate was done by adding 200 µL of block buffer (1% BSA in PBS) in each well and incubating at 37□ for 1 hour. The plates were washed 5 times with a wash buffer post-incubation. Dilution of the sample was done in sample diluent (1% BSA + 0.1% Brij 35 in PBS). 100 µL of diluted sample was added in each well and incubated at 37□ for 1 hour. The plates were washed 5 times with wash buffer post incubation and urea (8 M) was added (100 µL/well) to half the replicates of samples while a wash buffer was added to the other half. The plate was incubated for 10 minutes at 37□. The plates were washed 5 times with wash buffer post incubation and 100 µL per well of alkaline phosphatase tagged secondary antibody was added in the dilution of 1:10000 for IgG in sample diluent and incubated at 37□ for 30 minutes. The plates were washed 5 times with a wash buffer post-

incubation. The substrate (4-Nitrophenyl disodium hexahydrate {10 mg/mL}) was added and incubated at RT in the dark for 30 minutes. A stop solution of 3 M NaOH (50 µL/well) was added to stop the reaction, and the plates were read at 405 nm and 490 nm wavelengths with a microplate reader, and the OD value of 405 nm wavelength was deducted from the 490 nm wavelength OD value to nullify the background.

2.6 Antibody-dependent NK cell activation

Nunc MaxiSorp® flat-bottom plate was coated with streptavidin (5 µg/ml) and incubated overnight at 37°C. After 3 washes with PBS (200 µl/well), biotinylated Vi-polysaccharide (Deshpande & Jayakannan, 2018; Dj et al., 1984) (3 µg/ml) was added, and the plate was incubated at 37°C for 3 hours. After 5 washes with PBS, diluted plasma (plasma: PBS; 1:10) from typhoid-recovered patients was added and the plate was incubated at 37°C for 2 hours. NK cells obtained using Miltenyi MACS NK cell isolation kit (cat no. 130-092-657) were added to the plates after 5 PBS washes. 200 µl complete RPMI media was added, and the plate was incubated at 37°C for 12 hours. The cells from the microplate were carefully scraped off with 100 µl/well FACS buffer (PBS + 1% FBS) and transferred to a 96-well round bottom plate using a multichannel pipette. The step was repeated if required, to get most of the residual cells. The cells were washed twice by adding a FACS buffer and centrifuged at 3000 rpm for 5 minutes. Excess FACS buffer was decanted carefully, and a cocktail of antibodies was added (40 µl/well) **(Table 2)**. Samples were acquired at BDFACSCanto II and analyzed using FlowJo software (version v10). The gating strategy of the data analysis has been shown in **Supplementary Figure 1**.

2.7 Statistics

All the data visualization and statistical analyses were done using GraphPad Prism 10.4.2. Outliers were removed using the ROUT method (Q=1%). All the datasets were checked for normal distribution first. The Mann-Whitney test was used for statistical analysis to compare the two datasets. p value <0.05 was considered statistically significant.

3. Results

3.1 Polysaccharide antigens lead to a better IgA response

Anti-polysaccharide IgA (to LPS and Vi) was significantly higher in recovered participants than in healthy ones. For anti-LPS IgA, median OD was ~0.5852 in typhoid-recovered vs 0.4064 in healthy participants ($p=0.0087$). For anti-Vi IgA, median OD was ~0.406 vs 0.273 ($p=0.0140$) **[Fig. 1]**. IgA responses to H antigen, and OMP did not differ significantly; however, IgA response against HlyE showed significant difference among Typhoid recovered and healthy individuals **[Fig. 1]**.

3.2 IgM response could differentiate Typhoid recovered from healthy individuals

IgM levels were significantly elevated in the typhoid-recovered group for all five antigens **[Fig. 2]**. IgM to H antigen, HlyE, and OMP were all roughly twofold higher in the typhoid group (all $p<0.01$). This elevated IgM level among typhoid recovered individuals suggests durability of IgM response after typhoid, complicating the diagnosis in endemic regions. Notably, healthy participants samples showed detectable IgA and IgM levels against all the antigens, indicating that the healthy participants may have been exposed to the pathogen in the near past and may have remained asymptomatic.

