TRANSPLANTATION

Comment on Wang et al, page 166

Id3 keeps the PD-1 checkpoint in check in GVHD

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In this issue of *Blood*, Wang et al report that genetic ablation of inhibitor of DNA-binding 3 (Id3) attenuates murine graft-versus-host disease (GVHD) through upregulation of PD-1 expression in alloreactive donor T cells.[1]

Id3 is a helix-loop-helix transcription factor that acts as a negative regulator of the E-box binding family of transcription factors. Id3 plays a crucial role in T-cell biology, being involved in T-cell development[2] as well as in CD8[3,4] and CD4[5] memory T-cell differentiation. High expression of Id3 during T-cell activation has been reported to identify T cells with memory potential,[3,5] and genetic ablation of Id3 leads to defective CD8 T responses, resulting in failure to generate long-lived memory cells.[3,4]

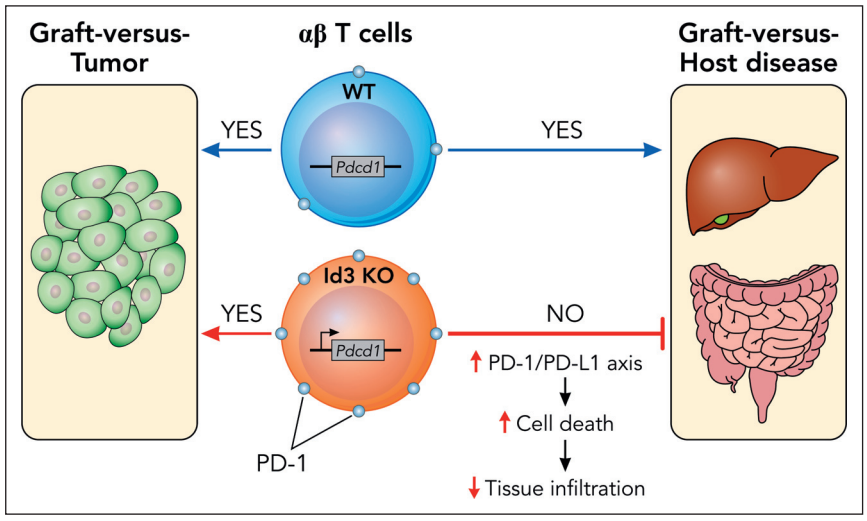
In the current study, Wang et al tested the hypothesis that Id3 controls T-cell responses during GVHD. Using a major and a minor histocompatibility complex–mismatch murine model of GVHD as well as a model of xenogeneic GVHD induced by human T cells, the authors demonstrated that genetic ablation of *Id3* prevents GVHD by modulating alloreactive T cells. Interestingly, the loss of Id3 did not impact engraftment, proliferation, expansion, and migration of donor T cells but dramatically decreased the infiltration of T cells in GVHD target tissues, namely the liver and intestine. Importantly, the authors showed that the decrease in T cells was associated with upregulation of PD-1 and PD-L1 on the T-cell surface (see figure).

Preclinical and clinical studies support a role for the PD-1/PD-L1 axis in the control of GVHD after allogeneic hematopoietic stem cell transplantation (HSCT).[6] PD-1 expression is upregulated and sustained at the T-cell surface after allogeneic HSCT,[7] and PD-1 blockade is associated with significant risk of GVHD exacerbation.[8] Wang et al showed that the PD-1/PD-L1 axis is finely regulated by Id3, which reduced the chromatin accessibility and thereby the transcription and surface expression of PD-1 in alloreactive Th1 cells. Such modulation of the PD-1/PD-L1 axis was associated with a decrease in total Th1 cells as well as in PD1^{+} TCF-1^{+} progenitor-like T cells, a crucial population in GVHD pathogenesis.[9] Importantly, PD-1 blockade reversed the protective effect of Id3 ablation, demonstrating the role of the PD-1/PD-L1 axis in Id3-mediated regulation of GVHD.

Given the role of Id3 in T-cell biology, a major question is whether *Id3* ablation might interfere with the graft-versus-tumor (GvT) effect post–allogeneic HSCT. Using 2 murine models of hematological malignancies, Wang et al show that Id3 is essential for GVHD induction but is dispensable for the GvT effect. Similarly, they demonstrate that the presence or absence of Id3 expression did not influence the antitumor effect of CRISPR-Cas9–edited ID3-KO CD19-CAR-T cells in a xenogeneic mouse model of lymphoma. Collectively, the data obtained in these murine models of cancer suggest that Id3 targeting does not interfere with the GvT effect, thus supporting the feasibility of this approach.

What are the important questions that need to be answered before studying Id3-targeting strategies in the clinic? First, Id3 is a well-established regulator of the cell cycle, and pathogenic *Id3* mutations have been shown to play a major role in several malignancies.[10] The long-term safety of *Id3* genetic ablation in adoptively transferred T cells remains a concern that can be only partially evaluated in murine models. Second, the role of Id3 on the immune reconstitution of different T-cell subsets, including antitumor but also pathogen-specific T cells, needs to be defined. Given the role of Id3 in memory T-cell differentiation,[3-5] Id3 ablation might lead to impaired immune-reconstitution of pathogen-specific T cells and increased risk of infection after allogeneic HSCT.

In summary, the work by Wang et al provides compelling evidence that Id3 plays an essential role in limiting PD-1



Id3 ablation mitigated GVHD through induction of PD-1 transcription and without interfering with the GvT effect. KO, knockout; WT, wild type. Professional illustration by Patrick Lane, ScEYEnce Studios.

expression on alloreactive T cells, therefore representing a potential target to favor PD-1–mediated suppression in GVHD. These findings provide a rationale for investigating genetic or pharmacological Id3-targeting approaches for GVHD prevention and treatment after allogeneic HSCT.

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