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A delicate balance

CD73-generated adenosine limits the severity of graft vs. host disease but also constrains the allogeneic graft vs. tumor effect

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Abbreviations: Ado, adenosine; AR, adenosine receptor; CML, chronic myelogenous leukemia; GvHD, graft vs. host disease; GvT, graft vs. tumor; mAb, monoclonal antibody

The utility of allogeneic stem cell transplantation for treating hematologic malignancies is enhanced by the graft vs. tumor (GvT) effect, but limited by graft vs. host disease (GvHD). Studies involving the inhibition of CD73 by genetic or pharmacologic means suggest that the levels of CD73-generated adenosine may be manipulated to control GvHD, while maintaining the GvT effect.

Allogeneic stem cell transplantation is the treatment of choice for hematologic malignancies such as chronic myelogenous leukemia (CML). Unfortunately, even with high-resolution HLA typing, graft vs. host disease (GvHD) causes significant morbidity and mortality in stem cell transplant recipients.[1] This complication occurs when donor T cells recognize minor histocompatibility antigens on host tissues, expand and attack them. Considerable effort has gone into developing strategies to limit the severity of GvHD. For example, beginning in the 1980s, stem cell transplants were performed with T cell-depleted bone marrow preparations. As hoped, the severity of GvHD was significantly reduced. However, together with this benefit came the unanticipated complication of too frequent graft failure due to graft rejection by residual T cells in the recipient.[1] In addition, patients with hematologic malignancies who received T cell-depleted bone marrow transplants sometimes experienced late relapses.[2] These observations strongly suggested that some T cells in the donor marrow inoculum are beneficial as they eliminate residual host T cells that have the potential to reject the stem cell graft and they also can control malignant cells in the recipient. The challenge remains how to limit GvHD while preserving the graft vs. tumor (GvT) effect.

Using CD73-deficient mice, we have recently shown that CD73-generated adenosine is a factor that can be manipulated to influence both the severity of GvHD and the strength of the GvT effect.[3] GvHD is enhanced by ATP released from dying cells and acting as a pro-inflammatory mediator to activate host antigen presenting cells through the P2X7 receptor.[4] In a compensatory pathway, ATP is metabolized by the sequential action of CD39 (ecto-nucleoside triphosphate diphosphohydrolase 1) to ADP and AMP, and by CD73, a cell surface ecto-5'-nucleotidase that converts AMP into adenosine, a largely potent anti-inflammatory mediator. Extracellular adenosine influences physiology by signaling through cell surface adenosine receptors (ARs), seven transmembrane-spanning G-protein coupled receptors (A_1, A_{2A}, A_{2B} and A_3) distinguished by their cell-type and tissue-specific expression pattern, affinity for adenosine and downstream signaling pathways.[5] We found that the severity of acute GvHD is significantly higher when mismatched bone marrow transplants are performed between *Cd73*$^{-/-}$ mice than between wild type mice. GvHD in *Cd73*$^{-/-}$ mice is characterized by a severe histopathology, increased levels of serum pro-inflammatory cytokines and increased accumulation of T cells in target tissues. We have further shown that CD73 expressed by both hematopoietic and non-hematopoietic cells exerts a protective effect, with that on non-hematopoietic cells playing a dominant role. These observations suggest that CD73-generated adenosine, especially that produced by non-hematopoietic (most likely endothelial) cells, limits the severity of GvHD. We have also demonstrated that the protective effect

