Multiparameter monitoring for optimal T-cell adoptive therapy

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Adoptive transfer of ex vivo-expanded antigen-specific T cells is a promising therapeutic approach for the treatment of cancer and infectious diseases. Clinical studies have proven the feasibility and potency of this procedure but several limitations need to be overcome before this form of immunotherapy reaches its full potential. For instance, the efficacy of the transferred cells is often impeded by terminal effector differentiation and exhaustion acquired during in vitro expansion. This would notably explain the lack of further in vivo proliferation and persistence in many trials. However, the factors that induce T-cell differentiation and functional impairment in culture remain poorly defined. Using the model antigen HA-1, we determined that phenotypic and functional features indicating T-cell exhaustion/dysfunction may not be detected simultaneously and depend on the method of expansion as well as the antigenic repertoire stimulated. Thus, our study has defined critical parameters to monitor in order to optimally differentiate and expand antigen-specific T cells in culture prior to adoptive transfer.

Keywords: T lymphocytes; T-cell exhaustion; adoptive immunotherapy; cancer; HA-1; CAR T cells


We have recently entered a new age in cancer therapy with the development of numerous effective antitumor immunotherapeutic approaches [¹, ²]. Pioneered in the 1970’s, allogeneic hematopoietic stem cell transplantation (HSCT) was among the first therapies demonstrating that the immune system could cure refractory blood cancers. In most instances, the potent anti-neoplastic effect of allogeneic HSCT hinges on genetically encoded donor-host proteome variations. As such, HLA molecules will present fragments from self-proteins that will differ in sequence between donor and host. These peptides are called minor histocompatibility antigen (MiHA) and are the cornerstone of both the graft-versus-leukemia (GVL) effect and graft-versus-host disease (GVHD) in HLA-identical, non-zygous allogeneic HSCT [³, ⁴]. The adoptive T-cell therapy (ACT) of hematopoietic-restricted MiHA-specific T cells that are absent on GVHD target tissues is an attractive approach to augment GVL effects without risking GVHD [⁵, ⁶]. While clinical studies have shown encouraging results with such approach, no long-term evidence of leukemia control or cure were recorded [⁷, ⁸]. One reason for such limited effect is the lack of persistence of the transferred T cells [⁹]. This might be influenced by the environment, repeated antigenic stimulations or culture duration prior to adoptive transfer. A common feature is that in vitro priming and expansion prior to transplantation can lead to terminal T-cell differentiation thereby limiting further T-cell expansion after transfer and leading to rapid apoptosis [¹⁰]. Although terminal T-cell differentiation and exhaustion have been studied at varying degrees of depth in animal models of chronic infections [¹¹-¹³] or in human immunodeficiency virus (HIV)-infected patients [¹⁴-¹⁶], the central question of ex vivo culture-driven human
T-cell differentiation has not been fully investigated and the critical variables influencing late T-cell differentiation and the acquisition of exhaustion features during in vitro expansion are incompletely understood. Thus, it is imperative to decipher the main mechanisms by which T-cell dysfunction occurs in order to properly control it in ex vivo expansion protocols.

Adoptive immunotherapy presents the opportunity to activate and expand T cells outside the tolerizing environment of the host or, in context such as HSCT, from healthy donors. A number of strategies have been developed to optimize the cellular product generated. The main idea across the field was that optimal therapeutic effects are achieved when the ex vivo generated T cells maintain features associated with early memory differentiation \cite{17, 18} or even T-cell “stemness” (i.e., capacity to further differentiate as effector or self-renew as memory cells and persist long-term in the host). Interestingly, evidence accumulates demonstrating that therapeutic efficacy might depend more on the antigen-specific T cells’ early’ differentiation phenotype as well as their ability to proliferate and/or persist in vivo rather than on the number of infused cell \cite{19}. Concretely, this means that central memory T cells (Tcm), generally described as expressing CD45RO, CD62L and CCR7 markers, are predicted to have increased in vivo efficacy relative to effector memory cells (Tem) showing a reduced expression of CD62L and CCR7 with a concomitant loss of proliferative capacity \cite{20-22}. However, there is also evidence that a fraction of effector memory T cells have the potential to revert back to a central memory phenotype when transferred into patient and persist, indicating that the acquisition of effector memory phenotype in culture may not always predict limited functionality in vivo \cite{23}. Thus, the analysis of additional phenotypic markers and functional properties of T cells prior to patient infusion could provide insights into optimal compositions of ACT for therapeutic efficacy \cite{19}.

