SLAMF7 self-ligation increases effector function of Tc-1 CD8+ T cells during anti-tumoral immune response

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ABSTRACT

Cancer remains one of the leading causes of mortality worldwide. Cytotoxic CD8 T cells are central to anti-tumor immunity and our research focuses on enhancing their activity through different signalling pathways. CD8 T cells are activated by (tumor) antigen-specific TCR stimulation and co-stimulation of CD28. Previous studies from our group emphasized the role of SLAMF7 in tumor-specific T cell activation and co-stimulation. SLAMF7, a self-ligand, is expressed on activated T and B lymphocytes as well as Antigen Presenting Cells (APCs). The precise role and potential of SLAMF7 on CD8 T cell differentiation remain uncertain and needs to be further elucidated.

We hypothesize that SLAMF7 signaling may enhances differentiation towards Tc1 CD8 T cells and promotes their effector function against tumor cells.To address this, we investigate the regulation of SLAMF7, its self-ligation, and its impact on regulatory molecules such as PD-1 and CTLA-4 on CD8 T cells. Our studies encompass both intra- and intercellular effects in the context of CD8 T cell activation. Our initial findings indicate that the cytokine milieu influences SLAMF7 expression. Furthermore, genetic depletion of SLAMF7 in APCs attenuate the expression of Eomes, Granzyme B and IFN-γ in activated CD8 T cells, highlighting the importance of SLAMF7 in cytotoxic effector function.

In conclusion, SLAMF7 signaling enhances T cell activation and promotes the differentiation into the Tc1 CD8 phenotype, leading to improved cytotoxic potential. This finding offers valuable insights to refine tumor immunotherapy strategies.

METHODS

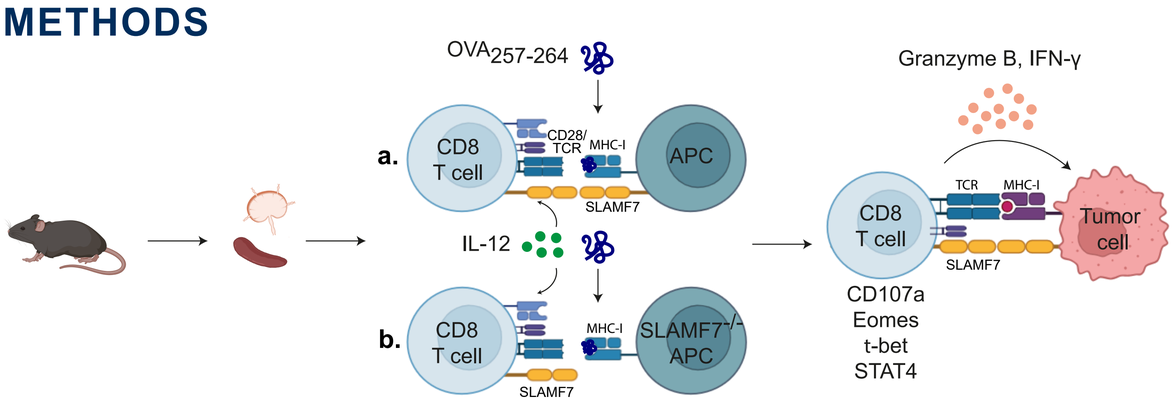

Fig. 1. Experimental setup of antigen-specific stimulation in vitro.
Schematic Illustration of the experimental set up: Lymphocytes were isolated from murine spleen and lymph nodes (LN). OVA-specific TCRtg CD8 T cells were stimulated under following conditions: a. Wildtype (WT) and b. SLAMF7$^{-/-}$ (KO) APCs. APCs were pulsed with 0.1 µg/ml SIIVFEKL peptide (V4, OVA$_{257-264}$), before co-cultured with OVA-specific TCRtg CD8 T cells. Cell culture medium was supplemented with 1 ng/ml IL-12 to induce Tc1 CD8 T cell differentiation. In all experiments CD8 T cells were co-cultured with unpulsed APCs as a control. The effector function of Tc1 CD8 T cells and the role of SLAMF7 were assed using Flow Cytometry.[1]

