SUPPLEMENTAL INFORMATION

ESAT-6-dependent cytosolic pattern recognition drives noncognate tuberculosis control \textit{in vivo}

Andreas Kupz, Ulrike Zedler, Manuela Stäber, Carolina Perdomo, Anca Dorhoi, Roland Brosch, Stefan H. E. Kaufmann
Supplemental Figure 1: Generation and expansion of memory CD8+ T cells and NK cells (Related to Figure 1). TCR transgenic effector memory OT-ITg and P14Tg CD8+ T cells were generated through in vitro activation of splenocytes with the cognate peptides SIINFEKL or KAVYNFATC, respectively. After 4 days cells were purified and 1×10^7 Tg cells were transferred i.v. into naïve Rag2−/− II2rg−/−, Ifnγr−/− or B6 (CD45.1+) mice. Mice were left untouched for at least 4 weeks to ensure that transferred Tg cells converted into central memory CD8+ T cells. For NK cell transfers, 1×10^6 purified and in vitro expanded NK cells from Rag1−/− or Ifnγ−/− donors were injected i.v. into naïve Rag2−/− II2rg−/− mice on day 5 and 6 after in vitro culture. Mice were infected 48 hours after NK cell transfer. Representative FACS plots are shown.
Supplemental Figure 2: Temporal and spatial characterization of innate IFN-γ secretion in response to Mtb (Related to Figure 2). (A) Percent of IFN-γ+ cells amongst total viable splenic and lung CD3+CD8+, CD3+CD4+, CD3+CD4–CD8– T cells and CD3–NK1.1+ cells at different time-points after injection of B6 mice with 1×10⁸ cfu of S. Typhimurium (Stm), BCG, heat-killed BCG (HKBCG), irradiated Mtb H37Rv (iMtb) or PBS. (B) Percent of IFN-γ+ cells amongst total viable CD3+CD8+, CD3+CD4+, CD3+CD4–CD8– T cells and CD3–NK1.1+ cells in the lung at different time-points after injection of B6 mice with 1×10⁸ cfu Mtb H37Rv. (C) Representative FACS plots and histograms of splenic CD3+CD8+, CD3+CD4+, CD3+CD4–CD8– T cells and CD3–NK1.1+ cells at 24 hours after B6 mice were injected with 1×10⁸ cfu Mtb H37Rv. (D) Representative FACS plots and histograms of endogenous (CD45.1+) and exogenous (CD45.2+, OT-Ig) splenic CD3+CD8+ cells at 24 hours after B6 mice were injected with 1×10⁸ cfu Mtb H37Rv, iMtb H37Rv or PBS. (F) Percent of IFN-γ+ CD3–NK1.1+ cells from either spleen, lung or draining lymph nodes 24 hours after infection with 1×10³ cfu Mtb H37Rv via aerosol (a.s.) route. Results are presented as pooled data means ± SEM (A – D) and representative FACS plots (C, E) of 4–9 (A), 5–10 (B), 4 (C), 3 (D), 5–7 (E) and 10 (F) mice per group from at least 2 pooled independent experiments.
Supplemental Figure 3: RD1-dependence is not organ specific (Related to Figure 3). (A–C) Percent of viable IFN-γ⁺ cells amongst total viable splenic CD3⁻NK1.1⁺ (A–C) and CD3⁺CD8⁺ (D–F) cells 24 hours after i.v. injection of purified mycobacterial ligands (A, D) or 1×10⁸ cfu wildtype or recombinant Mtb or BCG strains (B, C, E, F). (G, H) Percent of viable IFN-γ⁺ cells amongst total viable CD3⁻NK1.1⁺ cells from spleen (G) or draining lymph node (H) 24 hours after i.d. or s.c. injection of 1×10⁸ cfu BCG or BCG::RD1. Results are presented as individual data points of 3-15 mice per group from at least 2 pooled independent experiments. Statistical analyses: One-way ANOVA; significant differences are indicated by asterisks: * p<0.05; ** p<0.01; *** p<0.001; n.s. not significant.
Supplemental Figure 4: Rapid IFN-γ production by NK cells and Mtb-unrelated memory CD8+ T cells requires NLRP3-dependent IL-18 secretion (Related to Figure 4). (A–D) Percent of viable IFN-γ+ cells amongst total viable splenic CD3 NK1.1+ (A, C) and CD3+CD8+ (B, D) cells 24 h after i.v. injection of 1×10⁸ cfu Mtb H37Rv into different mouse strains lacking key components of the cytosolic PRR pathways (A, B), key surface receptors involved in Mtb recognition and innate immune responses (C, D) or B6 mice treated with different antibodies (E, F). Results are presented as individual data points (C - F) or pooled data means plus standard error of the mean (A, B) of 5–16 (A, B), 7–10 (C, D) or 6 (E, F) mice per group from at least two pooled independent experiments. Statistical analyses: One-way ANOVA; significant differences relative to B6 controls (A, D) are indicated by asterisks: * p<0.05; ** p<0.01; *** p<0.001; n.s. not significant.
**Supplemental Figure 5: CD11c+ cells are involved in early uptake of Mtb (Related to Figure 5).**

(A–F) Percent of IFN-γ+ cells amongst total splenic CD3− NK1.1+ (A, C, E) and CD3+CD8+ (B, D, F) cells of straight WT or Il18−/− (A, B) and CD11cDTR bone marrow chimeras (C, D) as well as DTX- and PBS-treated CD11cDTR mixed bone marrow chimeras (E, F) 24 hours after injection of 1×10⁸ cfu Mtb H37Rv.

(G, H) Number of viable PMNs, macrophages, DCs and NK cells per spleen (G) and lung (H) 24 hours after i.v. injection with 1×10⁸ cfu Mtb H37Rv of WT, Il18−/− and DTX- or PBS-treated CD11cDTR bone marrow chimeras 6 weeks after bone marrow transplantation.

(I) Uptake of Mtb H37Rv-GFP by individual lung myeloid cell populations 24 hours after i.v or i.t. delivery of 1×10⁸ cfu. Results are presented as individual data points (A–F) or pooled data means plus standard error of the mean (G–I) of 8-10 (A–H) or 6 (I) mice per group from 2 (I) or 3 (A–H) pooled independent experiments. Statistical analyses: One-way ANOVA; significant differences are indicated by asterisks: * p<0.05; ** p<0.01; *** p<0.001.