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Supplementary information

Supplementary Fig. 1

Supplementary Fig. 1. SAg-Sau stimulation expands Foxp3+ Treg population and induces IL-17A+Foxp3+ CD4+ T cells in tonsillar MNCs. Analysis of Treg expansion (a, b) and IL-17A-expressing Tregs (c) in isolated human tonsillar MNCs at 48hrs following bacterial CCS (1µg/ml) stimulation. a) Tonsillar MNCs were stimulated with CCS produced from *Spn*, *M. catarrhalis*, coagulase-negative staphylococcus (CNS, C4 and C5) and SAg-Sau, and the proportion of Tregs was analysed. Proportion of Tregs (b) and IL-17A-expressing Tregs (c) in CD4+ T cell population activated by NonSAg-Sau, SAg-Sau and Sau carriage strains (C1, C2 and C3). Results represent 8 (a), 5 (b) and 6 (c) independent experiments. Data displayed is median (center line), upper and lower quartile (box limits) and minimum to maximum range (whiskers). (*p <0.05, **p <0.01)
Supplementary Fig. 2. CD25+ cell depletion removes Foxp3+ Tregs in tonsillar MNCs. a) Foxp3+ CD4+ cells (Tregs) were gated out in the rectangular boxes with numbers on top indicating the percentage of Tregs in lymphocytes before and after CD25+ cell depletion. b) Unfractionated and CD25+ cell-depleted MNCs were stimulated with 1µg/ml of SAg-Sau CCS for 48hrs and activation of IL-17+ T cells was examined. CD4+ T cells were gated out in the representative dot plots and numbers in top right and left quadrants indicating percentages of IL-17A+ cells within Foxp3+ and Foxp3− T cells respectively. Results are representative of 3 individual samples.
Supplementary Fig. 3. Foxp3+ Tregs suppresses the Th1 but not Th17 responses in PBMCs activated by SAg-Sau. IL-17A and IFNγ expression in unfractionated PBMCs or CD25+ cell depleted PBMCs stimulated with SAg-Sau CCS (1µg/ml) at 48hrs. a) Zebra plots were gated on lymphocytes for IFNγ expression. Numbers in top left and right quadrants indicate the percentage of IFNγ+ CD4+ lymphocytes and IFNγ+ CD4+ T cells (Th1) respectively. For the expression of IL-17A, zebra plots were gated on CD4+ T cells and the percentage of Th17 cells within CD4+ T cell population was indicated in the top right quadrants.
Supplementary Fig. 4. IL-10 suppresses SAg-Sau-activated Th1 responses. Zebra plots were gated on CD4⁺ T cells and numbers in top right quadrants indicate the percentage of Th1 cell within CD4⁺ T cell population. Ctrl is the stimulation control without IL-10 treatment. Results represent 8 independent experiments and were analysed using paired t-test (**”p < 0.001).
Supplementary Fig. 5. Expression and secretion of native IL-35 by transfected CHO cells. Control CHO and IL-35 expressing CHO cells (Clone 7, 8, 14, 15) were cultured for 48hrs. a) Protein expression of IL-12A and EBI3 in cell lysates. b) Production of IL-35 heterodimer in cell culture supernatant as detected by co-immunoprecipitation.
Supplementary Fig. 6. SAg-Sau stimulation downregulates cell surface expression of CD39 on Foxp3+ Tregs.

Tonsillar MNCs were stimulated with 1µg/ml of Spn, NonSAg-Sau and SAg-Sau CCS respectively for 48hrs. Expression of CD39 was detected by cell surface staining and compared to media control (MC). a) Histogram plots were gated on Foxp3+ CD4+ cells, and the percentage of CD39+ cells within Tregs were analysed in (b). Results represent 6 independent experiments. Data displayed is median (center line), upper and lower quartile (box limits) and minimum to maximum range (whiskers). (\(^*\) p <0.05, \(**\) p <0.01)
Supplementary Fig. 7. β-actin expression in CD4⁺ T cell lysates. β-actin was detected by Western blot for the CD4⁺ T cell lysates prepared for IL-35 immunoprecipitation assay.