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Commentary

Spacing out dual checkpoint inhibition improves antigen-specific T cell manufacture

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The adoptive transfer of antigen-specific T cells is a promising therapy for treating infection, cancer, and autoimmunity. To generate large numbers of antigen-specific T cells, however, most protocols rely on *in vitro* culture with cytokines and repeated antigenic stimulation. This leads to terminal T cell effector differentiation and exhaustion, limiting the utility of expanded T cells.[1–3] In this issue of *Molecular Therapy – Methods and Clinical Development*, Lak et al. demonstrate that blocking both programmed death-ligand 1 (PD-L1) and T cell immunoglobulin and mucin-containing protein 3 (TIM-3) during the generation of antigen-specific $CD8^+$ T cells led to improved T cell expansion without inducing T cell dysfunction, but only when blockade was induced with specific timing.[4] This "delayed double blockade," or DDB, did not seem to induce changes in gene expression or T cell receptor (TCR) repertoire in expanded T cells. These findings suggest a translatable strategy for improving the manufacturing of adoptively transferred antigen-specific T cells.

The efficacy of adoptively transferred T cells is often limited by the dysfunction induced by continuous T cell stimulation and stimulatory cytokine treatment during the expansion process.[1–3] Therefore, determining conditions that minimize T cell dysfunction is crucial for the therapeutic potency of adoptive cell immunotherapy. T cell exhaustion is accompanied by the upregulation of cell surface inhibitory receptors known as immune checkpoint molecules. Blocking inhibitory receptor signaling using nonsignaling antibodies, also known as immune checkpoint blockade, enables T cells to overcome these inhibitory mechanisms to mitigate dysfunction.[5] Previously, it had been demonstrated that combined *in vivo* blockade of PD-1 and TIM-3, both of which are upregulated in exhausted CD8+ T cells, led to greater reduction in tumor burden than single blockade alone.[6] In the current work, the authors added anti-PD-L1 on day 1 of *ex vivo* expansion, followed by anti-TIM-3 on day 7. They demonstrated that DDB improved CD8+ T cell expansion and antigen-specific reactivity for both viral and tumor antigens, whereas single blockade of either PD-L1 or TIM-3 alone failed to achieve the same results. Interestingly, compared with double blockade at day 0, DDB showed improved T cell expansion and function. Intriguingly, DDB did not appear to change T effector differentiation or exhaustion marker expression, although DDB-expanded T cells were more proliferative and had improved cytotoxic activity compared with controls. The authors were also unable to identify differences in T cell transcription or in TCR clonotype evolution due to DDB, perhaps as a consequence of high interdonor variability. Moreover, the benefit conferred by DDB was not universal; for instance, neither short-term virus-specific T cell generation nor chimeric antigen receptor (CAR) T cell expansion benefitted from DDB. The authors hypothesize that the beneficial effects of DDB may be most pronounced in long-term culture settings with specific patterns of antigen stimulation; one additional notable difference may be the inclusion of monocyte-derived dendritic cells (DCs) in T cell expansion protocols benefitting from DDB. To explain the context-dependent benefit of DDB, more work is clearly needed to investigate the mechanistic effects of DDB across donors and clonotypes. Nonetheless, this work suggests that dual blockade of PD-L1 and TIM-3 may be useful for improving the *ex vivo* long-term expansion of some types of antigen-specific CD8+ T cells without inducing T cell dysfunction, identifying a potentially translatable improvement in the manufacture of antigen-specific T cell therapies.

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AUTHOR CONTRIBUTIONS

Conception, H.W. and L.D.W.; writing, H.W.; editing, H.W. and L.D.W.

DECLARATION OF INTERESTS

The authors have no conflicts of interest to disclose relevant to the information presented in this Commentary.

REFERENCES

1. Gattinoni, L., Klebanoff, C.A., and Restifo, N.P. (2012). Paths to stemness: building the ultimate antitumour T cell. Nat. Rev. Cancer *12*, 671–684.
2. Janelle, V., Carli, C., Taillefer, J., Orio, J., and Delisle, J.S. (2015). Defining novel parameters for the optimal priming and expansion of minor histocompatibility antigen-specific T cells in culture. J. Transl. Med. *13*, 123.
3. Janelle, V., and Delisle, J.S. (2021). T cell dysfunction as a limitation of adoptive immunotherapy: current concepts and mitigation strategies. Cancers *13*, 598.
4. Lak, S., Janelle, V., Djedid, A., Boudreau, G., Brasey, A., Lisi, V., Smaani, A., Carli, C., Busque, L., Lavallée, V.P., and Delisle, J.S. (2022). Combined PD-L1 and TIM3 blockade improves expansion of fit human $CD8^+$ antigen-specific T cells for adoptive immunotherapy. Mol. Ther. Methods Clin. Dev. *27*, 230–245.
5. Wei, S.C., Duffy, C.R., and Allison, J.P. (2018). Fundamental mechanisms of immune checkpoint blockade therapy. Cancer Discov. *8*, 1069–1086.
6. Zhou, Q., Munger, M.E., Veenstra, R.G., Weigel, B.J., Hirashima, M., Munn, D.H., Murphy, W.J., Azuma, M., Anderson, A.C., Kuchroo, V.K., and Blazar, B.R. (2011). Coexpression of Tim-3 and PD-1 identifies a CD8+ T cell exhaustion phenotype in mice with disseminated acute myelogenous leukemia. Blood *117*, 4501–4510.

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