Exhausting alloreactivity of donor-derived CAR T cells

Maksim Mamonkin & Helen E Heslop

A study in mouse models of allogeneic stem cell transplantation with donor-derived CD19 chimeric antigen receptor (CAR) T cells for the treatment of relapsed B cell malignancies indicates that T cell exhaustion might have a role in preventing allogeneic reactivity of CD19 CAR T cells.

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) from a closely matched donor is the only curative option for many patients with lymphoid malignancies. One risk of this procedure is graft-versus-host disease (GVHD), in which a donor's transferred lymphocytes recognize alloantigens on the recipient's tissues. Tumor relapse is also a leading cause of death among patients who receive these transplants. Adoptive transfer of HSCT donor-derived T cells expressing a tumor-specific chimeric antigen receptor (CAR) is an attractive therapeutic option for preventing or treating relapsed disease after transplant. In patients with B cell malignancies, the transfer of T cells bearing CARs targeted to the CD19 antigen (CD19 CAR T cells) is especially appealing because CD19 is widely present in malignant B cells and its expression is confined to lymphoid tissues, which limits 'on target, off tumor' toxicities.

Initial trials used CARs that were transferred into T cells specific to the most common post-transplant viruses (EBV, CMV and adenovirus) to avoid alloreactivity from any native T cell receptors[1,2]. More recently, however, several groups have induced the expression of CD19 CARs in allogeneic, polyclonally activated T cells and have observed complete remission in patients with B cell malignancies in the absence of notable GVHD[3–5]. In this issue, Ghosh *et al.*[6] offer a mechanistic explanation for the suppressed alloreactivity of donor-derived T cells expressing CD19 CARs with the CD28 costimulatory endodomain. They show in several mouse models of GVHD that alloreactivity is attenuated through the cumulative effect of activation of the allospecific T cell receptor (TCR) and the artificial CAR, which results in functional exhaustion of CD19 CAR T cells.

Ghosh *et al.*[6] modeled tumor relapse in mice that underwent-allo-HSCT in mouse models by inoculating bone marrow chimeric mice with recipient-matched CD19$^+$ lymphoma cells. The authors then adoptively transferred donor-derived T cells expressing murine CD19-specific CARs. In most of their experiments, the authors observed robust antitumor activity of CD19 CAR T cells but only limited GVHD, resulting in prolonged survival of mice. By contrast, mice that received the same numbers of T cells expressing a truncated nonsignaling CD19 CAR rapidly succumbed to lymphoma or developed lethal GVHD, which suggests that the expression of a functional CD19 CAR suppressed the development of allospecific T cell responses.

They found that diminished alloreactivity in mice injected with CD19 CAR T cells was not due to the poor persistence of CAR T cells because the recipient mice were able to sustain the elimination of lymphoma and had persistent B cell aplasia, reflecting continuous cytotoxic activity of the adoptively transferred T cells. The authors observed similar suppression of alloreactivity in several allo-HSCT models, although not in bone marrow chimeric mice lacking CD19$^+$ B cells. Similarly, reducing CAR activation by transferring excessive numbers of CD19 CAR T cells into bone marrow chimeric mice (where the numbers of endogenous CD19$^+$ cells were insufficient to activate all CD19 CAR T cells) restored alloreactivity and GVHD. This indicates that antigen stimulation via the CAR was required to inhibit alloreactivity. Furthermore, the authors showed that in mouse CD8$^+$ T cells, CAR stimulation suppressed TCR-mediated cytotoxicity and vice versa, which illuminates the interplay between the two targeting modalities. Whether CAR signaling promoted downregulation of TCR expression or dominantly inhibited TCR-induced signaling and/or degranulation remains to be elucidated. Nonetheless, these observations imply that saturating CAR signaling in allospecific T cells might be important for fully suppressing the TCR-mediated activation.

The authors' work suggests that the nature of CAR signaling has an important role in abating native TCR activation. They found that T cells expressing a second generation CD19 CAR with a CD28 costimulatory endodomain (m1928z) were the most refractory to allospecific activation. Removal of the CD28 endodomain or replacement of it with another commonly used costimulatory endodomain 4-1BB (CD137) resulted in increased severity of GVHD. The authors' analysis of the active signaling pathways and cell surface phenotypes of the T cells revealed increased activation of splenic m1928z CAR T cells as compared to control T cells expressing a nonsignaling CAR, and this activation was associated with upregulation of the inhibitory receptors PD-1, LAG3 and Tim3, hyperactivation of PKCα, STAT and MAPK signaling pathways and upregulation of the pro-apoptotic genes *Fas* and *Fasl*. Furthermore, transcriptomic profiling of allospecific m1928z CAR CD4$^+$ T cells identified changes in the expression of pro-apoptotic genes and transcription factors consistent with the induction of exhaustion and programmed cell death. This phenotype supports the authors' hypothesis that cumulative signaling from TCRs and CARs results in functional attrition and/or clonal deletion of alloreactive CD19 CAR T cells (**Fig. 1**).

