Superior expansion of T cells using NKG2D-targeted delivery of IL-2: Implications for adoptive T cell immunotherapy

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Abstract

Introduction: Infusion of activated autologous tumor-infiltrating lymphocytes or CAR-T cells is a promising clinical treatment for both solid and liquid tumors. Traditional methods for ex vivo T cell expansion have relied on transient stimulation of the T cell receptor in the presence of the common γ-chain cytokines. Although it has been routine for most clinical and experimental laboratories to rely on interleukin-2 (IL-2) to support T cell expansion, this cytokine can result in expansion of regulatory T cells (Tregs) and activation induced death of effector cells.

Furthermore prolonged exposure to IL-2 may result in differentiation of CD8+ T cells toward and effector phenotype, a terminally differentiated state that provides only short-lived and highly limited protection from malignancy. Alternate gamma chain cytokines, such as IL7 and IL15, do not expand Tregs and may offer an advantage for the expansion of central memory CD8+ T cells, that are superior in mediating tumor regression. Nevertheless optimal protocols for T cell expansion have yet to be defined.

We have recently demonstrated that IL2 targeted to NKG2D-expressing cells using the viral decoy ligand known as Orthopox Major Histocompatibility Complex Class I-like Protein (or OMCP for short), offers a superior method for in vivo NK cell expansion and NK mediated immunotherapy (1,2). Unlike the case for wild-type IL-2, our targeted cytokine approach relies on the activating receptor NKG2D for its high affinity interaction (Figure 1). The possibility of using this redirected cytokine (called OMCP-mutIL-2 for short) for ex vivo T cell expansion has not been explored.

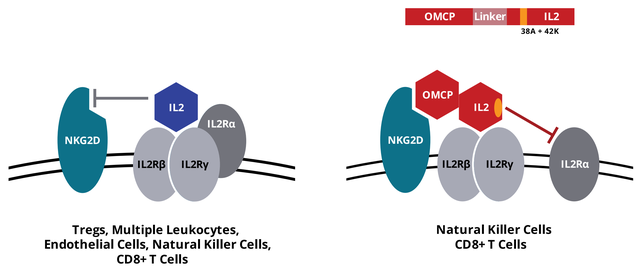

Figure 1: Unlike the case for wild-type IL-2, which uses the broadly-expressed IL2 receptor α chain (CD25) to mediate the high affinity interaction with the signaling portion of the IL-2 receptor (βχ chains), OMCP-mutIL-2 relies on NKG2D for its high affinity interaction

Methods: T cells were isolated from C57BL/6 splenocytes and stimulated with anti-CD3/CD28 agonistic antibodies for 72 hours in the presence of low or high dose cytokines. The antibodies were washed off after 72 hours but the T cells were expanded in cytokines for the duration of the study.

Results

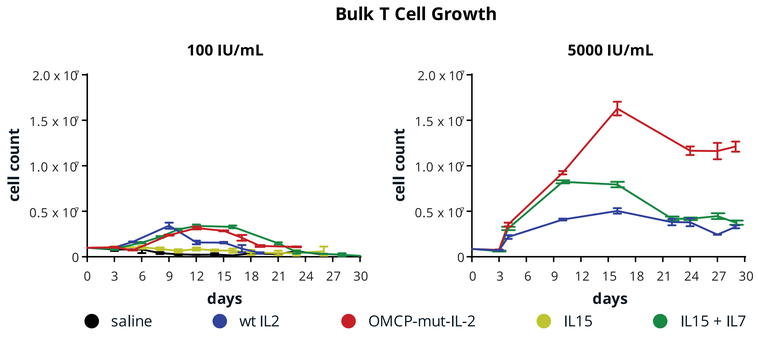

Figure 2: At low doses of 100U/ml wild-type IL2, OMCP-mutIL-2 and combination of IL7/15 resulted in similar T cell expansion while T cells in IL15 alone or in the absence of cytokines die over a period of three weeks. In high doses of 5000U/ml OMCP-mutIL2-treated T cells expand for as long as 35 days post stimulation.

