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Supplementary Information for

Siglec-7 Engagement by GBS β-protein Suppresses Pyroptotic Cell Death of Natural Killer Cells

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Supplemental: Materials and Methods

Bacterial strains and reagents

Streptococcus agalactiae or group B streptococcal (GBS) type Ia strain A909 (WT) and its isogenic ΔBAC mutants (ΔBAC) have been described previously (1). Anti-Siglec-7 antibody 194212 was obtained from R&D Systems. Anti-Siglec-7 neutralizing antibody (clone S7.7) was obtained from BioLegend. Anti-NLRP3 rabbit monoclonal antibody (D2P5E) was obtained from Cell Signaling and BioLegend.

Human NK cell isolation

Human NK cells derived from different donors were used in this study. To isolate the peripheral blood mononuclear cell fraction, whole blood was mixed with PBS at a 1:1 ratio and gently layered onto Ficoll-Paque solution (GE Healthcare). After centrifugation for 25 minutes in 4 degrees Celsius at 1000g, the enriched PBMC layer was removed and washed in PBS. Primary NK cells were isolated by the Human NK Cell Enrichment Kit (Miltenyi) using negative selection and subsequently cultured in RPMI 1640 supplemented with 10% FBS.

Human NK cell infection with GBS

GBS was grown in Todd-Hewitt broth (Oxoid) at 37 degrees Celsius without shaking and added to $2x10^5$ cells of NK cells at MOIs of 10:1 or 1:1 (GBS:NK) for 30 minutes (NK cell death assays and cytokine secretion) or 10 minutes (measuring NLRP3 foci formation). At the indicated time points, cells and supernatant were harvested for further analysis.

Siglec-Fc ELISA and Western blot binding assays

Siglec-7-Fc and purified β-protein, B6N, and IgA fragments were prepared as previously described (2,3). ELISA assays were performed by first adsorbing the β-protein (or negative controls) in sodium carbonate-bicarbonate buffer pH 9.2 onto the plate overnight at 4 degrees Celsius at the indicated concentrations. After washing with PBS-T (PBS + 0.1% Tween-20), the indicated Siglec-Fc protein was added to the plate for two hours. After three rounds of washing with PBS-T, an anti-human antibody conjugated to horseradish peroxidase was added to the well for one hour. After three hours of washing with PBS-T, the wells were exposed to the ELISA HRP Substrate 680 (Li-Cor) and scanned and analyzed by the Odyssey Imager and software (Li-Cor).

Western blots were performed similarly. After the proteins were separation by SDS-PAGE and transferred onto a PVDF membrane, the membranes were incubated with Siglec-Fc overnight at 4 degrees Celsius. The next day, the membranes were washed, incubated with an anti-human antibody conjugated with IRDye800 (Li-Cor), washed, and then scanned and analyzed by the Odyssey Imager and software (Li-Cor).

Flow cytometry

All flow cytometry analyses were performed on either a BD FACSCalibur or BD Canto II. Briefly, for flow cytometry assays that involve antibody or Siglec-Fc binding, the cells were incubated with the primary probe for 30 minutes on ice. The concentration used was 5 μg/ml for Siglec-Fc or as per manufacturer's instructions based on antibody used. After washing, a secondary fluorophore-conjugated

antibody was added and incubated on ice in the dark for an additional 30 minutes. After further washing, the cells were analyzed on the flow cytometer using the relevant channels based on fluorophore used. NLRP3 staining was performed on primary human NK cells enriched as described above. After permeabilization by BD Cytofix/Cytoperm kit, NK cells were probed with an anti-NLRP3 antibody, washed, and detected with a secondary AlexaFluor488 conjugated antibody.

Confocal microscopy

Primary human NK cells were incubated with FITC-labeled GBS as previously described (citation) for 15 minutes at 37 C, washed, and then fixed in 10% formalin at 4C for 30 minutes. NK cells were probed with a PE-conjugated anti-Siglec-7 antibody, and DNA was labeled with DRAQ5. To count NLRP3 foci, freshly purified NK cells were infected with GBS A909 WT or ΔBAC mutant at 37 ℃ for 30 min. NK cells were stained for NLRP3 (Clone D2P5E, BioLegend) following permeabilization, and NLRP3 foci were enumerated by counting at least 10 fields, and the average +/- SD is shown in the bar graph.