Our group has determined that the proportion of effector memory or central memory phenotype cells do not necessarily correlate with loss of antigen-specific cells or decline in their functionality \cite{24}. Thus, we decided to address the question of cell “fitness” for adoptive immunotherapy by...
finding complementary features that would better characterize specific T-cell lines to attest their differentiation and functional status. Figure 1 summarizes the system used to prime and expand T-cell lines against HA-1, a HLA-A0201-restricted MiHA. Briefly, donors predicted to generate an anti-HA-1 response were recruited and their T cells were co-cultured with HA-1 pulsed autologous monocyte-derived dendritic cells. Following three rounds of stimulation, the antigen-reactive cells were enriched using a cytokine capture system and further expanded in the presence of the anti-CD3 antibody OKT3 and interleukin (IL)-2. In order to study culture-driven exhaustion/dysfunction, both the co-culture and the expansion phases were prolonged.

Exhausted T cells express various inhibitory receptors, including Program cell death-1 (PD-1), 2B4, B and T-cell lymphocyte attenuator (BTLA), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), CD160, lymphocyte-activation gene 3 (LAG-3) and T cell immunoglobulin and mucin-domain-containing molecule-3 (Tim-3)\(^{[25-28]}\). Expression of PD-1 was among the first proposed marker for exhausted T cells\(^{[29]}\). It was further demonstrated that PD-1-positive T cells co-expressing Tim-3\(^{[30, 31]}\), LAG-3\(^{[25]}\) and BTLA\(^{[26, 27]}\) show a significant decrease in IL-2, interferon-γ (IFNγ), and tumor necrosis factor-α (TNFα) expression as well as cell cycle arrest, which also characterize T-cell exhaustion\(^{[32]}\). This phenomenon has been described through repeated antigenic T-cell activation in models such as chronic infections\(^{[25, 33]}\) and malignancies\(^{[30, 34, 35]}\). However, it is of importance to note that exhausted T cells may not automatically co-express all these inhibitory receptors, and it is still controversial whether their co-expression is always a determinant of T-cell functional state\(^{[36]}\).

In our hands, repeated antigenic stimulation of MiHA-specific T cells has indeed led to terminal differentiation, as evidenced by upregulation of specific markers such as PD-1 and Killer cell lectin-like receptor subfamily G member 1 (KLRG-1) predominantly on antigen-specific cells\(^{[24]}\) (and not on accompanying CD8\(^+\) T cells present in the same culture) with detection of Tim-3 at percentage similar to PD-1 (unpublished observations). By contrast, prolonging the expansion phase with OKT3 and IL-2 led to a decline in the antigen-specific population as well as a sharp decline in T-cell proliferation of both antigen-reactive and non-reactive CD8\(^+\) T cells. This occurred with little expression of PD-1 or KLRG-1 as well as with the preservation of polyfunctional cytokine secretion and antigen-specific granule exocytosis by the remaining antigen-reactive cells. We also observed these distinguishing features while using the same donors and protocol to expand T cells against the Epstein-Barr Virus (EBV)-derived antigen LMP2\(_{426-434}\) (also presented by HLA-A0201). However, we noted that the expression of KLRG1 and PD-1 occurred earlier during the co-culture with antigen-pulsed antigen-presenting cells (APC), suggesting that cells derived form a memory repertoire (all donors were EBV-seropositive) might be more susceptible to exhaustion following repeated antigen exposure.

