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CORRESPONDENCE

Challenges of iNKT cell-based antitumor immunotherapies

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Invariant natural killer T (iNKT) cells are good candidates for antitumor immunotherapies due to their functional advantages and low toxicity. Their promising utility has been exhibited by studies in animal models and clinical trials. Here, we discuss the strategies and challenges of iNKT cell-based immunotherapies.

iNKT cells express a semi-invariant T cell antigen receptor (TCR), with an α chain variable region encoded by *Va24Ja18* in humans and *Va14Ja18* in mice. Unlike conventional T cells, iNKT cells show an effector memory phenotype and recognize lipid antigens presented by nonpolymorphic CD1d molecules.[1] Due to the broad expression of CD1d by professional and nonprofessional antigen-presenting cells (APCs), iNKT cells are able to directly regulate the function of multiple immune cells, including dendritic cells (DCs), B cells, and macrophages, in a reciprocal manner. Upon activation, iNKT cells can rapidly produce large amounts of Th1 and Th2 cytokines, including IFNγ, TNFα, IL2, IL4, and IL13, and thus indirectly modulate the function of bystander cells, including natural killer (NK) cells and T cells. These properties make iNKT cells important immunoregulatory cells in vivo. Additionally, iNKT cells can kill target cells by perforin- and granzyme-mediated cytotoxicity, similar to NK and CD8 T cells. The immunosurveillance role of iNKT cells against tumors has been demonstrated by studies in both mice and humans, as indicated by the increased tumor growth in iNKT cell-deficient mice as well as the beneficial effects seen in the clinic after activating iNKT cells in vivo.[2] Mechanistic studies revealed that CD1d-expressing tumor cells can be directly killed by iNKT cells via recognition of lipid antigens. For tumor cells without CD1d expression, activation of iNKT cells promotes tumor clearance by transactivating NK and CD8 T cells, as well as by depleting tumor-associated macrophages.[1] These direct and indirect tumor clearance strategies, in addition to the low risk of graft-versus-host disease (GVHD) in allogeneic cell infusion due to the nonpolymorphism of CD1d, make iNKT cells ideal candidates for antitumor immunotherapies.

Previous clinical studies have shown that strategies transferring expanded iNKT cells and activating iNKT cells with APCs that have been pulsed with the lipid antigen α-galactosylceramide (αGalCer) or αGalCer alone are safe and feasible. Interestingly, administration of αGalCer-pulsed DCs better induces Th1 responses than αGalCer administration and thus exhibits stronger antitumor effects.[3] Although iNKT cells release both Th1 and Th2 cytokines, some αGalCer variants, such as AH10-7 and α-C-GalCer, induce Th1-biased immune responses.[1,4] Therefore, modifying the lipid antigens to skew toward Th1 responses would presumably augment iNKT cell-mediated antitumor responses. On the other hand, activation of iNKT cells promotes CD8 T cell responses by enhancing the maturation of DCs. Based on the adjuvant activity of iNKT cells, some new strategies are designed to augment adaptive T cell responses by forcing DCs, tumor cells or artificial APCs to simultaneously present the lipid antigen αGalCer and intracellular tumor antigens.[2] Recently, the application of chimeric antigen receptor (CAR)-T cells in immunotherapy has achieved remarkable therapeutic effects. A similar strategy has been used to generate CAR-iNKT cells, and these CAR-iNKT cells directly kill tumor cells by targeting tumor cell surface antigens.[5,6] Clinical trials using CAR-iNKT cells specific for the ganglioside GD2 (NCT02439788 and NCT03294954) and CD19-specific CAR-iNKT cells (NCT03774654) have been launched against neuroblastoma and B cell lymphoma, respectively.

Similarly, remarkable progress has been achieved in generating functional iNKT cells. iNKT cells can be efficiently expanded in vitro. The Metelitsa group has demonstrated that antigen-induced expansion of iNKT cells generates $CD62L^+$ and $CD62L^-$ populations. $CD62L^+$ iNKT cells but not $CD62L^-$ iNKT cells have prolonged persistence and strong antitumor activity.[6] Combined IL2 and IL21 treatments could increase the frequency of $CD62L^+$ iNKT cells during their expansion.[7] In addition, the Yang group recently developed a way to generate iNKT cells in vivo by engineering hematopoietic stem cells (HSCs) with an iNKT TCR gene.[8] This engineered TCR instructs the development of iNKT cells in humanized mouse models, which efficiently suppress tumor growth. Due to the self-renewal and longevity of HSCs, this approach presumably could provide patients with large numbers of iNKT cells throughout their lifetime.

