SUPPLEMENTAL MATERIALS

Human serum mannose-binding lectin senses wall teichoic acid glycopolymer of *Staphylococcus aureus*, which is restricted in infancy

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This file includes supplemental Figures S1 –S8.

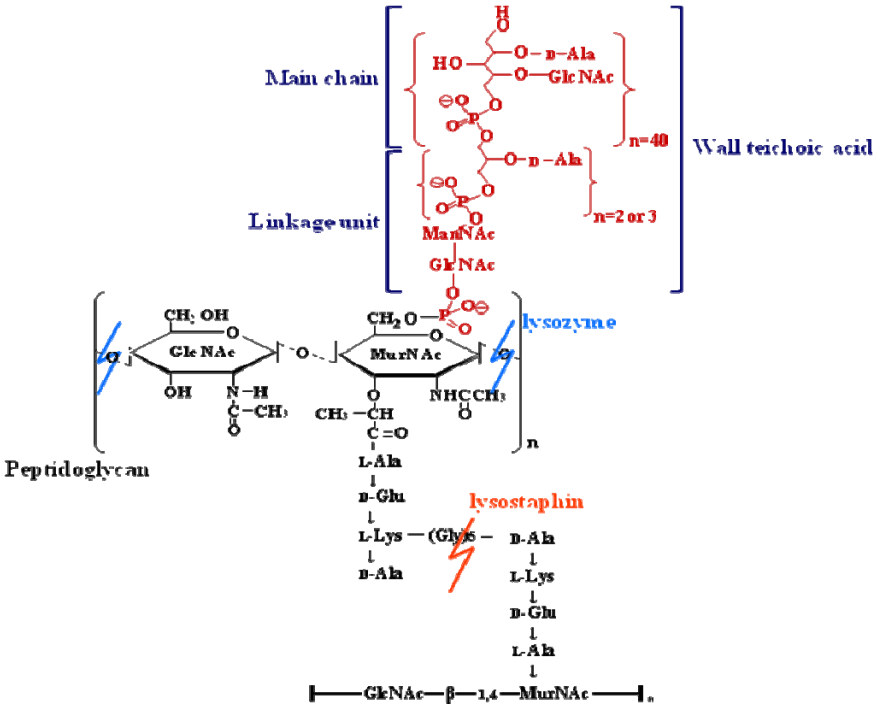

Figure S1. *S. aureus* WTA was obtained from *S. aureus* PG by two PG-degrading enzyme treatments.

The purified WTA coupled to GlcNAc and MurNAc was obtained from insoluble *S. aureus* PG by two PG-degrading enzymes treatments, lysozyme and lysostaphin. The cleavage sites by two enzymes are shown as zigzag lines. Lysozyme is a family of enzymes which catalyzes hydrolysis of 1,4-β-linkages between *N*-MurNAc and *N*-GlcNAc residues in a PG. Lysostaphin is known to preferentially hydrolyze the pentaglycine bridge of the *S. aureus* PG. The most common structures of WTAs are composed of a *N*-acetylmannosamine (ManNAc)-(β1→4)-*N*-(GlcNAc) disaccharide with one to three glycerol phosphates attached to the C4 hydroxyl of the ManNAc residue (the "linkage unit") followed by a much longer chain of glycerol- or ribitol phosphate repeats (the "main chain"). The hydroxyls on the glycerol- or ribitol phosphate repeats are tailored with cationic D-alanine esters and monosaccharides, such as glucose or *N*-GlcNAc. WTA is covalently linked to PG at the C6 position of *N*-acetylmuramic acid (MurNAc).