Analysis of cytokine and cytolytic molecule secretion

Cell-culture supernatants were harvested for ELISA to determine the amount of IL-1β (R&D Systems catalog# DY201-05) or granzyme B (BioLegend catalog# 439207) as per manufacturers' instructions. Cell death was measured by LDH release in supernatant as per manufacturer's instructions (Promega). Caspase-1 activity in culture supernatants was quantitated using Caspase-1 colorimetric assay kit as per manufacturer's instructions (BioVision). For detecting bioactive IL-1β, the HEK-Blue IL-1β reporter cell line (InVivoGen, catalog# HKB-IL1B) was

used. Supernatants from the GBS-NK cell co-incubation were centrifuged to remove cellular contaminants, and then applied to the cell lines as per manufacturer's instructions.

Statistical analysis

Statistical significance was analyzed by the unpaired *t* test. Data are expressed as mean ± SD. Significance of *p* values was as follows: **p*<0.05, ***p*<0.005, ****p*<0.001.

Figure S1: Direct interaction between Siglec-7 and GBS β-protein

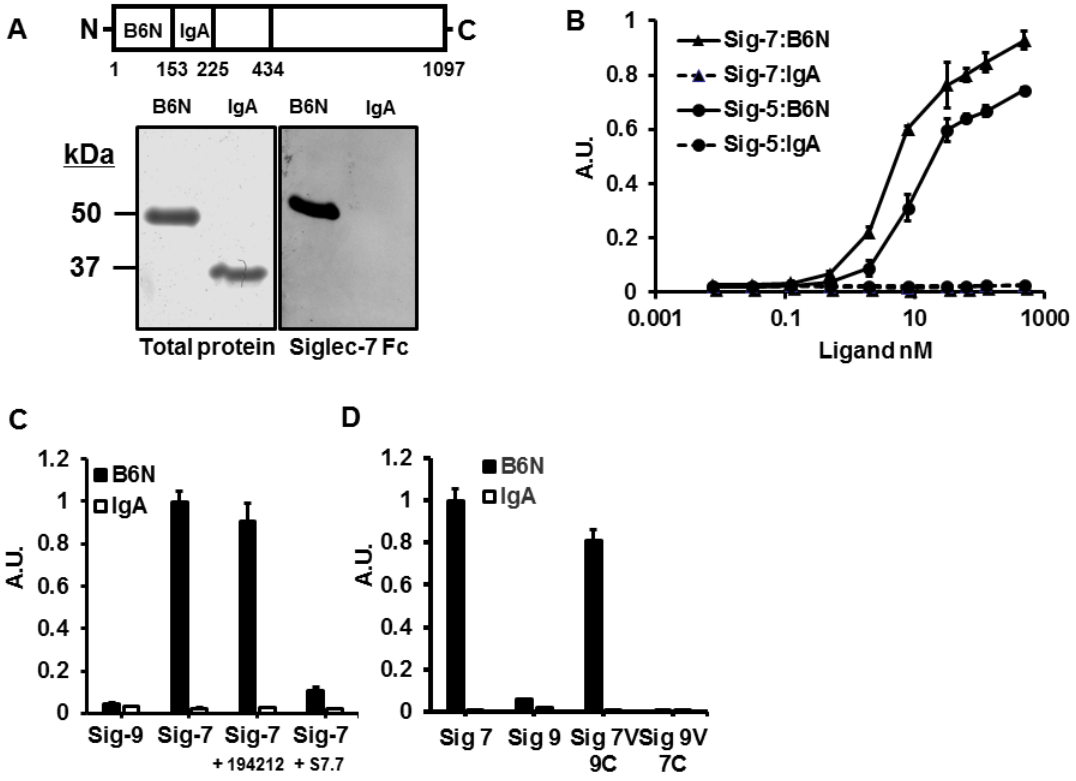