Globally, our findings reveal the importance of context in the acquisition of features indicative of T-cell dysfunction. While co-culture with antigen-loaded dendritic cells in the presence of cytokines leads to PD-1 and KLRG-1 expression on antigen-reactive cells without altering central memory marker expression or proliferation (as indicated by Ki-67 staining, a nuclear protein associated with cellular proliferation) in function of time, the use of OKT3 and IL-2 leads to another type of impairment. In a context independent of APC and repeated antigen exposure\(^{[24]}\), the prolongation of culture led to proliferation arrest. This suggests that dysfunction occurring during in vitro expansion phase, where no APC is required, is influenced by pathways that selectively target cell division without affecting polyfunctionality and cytotoxicity. It may still be controversial that PD-1 is a marker of exhausted cells (as it is expressed on recently activated T cells) whereas Tim-3 and KLRG-1 are markers for terminally differentiated and/or senescent cells, but one have to consider that they might not be mutually exclusive nor inclusive\(^{[39]}\). Furthermore some markers may act synergistically or additively to mediate T-cell dysfunction according to different kinetics and thus collectively induce pathways that affect T-cell proliferation and survival\(^{[32, 38, 39]}\).

In our experience, the use of CD45RO/CD62L/CCR7 expression was not helpful at determining the state of T-cell differentiation in culture. Hence, in an attempt to better predict T-cell fitness, a multiparameter approach was essential for a rigorous follow up of our cultures. The differential regulation of phenotypic and functional T-cell exhaustion according to culture conditions and duration argues for the implementation of a more comprehensive monitoring of in vitro expanded cells to optimally predict the in vivo fitness of an immunotherapeutic product. We determined that the expression of several extracellular as well as intracellular markers such as PD-1, KLRG-1, IFNγ and Ki67 provided substantial information about T-cell quality and will hopefully predict in vivo persistence of cultured specific T cells (Figure 1).

Understanding and curtailing CD8\(^+\) T-cell terminal effector differentiation is a central issue in adoptive immunotherapy using either the natural T-cell receptor repertoire or genetically modified T cells. As such, chimeric
antigen receptor (CAR) T cells, another promising approach for immunotherapy, have provided limited success against a broad variety of cancer types while showing spectacular results in others [40]. These cells, genetically engineered to express antibody binding domains fused to T-cell signaling domains, are generally polyclonally expanded with anti-CD3/CD28 antibodies or coated beads with or without IL-2 [40-42] which may eventually drive them towards an exhaustion state [43]. The persistence of CAR T cells at the tumor site is one of the major principles for effective tumor eradication [44, 45] and poor T-cell homing is thought to be one reason for the reduced efficiency of adoptive immunotherapy based on CAR-engineered T cells in certain cancer types. However, in vivo proliferation of transferred cells is a key feature that also predicts the success of the therapy and several studies have shown that the lack of survival of the infused CAR T cells greatly limits the efficiency of adoptive immunotherapy [9, 46, 47]. To solve this problem, modulating co-stimulatory signaling during T-cell culture or modifying the cytokine environment, which greatly influences the human T-cell differentiation processes, have been suggested to improve the persistence of infused T cells [40, 43, 48-50]. The central issue of T-cell proliferation following transfer might also be dependent on the exhaustion status of T cells. Unfortunately, to our knowledge, there are no markers that can reliably attest of cell quality (neither for classical ACT nor for CAR T-cell studies) prior to infusion. Thus, determining molecular targets leading to exhaustion/dysfunction characteristics could help us enhance T-cell culture for clinical-scale expansion of a healthier non-exhausted product. Generating strategies to manipulate these targets to limit/reverse exhaustion in a clinical-grade culture setup will be of great interest for T-cell biologists and adoptive immunotherapists.

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List of abbreviations

ACT: Adoptive cell therapy; APC: antigen-presenting cells; BTLA: B and T-cell lymphocyte attenuator; CAR: chimeric antigen receptor; CTLA-4: cytotoxic T-lymphocyte-associated protein 4; EBV: Epstein-Barr Virus; GVL: graft-versus-leukemia; GVHD: graft-versus-host disease; IFNγ: interferon-γ; IL-2: interleukine-2; LAG-3: lymphocyte-activation gene 3; MiHA: minor histocompatibility antigen; PD-1: Program cell death-1; HSCT: hematopoietic stem cell transplantation; Tcm: central memory T cell; Tem: effector memory T cell; Tim-3: T cell immunoglobulin and mucin-domain-containing molecule-3; TNFα: tumor necrosis factor-α.

Authors’ contributions

VJ, CC and JSD wrote the manuscript. All authors read and approved the final manuscript.

References


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