SLAMF7 on CD8+ T cells adjusts cytotoxic Effector Populations in Responses to low antigenic Activation

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ABSTRACT

CD8+ T-cell activation is a highly coordinated process that is regulated by receptor-mediated cellular interactions. These interactions play a critical role in controlling the dynamics of cytotoxic T-cell populations, which are crucial for overcoming threats like viral infections or cancer. However, the mechanisms governing these dynamics are not yet completely understood. Here, we identified a previously unknown function of the self-ligating surface receptor SLAMF7 (CD319) on CD8+ T cells as a novel regulator of CD8+ T-cell priming and response decisions. We found abundant expression of SLAMF7 on the surface of cytotoxic T lymphocytes, suggesting that it may function to modulate their effector differentiation. Pro-inflammatory cytokines, such as IL-12, and the strength of the initial stimuli directly up-regulated its expression, suggesting that CD8+ T cells could use SLAMF7 to transduce inflammatory cues into cellular interactions and information exchange. Specifically, SLAMF7 promoted the dose-dependent formation of stable homotypic contacts that lead to priming, quorum populations, and differentiation commitment. By conducting pull-down assays and network analysis, we identified novel SLAMF7-binding intracellular signaling molecules, including the CRK, CRKL and Nck adaptors, that may mediate SLAMF7 functions in sensing and adhesion. Therefore, amplification of SLAMF7 signaling during activation of CD8+ T cells increased their overall size, particularly in response to low affinity antigens, resulting in a significant increase in their proliferation and cytotoxic capacity.

METHODS

The impact of SLAMF7 on early CD8+ T-cell activation and differentiation events was assessed by *in vitro* assays and analyzed by flow cytometry or monitored *in vivo* by Multi-Epitope-Ligand-Cartography (MELC). Using SLAMF7 peptide pull-down combined with mass spectrometry identified putative interacting intracellular signaling molecules of SLAMF7 in CD8+ T cells.

