IL-2 INDUCES A REVERSIBLE EXPRESSION OF TIM-3 IN THE EXPANDED CD8+ T CELLS AND AFFECTS THEIR PHENOTYPE AFTER THE ANTIGEN RE-CHALLENGE

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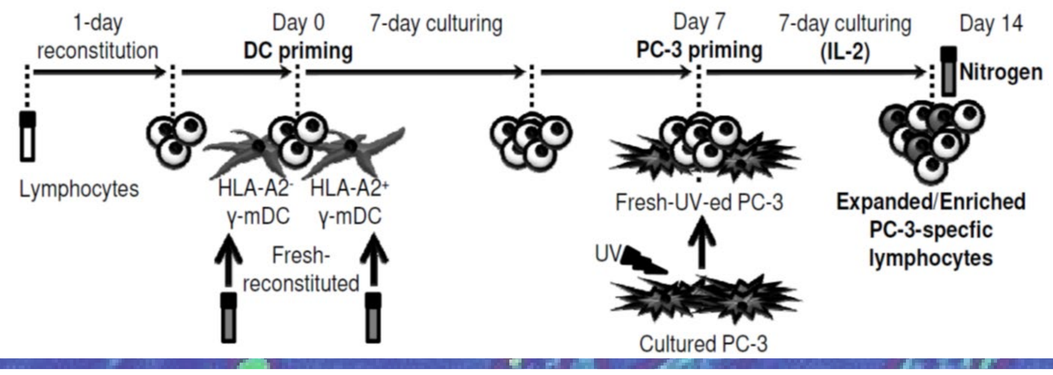
SUMMARY

IL-2 is a widely used cytokine for the expansion of T cells ex vivo. This cytokine induces expression of Tim-3 on the surface of T cells and differentiation of T cells into short-lived effector memory T cells. However, it is not known how IL-2 affects T cell differentiation after the antigen re-challenge. We found that acute cytokine starvation substantially reversed IL-2-elicited Tim-3 expression in the antigen-expanded CD8+ T cells and downregulated immediate IFNγ and TNFα production after the antigen re-challenge. Following their re-expansion, the antigen-stimulated cells were, however, able to produce significantly more TNFα than their IL-2-non-starved counterpart. Similar to the cytokine starvation, acute conditioning of antigen-expanded CD8+ T cells with the GSK3β inhibitor TWS119 or mTORC1 inhibitor Rapamycin before the antigen re-challenge also significantly enhanced inflammatory responses of the re-expanded CD8+ T cells. Our results demonstrate that the presence of inflammatory cytokines before and during the antigen re-challenge can have a split impact on the immediate and post-re-expansion responsive phenotype of antigen-experienced CD8+ T cells and that this impact can be modulated by acute conditioning of the cells with GSK3β or mTORC1 inhibitors.

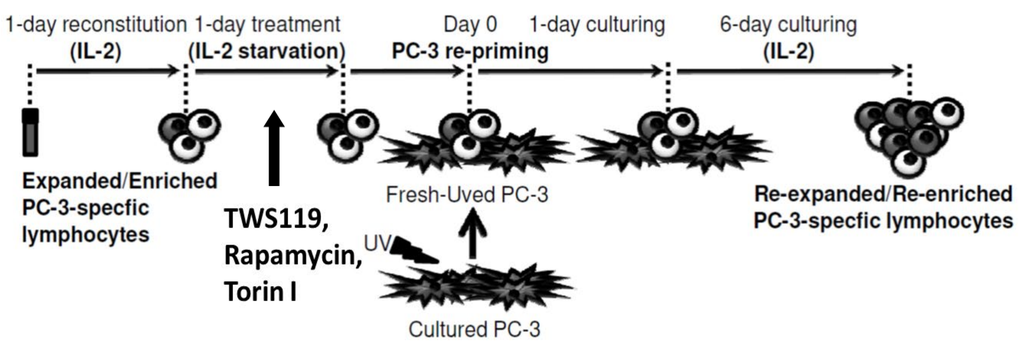
METHODS

The *ex vivo* expanded T cells were generated from lymphocytes using allogeneic monocyte-derived matured dendritic cells (DCs), inactivated PC-3 prostate cancer (PCa) cell line, and IL-2 (**A**). The expanded cells were cultured overnight in the presence or absence of IL-2 (80 IU/ml). Alternatively, the cells were cultured overnight in the presence of IL-2 and GSK3β or mTORC1 inhibitors. The cells were analyzed for Tim-3 expression or re-challenged with inactivated PC-3 for 1 day. Then the cells were supplemented with IL-2 (4000 IU/ml) and cultured for 6 days. (**B**) The inflammatory responses of the cells were analyzed by FACS using IFNγ and TNFα -specific antibodies.