Both the direct killing driven by the iNKT TCR or CAR and the indirect tumor clearance mediated by the immunoregulatory role of iNKT cells depend on the normal function of iNKT cells in tumors. However, dysfunction of iNKT cells in tumors, particularly their reduced IFNγ production, has been reported and hinders the efficacy of iNKT cell-based immunotherapies.[9] Notably, the function of iNKT cells can be modulated by many factors in the tissue microenvironment, including the type of APC, antigen variants, cytokines, and metabolites.[1] Compared to the normal tissue microenvironment, the tumor microenvironment differs in these factors and consequently alters the function of tumor-infiltrating iNKT cells. Due to the active glycolysis of tumor cells, the tumor microenvironment is characterized by glucose restriction and lactic acid accumulation, both of which have been recently reported to interfere with iNKT cell function.[9–11] We found that glycolysis promotes TCR vesicle recycling and thus maintains TCR signaling in iNKT cells. Sustained TCR signaling is required for optimal IFNγ production. Therefore, glucose restriction reduces IFNγ production in iNKT cells by interfering with TCR signaling.[11] Due to the lower dependence of IL4 production on TCR signaling duration, glucose restriction has a minor effect on IL4 production and leads to Th2 polarization of iNKT cells, which

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favors tumor growth. Additionally, we revealed that lactic acid inhibits IFNγ production in intratumoral iNKT cells by interfering with their lipid synthesis. Lactic acid reduces PPARγ expression in iNKT cells by inhibiting the activation of mTORC1. In cooperation with PLZF, the master transcription factor of iNKT cells, PPARγ promotes transcription of SREBP1, which controls lipid synthesis. In addition, cholesterol augments TCR signaling and IFNγ production. Therefore, reduced PPARγ expression as a result of lactic acid accumulation in the tumor microenvironment inhibits cholesterol synthesis and IFNγ production in tumor-infiltrating iNKT cells in both mouse models and hepatocellular carcinoma patients.[9] In addition to metabolic reprogramming, impaired crosstalk between iNKT cells and DCs has been indicated in tumors and is related to impaired antitumor immune responses. iNKT cell-DC crosstalk enhances Th1 immune responses and is promoted by polarized secretion of IL4 by iNKT cells under the control of Cdc42.[12] Notably, the Cdc42 molecule also controls cell migration and interactions. Considering the reduced Cdc42 expression of intratumoral iNKT cells, impaired cell interactions, and activation are likely and would also contribute to their dysfunction. In addition, other factors might also contribute to the dysfunction of intratumoral iNKT cells, and more studies are required to reveal the mechanisms.

As mentioned above, dysfunction of intratumoral iNKT cells hinders their immunosurveillance role against tumor cells, and therefore, resetting the function of intratumoral iNKT cells is another direction to further improve the efficacy of iNKT cell-based immunotherapies, in addition to identifying better Th1-biased lipid ligands, engineering APCs, and engineering CAR. For example, coexpressing IL-15 in CAR-iNKT cells increases their localization to tumor sites and improves tumor control without significant toxicity.[13] In our study, activating PPARγ with its agonist while administrating αGalCer restored cholesterol synthesis and IFNγ production in tumor-infiltrating iNKT cells and thus augmented antitumor immune responses and resulted in superior tumor control.[9] It is rational that targeting intratumoral iNKT cell migration, interactions, and activation to increase cell numbers and reset their function would result in superior clinical outcomes.

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ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

REFERENCES

1. Zhu, S. S. et al. NKT cells in liver diseases. *Front. Med.* **12**, 249–261 (2018).
2. Fujii, S. I. et al. Immune networks and therapeutic targeting of iNKT cells in cancer. *Trends Immunol.* **40**, 984–997 (2019).
3. Kunii, N. et al. Combination therapy of in vitro-expanded natural killer T cells and alpha-galactosylceramide-pulsed antigen-presenting cells in patients with recurrent head and neck carcinoma. *Cancer Sci.* **100**, 1092–1098 (2009).
4. Chennamadhavuni, D. et al. Dual modifications of alpha-galactosylceramide synergize to promote activation of human invariant natural killer T cells and stimulate anti-tumor immunity. *Cell Chem. Biol.* **25**, 571–84.e8 (2018).
5. Heczey, A. et al. Invariant NKT cells with chimeric antigen receptor provide a novel platform for safe and effective cancer immunotherapy. *Blood* **124**, 2824–2833 (2014).
6. Tian, G. W. et al. CD62L(+) NKT cells have prolonged persistence and antitumor activity in vivo. *J. Clin. Invest* **126**, 2341–2355 (2016).
7. Ngai, H. et al. IL-21 selectively protects CD62L(+) NKT cells and enhances their effector functions for adoptive immunotherapy. *J. Immunol.* **201**, 2141–2153 (2018).
8. Zhu, Y. N. et al. Development of hematopoietic stem cell-engineered invariant natural killer T cell therapy for cancer. *Cell Stem Cell* **25**, 542 (2019).
9. Fu, S. et al. Impaired lipid biosynthesis hinders anti-tumor efficacy of intratumoral iNKT cells. *Nat. Commun.* **11**, 438 (2020).
10. Xie, D. et al. Lactic acid in tumor microenvironments causes dysfunction of NKT cells by interfering with mTOR signaling. *Sci. China Life Sci.* **59**, 1290–1296 (2016).
11. Fu S. C. et al. Immunometabolism regulates TCR recycling and iNKT cell functions. *Sci Signal* **12**, eaau1788 (2019).
12. Wang, L. et al. Spatial distribution of IL4 controls iNKT cell-DC crosstalk in tumors. *Cell Mol. Immunol.* **17**, 496–506 (2020).
13. Xu, X. et al. NKT cells coexpressing a GD2-specific chimeric antigen receptor and IL15 show enhanced in vivo persistence and antitumor activity against neuroblastoma. *Clin. Cancer Res.* **25**, 7126–7138 (2019).