RESULTS

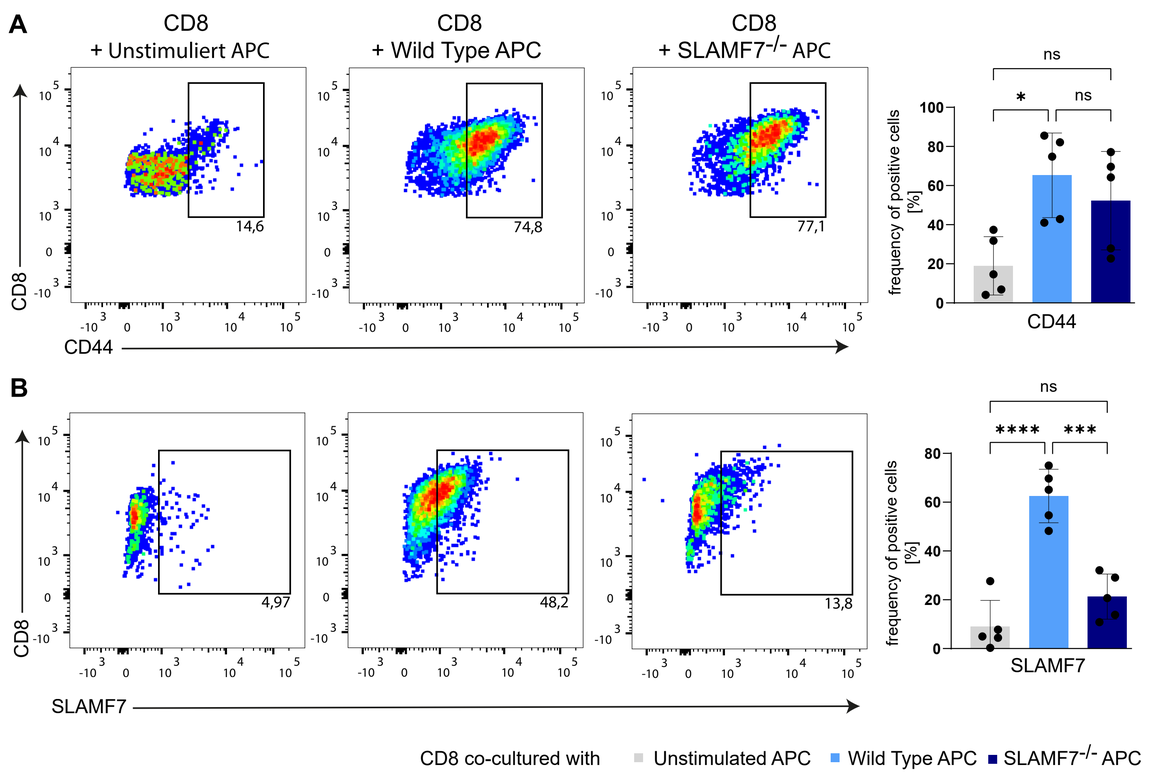

Fig. 2. Expression of SLAMF7 on activated CD8 T cells after antigen specific stimulation.
Flow cytometric analysis was performed on CD8 T cells activated by either WT APCs or KO APCs three days post-stimulation. A) The expression of the activation marker CD44. B) The expression of SLAMF7. The frequency of CD44^{+} and SLAMF7^{+} CD8^{+} T cells from five independent experiments is depicted in the histogram. Statistical analysis: One-way ANOVA followed by Tukey's multiple comparison test: *p < 0.05; **p < 0.005; ***p < 0.0005; ****p > 0.0001.

[1] Schematic Illustration: BioRender

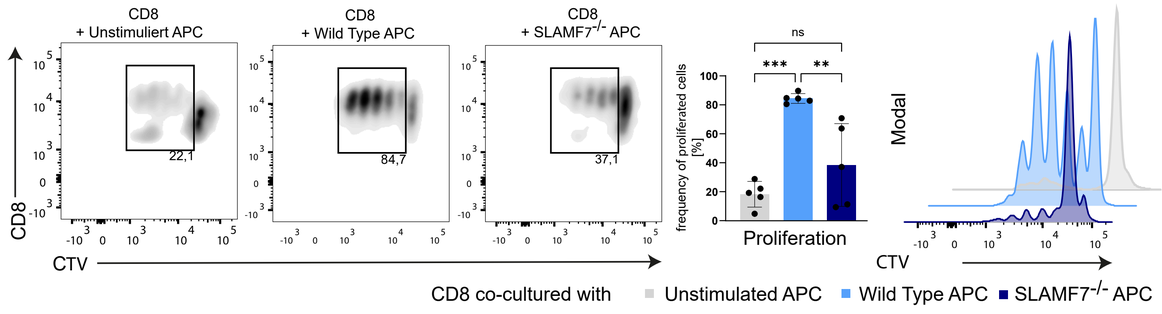

Fig. 3. Reduced SLAMF7 signal impairs proliferation of Tc1 CD8 effector T cells.
Flow cytometric analysis of stimulated CD8 T cells co-cultured with either WT APC or KO APC. CD8 T cells were stained with 1 µg/ml CTV to assess the proliferation three days post stimulation. The frequency of proliferated cells across generations 1 to 5 was determined from five independent experiments and is depicted in the histogram. Statistical analysis: One-way ANOVA followed by Holm-Šídák's multiple comparisons test: *p < 0.05; **p < 0.005; ***p < 0.0005.

