

Supplemental information

Immune responses against group B *Streptococcus* monovalent and pentavalent capsular polysaccharide tetanus toxoid conjugate vaccines in Balb/c mice

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SUPPLEMENTARY INFORMATION

Supplementary Figures

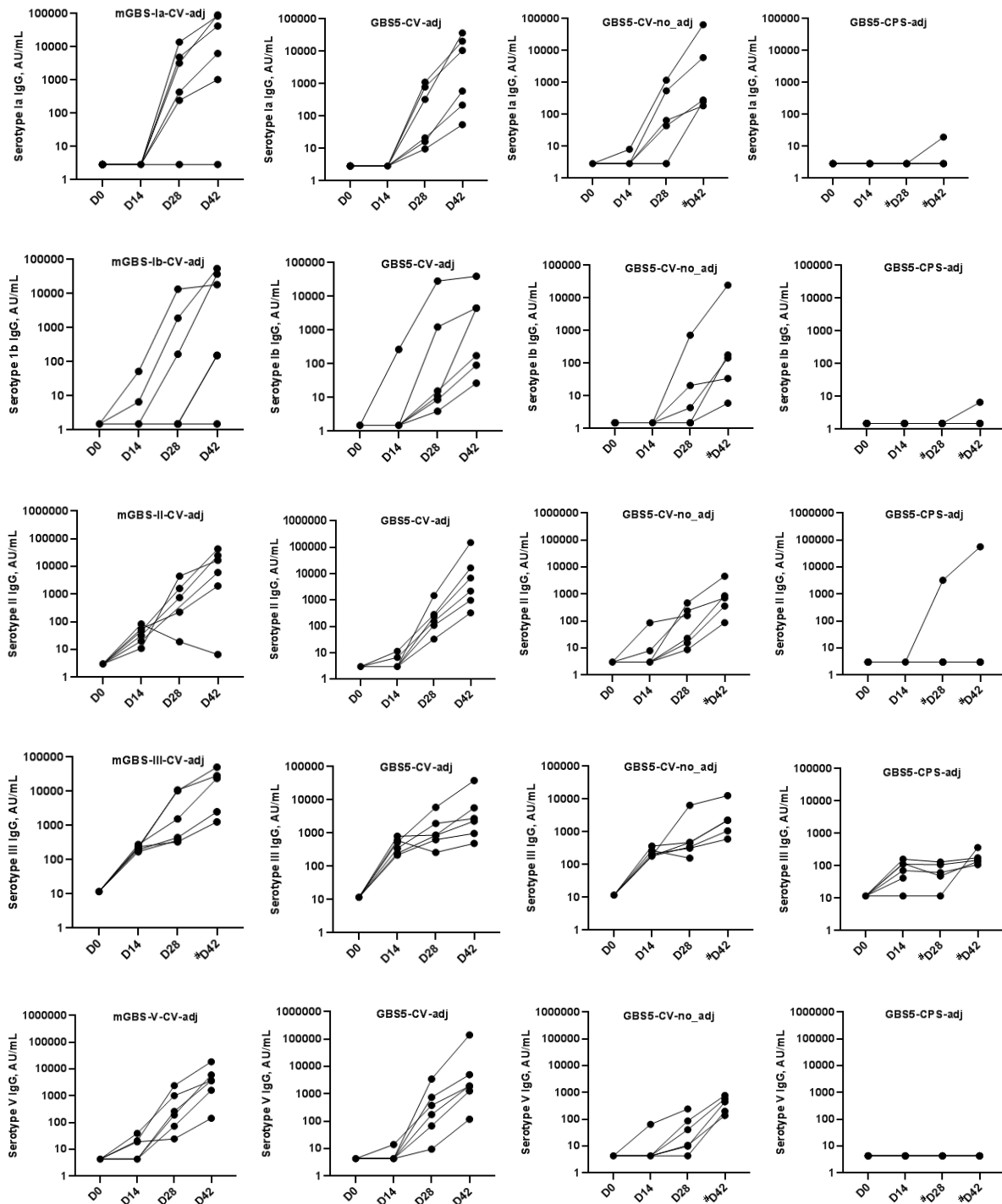


Figure S1: Serotype specific IgG responses among vaccination groups, related to Figure 2. Represented are pre-immunization day 0 (D0) and post-immunization day 14 (D14), day 28 (D28), and day 42 (D42) on X axis, and serotype specific IgG concentrations (black dots) in arbitrary units (AU/ml) on Y axis among monovalent conjugate vaccines with adjuvant (mGBS-Ia-CV-adj, mGBS-Ib-CV-adj, mGBS-II-CV-adj, mGBS-III-CV-adj, mGBS-V-CV-adj), pentavalent conjugate vaccine with adjuvant (GBS5-CV-adj) and without adjuvant (GBS5-CV-no_adj), and unconjugated pentavalent CPS vaccine with adjuvant (GBS5-CPS-adj) study groups. # indicates observation based on 5 mice.