RESULTS

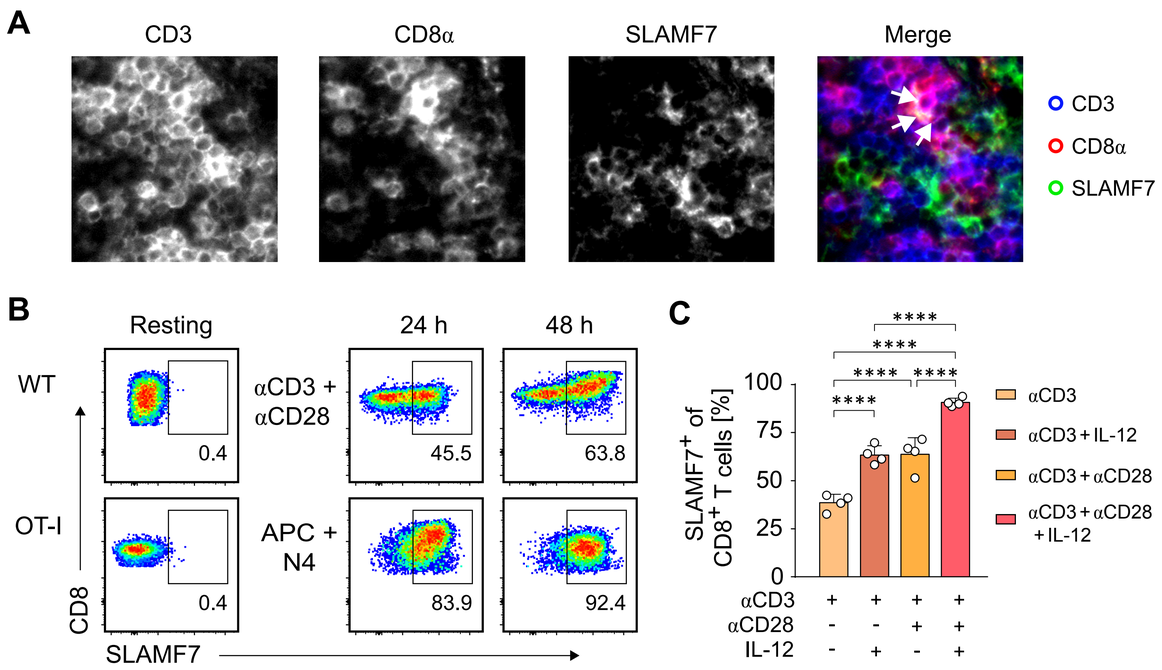

Fig. 1. SLAMF7 expression on CD8+ T cells is dependent on T-cell activation signals (A) Multi-Epitope-Ligand-Cartography (MELC) analysis of mouse spleen sections. **(B)** WT or OT-I CD8+ T cells were activated with αCD3 and αCD28 coated microspheres or $OVA_{257-264}$ (N4) peptide pulsed APCs, respectively, or not (resting) and SLAMF7 abundancies on CD8+ T cells were analyzed by flow cytometry. **(C)** WT CD8+ T cells were activated by microspheres coated with αCD3 with or without αCD28 and/or IL-12 and SLAMF7 expression on CD8+ T cells was assessed after 48 h.