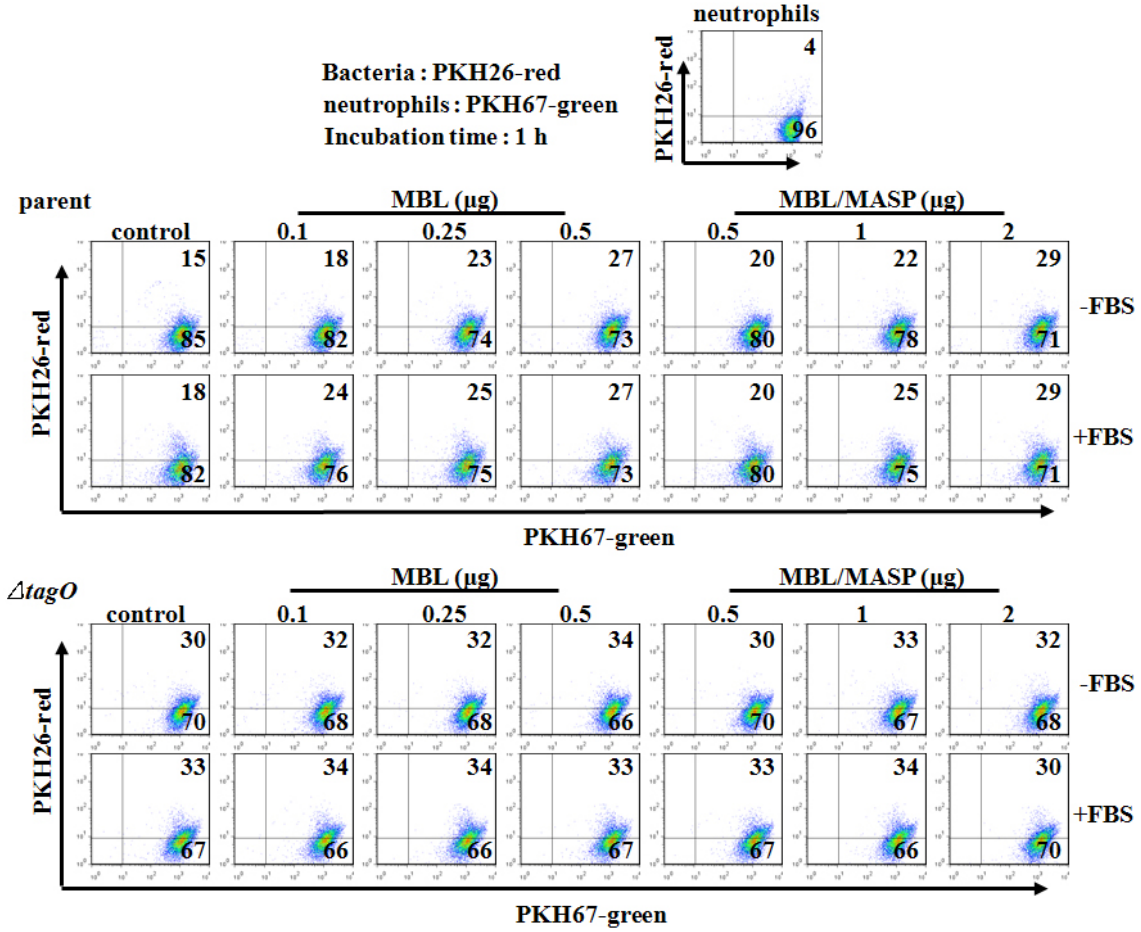

Figure S2. The neutrophilic phagocytosis was enhanced by MBL bound to *S. aureus* WTA.

Ethanol-fixed *S. aureus* parental (M0107) or Δ*tagO* mutant (T258) cells labeled with red PKH26 dye (1.0×10^6 cells) were incubated with rMBL (250 ng) or MBL/MASP complex (1 μg) at 4°C for 2 h. Bacterial cells were washed, and further incubated with human neutrophils labeled with green PKH67 dye (1.0×10^5 cells) at MOI of 10:1 at 37°C for 1 h. The phagocytosis of *S. aureus* cells by neutrophils was analyzed by flow cytometry in the presence and absence of fetal bovine serum, indicated as FBS(+) and FBS(-), respectively.

