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Key role of CD4+ T cells in determining CD8 function during CAR-T cell manufacture

J Joseph Melenhorst,[1,2,3] Beatriz C Oliveira[1]

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[1]Cell Therapy & ImmunoEngineering Program, Cleveland Clinic Lerner Research Institute, Cleveland, Ohio, USA
[2]University of Pennsylvania, Philadelphia, Pennsylvania, USA
[3]Department of Pathology and Laboratory Medicine, Parker Institute for Cancer Immunotherapy at University of Pennsylvania, Philadelphia

Correspondence to
Dr J Joseph Melenhorst; melenhj@ccf.org

ABSTRACT

T-cell-based immunotherapies have revolutionized the treatment landscape of blood cancers. Introduced into the clinic in the last decade, chimeric antigen receptor (CAR)-T cells have shown remarkable efficacy in relapsed and refractory B cell malignancies. In pursuing standardized CAR-T cell production, multiple groups have chosen to premanufacture T cells through selective culture of CD4+ and CD8+ T cells. These cells are separately grown, engineered with the CAR, and administered to patients. In this issue of the *Journal of ImmunoTherapy*, Lee *et al* at the Fred Hutchinson center in Seattle, USA, report on the suboptimal expansion and reduced functionality in CD8+CART cells when grown in the absence of CD4+T cells, prompting further investigation that culminated in their paper. Their research demonstrates that the successful production of functional, memory CD8+CART cells is significantly dependent on the activation of CD4+T cells during CAR-T cell generation. Notably, CD8+CART cells produced without CD4+T cells displayed signs of dysfunction, both transcriptionally and immunophenotypically. The paper also reveals key molecules involved in this interaction. This work highlights the crucial role of T cell help in the functionality of CD8+T cells, especially in contexts lacking antigen-presenting cells.

BACKGROUND

Chimeric antigen receptor (CAR)-T cell therapies have led to durable remissions in cancer patients who are at high risk of relapse and death.[1 2] However, despite great success, there is still a significant fraction of patients who do not achieve response. It has been a common goal of various research groups to identify which T cell subsets contribute to optimal clinical responses and to characterize the mechanisms that determine their effective antitumor properties.[3 4] One of the major challenges is the fact that cells obtained from some patients with hematological malignancies, particularly with chronic lymphocytic leukemia, might display dysfunctional phenotypes. Apheresed cells from these patients are characterized by a lower frequency of naïve, and a higher frequency of effector memory and exhausted T cells, which leads to poor efficacy of their CAR-T cell products.

Preclinical models provide the proof of concept required to translate novel findings into clinical trials and are essential to predict the potency and/or efficacy of a given treatment. It has been previously shown that the concurrent utilization of $CD4^+$ and $CD8^+$ T cells demonstrates synergistic antitumor effects, both in vitro and in vivo.[5 6] Those studies have also reported a higher in vivo potency when using defined CD4:CD8 ratios in the CAR-T products as opposed to unselected T cells. A clinical trial involving 29 adult B-ALL patients used equal proportions of $CD4^+$ and $CD8^+$ T cells to manufacture anti-CD19 CAR-T cells, which yielded remarkable effects even though these subsets were manufactured separately (NCT01865617).[7] Nevertheless, these studies highlight the importance of maintaining CAR-T cells in a less differentiated state and having both subsets present within the product to more likely achieve long-term functionality and avoid T cell dysfunction.

COMMENTARY

One question that Lee *et al*'s group[8] raised was that the separate manufacture of CD4+ and CD8+ CART cells adds cost, complexity as well as risks of having a product that does not fulfill the ideal requirements in terms of phenotype and function. They observed that separately cultured CD8+T cells were hypofunctional when challenged with CD19+ target cells, even after they are mixed with CD4+ after infusion. In addition, the preselection of CD4+ and CD8+ T cells takes away one variable which may affect clinical outcome, but this approach will inadvertently remove T cells not expressing either coreceptor. While much remains unclear regarding the nature and function of such cells, they likely to play a role in CAR-T cell response as we recently demonstrated.[9] It has been proven possible

by Lee *et al*[8] and others that it is possible to manufacture and grow both $CD4^+$ and $CD8^+$ T cells together, and then formulate the defined ratio that will be infused. However, a commercial product (lisocabtagene maraleucel), which provides equal target doses of $CD4^+$ and $CD8^+$ T cells that are cultured separately, is an example that this strategy can similarly provide high response rates in patients with relapsed/refractory hematological malignancies. Importantly, on the other hand, some factors that are intrinsic to the patient and vary from one to the other still remain unclear, such as the maintenance of memory and effector function, lack of costimulatory molecules on the surface of some tumor cells, the role of metabolic and epigenetic regulators, and the relationship between lentiviral integration sites and CAR-T function. Another important point raised was regarding the manufacturing conditions of CAR-T cell products that until the moment are not optimized, and vary from one place to the other. During this process, many variables contribute to the function of the final product, such as the T cell selection method, activation with beads versus antibody cocktails (supplemental cytokines) (IL-2, IL-7/15), and culture duration. Insights were provided on how $CD8^+$ T cells become hypofunctional if cultured in the absence of $CD4^+$ T cells in the ex vivo context. This was explained by the $CD4^+$ T cell-mediated cytokine production (elevated IFN-γ, TNF and IL-2 in mixed cultures) and the contact-dependent mechanisms of these cells mediated by the CD40-CD40L and CD27-CD70 axes, that increase $CD8^+$ T cell proliferation and memory formation contributing to their overall effector function. In other words, the ligation on CD8+CAR T cells with CD4+CAR T cells by CD4-CD40L or CD27-CD70 (respectively) provides a costimulatory signal that, paired with cytokines produced by CD4+T cells, increases ex vivo expansion and contributes to a more functional phenotype. This corroborates with data from a previous study which investigated the 'help' $CD4^+$ T cells provide to $CD8^+$ T cells through the CD27-CD70 axis.[10] Cost and time were factors pointed out by the authors that have to be considered during CAR-T cell production. They modified their protocol considering their findings of improved ex vivo expansion and a similar CD4:CD8 ratio at the end of manufacture compared with culture initiation, in order to design a clinical trial. The expected reduced culture times and improved efficacy of this new product can potentially result in a more cost-effective approach to manufacture CAR-T cells.

CONCLUSION

Identifying the ideal composition of CAR-T-cells requires careful evaluation in preclinical models and mechanistic studies, as they provide important information about the biological and molecular components of the various subtypes of T cells and tumor cells, which must be taken into account when designing new or improved products. The authors translated their findings into a clinical trial which is currently ongoing. Despite the fact that there was no significant gap to be filled regarding the role of $CD4^+$ T cells in helping $CD8^+$ T cells in preserving their function and proliferation during CAR-T manufacture, this study confirmed previous observations on in vitro and in vivo contexts. This cycle of treating patients to performing mechanistic studies, going back to therapeutic improvements, will continue to bring great impacts to the field of translational immunotherapies.

Twitter J Joseph Melenhorst @j_melenhorst

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