3.3 IgG antibody responses and IgG antibody avidity (against Vi PS) did not differ between typhoid-recovered and healthy individuals

No significant differences were observed in antigen-specific IgG levels between the groups. Mean IgG ODs were similar in recovered vs healthy participants for all antigens ($p>0.1$). This indicates that by >3 months post-infection, IgG levels against these antigens had declined to the background levels **[Fig. 3]**. The chaotropic agent-based modified ELISA assay for testing the avidity of the IgG antibodies yielded interesting results. The median IgG antibody avidity index for both participant groups exceeded 70%, substantiating the presence of high-affinity antibodies targeting the Vi polysaccharide antigen **[Fig. 3(i)(b)]**. Serological data are represented in **Table 3**, providing a summary of these findings.

3.4 No sex-specific disparities observed in serological immune responses

The study cohort demonstrated a balanced representation of both sexes, as evident from **Table 1**. Specifically, the participant groups included approximately 55% females among typhoid-recovered individuals and 43% females in the healthy group. Sex-disaggregated analysis of the immune responses was performed to evaluate any potential disparities between immune responses in males and females against *S.* Typhi. Our analysis revealed that there is no statistically significant difference in serological immune responses between these participants **[Supplementary Figures 2-6]**. This data indicates that sex does not appear to influence the immune response at recovery to typhoid infection.

3.5 NK cell response to antibody-dependent activation

We investigated the potential of anti-Vi polysaccharide (Vi-PS) antibodies present in the plasma samples of typhoid-recovered individuals to be able to activate natural killer (NK) cells isolated from the PBMCs of the healthy participants. The primary hypothesis was that the NK cells isolated from healthy participants would exhibit distinct immune responses when exposed to anti-Vi PS antibodies. To test this, we measured NK cell activation and proliferation using molecular markers CD16, CD25 and CD69 after incubating NK cells with typhoid-recovered participant's plasma and culturing the cells for 12 hours. Cells incubated with plasma showed similar levels of activation and proliferation markers as those cultured without any plasma **[Fig. 4]**. Probably, due to the endemicity of the disease in India, the control samples also behaved like the plasma treated ones. Another possibility is that the interaction of the antigen with antibodies present in the plasma of the typhoid participants is needed to activate the NK cells.

4. Discussion

The findings of this study provide insight into the humoral response against different classes of antigens and NK cell responses against *S.* Typhi in individuals from an endemic region. The most notable observation is the robust IgM response three months post-infection against all the antigens we tested. A previous study found IgM responses against *S.* Typhi to be non-specific, contrary to our findings (Andrews et al., 2019). It could be because the samples tested in their study were from acute patients. In general, the presence of IgM antibodies suggests a recent infection while IgG antibodies imply long-term exposure. However, in certain viral and bacterial infections, long-lasting IgM response has been observed (Athavale et al., 2024; Costa et al.,

2021; Racine & Winslow, 2009). Another possibility for a prolonged IgM antibody response is possibly due to a continued presence of the pathogen or re-exposure which is common in endemic areas. The persistence of IgM antibodies further complicates the interpretation of immune status in endemic populations (House et al., 2001).

Interestingly, while the IgM antibody levels were elevated in case of the typhoid-recovered individuals as compared to the healthy group, IgG levels were not substantially different. These results imply that IgG mediated humoral immunity against *S.* Typhi returned to its baseline level during the post three-month period. Alternatively, healthy participants likely have background IgG from asymptomatic exposures which is leading to the underestimation of the IgG levels present in recovered individuals.

Another key finding of this study is the better IgA response among typhoid-recovered individuals against polysaccharide antigens (Vi-PS and LPS). Vi capsule and LPS are the antigens that are heavily expressed on *S.* Typhi and engage with immune cells. IgA antibodies are present in the mucosal secretions and are known to contribute to the local immunity in the gut, potentially preventing *S.* Typhi from establishing the infection (Dahora et al., 2019; Sztein et al., 2014). IgA response against LPS was also found to be an accurate diagnostic test in a human challenge study (Darton et al., 2017). These results suggest that we may need to test the immune responses against other antigens of *S.* Typhi to expand the repertoire of the options available to be used for multigenic screening for Typhoid.

Polysaccharides are classic T cell independent antigens and primarily trigger IgM and IgG2 responses but generate little immunological memory (Mond et al., 1995). In regions with frequent exposure, repeated encounters with antigens tend to sustain IgM levels. In contrast, immune response against protein antigens are T cell dependent (El Shikh et al., 2009). They usually induce IgG antibodies and promote long-term memory. In this study, IgG levels to HlyE, H protein and OMP showed no significant difference between groups after three months of disease. This could reflect the waning of IgG over time towards the basal level which is significantly high in endemic regions.