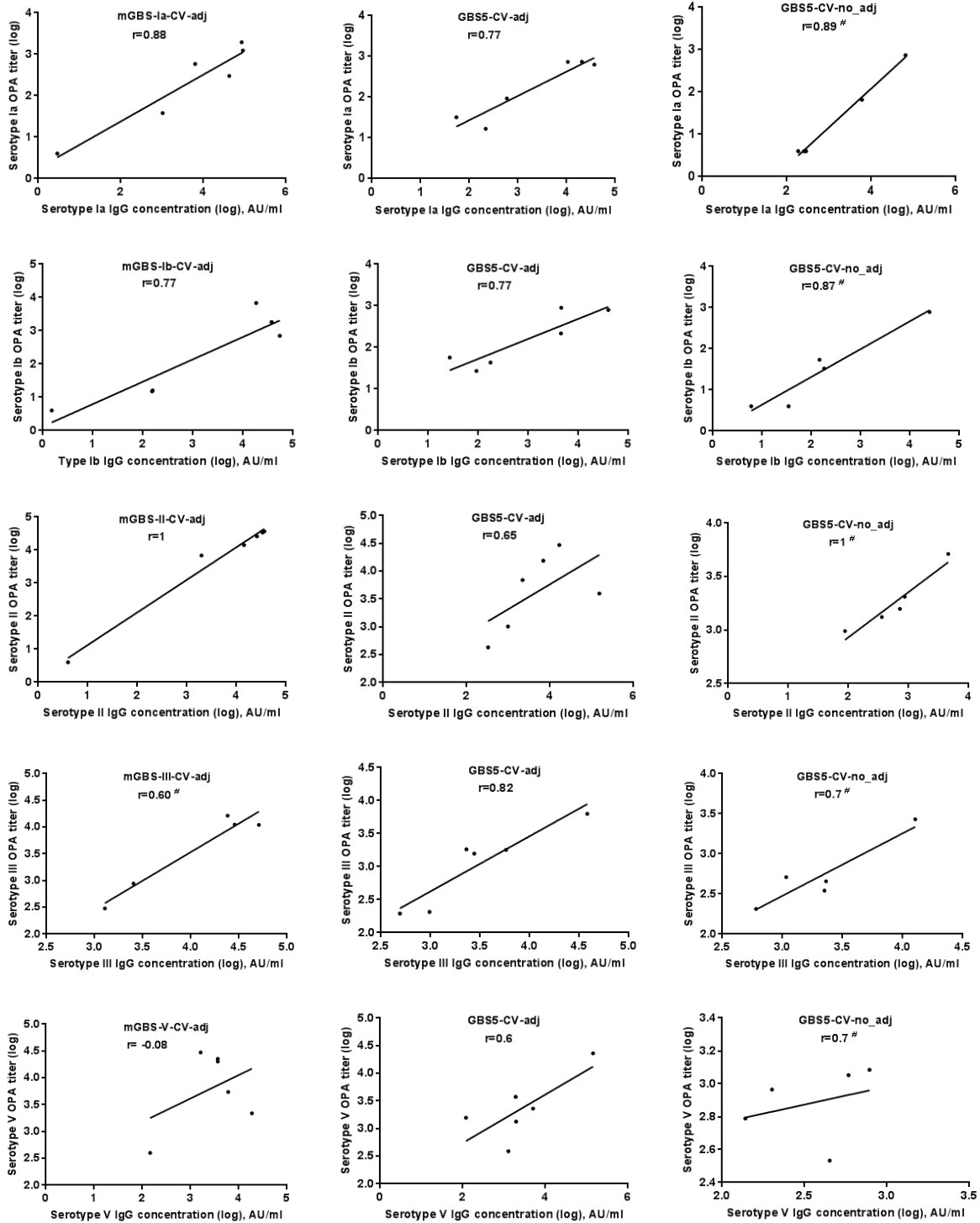


Figure S2: Correlation between serotype-specific IgG concentrations and opsonophagocytic titers among conjugate vaccine groups, related to Figure 4. Represented are serotype specific IgG concentrations in arbitrary units (AU/ml) on X axis, opsonophagocytic (OPA) titers on Y axis among monovalent conjugate vaccines with adjuvant (mGBS-Ia-CV-adj, mGBS-Ib-CV-adj, mGBS-II-CV-adj, mGBS-III-CV-adj, mGBS-V-CV-adj), pentavalent conjugate vaccine with adjuvant (GBS5-CV-adj) and without adjuvant (GBS5-CV-no_adj) study groups, Spearman correlation coefficient (r), # indicates analysis based on 5 mice.

Supplementary tables

Table S1: Summary of the analytical data generated for the capsular polysaccharide used for the generation of the conjugates, related to STAR methods.

Serotype	Residual protein	Residual nuclei acid	Molecular weight (kDa)
Ia	< 3 %	< 3 %	138
Ib	< 3 %	< 3 %	124
II	< 3 %	< 3 %	109
III	< 3 %	< 3 %	128
V	< 3 %	< 3 %	169

Table S2: Summary of the analytical data generated for the monovalent conjugates used in the vaccine formulations, related to STAR methods.

Serotype	CPS concentration (mg/mL)	TT concentration (mg/mL)	Free sugar (%)	CPS:TT ratio	MW (kDa)	Endotoxin
Ia	0.70	0.71	4.94	0.94	276 & 595	< 0.1 EU/μg
Ib	0.82	0.86	7.92	0.88	266 & 575	< 0.1 EU/μg
II	0.40	0.60	5.57	0.63	213	< 0.1 EU/μg
III	0.34	0.49	17.01	0.58	242	< 0.1 EU/μg
V	0.85	0.77	5.41	1.04	288 & 494	< 0.1 EU/μg