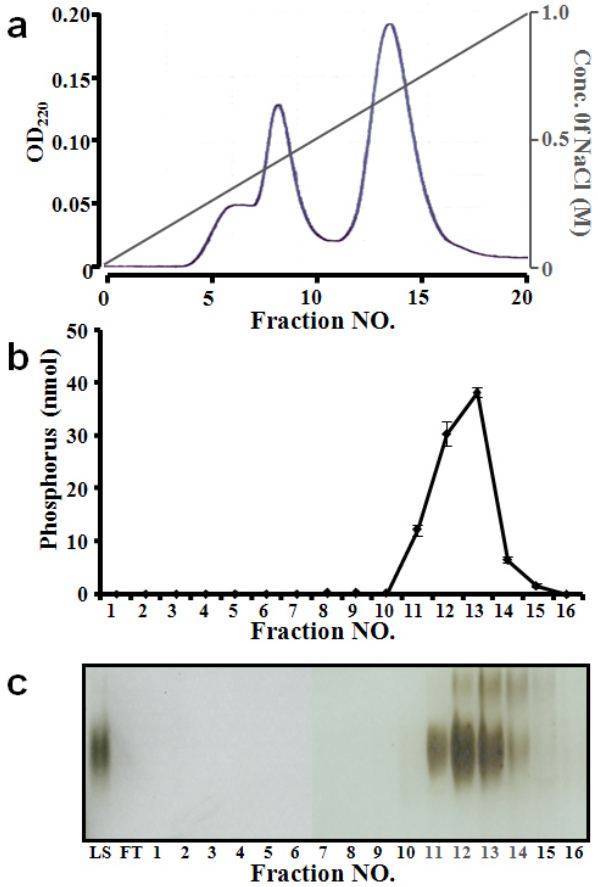

Figure S3. The purification of *S. aureus* WTA.

Two-enzymes-treated *S. aureus* PG was separated on HiTrap-Q column and detected by OD_{220} (a). The released WTA harboring disaccharide with tetrapeptide is monitored by inorganic phosphorus (Pi) assay for phosphate group of WTA (b) and is also identified by silver staining of PAGE (c). The faint upper bands in (c) were assumed as dimeric-WTA due to insufficient cleavages by enzymes.

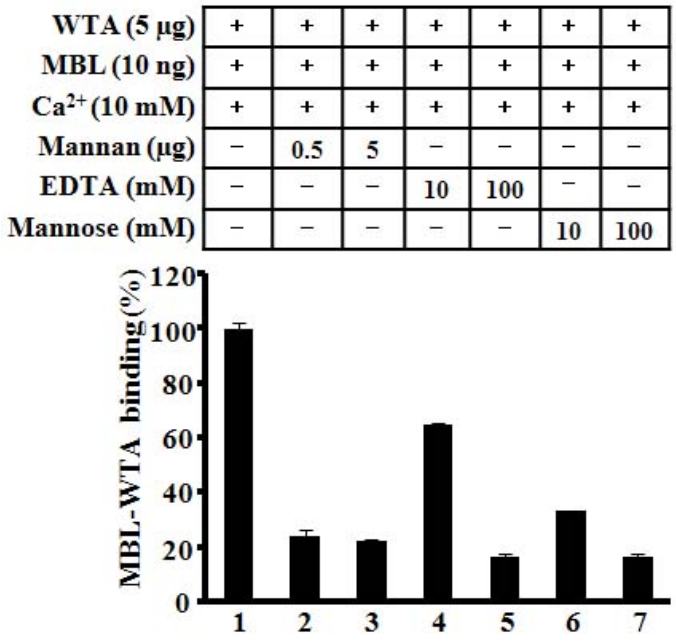

Figure S4. MBL binding to *S. aureus* WTA is mediated via carbohydrate recognition domain of MBL.

Inhibitory effect of mannan, mannose and EDTA on MBL binding to WTA was determined in ELISA. In brief, 5 μg of WTA in 50 μl of PBS (pH 7.5) was applied to F96 Cert. maxisorp immuno plates (duplicate, Nunc) and absorbed overnight at room temperature. Wells were washed with washing buffer [10 mM Tris-HCl (pH7.4), 140 mM NaCl, 0.05% tween20, 1 mM $CaCl_2$] and blocked with 200 μl of buffer D [20 mM Tris-HCl (pH 7.4) containing 150 mM NaCl and 1% BSA] for 1 h at room temperature. After washing, the wells were incubated with 100 ng of MBL in 50 μl of buffer C [10 mM Tris-HCl (pH 7.4), 150 mM NaCl, 10 mM $CaCl_2$, 1% BSA, and 0.05% Tween20] for 2 h at 4˚C. After washing the wells with washing buffer 2 times, mouse anti-human MBL mAb (Dobeel, Korea, diluted 1:1,000) was added used to detect bound MBL. Secondary antibodies were goat anti-mouse IgG (H+L) conjugated with horseradish peroxidase (HRP) (Beckman coulter, 1:10,000 dilution). The resulting plates were developed with the substrate, 3,3′,5,5′-tetramethylbenzidine (Zymed Laboratories) in the dark and stopped by 2N H_2SO_4. Absorbance at 450 nm was recorded using a microplate reader (Thermo Scientific USA). To monitor the inhibitory effects of MBL binding to WTA by mannan, mannose or EDTA, we prepared the mixture of MBL (10 ng) with