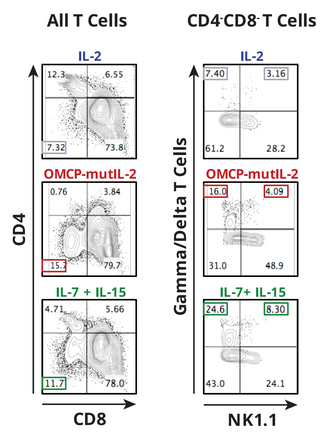

Figure 3: Sixteen days after start of expansion IL-2-treated cultures contain CD8+ and CD4+ T cells while OMCP-mutIL-2 and IL-7/15 cultures contain 10-15% CD4-CD8- T cells (left). CD4-CD8- T cells are comprised of gamma/delta and NKT cells (right panel).

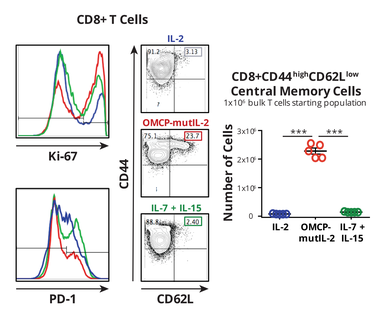

Figure 4: Sixteen days after start of expansion OMCP-mutIL-2-treated CD8+ T cells demonstrate higher expression of the early proliferation antigen KI-67 and have lower surface levels of PD-1 than CD8+ T cells from IL-2 and IL-7/15 treated cultures (left). OMCP-mutIL-2 treated cultures contain more $CD62L^{high}CD44^{high}$ central memory T cells than IL-2 and IL7/15 treated cultures where all T cells develop the $CD62L^{low}CD44^{high}$ effector phenotype (middle and right panels).

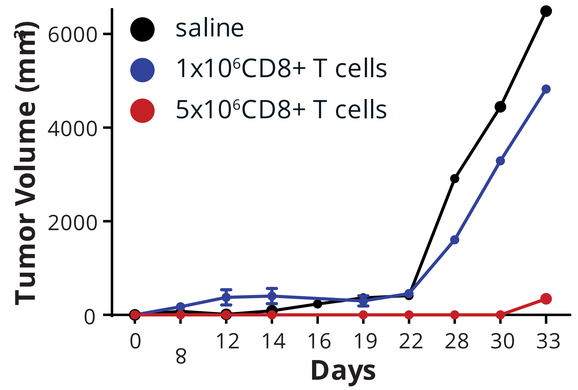

Figure 5. To test the efficacy of our system in an in vivo model we expanded OT-1 ovalbumin TCR transgenic T cells in OMCP-mutIL-2 and 16 days later transferred either none, $1x10^6$ or $5x10^6$ CD8+ T cells to B16-ova bearing mice. $5x10^6$ CD8+ T cells expanded in OMCP-mutIL-2 significantly ameliorated B16-ova growth.

Conclusions

In summary we now demonstrate that the use of an NKG2D-targeted form of IL2 provides a significant quantitative and qualitative advantage for T cell expansion in vitro. It leads to more robust T cell expansion, generation of NKT cells and gamma delta T cells, as well as higher numbers of central memory CD8+ T cells. Since the ability to obtain a significant number of CAR T cells as well as tumor infiltrating lymphocytes limits the clinical applicability of both of these technologies, the possibility of quickly and efficiently expanding T cells using an NKG2D-targeted approach may offer novel solutions to improve clinical outcomes.

References

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2) Lazear E, Ghasemi R, Hein SM, Westwick J, Watkins D, Fremont DH, Krupnick, AS. Targeting of IL-2 to cytotoxic lymphocytes as an improved method of cytokine-driven immunotherapy. Oncoimmunology, 6(2):e1265721, 2017.