CpG-DNA induces bacteria-reactive IgM enhancing phagocytic activity against *Staphylococcus aureus* infection

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**Running title:** Bacteria-reactive IgM enhances phagocytic activity  **Keywords:** CpG-DNA, IgM, phagocytosis, *Staphylococcus aureus*, TLR9

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Supplementary Methods

Mice

Eight-week old BABL/c mice (Nara Biotech Inc.) and BALB/c TLR9 knockout mice (TLR9<sup>-/-</sup>) (Oriental BioService Inc.) were purchased for the experiments. The mice were kept under specific-pathogen-free (SPF) conditions with the optimal temperature (20-25°C) and humidity (32-37%). Sera were obtained by retro-orbital bleeding under isoflurane (2-3%, JW Pharmaceutical Inc.) inhalation anesthesia to minimize pain. After the experiments were terminated, the mice were sacrificed by CO<sub>2</sub> inhalation and all efforts were made to minimize suffering. All animal study protocols were approved by the Hallym University Institutional Animal Care and Use Committee (Permit Number: Hallym 2017-43).

Bacteria

*Staphylococcus aureus* (S. aureus, KCCM 12103), *Staphylococcus epidermidis* (S. epidermidis, KCCM 40416), *Streptococcus pyogenes* (S. pyogenes, KCCM 11873), *Klebsiella pneumoniae* 11418 (K. pneumoniae, KCCM 11418), *Klebsiella pneumoniae* 40145 (K. pneumoniae, KCCM 40145), *Klebsiella pneumoniae* 41293 (K. pneumoniae, KCCM 41293), *Escherichia coli* (E. coli K1, KCCM 12119), *Acinetobacter baumannii* (A. baumannii, KCCM 40203), and *Pseudomonas aeruginosa* (P. aeruginosa, KCCM 11803) were purchased from the Korean Culture Center of Microorganisms (KCCM). All bacteria were grown at 37°C in Lysogeny broth (LB). The S. aureus strain MW2 (S. aureus MW2, MRSA) was grown at 37°C in Columbia broth supplemented with 2% NaCl (1). All bacteria were grown overnight, re-cultured in fresh media at a 1/50 dilution until the mid-log phase (OD<sub>600</sub> 0.5-0.6) and harvested, and colony forming units (CFU) were determined on agar plates.
ELISA

To determine the production of bacteria-reactive antibodies following CpG-DNA 1826 administration in mice, we used poly-L-lysine coated plates (Corning Inc.). The bacteria were grown overnight, washed twice with PBS by centrifugation at 10,000 rpm for 15 min, and re-suspended in conventional ELISA coating buffer. Each well was coated with 100 μl of re-suspended bacteria (5 × 10⁷ CFU /well) and incubated overnight at 4°C. After incubation, the bacteria were fixed with 0.5% glutaraldehyde in PBS for 15 min at room temperature. After washing twice with PBS, each well was incubated with RPMI 1640 medium containing 100 mM glycine and 0.1% BSA for 30 min at room temperature to block the glutaraldehyde and washed twice with PBS. The bacteria-coated wells were then blocked with PBS containing 1% BSA for 1 h at room temperature. Serum, peritoneal cavity fluid, and culture supernatants of cells from primary tissues such as peritoneal cells (PerC), spleen, mesenteric lymph nodes (MLN), and cervical lymph nodes (CLN) or purified antibodies were serially diluted, added to each well, and incubated for 1 h at room temperature. The samples were washed three times with PBS-T (0.2% Tween-20 in PBS) and antibodies, including horseradish peroxidase (HRP)-labeled goat anti-mouse IgM (Southern Biotech Inc.) were added to the wells and incubated for 1 h at room temperature. After washing with PBS-T four times, the TMB Microwell Peroxidase Substrate Kit (KPL Inc.) was used to detect the peroxidase-labeled conjugates (blue-color expression), followed by the addition of the TMB Stop solution (KPL) (yellow-color), and the absorbance was measured at 450 nm using a SpectraMax 250 microplate reader (Molecular Devices Inc.). To determine the concentrations of the total IgM, the plates were coated with goat anti-mouse Ig (Southern Biotech Inc.) and analyzed using anti-mouse IgM antibody by ELISA as previously described (2).
Production of hybridoma cells from B cells in the peritoneal cavity

BALB/c mice were intraperitoneally injected with 50 µg of CpG-DNA 1826. After 7 days, the peritoneal cells were harvested from the mice, and fused with mouse SP2/0 myeloma cells, and then, bacteria-reactive IgM antibody-producing hybridoma clones were isolated using a standard hybridoma technique (2).

Fluorescent labeling of bacteria

*S. aureus* MW2 was cultured to OD$_{600}$ 0.5-0.6 ($3 \times 10^8$ CFU), washed with PBS, and fixed with 70% ethanol in PBS for 1 h. The fixed bacteria were dissolved in 0.1 M Na$_2$CO$_3$ buffer (pH 8.5) with 0.02 mM FITC (Sigma-Aldrich Co.) for 30 min at room temperature, washed with serum-free Hank’s Balanced Salt Solution (HBSS), and re-suspended in HBSS containing 2 mM CaCl$_2$, 1 mM MgCl$_2$, 10 mM HEPES, 150 mM NaCl, and 0.4% BSA.

References
