Cytokine responses against two respiratory pathogens in children are dependent on IL-1β
— Data Supplement

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SUPPLEMENTARY MATERIALS AND METHODS

Stimulation with nontypeable *Haemophilus influenzae*. A single clinical isolate of NTHi was used for all experiments and prepared as previously described (1). Briefly, the NTHi strain was grown for 8 h in brain heart infusion broth supplemented with NTHi growth factors. Single use aliquots were stored in 20% heat-inactivated fetal bovine serum at -80°C. Low-dose NTHi stimulation using 0.33 MOI (0.2 x 10^6 cfu/mL) for 24 h, 72 h or 5 days was applied for all experiments using PBMC.

Cytokine quantification using ELISA. Commercially available ELISA kits were used to measure concentrations of IL-1β (lowest detection limit was 3.9 pg/mL) (BD Biosciences, Franklin Lakes, NJ), IL-1Ra (lowest detection limit was 39 pg/mL) (R&D systems, Minneapolis, MN), and IL-18 (lowest detection limit was 93 pg/mL) (R&D systems, Minneapolis, MN) in culture supernatants. In-house ELISA using paired antibodies were used to measure concentrations of IL-6 (BD Biosciences), IFN-γ (BD Biosciences), and IL-10 (BD Biosciences). Lowest detection limit was 3.9 pg/mL for all in-house ELISA.

Cell surface staining and intracellular cytokine staining. Cultured cells were incubated with Brefeldin A (BioLegend, San Diego, CA) for 3.5 h before staining, the cells were then washed with FACs buffer (2% HI-FCS/ PBS) and blocked with normal goat IgG (Sigma-Aldrich, St. Louis, MO) for 15 min on ice. The cells were surface stained with CD3-APC-Cy7, CD14-PerCP, CD56 AlexaFluor488, CD4-PerCP, or CD25-APC for 30 min on ice. After fixation and permeabilisation (Foxp3 Staining Buffer Set, eBioscience, San Diego, CA), the cells were incubated with intracellular cytokine IFN-γ-FITC and transcription factor Foxp3-PE for 30 min at room temperature. The cells were finally fixed in 2% paraformaldehyde (PFA) prior to analysis.

Reference

1. Pizzutto SJ, Yerkovich ST, Upham JW, Hales BJ, Thomas WR, Chang AB. Children with chronic suppurative lung disease have a reduced capacity to synthesize interferon-gamma in vitro in response to non-typeable Haemophilus influenzae. *PloS one* 2014; 9: e104236.

SUPPLEMENTARY TABLES

TABLE S1 Correlation between clinical measurements and cytokine produced by unstimulated PBB PBMC (n=19). Table show *r* (*P*-value); **P*<0.05 by Spearman's correlation.

	IL-18	**IL-10**
Univariable		
Age	0.528 (0.020) *	-0.075 (0.761)
Cough score	-0.023 (0.926)	0.082 (0.739)
Number of organisms	0.388 (0.101)	0.071 (0.773)
*H. influenza*e	0.034 (0.889)	-0.004 (0.988)
Multivariable		
Age	0.218 (0.205)	

