NOVEL WAYS TO EXPLOIT IL-21 TO AUGMENT ADOPTIVE T CELL TRANSFER THERAPY AGAINST TUMORS

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Background IL-21 enhances the anti-tumor capacity of adoptively transferred CD8+ T cells, while IL-2 and IL-15 impair T cell immunity by driving their expansion to a more differentiated status. Yet, these cytokines can act on many different immune cells. Given the potency of IL-21, we tested if this cytokine directly augments T cells or rather if it enhances other immune cells in the culture that indirectly improves T cell therapy.

Methods To test this question, splenocytes from pmel-1 transgenic mice were used, as all CD8+ T cells express a transgenic TCR specific for tumor-antigen gp10025–33 overexpressed on melanoma. We then peptide activated naïve CD8+ T cells enriched or not from the spleen of pmel-1 mice and expanded them in the presence of IL-21 or IL-2 (10 ng/mL) for four days. Expanded pmel-1 from these various cultures were then restimulated with irradiated splenocytes pulsed with gp10025–33 and grown an additional seven days with IL-2 (10 ng/mL), irrespective of their initial cytokine condition. The in vitro memory phenotype, exhaustion profile, and cytokine secretion of these cultures were then assayed. Furthermore, mice bearing B16KVP melanoma tumors were infused with pmel-1 T cells expanded via these various approaches and compared for their relative capacity to engraft, persist, and regress tumor in vivo.

Results Interestingly, we discovered that IL-21-treated T cells generated from bulk splenocytes are phenotypically and functionally distinct from IL-21-treated isolated T cells. Upon restimulation, IL-21-treated T cells from bulk splenocytes exhibited an exhausted phenotype that was like anergic IL-2-treated T cells. Moreover, few cells expressed CD62L but expressed heightened markers of suppression, including TIM3, PD-1, and EOMES. Moreover, they produced more effector molecules, including granzyme B and IFN-gamma. In vivo IL-21-treated T cells expanded from bulk splenocytes engrafted and persisted poorly, in turn mediating suboptimal regression of melanoma. Conversely, IL-21 dramatically bolstered the engraftment and antitumor activity of T cells only if they were first isolated from the spleen prior to their expansion and infusion into the animal.

Conclusions Collectively, our data shows that IL-21 may improve ACT therapy best when used directly on antitumor CD8+ T cells. Further studies will illuminate the mechanism behind this striking difference and determine whether other cell subsets reactive to IL-21 cause T cell dysfunction and/or reduced bioavailability. These findings are important for defining the best culture conditions in which to use IL-21 for ACT.

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Ethics Approval All animal procedures were approved by the Institutional Animal Care and Use Committee of Emory University, protocol number 201900225.