Table S3: Serotype Ia specific IgG concentrations pre-immunisation (day 0), and at three post-immunization intervals (day 14, 28, and 42), and opsonophagocytic (OPA) titers post-third immunization dose (day 42) among study groups, related to Figure 2 and 3.

Study group	Day 0 Serotype Ia-IgG, AU/ml	Day 14 Serotype Ia-IgG, AU/ml	Day 28 Serotype Ia-IgG, AU/ml	Day 42 Serotype Ia-IgG, AU/ml	Day 42 Serotype Ia-OPA titers
	GMC (95% CI)	GMC (95% CI), fold change*	GMC (95% CI), fold change*	GMC (95% CI), fold change*	GMT (95% CI)
Placebo	2.90 §	2.90 § 0	2.90 § 0	2.90 § 0	4 §
Monovalent serotype Ia CPS-TT conjugate vaccine with adjuvant (mGBS-Ia-CV-adj)	2.90 §	2.90 § 0	639.1 (26.12-15639.0) 220.37	4294 (66.36-277812) 1480.68	199 (17-2369)
Pentavalent CPS-TT conjugate vaccine with adjuvant (GBS5-CV-adj)	2.90 §	2.90 § 0	99.34 (10.63-928.6) 34.25	1963 (116.6- 33067) 676.89	160 (27-957)
Pentavalent CPS-TT conjugate vaccine without adjuvant (GBS5-CV-no_adj)	2.90 §	3.43 (2.22-5.31) 1.18	50.09 (3.50-714.9) 17.27	1395 (57.61-33780.0) 481.03 #	20 (1-370) #
Unconjugated pentavalent CPS vaccine with adjuvant (GBS5-CPS-adj)	2.90 §	2.90 § 0	2.90 § 0 #	4.24 (1.47-12.2) 1.46 #	4 §#

*calculated from day 0

indicates analysis based on 5 mice

§ 95% CI not applicable- all sample IgG titers were below limit of detection

geometric mean concentrations (GMCs)

Table S4: Serotype 1b specific IgG concentrations pre-immunisation (day 0), and at three post-immunization intervals (day 14, 28, and 42), and opsonophagocytic (OPA) titers post-third immunization dose (day 42) among study groups, Figure 2 and 3.