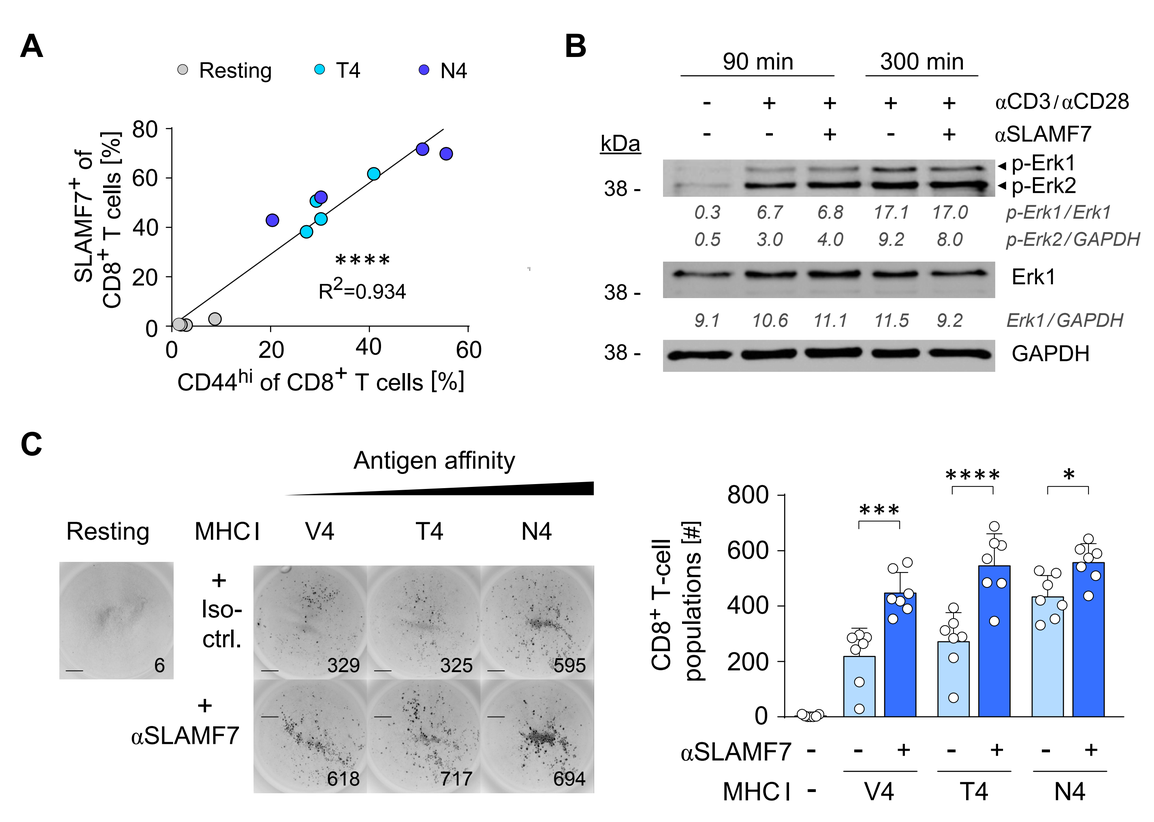

Fig. 2. SLAMF7 promotes initial population formation of activated CD8+ T cells. (A) OT-I CD8+ T cells were activated with microspheres coated with T4- or N4-peptide pulsed MHC I comlexes, or not (resting) and correlation of CD44 and SLAMF7 expression on CD8+ T cells was assessed by flow cytometry after 24 h. **(B)** Western Blot analysis of Erk phosphorylation in resting WT CD8+ T cells or 90 and 300 min after activation by microspheres coated with αCD3 and αCD28 antibodies in combination with or without agonistic αSLAMF7 antibodies, respectively. **(C)** Inverted grayscale images of cell culture wells (left panel) and numbers (right) of CFSE-labelled population-forming OT-I CD8+ T cells activated by microspheres coated with CD80 and V4-, T4-, or N4-peptide pulsed MHC I complexes in combination with or without agonistic αSLAMF7 antibodies, respectively, or not (resting).

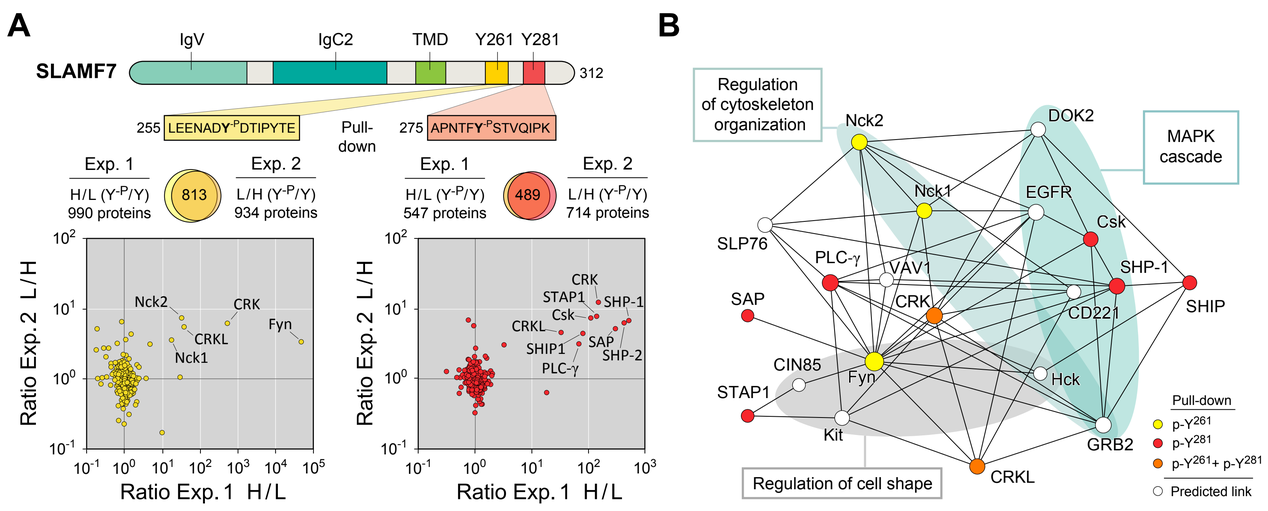

Fig. 3. SLAMF7 signaling impinges on contact formation. (A) Pull-down experiments using phosphorylated (Y-p) or non-phosphorylated (Y) SLAMF7 Y261 (yellow) or Y281 (red) peptid motifs. Plots show the isotope ratios of all quantified proteins determined in two independent experiments with switched labeling by heavy (H) or light (L) water. **(B)** Network of possible protein interactions of identified SLAMF7-bound proteins (A) analyzed by NetworkAnalyst software. Significantly (p<0.001) affected functional subsets are highlighted as calculated by GO:BP enrichment analysis.

