TRANSPLANTATION

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Allo-HCT: damaged T cells don't bite

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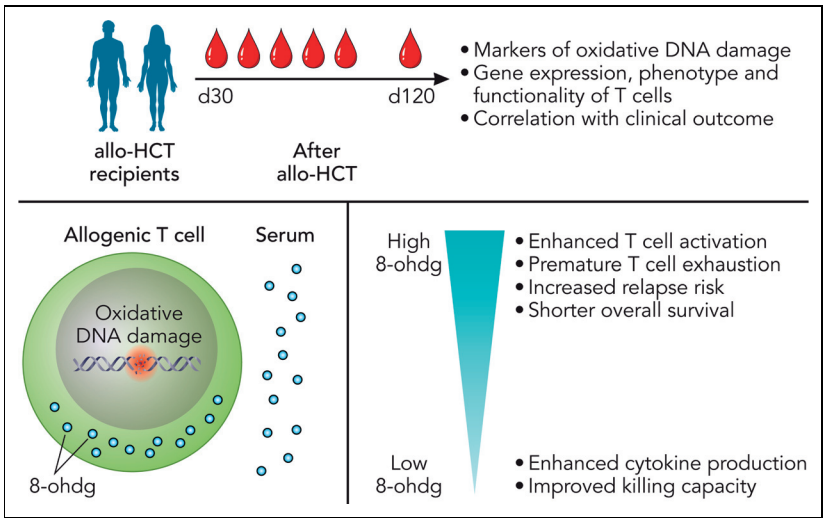
In this issue of *Blood*, Karl et al[1] report that oxidative stress markers are elevated in the serum and immune cells of allogeneic hematopoietic cell transplantation (allo-HCT) recipients. High levels of oxidative DNA damage in circulating T cells correlated with impaired graft-versus-tumor (GVT) activity and inferior survival.[1]

Allo-HCT, an effective treatment for hematological malignancies, represents one of the oldest standard-of-care forms of tumor immunotherapy. Its success relies on the capacity of donor T cells to eliminate residual malignant cells in the host and exert lifelong immunosurveillance. However, primary disease relapse occurs in 25% to 30% of allo-HCT recipients and is the predominant cause of death.[2] As novel classes of immunotherapy advance across diseases, and we gain a deeper understanding of the intricate cross talk between tumor and immune cells, some of the central questions remain: Which events drive alloreactive T-cell dysfunction? What druggable targets could potentially boost antitumor immunity after allo-HCT? Established mechanisms of tumor immune escape include enhanced immune checkpoint signaling and harmful changes in the cytokine and metabolic milieu.[3] Karl et al add a new piece to the puzzle by identifying that a high degree of oxidative DNA damage, particularly in allogeneic T cells, is associated with premature exhaustion, reduced antitumor activity, and, thereby, inferior clinical outcomes of allo-HCT recipients.

Oxidative DNA damage results from the accumulation of reactive oxygen species (ROS) in the cell. ROS are highly reactive oxygen-containing molecules produced mainly in the mitochondria that can damage the DNA when in excess. Cells are equipped with mechanisms to scavenge ROS, such as glutathione and thioredoxin. When ROS production exceeds the cell's capability to eliminate them, oxidative DNA damage may occur.[4] Karl et al analyzed serum and circulating immune cells from allo-HCT recipients at 6 standardized time points after transplantation for signs of oxidative stress. Healthy donors and patients undergoing an autologous stem cell transplantation (auto-SCT) served as controls. The authors found that allo-HCT recipients had the highest amounts of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in serum, T cells, and natural killer cells. 8-OHdG is the product of the oxidation of 2′-deoxyguanosine and a commonly used marker for oxidative DNA damage. Earlier studies have reported increased oxidative stress markers, such as malondialdehyde, in the serum of patients undergoing allo-HCT.[5,6] A common hypothesis has been that the conditioning regimen induces oxidative stress.[5,6] The current study expands our knowledge by focusing on intracellular indicators of oxidative DNA damage (in addition to serum analysis) in a longitudinal manner. Notably, the authors provide evidence that the process of allorecognition in itself contributes to oxidative DNA damage. T cells from patients undergoing allo-HCT had elevated concentrations of 8-OHdG until day 60 (d+60) after transplantation. In contrast, the concentrations in T cells of auto-SCT recipients (who had also received high-dose chemotherapy) were equal to those of healthy donors. Furthermore, T cells cocultured with allogeneic dendritic cells displayed signs of oxidative DNA damage. These observations align with a study implying allorecognition as an essential inducer of ROS and oxidative DNA damage in vitro.[7] Together, the findings convey that oxidative DNA damage occurs in reconstituting immune cells and is at least partly a direct consequence of alloreactivity.