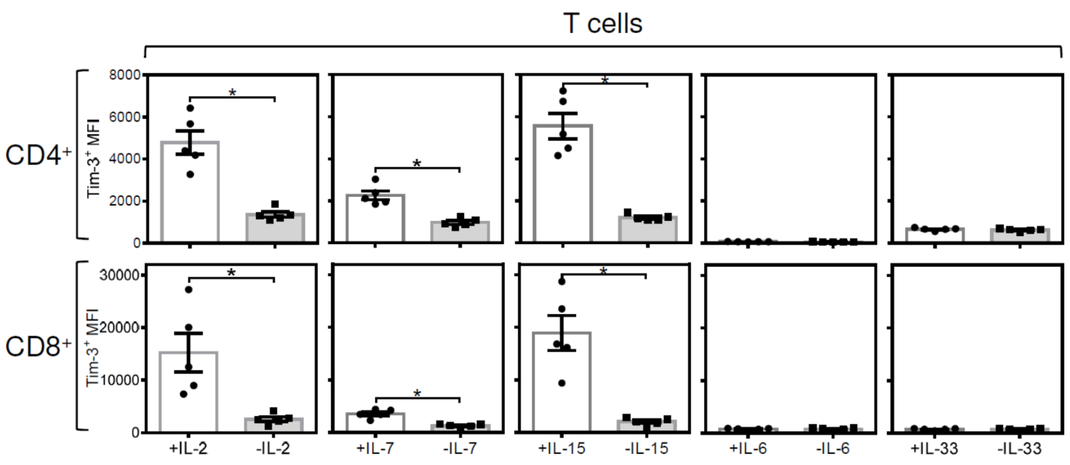
A. Enrichment and expansion of antigen-specific CD8+ T cells.



B. Acute starvation of IL-2 or GSK3β/mTORC1 inhibition before antigen re-challenge and re-expansion of CD8+ T cells



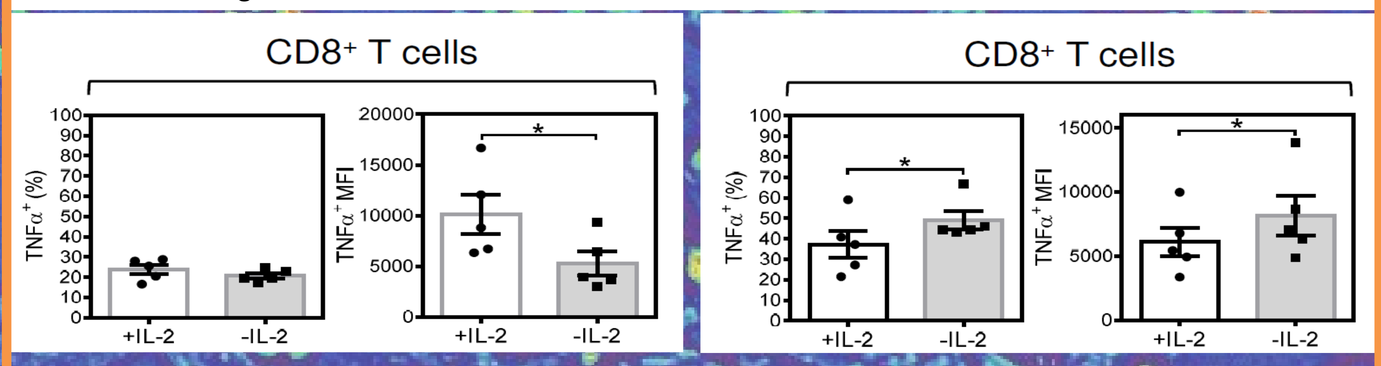
4 IL-2, IL-7, and IL-15 induce reversible surface expression of Tim-3 in antigen-expanded T cells.



