

BCG Cell Wall Skeleton As a Vaccine Adjuvant Protects Both Infant and Old-Aged Mice from Influenza Virus Infection

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Supplementary Information

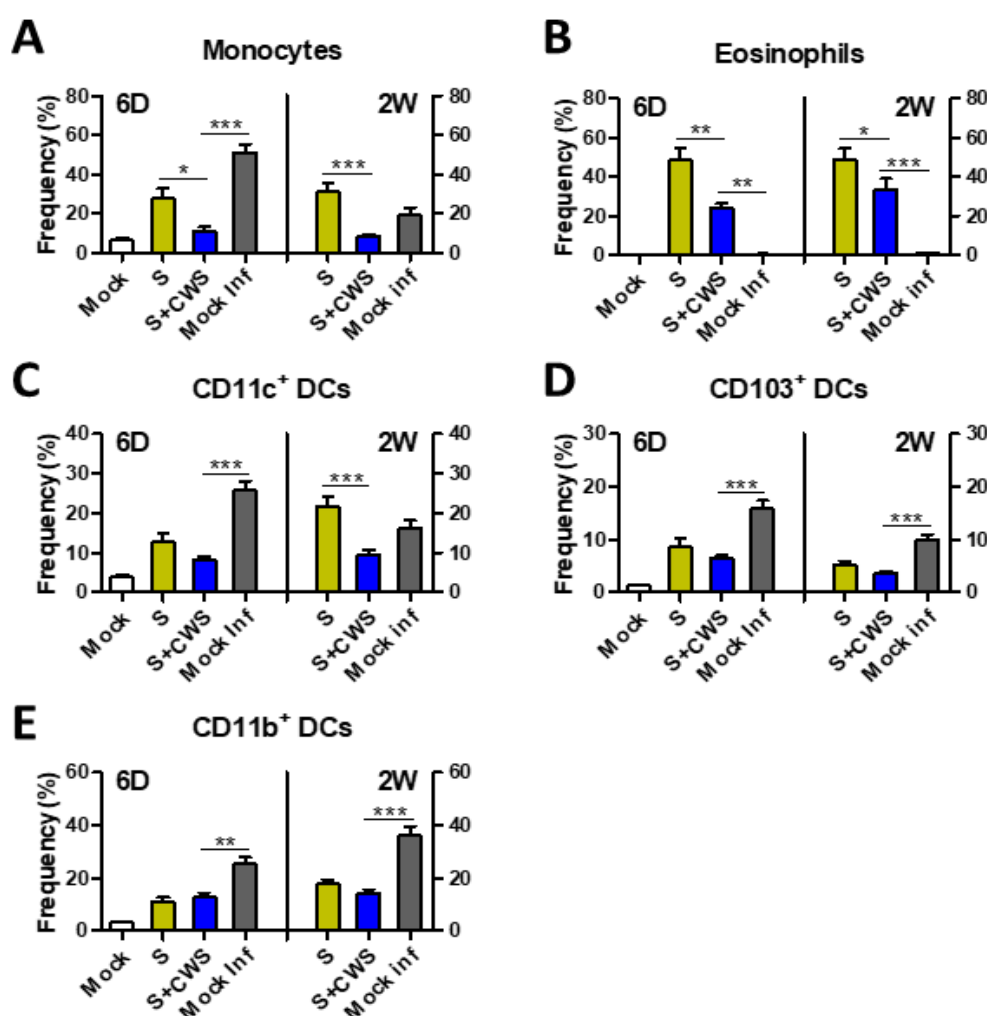


Figure S1. The frequency of inflammatory and antigen presenting cells in the lungs after challenge infection. Cellular phenotypes were determined in the lung samples collected at 7 dpi with A/Cal virus by flow cytometry. (A-B) Both monocytes and eosinophils are presented in percentages out of the total gated CD11b⁺ cells in lung cells. (A) Monocytes (CD11b⁺Ly6c^{hi}F4/80⁺), (B) Eosinophils (CD11b⁺CD11c⁺SiglecF⁺). (C) CD11c⁺ dendritic cells (DCs, CD45⁺F4/80⁺CD11c⁺MHCII^{hi}) presented in percentages out of total gated CD45⁺F4/80⁺ cells in lung cells. (D and E) CD103⁺ DCs (CD45⁺F4/80⁺CD11c⁺MHCII^{hi}CD11b⁺CD103⁺) and CD11b⁺ DCs (CD45⁺F4/80⁺CD11c⁺MHCII^{hi}CD11b⁺CD103⁺) are presented in percentages out of the total gated CD45⁺F4/80⁺CD11c⁺MHCII^{hi} cells in the Lung of 6D and 2W age mice. Statistical significance was calculated by using one-way ANOVA and a Dunnett's multiple-comparison test. Error bars indicate the mean \pm SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

Table S1. Cytokine levels after *in vitro* stimulation of BMDMs and BMDCs from wild type BALB/C and C57BL/6 mice. Cytokines in culture supernatants were presented in concentrations (pg/ml) after incubation of BMDCs and BMDMs with a stimulator, medium control or BCG-CWS (10 µg/ml) for 24 hours.

Table 1. Cytokine levels after *in vitro* stimulation of BMDCs and BMDMs

Mice	BALB/c								C57BL6		
Cell	BMDCs				BMDMs				BMDMs		
Cytokines (pg/mL)	TNF-α	IL-6	IL-12	IL-10	TNF-α	IL-6	IL-12	IL-10	TNF-α	IL-6	IL-12
Control	-	-	-	-	-	-	-	-	-	-	-
CWS (10µg/ml)	4,597.4 ± 563.1	4,163.8 ± 465.4	1,301.3 ± 197.2	1,190.4 ± 184.2	5,457.9 ± 66.6	2,298.8 ± 204.8	472.8 ± 89.8	241 ± 10.4	6471.2 ± 800.7	3586.7 ± 104.8	874.7 ± 35.2