The antibody-dependent NK cell activation (ADNKA) assay showed that the plasma from typhoid-recovered individuals did not significantly enhance NK cell activation and proliferation

markers (CD16, CD25 & CD69 respectively). These findings are at variance with the existing literature (Jin et al., 2021), probably because of the modified protocol we followed for this study or due to the possibly exposed healthy individuals from whom PBMCs were used for this assay. The mature, $CD56^{dim}$CD16+ NK cells are identified as a highly differentiated subset known for their potent cytotoxicity and capacity to induce antibody-dependent cellular cytotoxicity (ADCC); on the other hand, less mature $CD56^{bright}$CD16- NK cells are major cytokine producers with regulatory functions (Petrov et al., 2024). The enigmatic behavior of NK cell responses in this study could be attributed to several factors. One possibility is that the plasma from typhoid-recovered individuals possibly contains immunomodulatory factors that suppress NK cell activity, similar to what is observed in other chronic infections like Hepatitis C and HIV (Lang et al., 2023; Lanier, 2008). Moreover, the healthy population at a larger sample size may need to be screened against different antigens and antibodies to draw a cut-off to differentiate the symptomatic from exposed individuals, particularly for endemic regions. Additionally, the pre-existing immunity in healthy individuals, as reflected by their antibody levels, could be a result of prior, subclinical infections, contributing to the overall immune landscape of the population (Bharmoria, 2017).

A limitation of this study is the lack of acute-phase samples, which might have given clear differentiation of the immune parameters tested between healthy and infected individuals. Another limitation is the age group of our participants which are mainly in the adult category and hence even healthy participants are probably exposed to the pathogen due to its endemicity in the region. Therefore, understanding the impact of endemicity on the immune response to typhoid with a larger sample size is essential for developing effective prevention and control strategies. Despite these limitations, this study provides critical insights into immune responses to *S.* Typhi antigens in endemic regions, offering an understanding of the immune correlates which could be protective. Since we have compared five antigens and their response in terms of different antibodies, the data will be invaluable for designing vaccine trials and exploring antibody-mediated cellular mechanisms in future research. Further, many of the recent vaccines against typhoid are having Vi-polysaccharide as a component, this study could provide a hindsight for the kind of immune response one can expect in an endemic region against the Vi-antigen. Together, these results emphasize the need for tailored vaccine strategies that considers pre-existing immunity and endemic exposure.

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6. Author's contribution

Atharv Athavale□ collected samples, performed experiments, made tables and figures, wrote, reviewed and edited the different versions of the manuscript; Adarsh Subramaniam and Aftab Hussain- collected samples and performed experiments; Indu Rani- performed experiments; Deepak Kumar Rathore and Santosh K. Upadhyay- edited and reviewed the different versions of the manuscript; Anil Kumar Pandey and Praveen Kumar Malik □ provided samples for the study, reviewed the different versions of the manuscript; Amit Awasthi□ conceptualized the study, and reviewed the different versions of the manuscript; Ramesh Chandra Rai□ conceptualized the study, wrote the manuscript, reviewed and edited the different versions of the manuscript, tables and figures. All authors read and approved the final version of the manuscript.

7. Declaration of interest statement

The authors declare no conflict of interest.

8. Data Availability

Data related to the article has been presented in the form of figures and tables. Keeping confidentiality of the participants the details could be shared as per the journal's policy.

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Figure legends

Figure 1: IgA ELISA against Vi PS, LPS, H protein, HlyE and OMP

Figure 2: IgM ELISA against Vi PS, LPS, H protein, HlyE and OMP

Figure 3: IgG ELISA against Vi PS, LPS, H protein, HlyE and OMP; IgG Avidity against Vi PS

Figure 4: Percentage of NK cell proliferation and activation markers after antibody-dependent activation

Table 1: Participant demographics

		Typhoid Recovered	**Healthy**
Sex	Male	15	27
	Female	18	20
Age (years)	Range	13-53	12-58
	Median	25	24
Months since infection	Range	3-10	-
	Median	3	-

Table 2: Molecular markers used for estimation of NK cell proliferation and activation

Sr. No.	Molecular marker	Fluorophore	Make and Catalogue number
1	Fixable viability dye	Violet Dye	Invitrogen #L34964
2	CD3	PerCP	BioLegend #344814
3	CD19	PE	BioLegend #302208
4	CD25	APC	BioLegend #302610
5	CD56	FITC	BioLegend #318304
6	CD69	BV510	BioLegend #310936
7	CD14	APC-Cy7	BioLegend #301820

