Selection bias: maintaining less-differentiated T cells for adoptive immunotherapy

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The clinical application of T cell immunotherapy depends on ex vivo modification and expansion of T cells for adoptive transfer. In preclinical models, the use of a purified, naive T cell subset enhances persistence and antitumor immunity; however, the majority of clinical studies rely on modification of mixed populations of T cells that contain only a small subset of highly functional T cells with less-differentiated phenotype. In this month’s issue of the JCI, Klebanoff and colleagues uncover a Fas-mediated interaction between naive T cells and antigen-experienced T cells that drives differentiation and impairs adoptive immunotherapy. Further, they show that blockade of Fas signaling enhances antitumor immunity and increases survival in a mouse model of melanoma. Their work supports a growing body of evidence that the use of naive T cells enhances the efficacy of adoptive T cell therapy and suggests a new therapeutic strategy for preserving less-differentiated T cell populations.

Generating T cells for immunotherapy

Adoptive transfer of T lymphocytes engineered to achieve tumor specificity through the genetic insertion of either T cell receptors or chimeric antigen receptors (CARs) allows remarkable control of disseminated tumors across multiple phase I/II clinical studies in the academic setting (1, 2). Some of these approaches are now further developed in multicenter studies supported by pharmaceutical companies. Manufacturing of engineered T cells is a key element for the realistic execution of multicenter clinical studies and for the future use of these cells in the clinical practice. Manufacturing of T cells is generally centralized to ensure reproducibility and aims at implementing standard operating procedures with minimized complexity. To this end, peripheral blood mononuclear cells (PBMCs) are frequently preferred for generating engineered T cell products. PBMCs are indeed readily obtained by gradient centrifugation and contain all circulating T cell subsets, from naive T cells to memory T cells, susceptible to effective transduction by viral vectors encoding the transgene following activation with CD3/CD28 crosslinking antibodies and cytokines. Starting from PBMCs, T cell products can be obtained within two weeks of ex vivo culture, a time schedule compatible with an effective clinical application in many patients with refractory and resistant malignancies.

To select or not to select — that is the question

In the current issue of the JCI, Klebanoff et al. highlight that the complexity of the immune system may not be easily recapitulated using simplified approaches and that a more sophisticated manipulation of T cell subsets within the infused T cell products may affect clinical outcome (3). This last observation stems from a long history of mouse model studies showing how less-differentiated T cell subsets from naive T cells (T_N cells) to central memory T cells (T_CM cells) display superior antitumor capacity and persistence compared with more-differentiated T cells, such as effector-memory T cells (T_EM cells) or effector T cells (T_E cells) (4). More recently, another subset of memory T cells, identified as T stem cell memory cells (T_SCM cells), claims properties superior even to T_N and T_CM cells (5). Clinical observations corroborate the relevance of preserving primitive T cell subsets within infused T cell products to ensure T cell expansion and potentially long-term engraftment (6, 7).

The composition of T cell subsets found in PBMCs of cancer patients is largely affected by several factors, including age, disease, and type of chemotherapy treatments received before blood procurement. All these factors contribute to creating very heterogeneous T cell products when whole PBMCs are used as a starting material. One could argue that the high response rate in both adult and pediatric patients with acute lymphoblastic leukemia infused with CD19-specific CAR–T cells supports the use of PBMCs as a starting source of T cells, regardless of their heterogeneity, as they contain all the T cell subsets needed for an effective immune response (2, 8, 9). However, a more precise composition of the T cell products and in particular the enrichment in T cells with the highest potential for engraftment may become particularly relevant in other clinical settings, specifically in most solid tumors, in which the response rate to adoptive T cell therapies is currently significantly inferior compared with that for lymphoblastic leukemia.

Maintaining undifferentiated T cell populations

How can we preserve more immature and bona fide highly functional T cell subsets within infusion products without applying complex standard operating procedures? Activation, genetic manipulation, and ex vivo expansion of T cells inevitably induce their differentiation.
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Commentary

pathways that promote the precocious differentiation of T<sub>N</sub> cells. They found indeed that T<sub>MEM</sub> cells mediate the differentiation of T<sub>N</sub> cells through Fas ligand (FasL) expressed by activated T<sub>MEM</sub> cells interacting with Fas on activated T<sub>N</sub> cells. Preventing Fas-FasL interaction by FasL-blocking antibodies or genetic ablation of Fas on T<sub>N</sub> cells restores the primitive T<sub>SCLM</sub> and T<sub>CM</sub> cell populations in the T<sub>N</sub> cell progeny. In addition, provision of FasL alone is sufficient to promote T<sub>N</sub> cell precocious differentiation in the absence of T<sub>MEM</sub> cells. Fas-FasL interaction induces the extrinsic apoptosis pathway via caspase 8. Interestingly, the precocious differentiation of T<sub>N</sub> cells induced by T<sub>MEM</sub> cells does not induce a cell death pathway, but instead depends on activation of Akt signaling and metabolic modulation. TN cells expanded with T<sub>MEM</sub> cells show elevated activation of Akt and glycolytic activity, a phenomenon that can be recapitulated by the addition of FasL. Inhibition of Akt activation reverses the precocious differentiation, raising the possibility of pharmacologic modulation of this process.

Conclusions and future directions

It remains unknown whether there is crosstalk among the mixed populations of T<sub>EM</sub>, T<sub>CM</sub>, and T<sub>SCLM</sub> cells present in the infused products. Moreover, since CD4+ T cells are also present in unfractionated PBMCs and play a role in promoting the survival of engineered T cells (14), determining whether this quorum-sensing phenomenon exists within the CD4 subset would greatly benefit the manufacturing of T cell products.
products. In addition to the application in cancer immunotherapy, this uncovered crosstalk between $T_{\text{MEM}}$ and $T_N$ cells may represent a physiologic immunoregulatory mechanism to favor $T_E$ cell differentiation over immune memory formation. Priming of $T_N$ cells in the presence of preexisting antigen-experienced T cells may be a signal for chronic persistent infection. Clearance of antigen by $T_N$ cells in this situation may be the priority over establishment of long-lived memory T cells. Moreover, since the ratio of $T_{\text{MEM}}$ to $T_N$ cells gradually increases with age, due to reduced output of $T_N$ cells from thymus, the discovered crosstalk between these subsets could be exploited to improve vaccination approaches in aged individuals.

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