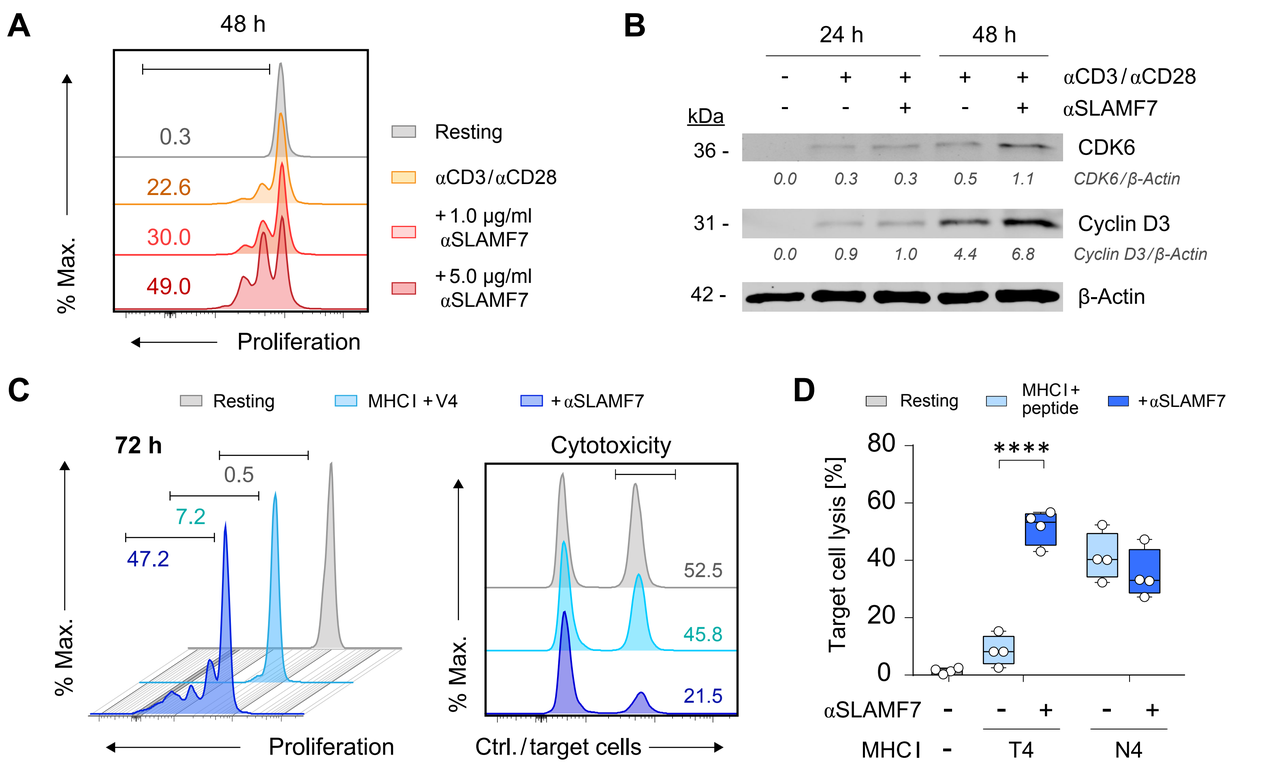

Fig. 4. SLAMF7 signals enhance CD8+ T-cell responses towards low-affinity antigens. (A) WT CD8+ T cells were activated by microspheres coated with αCD3 and αCD28 antibodies in combination with or without increasing concentrations of agonistic αSLAMF7 antibodies, respectively, or not (resting). Proliferation of cells was monitored by CFSE dilutionafter 48 h. **(B)** Western Blot analysis of cell cycle regulators CDK6 and Cyclin D3 in WT CD8+ T cells at 24 h and 48 h after activation as desceibed in (A). **(C)** OT-I CD8+ T cells activated by microspheres coated with CD80 and V4-pulsed MHC I complexes in combination with or without agonistic αSLAMF7 antibodies, respectively, or not (resting). Proliferation (left) of cells was analyzed after 72h. Cytotoxic capacity (right) of accordingly pre-activated OT-I CD8+ T cells was assessed by decrease of N4-pulsed high-fluorescent target cells in relation to unpulsed low-fluorescent control cells. **(D)** Cytotoxicity assay (C) of OT-I CD8+ T cells 48h pre-activated by microspheres coated with CD80 and T4- or N4-peptide pulsed MHC I complexes in combination with or without agonistic αSLAMF7 antibodies, respectively, or not (resting).