Table 3: Antigen-wise seropositivity among individuals after 3 months of recovery from typhoid (ELISA)

Antigens	**Vi polysaccharide**			**Lipopolysaccharide**		
Antibody class	IgA	IgG	IgM	IgA	IgG	IgM
Relative Antibody response among Typhoid-recovered individuals over the healthy participants	* p=0.0140 (p<0.05)	ns	* p=0.0107 (p<0.05)	* p=0.0227 (p<0.05)	ns	* p=0.0222 (p<0.05)

Antigens	**H Protein**			**Hemolysin E**			**OMP**		
Antibody class	IgA	IgG	IgM	IgA	IgG	IgM	IgA	IgG	IgM
Relative Antibody response among Typhoid-recovered individuals over the healthy participants	ns	ns	**** (p<0.0001)	*** p=0.0009 (p<0.001)	ns	** p=0.0022 (p<0.01)	ns	ns	** p=0.0025 (p<0.01)

Study subjects

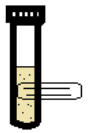
n = 33

Typhoid-recovered participants

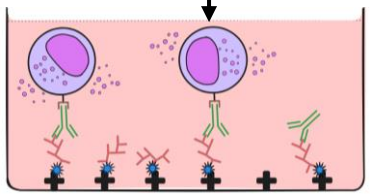
n = 47

Healthy participants

Study Design

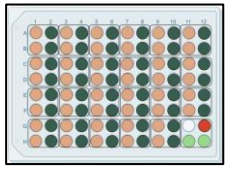


PBMCs



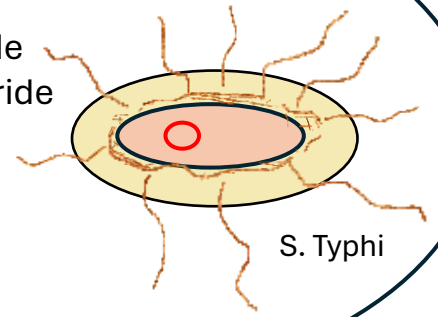
NK cell activation assay

Plasma



ELISA

- Vi polysaccharide
- Lipopolysaccharide
- H-Protein
- OMP
- Hemolysin E



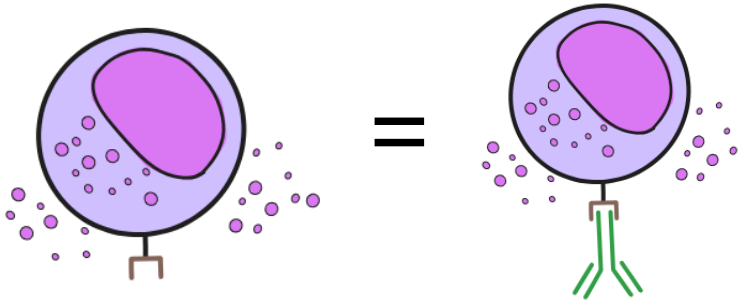
Outcomes

Statistical significance between immune responses of typhoid recovered and healthy participants (ELISA)

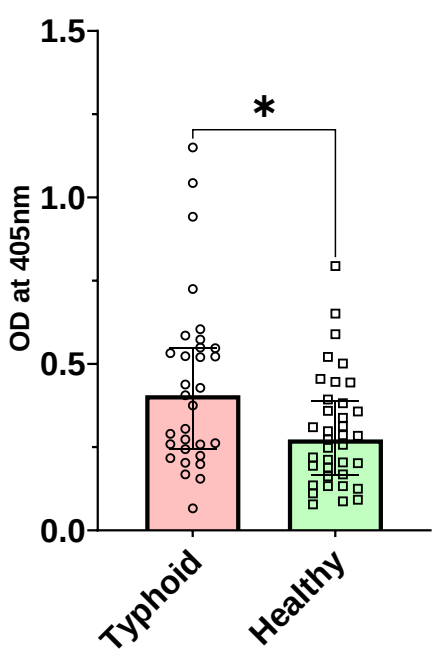
	LPS	Vi	H-Ag	HlyE	OMP
IgA	*	*	-	***	-
IgG	-	-	-	-	-
IgM	*	*	****	**	**