Of note, in a recent report, Jacoby *et al.*[7] show in a minor-mismatch bone marrow chimera model the ability of donor-derived mouse T cells expressing a similar CD19 CAR (similar m1928z, with the same scFv and signaling domains) to promote rapid lethal GVHD in the presence of donor-matched CD19$^+$ leukemia. The GVHD in the authors' model was dependent on CD4$^+$CD19 CAR T cells and exaggerated by interleukin (IL)-6. Notably, switching the donor and recipient

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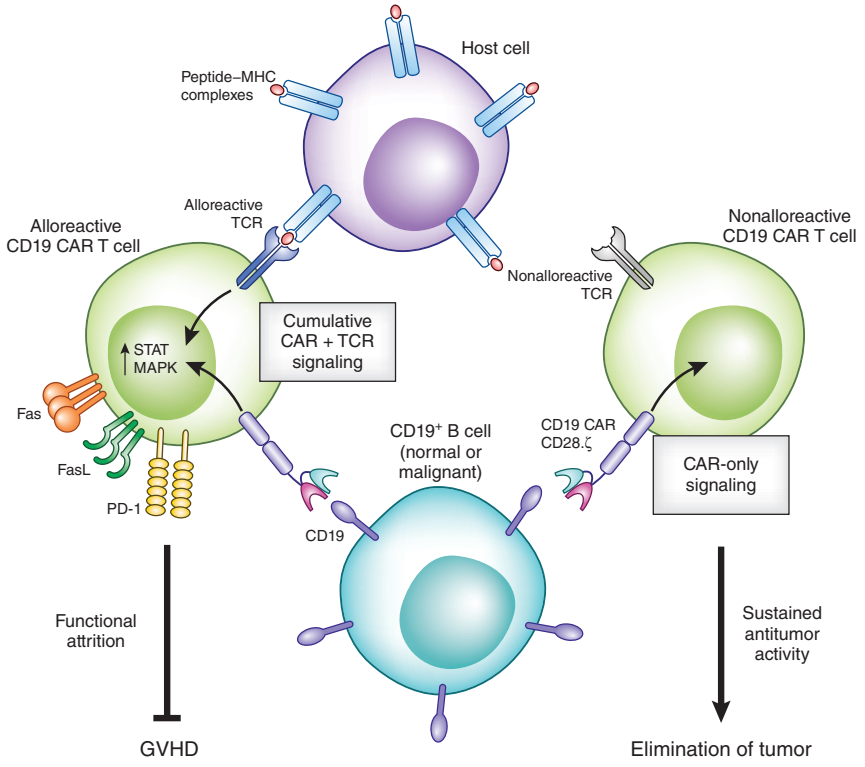

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Figure 1 Ghosh *et al.*[6] show in mouse models of allogeneic stem cell transplant of donor-matched CD19 CAR T cells for the treatment of B cell malignancies that excessive stimulation of alloreactive CD19 CAR T cells through cumulative CAR and TCR signaling via STAT and MAPK upregulates inhibitory receptors and pro-apoptotic molecules such as PD-1, Fas and FasL. This leads to exhaustion or clonal deletion and prevents the development of GVHD. CAR signaling in nonalloreactive T cells permits long-term persistence and sustained cytotoxicity against normal and malignant B cells.

strains resulted in equally potent antitumor activity of CD19 CAR T cells in the absence of fulminant GVHD, which suggests that the magnitude of allospecific responses might be dependent on factors such as the activity and availability of antigen-presenting cells, mechanisms of peripheral tolerance and the capacity of host cells to amplify the production of key cytokines. Additional factors, such as the inflammatory milieu at the time of GVHD onset, the availability and tissue distribution of $CD19^+$ target cells and the cellular composition of the infused CAR T cell product, could also influence the outcome. Finally, the differences in the structural components of the CD19 CAR that lead to changes in the level of its surface expression, the magnitude of ligand-driven signaling or the propensity for ligand-independent tonic signaling[8,9] could ultimately have a role in regulating the potency of CAR-mediated activation of T cells and the concomitant changes in the cell phenotype.

Using donor-derived T cells for CD19 CAR transfer is an attractive option because it allows for the manufacturing of CD19 CAR T cells from a normal donor rather than a recipient, who might have low numbers and functionally impaired allograft-derived T cells, after HSCT and/or chemotherapy. So far, clinical trials using donor-derived CD19 CAR T cells in patients with relapsed B cell malignancies post-allo-HSCT have shown potent antitumor responses without substantial alloreactivity[3,4]. The results of the present study provide mechanistic data to explain these clinical observations. The authors show that the alloreactivity of donor-derived T cells expressing a CD19 CAR with a CD28 costimulatory endodomain is suppressed when these cells are given in limited numbers and in the continuous presence of the $CD19^+$ cells that are required for sustained CAR signaling. These findings also highlight the potential interaction between TCR and CAR signaling in T cells, and thus have broad implications for the rational design of CARs expressed on virus- or tumor-antigen-specific T cells, wherein the activity of both targeting modalities might be important to achieve full functionality.

COMPETING FINANCIAL INTERESTS
The authors declare competing financial interests: details are available in the online version of the paper.

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HDAC3 sets the timer on muscle fuel switching

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In a recent study in mice, it is shown that circadian oscillations in genomic histone deactylase 3 (HDAC3) occupancy influence fuel switching and carbon flux in muscle to regulate glucose homeostasis and exercise performance.

Skeletal muscle has a key role in whole-body energy metabolism and serves as the major site of glucose uptake upon feeding and physical activity. In general, glucose and fatty acids serve as the principal carbon fuels, whereas protein-derived amino acids are used as a last resort to conserve lean body mass. Because tight control of normoglycemia is critical to survival, evolution has favored biological processes that give peripheral tissues (for example, muscle and liver) flexibility to choose the fuel that is most appropriate for a particular physiological state[1]. Peripheral use of glucose during the fasted-to-fed transition prevents hyperglycemia, whereas a switch to alternative fuels (lipids and amino acids) defends against hypoglycemia during food restriction or prolonged exercise. Studies attempting to map the regulatory circuits that govern these fuel transitions have remained at the forefront of metabolic research for decades. Whereas current models of metabolic flexibility

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