mannan (0.5 μg and 5 μg), mannose (final concentration 10 mM and 100 mM) or EDTA (10 mM and 100 mM) and then the mixtures were added to the WTA-coated microplate wells. The binding ability of MBL to WTA was estimated as the same method as described above. Data were representative of at least three independent experiments.

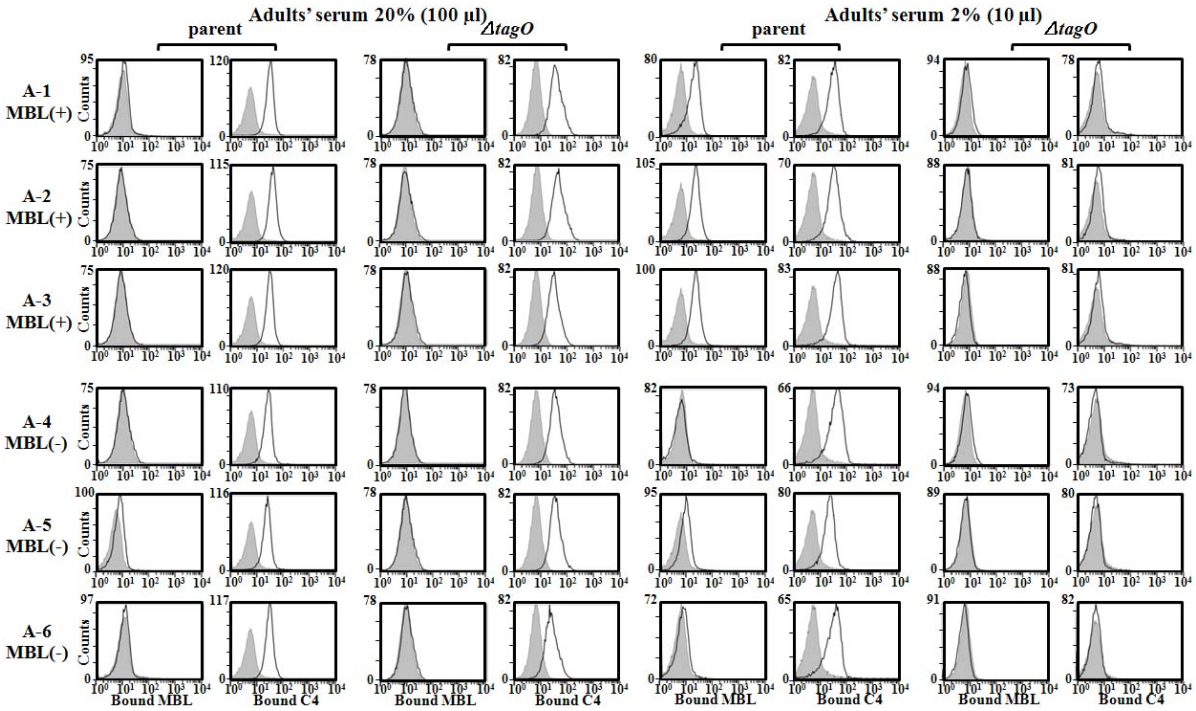

Figure S5. Estimation of MBL binding and C4 deposition of six adults' sera.
S. aureus M0107 (parent) or T258 (Δ*tagO*) cells were incubated with adult serum of MBL-sufficient (A1-A3) or MBL-deficient (A4-A6), and bound MBL or C4 on *S. aureus* cells were detected by flow cytometry as described in the legend of Fig. 3c. Serum concentration used was 20% in left 24 columns or 2% in right 24 columns. Gray area represents data without serum.