Study group	Day 0 Serotype 1b-IgG, AU/ml	Day 14 Serotype 1b-IgG, AU/ml	Day 28 Serotype 1b-IgG, AU/ml	Day 42 Serotype 1b-IgG, AU/ml	Day 42 Serotype 1b-OPA titers
	GMC (95% CI)	GMC (95% CI), fold change*	GMC (95% CI), fold change*	GMC (95% CI), fold change*	GMT (95% CI)
Placebo	1.50 §	1.50 § 0	1.50 § 0	1.50 § 0	4 §
Monovalent serotype 1b CPS-TT conjugate vaccine with adjuvant (mGBS-1b-CV-adj)	1.50 §	3.47 (0.75-16.05) 2.31	121.7 (2.42-6099) 81.13	1048 (13.23-82947) 698.66	142 (6-3504)
Pentavalent CPS-TT conjugate vaccine with adjuvant (GBS5-CV-adj)	1.50 §	3.56 (0.38-32.85) 2.37	76.72 (1.86-3153) 51.14	839.0 (43.51-16180) 559.33	147 (30-724)
Pentavalent CPS-TT conjugate vaccine without adjuvant (GBS5-CV-no_adj)	1.50 §	1.50 § 0	7.78 (0.59-101.9) 5.18	167.8 (3.53-7959) 111.86 #	29 (2-444) #
Unconjugated pentavalent CPS vaccine with adjuvant (GBS5-CPS-adj)	1.50 §	1.50 § 0	1.50 § 0 #	2.02 (0.88-4.63) 1.34 #	4 §#

*calculated from day 0

indicates analysis based on 5 mice

§ 95% CI not applicable- all sample IgG titers were below limit of detection
geometric mean concentrations (GMCs)

Table S5: Serotype II specific IgG concentrations pre-immunisation (day 0), and at three post-immunization intervals (day 14, 28, and 42), and opsonophagocytic (OPA) titers post-third immunization dose (day 42) among study groups, Figure 2 and 3.

Study group	Day 0 Serotype II-IgG, AU/ml	Day 14 Serotype II-IgG, AU/ml	Day 28 Serotype II-IgG, AU/ml	Day 42 Serotype II-IgG, AU/ml	Day 42 Serotype II-OPA titers
	GMC (95% CI)	GMC (95% CI), fold change*	GMC (95% CI), fold change*	GMC (95% CI), fold change*	GMT (95% CI)
Placebo	3.05 §	3.05 § 0	3.05 § 0	3.05 § 0	4 §
Monovalent serotype II CPS-TT conjugate vaccine with adjuvant (mGBS-II-CV- adj)	3.05 §	34.15 (15.79-73.84) 11.19	472.4 (35.0-6377) 154.88	3360 (112.3-100578) 1101.63	4825 (119-196516)
Pentavalent CPS-TT conjugate vaccine with adjuvant (GBS5-CV-adj)	3.05 §	4.350 (2.38-7.96) 1.42	197.7 (53.37-732.6) 64.81	4808 (484.1-47742) 1576.39	4199 (773-22823)
Pentavalent CPS-TT conjugate vaccine without adjuvant (GBS5-CV- no_adj)	3.05 §	6.25 (1.52-25.66) 2.04	62.10 (11.0-356.6) 20.36	617.4 (103.9-3669) 202.42 #	1848 (842-4054) #
Unconjugated pentavalent CPS vaccine with adjuvant (GBS5-CPS-adj)	3.05 §	3.05 § 0	12.31 (0.255-592.0) 4.03 #	21.77 (0.09-5099.0) 7.13 #	17 (1-261) #

*calculated from day 0

indicates analysis based on 5 mice

§ 95% CI not applicable- all sample IgG titers were below limit of detection

geometric mean concentrations (GMCs)

Table S6: Serotype III specific IgG concentrations pre-immunisation (day 0), and at three post-immunization intervals (day 14, 28, and 42), and opsonophagocytic (OPA) titers post-third immunization dose (day 42) among study groups, Figure 2 and 3.