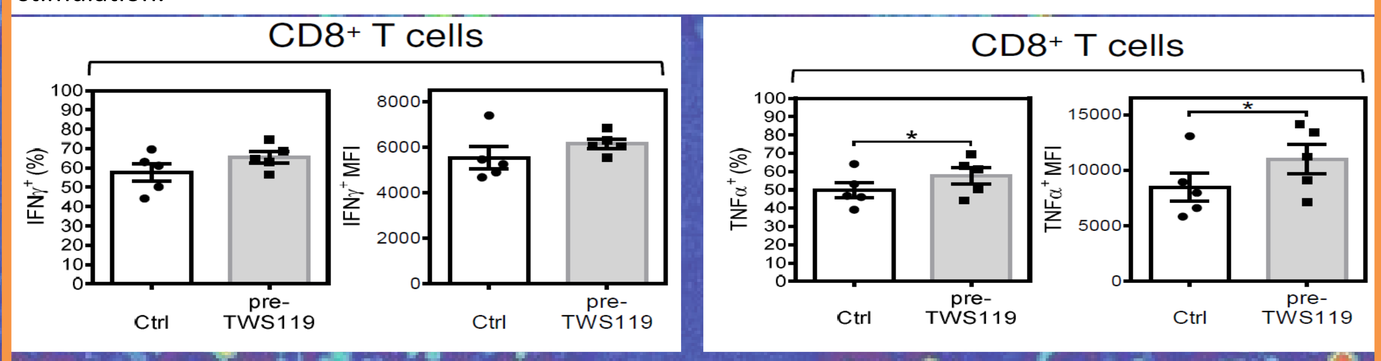
5 Acute conditioning of the expanded CD8+ T cells with GSK-3β inhibitor TWS119 downregulate expression of Tim-3.

RESULTS

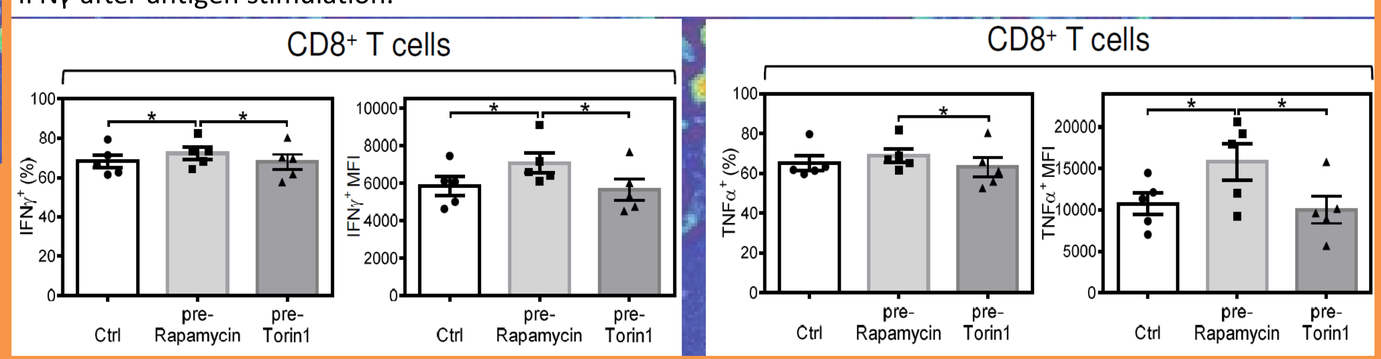
1 Acute starvation of IL-2 diminishes the robustness of the immediate response of expanded CD8+ T cells after their stimulation with antigen but promotes re-expansion of T lymphocytes with enhanced production of TNFα after antigen re-stimulation.



2 Acute conditioning of the expanded CD8+ T cells with GSK-3β inhibitor TWS119 prior to antigen re-challenge promotes re-expansion of CD8+ T cells with enhanced production of TNFα after antigen stimulation.



3 Acute conditioning of the expanded CD8+ T cells with mTOR complex inhibitor Rapamycin but not Torin1 prior to antigen re-challenge promotes re-expansion of CD8+ T cells with enhanced production of TNFα and IFNγ after antigen stimulation.



CONCLUSIONS

- Expression of Tim-3 on the surface of antigen-experienced CD8+ T cells was highly reversible and sensitive to inflammatory cytokine concentration in the media.
- Acute interventions with GSK-3β inhibitor TWS119 downregulate the expression of Tim-3 on CD8+ T lymphocytes.
- Preconditioning of expanded antigen-specific CD8+ T cells with GSK-3β or mTORC1 inhibitors enhances the distal inflammatory response of re-expanded CD8+ T cells

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