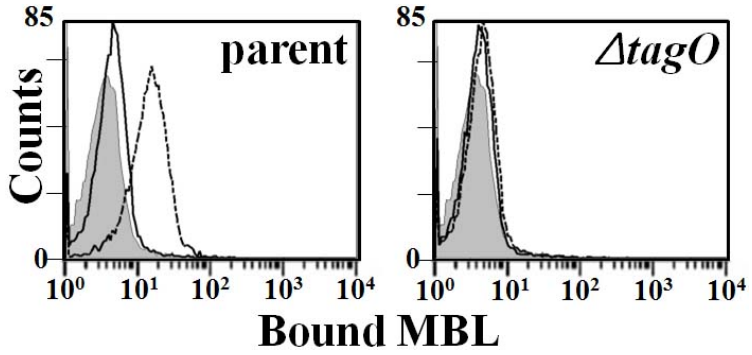

Figure S6. MBL binding to *S. aureus* parental cells was restored in IgG-depleted adult serum.

S. aureus M0107 (parent) or T258 (Δ*tagO*) cells were incubated with 50 µl of adult serum of MBL-sufficient (full line) or IgG-depleted serum (IgG(-), dotted line). Amount of IgG-depleted serum used was adjusted by MBL concentrations. Bound MBL on *S. aureus* cells were detected by flow cytometry as described in the legend of Fig. 1b. Gray area represents data without serum.

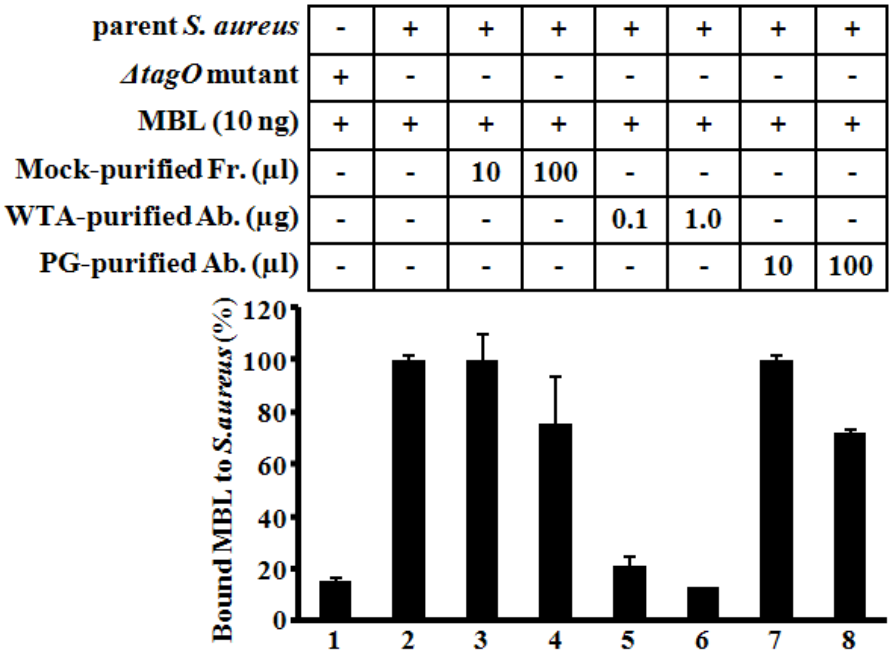

Figure S7. Affinity purified anti-WTA antibody inhibits MBL binding to *S. aureus* cells.