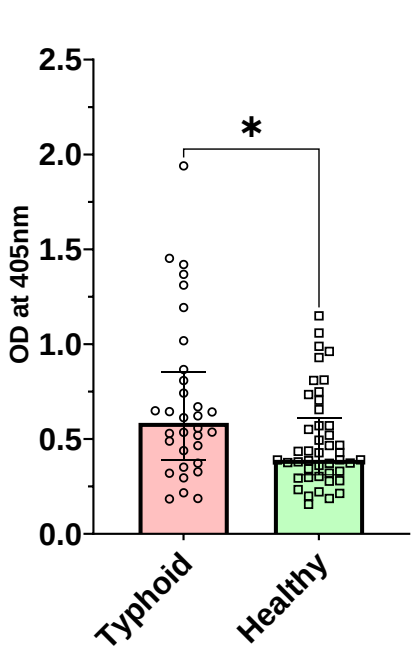
[*p < 0.05; **p < 0.01; ***p< 0.001; ****p<0.0001]

No difference between the activation markers on NK cells unexposed or exposed to plasma containing anti-Vi PS antibodies

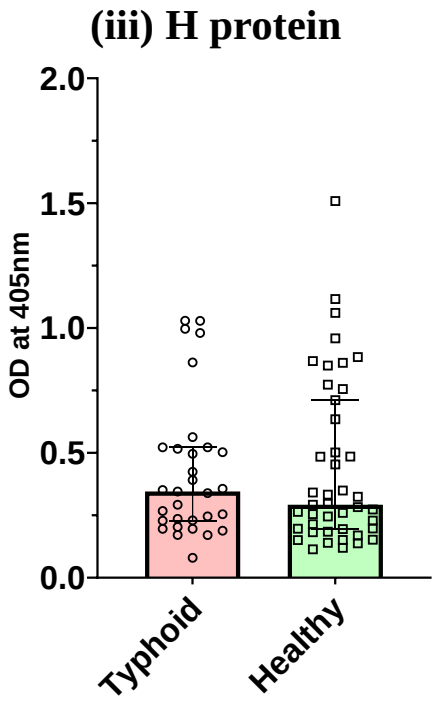


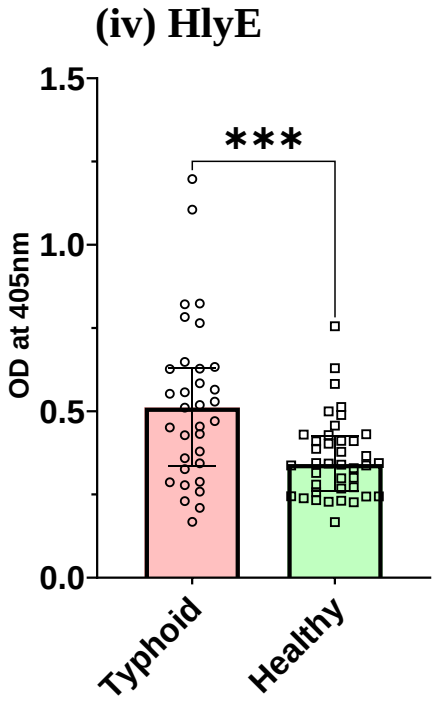
Polysaccharide antigens

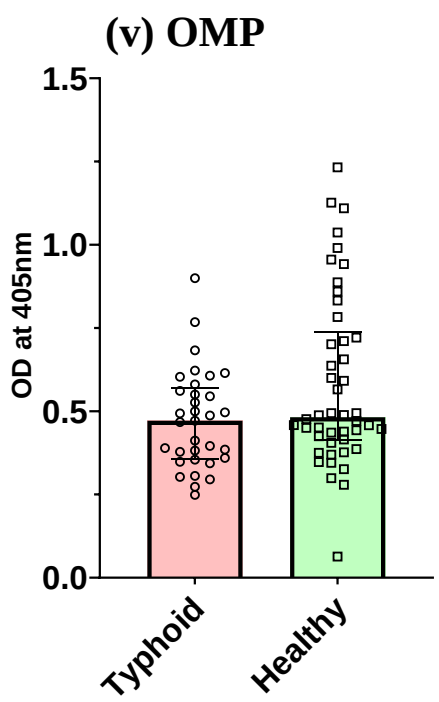




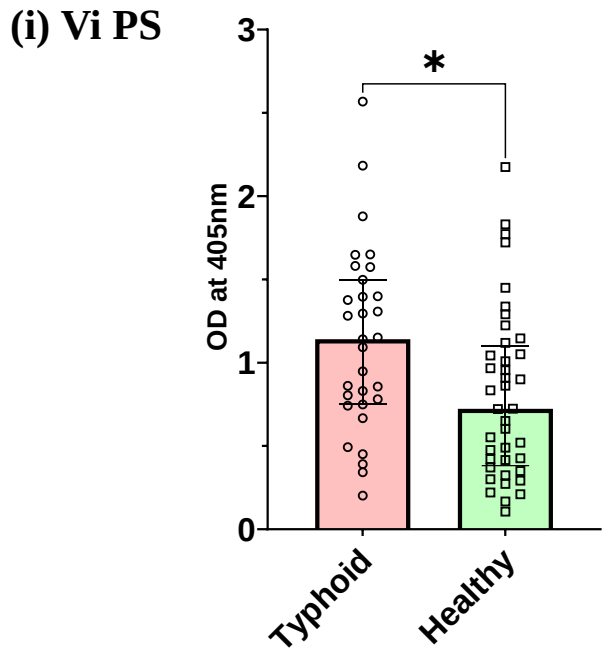
Protein antigens