CONCLUSIONS

- SLAMF7 acts as novel regulator of CD8+ T-cell priming and response decisions
- SLAMF7 is a target to adjust CD8+ T-cell responses against weak viral or tumor-associated antigens in advanced immunotherpeutic approaches

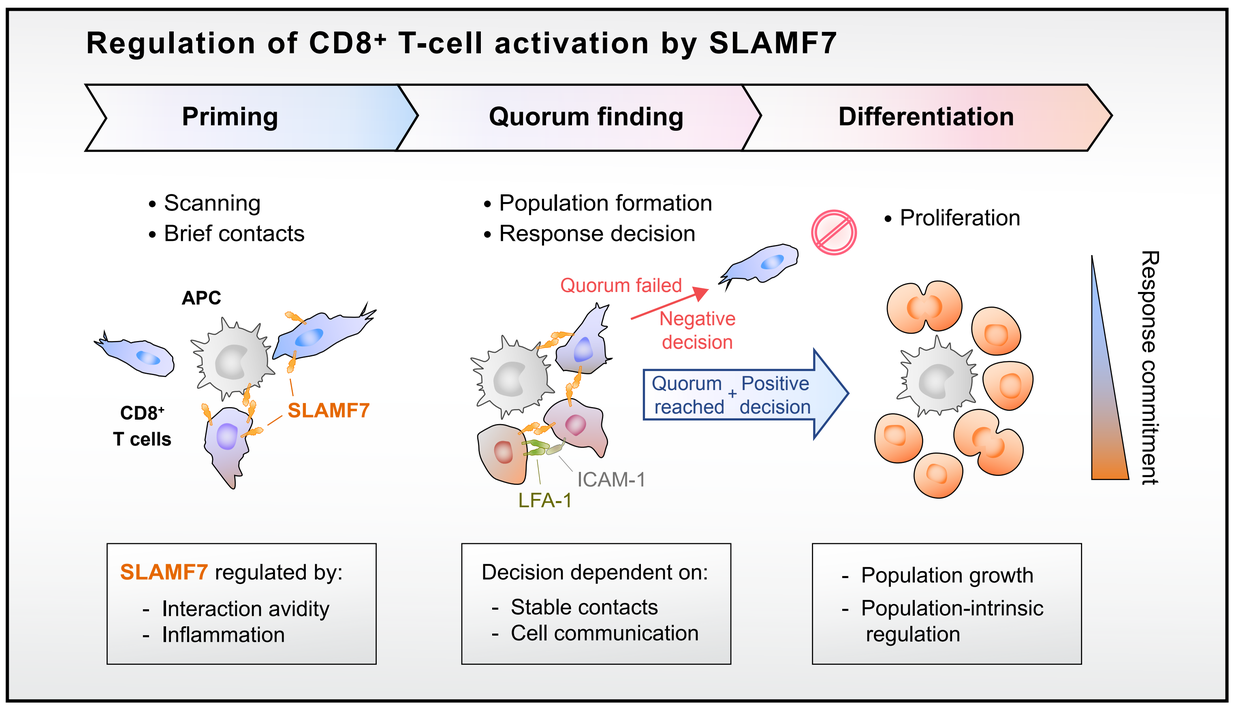

Fig. 5. Model of SLAMF7-mediated functions during CD8+ T-cell activation. During priming phase T-cell-initiated antigen recognition induces SLAMF7 expression that integrates the activation strenght of stimuli as well as inflammatory cues. SLAMF7 signals then transmit these information to facilitate the formation of T-cell populations through stable homotypic contacts that are neccessary for Quorum regulation and cell communication. After a Quorum has been reached, T-cells could collectively decide wether the CD8+ T-cell expansion and differentiation program is started or not.

This study was funded by the German Research Foundation (DFG, CRC854 B14 and Br1860/12).