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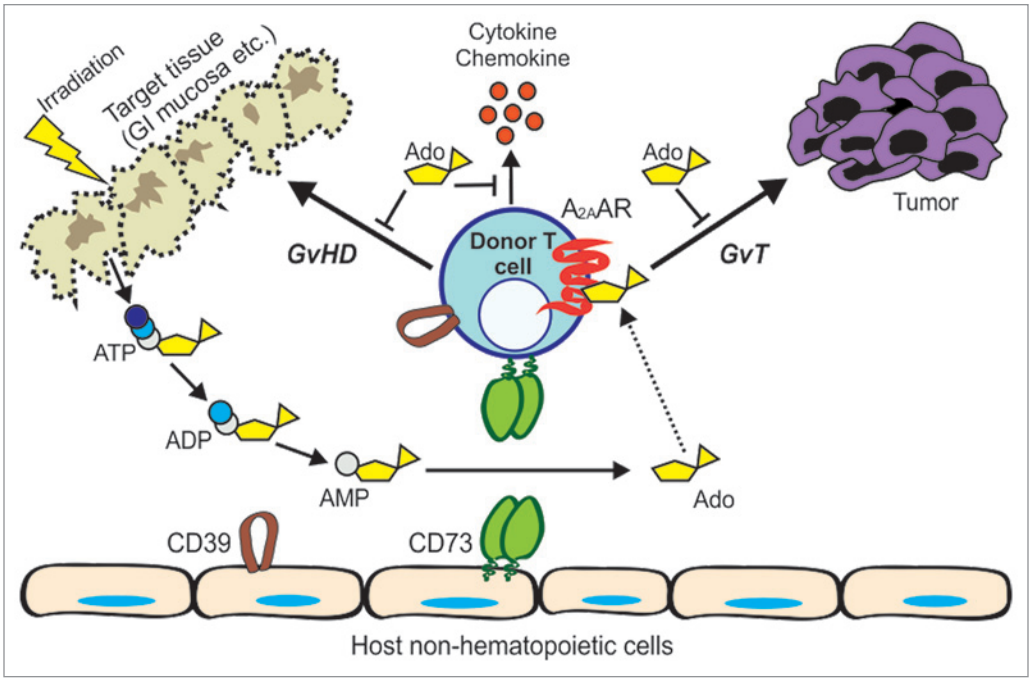

Figure 1. CD73-generated adenosine inhibits alloreactive T cell activation. ATP released from dying or damaged host cells by irradiation or graft vs. host disease (GvHD) is converted to adenosine (Ado) via ADP and AMP by CD39 and CD73 on donor and host cells. Extracellular Ado engages the A_{2A} adenosine receptor (AR) on donor alloreactive T cells and inhibits their activation by host-derived antigen-presenting cells (APCs). Limitation of alloreactive T cell activation by extracellular Ado provides a benefit in preventing GvHD but constrains the graft vs. tumor (GvT) effect.

of CD73 is likely to be mediated by AR signaling, as the increased GvHD severity seen in *Cd73*$^{-/-}$ mice can be recapitulated in wild type mice upon exposure to the general AR antagonist caffeine. We identified the A_{2A} AR as an important player through the use of donor T cells from A_{2A} AR-deficient mice. Our work extends previously findings indicating that the severity of GvHD can be lessened by an exogenous A_{2A} AR agonist[6] by demonstrating that physiologic signaling though the A_{2A} AR with endogenous levels of adenosine is beneficial.

In addition, we have examined the role of CD73 enzymatic activity in the GvT effect using BALB/c mice inoculated with syngeneic B cell leukemia A20 and allogeneic T cells. Treatment of tumor-bearing mice with α,β-methylene adenosine 5'-diphosphate, a specific ecto-5'-nucleotidase inhibitor, slowed tumor growth and improved survival. We interpreted these results to mean that CD73-generated adenosine can inhibit the function of antitumor T cells, leading to increased tumor growth and rapid mortality.

The important question now is whether these results with murine models can be translated into benefits for human patients. We propose that increasing AR signaling in GvHD target tissues would be beneficial for recipients of allogeneic stem cell transplants. In principle, this could be accomplished by administering an AR agonist. However, such treatments can have deleterious side effects such as a lowering of blood pressure.[7] An alternative would be to administer recombinant CD73. The enzyme would increase adenosine levels only at sites where its substrate, AMP, is available, such as sites of inflammation in GvHD target tissues. Another exciting alternative would be to administer a pro-drug that is a substrate of CD73 and can be converted into an AR agonist only at sites where CD73 is available, again at sites of inflammation. This strategy has recently been shown to be effective by Flögel and colleagues in a murine model of collagen-induced arthritis.[8]

On the other hand, inhibiting CD73 has the potential to enhance the GvT effect in patients with malignancies who have been treated by allogeneic stem cell transplants. This could be accomplished with an ecto-5'-nucleotidase enzyme inhibitor, as we did in our preclinical studies. Alternatively, the administration of an anti-CD73 monoclonal antibody (mAb) or an AR antagonist may also be beneficial. Recent publications have shown the ability of CD73-targeting mAb therapy to enhance the antitumor immune response and improve the survival of mice bearing several different types of tumors.[9,10] In this case, there is a need for a delicate balance—extracellular adenosine needs to be lowered enough to promote antitumor immunity, but not so much as to exacerbate GvHD. Our studies with tumor-bearing mice suggest that such a delicate balance can be achieved and provide optimism that similar approaches may provide therapeutic benefits in patients (**Fig. 1**).

Notice of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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