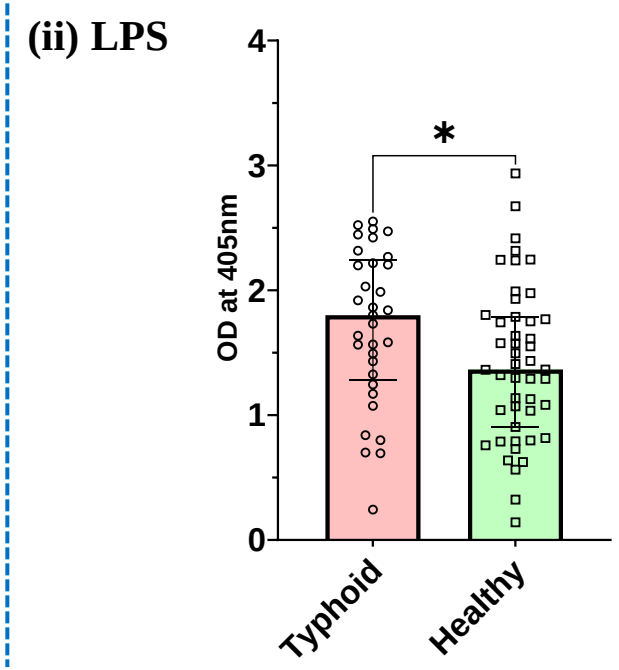




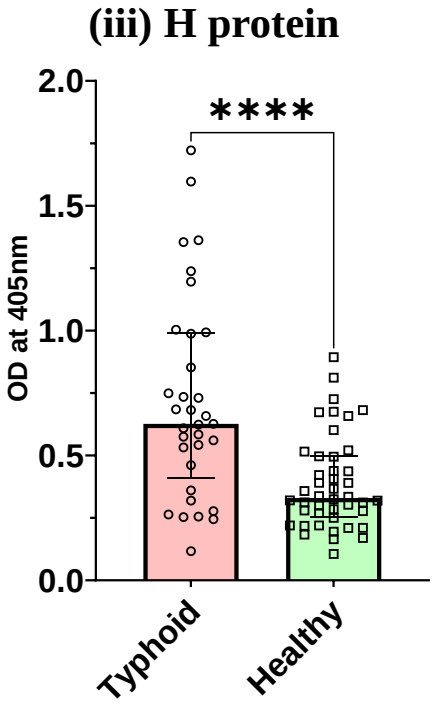


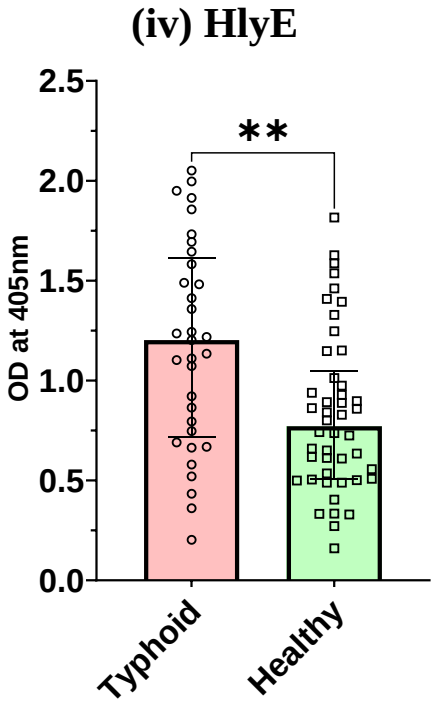
Polysaccharide antigens

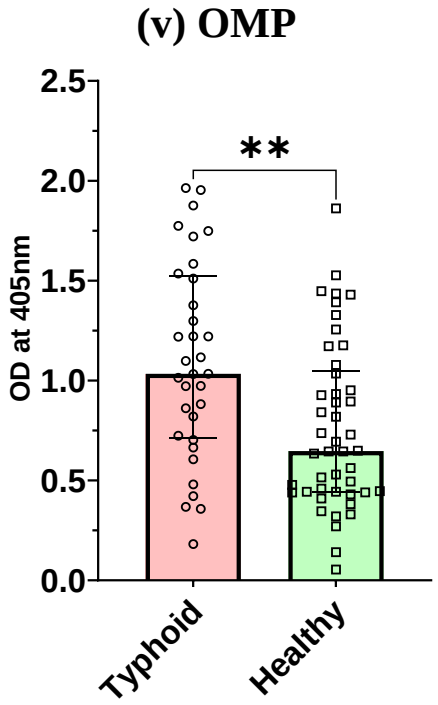




Protein antigens