MBL binding to *S. aureus* whole cells and its inhibition by affinity-purified immunoglobulin fractions were evaluated using ELISA, as described in Fig. S4 and Fig. 4d. Briefly, ethanol-fixed *S. aureus* parental (M0107) or Δ*tagO* mutant (T258) cells (8 x10^5 cells) in 50 μl of PBS (pH 7.5) was absorbed on maxisorp immuno 96-well plates (duplicate, Nunc). After washing and blocking, affinity purified immunoglobulin fraction from nitrocellulose membrane-immobilized WTA, PG, or mock from IVIG (SK Chemicals, Seoul) were added to immobilized cells. An aliquot containing 0.1 or 1 μg of anti-WTA antibodies or one of equivalent fraction for others was used. After incubation in buffer C [10 mM Tris-HCl (pH 7.4), 150 mM NaCl, 10 mM $CaCl_2$, 1% BSA, and 0.05% Tween20] for 2 h at 4°C and washing, wells were incubated with 10 ng of rMBL in 50 μl of buffer C for 2 h at 4°C. Bound MBL was detected using mouse monoclonal Abs for human MBL (Dobeel, Korea) and goat anti-mouse IgG (H+L) Abs conjugated with HRP (Beckman coulter), followed by developing with the substrate, 3,3′,5,5′-tetramethylbenzidine (Zymed Laboratories). Data were representative of at least two independent experiments.

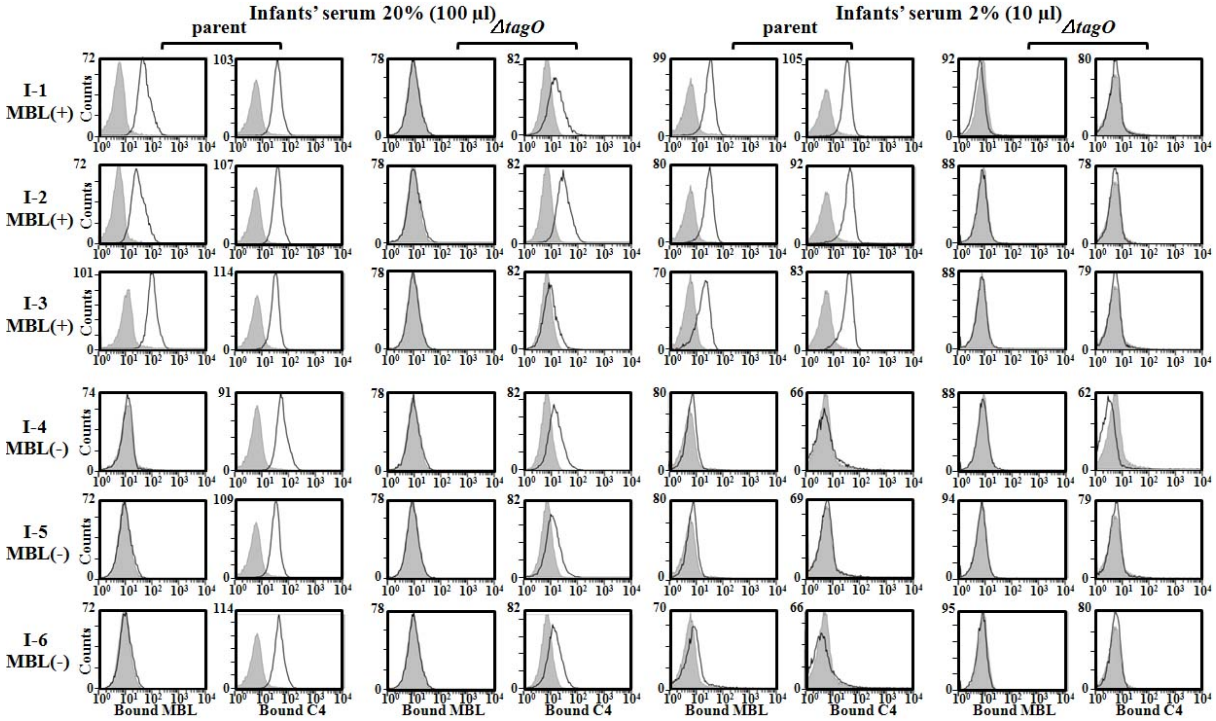

Figure S8. Estimation of MBL binding and C4 deposition of six infants' sera.
S. aureus M0107 (parent) or T258 (Δ*tagO*) cells were incubated with adult serum of MBL-sufficient (I1-I3) or MBL-deficient (I4-I6), and bound MBL or C4 on *S. aureus* cells were detected by flow cytometry as described in the legend of Fig. 3c. Serum concentration used was 20% in left 24 columns or 2% in right 24 columns. Gray area represents data without serum.