SUPPLEMENTARY FIGURES

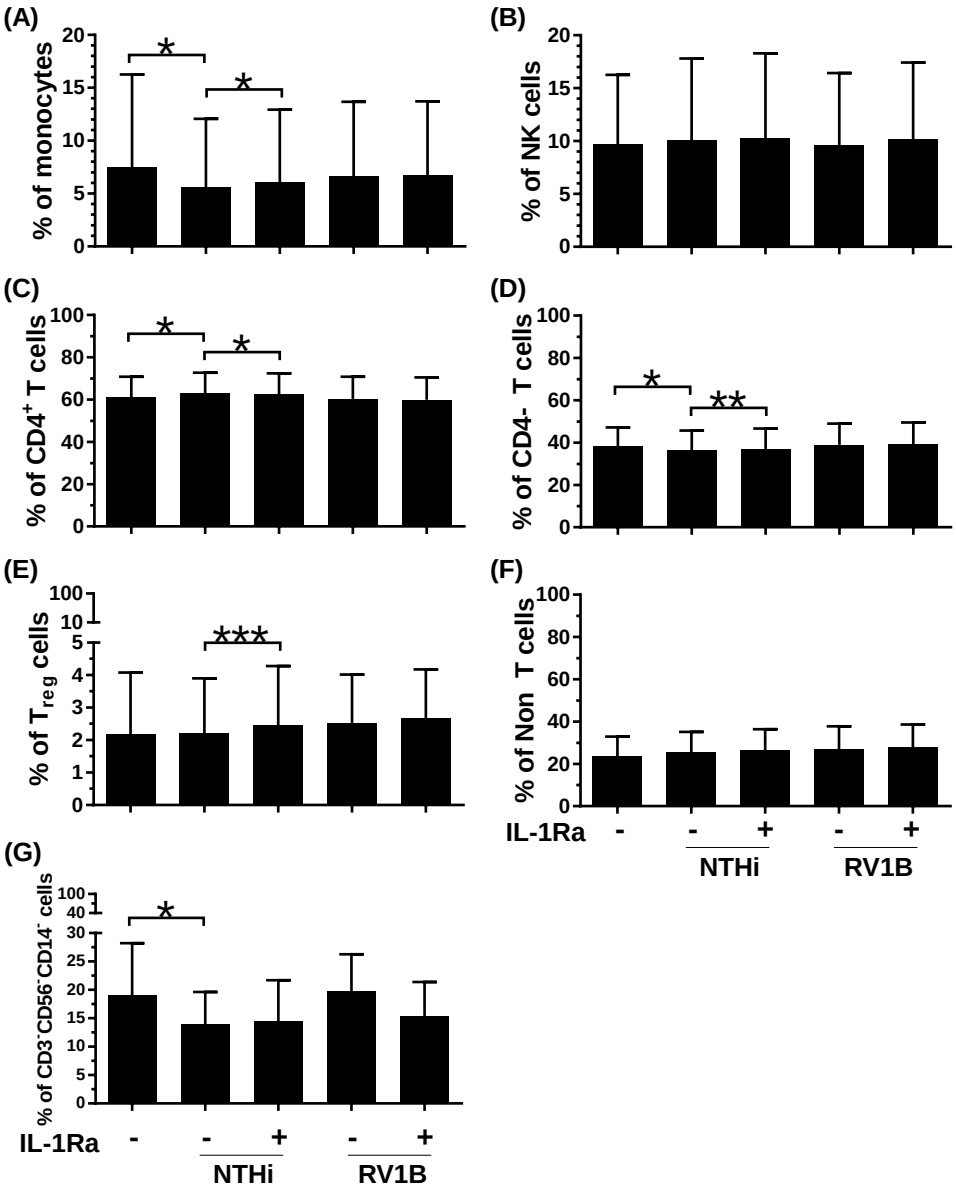

FIGURE S1 — Effects of IL-1Ra on NTHi and RV1B stimulated immune cell number. (A) Percentage of NTHi and RV1B stimulated monocytes ($CD3^-CD14^+$) in the absence and presence of IL-1Ra at 24 h post stimulation (n=10). (B) Percentage of NK cells ($CD3^-CD56^+$) as described in (A). (C) Percentage of NTHi and RV1B stimulated $CD4^+$ T cells ($CD3^+CD4^+$) in the absence and presence of IL-1Ra at 5 days post stimulation (n=11). (D) Percentage of $CD4^-$ T cells ($CD3^+CD4^-$) as described in (C). (E) Percentage of T_{reg} cells ($CD4^+CD25^+Foxp3^+$) as described in (C). (F) Percentage of non T cells ($CD3^-$) as described in (C). (G) Percentage of $CD3^-CD56^-CD14^-$ cells as described in (A). Mean and standard deviations are shown. **P*<0.05, ***P*<0.01 and ****P*<0.001 by Wilcoxon matched-pairs signed rank test. IL-1Ra, interleukin 1 receptor antagonist (Anakinra).