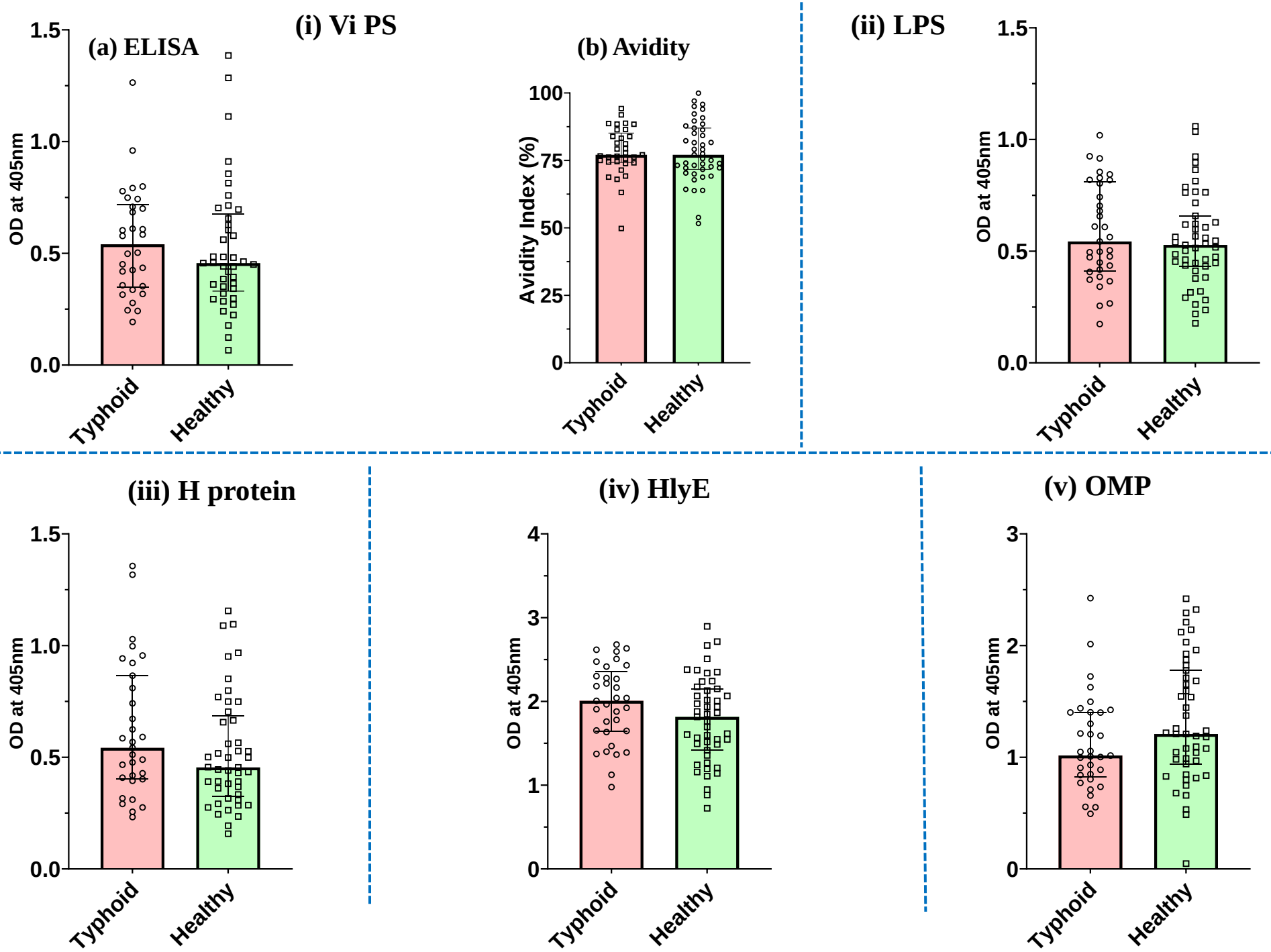


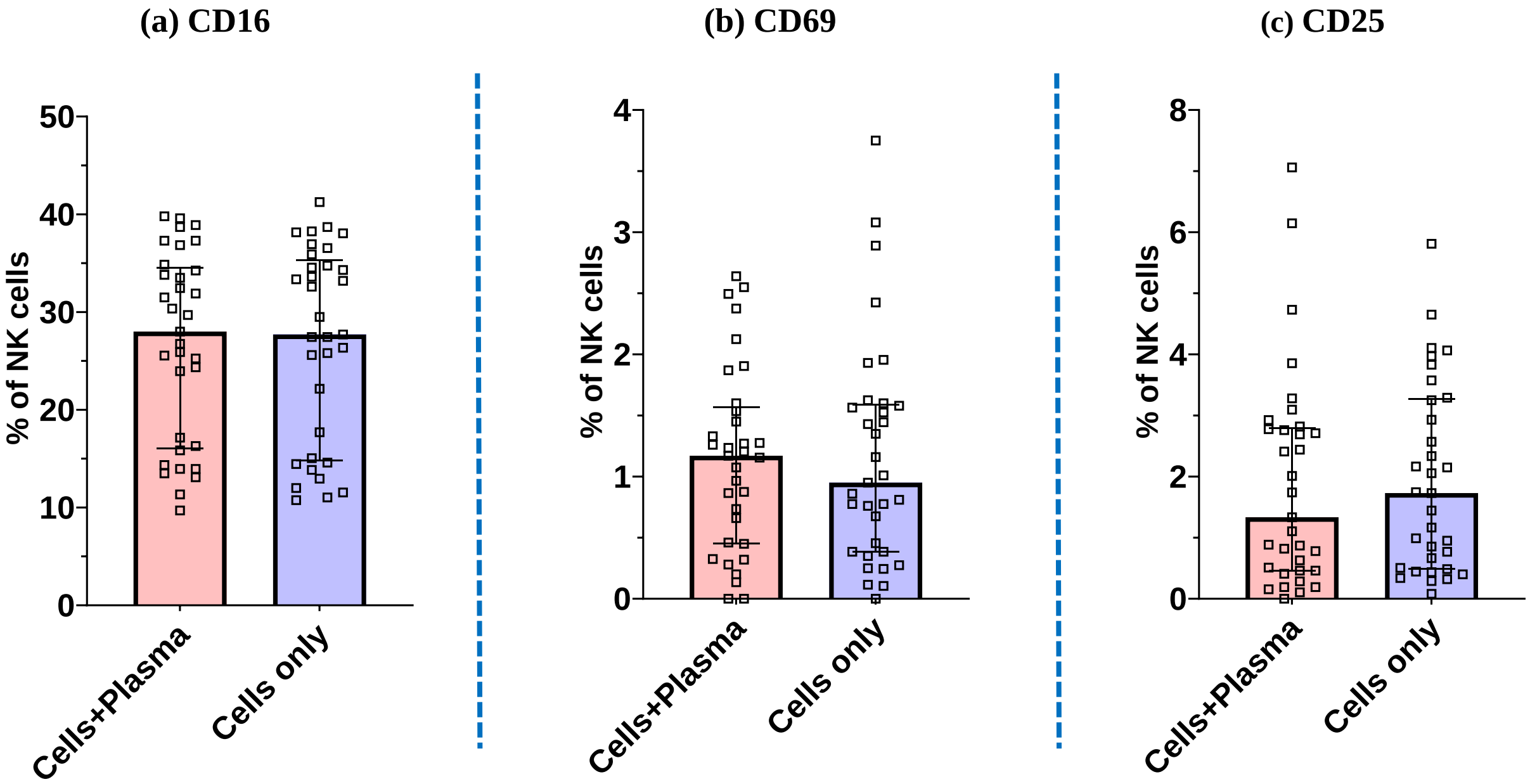




Polysaccharide antigens

Protein antigens




(a) CD16
(b) CD69
(c) CD25
% of NK cells
50
40
30
20
10
0
4
3
2
1
0
8
6
4
2
0
Cells+Plasma
Cells only