Study group	Day 0 Serotype III-IgG, AU/ml	Day 14 Serotype III-IgG, AU/ml	Day 28 Serotype III-IgG, AU/ml	Day 42 Serotype III-IgG, AU/ml	Day 42 Serotype III-OPA titers
	GMC (95% CI)	GMC (95% CI), fold change*	GMC (95% CI), fold change*	GMC (95% CI), fold change*	GMT (95% CI)
Placebo	11.75 §	11.75 § 0	11.75 § 0	11.75 § 0	4 §
Monovalent serotype III CPS- TT conjugate vaccine with adjuvant (mGBS-III-CV-adj)	11.75 §	220.1 (185.0-261.8) 18.73	1458 (260.6-8160) 124.08	10266 (1354-77855) 873.70 #	3507 (375-32791) #
Pentavalent CPS-TT conjugate vaccine with adjuvant (GBS5-CV-adj)	11.75 §	411.0 (238.3-709.0) 34.97	1063 (347.8-3248) 90.46	2958 (602.2-14533) 251.74	1045 (246-4428)
Pentavalent CPS-TT conjugate vaccine without adjuvant (GBS5-CV-no_adj)	11.75 §	225.1 (167.1-303.4) 19.15	541.4 (141.3-2075) 46.07	2117 (510-8792) 180.17 #	537 (161-1787) #
Unconjugated pentavalent CPS vaccine with adjuvant (GBS5-CPS-adj)	11.75 §	65.10 (23.75-178.4) 5.54	55.07 (16.85-180) 4.68 #	170.1 (95.48-302.9) 14.47 #	48 (29-79) #

*calculated from day 0

indicates analysis based on 5 mice

§ 95% CI not applicable- all sample IgG titers were below limit of detection

geometric mean concentrations (GMCs)

Table S7: Serotype V specific IgG concentrations pre-immunisation (day 0), and at three post-immunization intervals (day 14, 28, and 42), and opsonophagocytic (OPA) titers post-third immunization dose (day 42) among study groups, Figure 2 and 3.

Study group	Day 0 Serotype V-IgG, AU/ml	Day 14 Serotype V-IgG, AU/ml	Day 28 Serotype V-IgG, AU/ml	Day 42 Serotype V-IgG, AU/ml	Day 42 Serotype V-OPA titers
	GMC (95% CI)	GMC (95% CI), fold change*	GMC (95% CI), fold change*	GMC (95% CI), fold change*	GMT (95% CI)
Placebo	4.35 §	4.35 § 0	4.35 § 0	4.35 § 0	4 §
Monovalent serotype V CPS-TT conjugate vaccine with adjuvant (mGBS-V-CV- adj)	4.35 §	10.48 (3.68-29.78) 2.40	246.6 (42.52-1430) 56.68	2668 (478.4-14882) 613.33	6330 (1081-37072)
Pentavalent CPS-TT conjugate vaccine with adjuvant (GBS5-CV-adj)	4.35 §	5.29 (3.19-8.78) 1.21	220.3 (26.10-1859) 50.64	2728 (242.5-30688) 627.12	2335 (565.4-9644)
Pentavalent CPS-TT conjugate vaccine without adjuvant (GBS5-CV- no_adj)	4.35 §	6.82 (2.14-21.68) 1.56	27.25 (5.51-134.6) 6.26	356.2 (143.5-884.4) 81.88 #	766 (400-1470) #
Unconjugated pentavalent CPS vaccine with adjuvant (GBS5-CPS-adj)	4.35 §	4.35 § 0	4.35 § 0 #	4.35 § 0 #	4.24 (1-12) #

*calculated from day 0

indicates analysis based on 5 mice

§ 95% CI not applicable- all sample IgG titers were below limit of detection

geometric mean concentrations (GMCs)