Fig. S1. Direct interaction between Siglec-7 and the B6N domain of the □-protein. (A) Schematic of the β-protein detailing the regions containing the B6N and IgA domains and western blot of B6N and adjacent IgA peptide fragments derived from the b-protein probed by recombinant chimeric Siglec-7-Fc. (B) ELISA analysis comparing relative binding of Siglec-5-Fc and Siglec-7-Fc to B6N or IgA peptide fragments immobilized to the plate surface. (C) ELISA analysis of immobilized B6N or IgA peptide fragments by Siglec-9-Fc or Siglec-7-Fc in the absence or presence of anti-Siglec-7 antibody clone 194212 or clone S7.7. (D) ELISA analysis of immobilized B6N or IgA peptide fragments by Siglec-9-Fc, Siglec-7-Fc, or domain swapped Siglec-7 V-set + Siglec-9 C2-set-Fc, and Siglec-9 V-set + Siglec-7 C2-set-Fc.

Figure S2: NLRP3 foci formation in NK cells after exposure to GBS

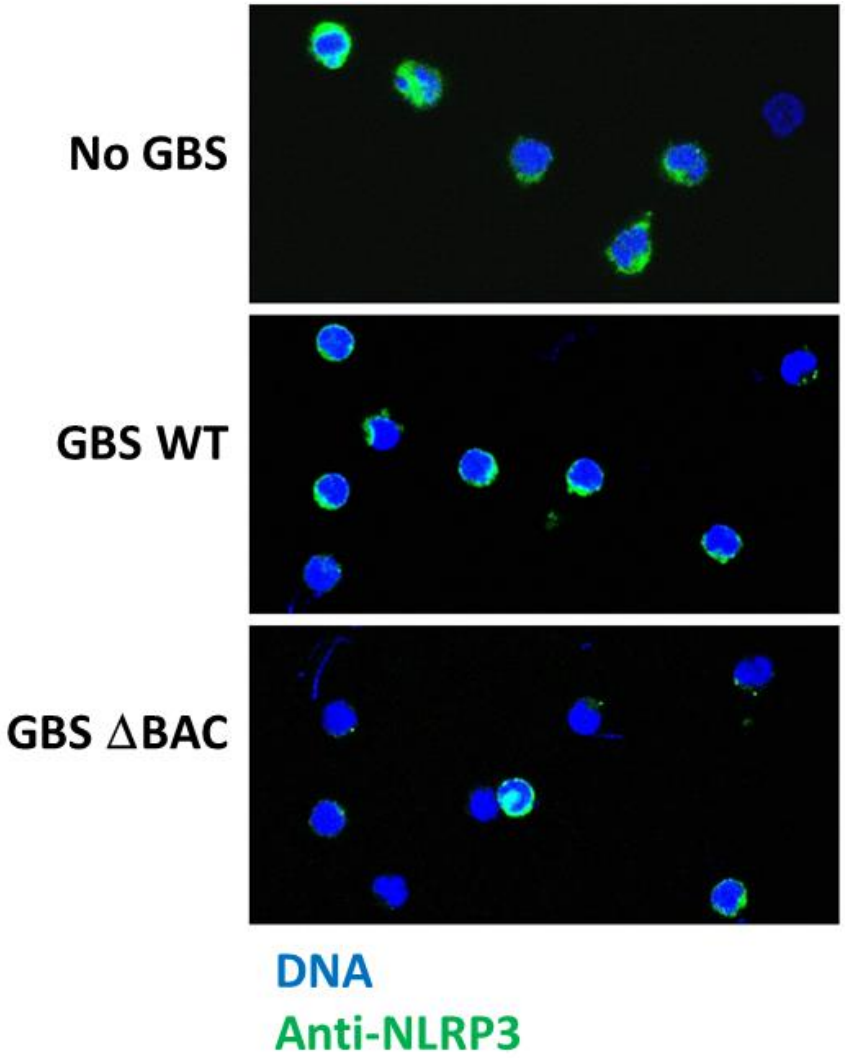

Fig. S2. Formation of NLRP3 foci in human NK cells after exposure to GBS A909 WT or ΔBAC. Lower magnification representative photos demonstrating NLRP3 foci formation in human NK cells after exposure to GBS WT (A909) or ΔBAC, or without exposure to GBS.

References

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