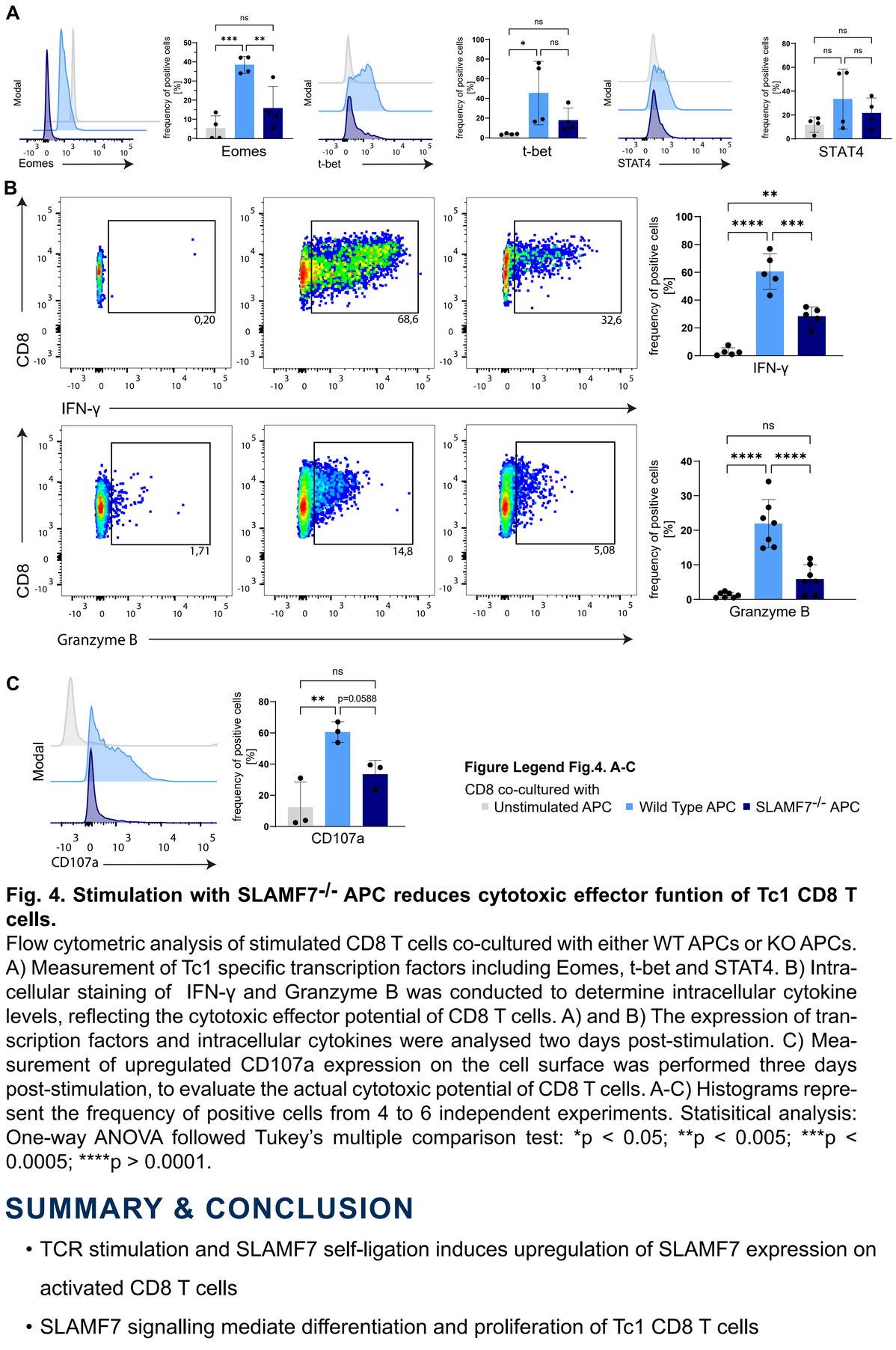

Fig. 4. Stimulation with SLAMF7$^{-/-}$ APC reduces cytotoxic effector funtion of Tc1 CD8 T cells.
Flow cytometric analysis of stimulated CD8 T cells co-cultured with either WT APCs or KO APCs. A) Measurement of Tc1 specific transcription factors including Eomes, t-bet and STAT4. B) Intracellular staining of IFN-γ and Granzyme B was conducted to determine intracellular cytokine levels, reflecting the cytotoxic effector potential of CD8 T cells. A) and B) The expression of transcription factors and intracellular cytokines were analysed two days post-stimulation. C) Measurement of upregulated CD107a expression on the cell surface was performed three days post-stimulation, to evaluate the actual cytotoxic potential of CD8 T cells. A-C) Histograms represent the frequency of positive cells from 4 to 6 independent experiments. Statisitical analysis: One-way ANOVA followed Tukey's multiple comparison test: *p < 0.05; **p < 0.005; ***p < 0.0005; ****p > 0.0001.

SUMMARY & CONCLUSION

- TCR stimulation and SLAMF7 self-ligation induces upregulation of SLAMF7 expression on activated CD8 T cells
- SLAMF7 signalling mediate differentiation and proliferation of Tc1 CD8 T cells
- Activation of SLAMF7 on CD8 T cells enhances their cytotoxic effector function, as evidenced by increased intracellular levels of IFN-γ and Granzyme B
- CD107a upregulation on CD8 T cells stimulated with WT APC reflects an elevated cytotoxic capacity of Tc1 CD8 T cells

Conflict of interest declaration

The authors declare that they have no conflict of interest.