Precise regulation of intracellular ROS levels is essential for optimal T-cell activation, as both failure to produce ROS and insufficient presence of molecules to eliminate them can impair T-cell function.[4] To study how oxidative DNA damage might impact T cells in allo-HCT recipients, the authors performed bulk RNA sequencing on sorted 8-OHdG^{hi} and 8-OHdG^{lo} cells. The 8-OHdG^{hi} T cells upregulated genes belonging to cell cycle– and metabolism-related gene sets, such as "cell cycle," "oxidative phosphorylation," "tricarboxylic acid cycle," and "glycolysis." In line with the notion that 8-OHdG^{hi} T cells have an enhanced cell cycle progression, proliferation rates (as measured by Ki-67 staining) were higher in T cells from patients belonging to the 8-OHdG^{hi} group than the 8-OHdG^{lo} group. T cells from 8-OHdG^{hi} patients also had higher expression of the activation markers CD25, CD69, and CD137 than T cells from 8-OHdG^{lo} patients. However, the authors observed that a high degree of oxidative DNA damage was also associated with an exhaustion phenotype. The 8-OHdG^{hi} T cells had higher expression of programmed cell death protein 1 and killer cell lectin-like receptor G1 than 8-OHdG^{lo} cells. The presence of T cells with an exhaustion signature in the bone marrow of allo-HCT recipients has been shown to predict relapse.[8] Karl et al observed that 8-OHdG^{hi} T cells were less capable of killing acute myeloid leukemia (AML) cell lines and primary AML blasts than 8-OHdG^{lo} T cells, which was also mimicked by in vitro pretreatment with ROS. Accordingly, patients with 8-OHdG^{hi} T cells had a more than twofold increased risk of relapse compared with patients with low 8-OHdG concentrations, resulting in an inferior overall survival. Notably, levels of 8-OHdG did not correlate with graft-versus-host disease (GVHD), cytomegalovirus, or Epstein-Barr virus reactivation. Together, the results support the hypothesis that high levels of oxidative DNA damage in allogeneic T cells correlate with premature exhaustion and an impaired GVT effect (see figure).

Improving antitumor immunity in patients undergoing allo-HCT remains—in many cases—an unresolved challenge. Can oxidative stress be targeted to improve the longevity and cytotoxicity of allogeneic T cells? Further studies will be required to understand whether the



Karl et al[1] analyzed serum and peripheral blood mononuclear cells collected at serial time points (between d+30 and d+120) from a cohort of 66 allo-HCT recipients. Signs of oxidative stress, T-cell gene signatures, and phenotypes were evaluated in relation to the clinical outcome. Elevated concentrations of 8-OHdG as a marker for oxidative DNA damage were detected in the serum and circulating immune cells of allo-HCT recipients. T cells with high concentrations of 8-OHdG were characterized by enhanced activation and premature exhaustion, whereas their cytokine production and killing capacity were reduced compared with 8-OHdG^{lo} cells. Thereby, high concentrations of 8-OHdG were associated with an increased risk of malignancy relapse and a shorter overall survival. Professional illustration by Patrick Lane, ScEYEnce Studios.

variable degree of oxidative DNA damage between patients results from ROS production differences or an altered capacity to synthesize molecules to eliminate ROS. The current study provides a rationale for testing the efficacy of antioxidants after allo-HCT. An earlier study showed that in vitro culture with the antioxidant N-acetylcysteine yielded a high proportion of T cells with a stem cell memory-like phenotype that had superior antitumor efficacy in a mouse model of chimeric antigen receptor T-cell transfer.[9] Administration of the ROS scavenger thioredoxin 1 in mouse models of allo-HCT resulted in impaired early activation of allogeneic T cells.[10] Subsequently, it reduced the development of GVHD, whereas the GVT effect was maintained.[10] The study by Karl et al paves the way to further assessments of how we can target oxidative stress in T cells to improve the outcome of allo-HCT recipients.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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https://doi.org/10.1182/blood.2022019242