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Commentary

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Mechanism of action of posttransplantation cyclophosphamide: more than meets the eye

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For high-risk and refractory hematological malignancies, allogeneic hematopoietic stem cell transplantation (alloHSCT) is the only available curative therapy, with benefits derived from the antigenic disparity between recipient cancer and the incoming immune system. This immunologic mismatch can also lead to lethal graft-versus-host disease (GVHD), and immunosuppression strategies, including high-dose posttransplantation cyclophosphamide (PTCy), have been developed to allow for safe alloHSCT delivery. In this issue of *JCI*, Wachsmuth et al. present the results of preclinical studies designed to evaluate the mechanisms that underlie efficacy of PTCy after alloHSCT. The results of this study challenge previous reports indicating that alloreactive T cell elimination and thymic clonal deletion are primary mediators of PTCy efficacy and provide strong evidence to support FoxP3+CD4+ Tregs as important effectors of PTCy benefits.

PTCy improves allogeneic bone marrow transplant outcomes

Allogeneic hematopoietic stem cell transplantation (alloHSCT) remains the most effective curative therapy for patients with high-risk hematologic malignancies. Unfortunately, the development of acute and chronic graft-versus-host disease (GVHD) continues to affect the majority of transplant patients, causing high morbidity and mortality and consequent therapy failure. Administration of high-dose posttransplantation cyclophosphamide (PTCy) following alloHSCT has dramatically changed the therapeutic landscape of alloHSCT. PTCy has allowed almost universal access to the alloHSCT procedure through haploidentical donor use without a parallel increase in GVHD incidence or other immunosuppression-related toxicities (1, 2). Moreover, regardless of the degree of HLA matching or stem cell source for alloHSCT, PTCy effectively ablates chronic GVHD (1–5), thus enabling permanent discontinuation of immunosuppression in the majority of patients within the first year after transplantation (2, 6). In addition to being protected against GVHD, PTCy recipients maintain antiinfectious and antitumor responses (6–9), thus not compromising survival outcomes (10–12).

Despite clinical advances with the use of PTCy to improve alloHSCT outcomes, the mechanistic underpinnings of PTCy efficacy are not fully understood. Until recently, insight into PTCy action has been primarily driven by decades-old data obtained from experiments in MHC-matched murine models of skin allograft rejection (13, 14), in which selective elimination of alloantigen-reactive CD4+ but not CD8+ T cells was critical for preventing rejection (15). These highly contextual observations have not been tested in alloHSCT models, in which more complex immune interactions are at play, and Tregs have recently been described as essential for the GVHD-protective benefits of PTCy (16, 17). Now, in this issue, Wachsmuth et al. demonstrate that previously proposed preferential elimination and clonal deletion of alloreactive T cells following cyclophosphamide use (13, 14) are not the dominant mechanisms needed for the beneficial effects of PTCy after alloHSCT, while solidifying the evidence supporting Treg importance in mediating long-term posttransplant tolerance and GVHD control with PTCy (18).

Tregs are essential

Wachsmuth et al. performed meticulously executed studies in multiple models of murine transplantation, with a particular focus on alloantigen-specific responses following PTCy to reach their conclusions. However, some limitations to the study remain. Authors base their findings mostly on a model of haploidentical transplantation (B6C3F1→B6D2F1) in which the pathogenic role of CD4+ versus CD8+ T cells remains undefined, thus limiting complete interpretation of study results. This is particularly relevant when superantigen responses are considered, as CD8+ T cell responses are of questionable significance in mice and more so in humans. Nevertheless, experiments investigating the fate of transgenic 2C and 4C T cells unequivocally demonstrated the limited impact PTCy has on alloreactive T cell proliferation, expansion, and persistence, despite parallel GVHD protection (though the latter was documented only for the 2C T cells). Wachsmuth et al. did not observe any measurable effects of PTCy on the posttransplant profile or recovery kinetics of 2C CD8+ T cells, while only proliferation of 4C CD4+ T cells was affected by the drug. The similarities between this work and the original studies of cyclophospha-

mide and allograft tolerance end here, as the study by Wachsmuth et al. unquestionably dispels the notion that thymic function is necessary for PTCy-mediated GVHD protection and subsequently reinforces previous work to establish a role for Tregs in mediating PTCy benefits (16, 17). While the thymic independence of PTCy was hinted at in previous work describing the impact of distinct Treg subsets on PTCy outcome (16) and based on the observation that clinical PTCy benefits are maintained in adult human patients with limited thymic function, the current study by Wachsmuth and colleagues provides the definitive experimental proof. Several aspects regarding the role of Tregs in PTCy-mediated tolerance still require additional clarification, particularly regarding the duration of Treg benefit mediated by PTCy. Experiments in which Treg depletion can be induced validate the requirement for Tregs in mediating long-term alloHSCT tolerance; however, the precise impact of PTCy on the observed GVHD-like disease remains unclear. Notably, a GVHD-like condition that was distinct from the model-specific GVHD phenotype was also seen with Treg depletion following murine alloHSCT when GVHD was prevented without PTCy use (19).

Conclusions and future directions

The observations reported by Wachsmuth et al. lay particularly strong foundations for future studies, as their work provides decisive evidence that PTCy-dependent benefits are not mediated via alloreactive T cell elimination, while solidifying the evidence of Treg importance in delivering the PTCy outcome. Indeed, the sparing of the alloantigen-specific T cells and an overall negligible impact on T cell proliferation and expansion is the likely foundation behind the robust immune reconstitution and limited impact of PTCy on antiinfectious and antitumor responses. Moreover, the documented ability of PTCy-exposed T cells to respond to alloantigen further hints that immune responses remain preserved, though dedicated in vivo or in vitro experiments are needed to confirm this finding and to investigate its relevance for graft-versus-tumor responses.

Several of the observations made by Wachsmuth et al. will require careful attention while providing leads for future studies. For example, a clear difference between CD8+ and CD4+ T cell responses with PTCy use after allotransplantation — in both hematopoietic cell and solid organ transplants — has now been repeatedly observed; however, the significance of this finding has not been fully queried. It is plausible that PTCy has distinct effects on T cell subsets and can thereby differentially mediate their functions after transplantation (e.g., antitumor or antiinfectious immunity vs. GVHD). Therefore, identification of these individual T cell roles could open new approaches to modulating post-transplant alloreactivity and optimizing PTCy-based GVHD prevention as a platform for cellular therapies after alloHSCT. These observations are highlighted in data demonstrating the impaired proliferation of PTCy-treated CD8+ and CD4+ T cells in a mixed-lymphocyte reaction assay and the ability to transfer PTCy-mediated GVHD protection in the absence of Tregs (see Figures 6 and 7 in ref. 18), suggesting that T cell–intrinsic effects, which have thus far been largely ignored, may be essential for the long-term benefits of PTCy administration. In addition, the results of the Wachsmuth et al. study provide further guidance about when to query post-PTCy immune responses, as tolerizing imprinting occurs within a very short window after cyclophosphamide administration. These results provide clear fodder for dedicated investigation and further narrow down the time line within which PTCy modulates the alloresponse. Early control of the alloresponse is increasingly proving to be critical for long-term success of immunosuppression strategies, yet no other approaches are further reaching than PTCy. The robust chronic GVHD control, which is not seen with other novel acute GVHD-controlling strategies, such as siro-limus or IL-6 receptor inhibition (20–22), coupled with the ability to break the tolerance barriers, ease of use, and unequiv-ocal evidence of outcomes comparable to standard alloBMT approaches, all provide for an enhanced access to transplantation across the globe. These clinical developments are made possible by the years of work aimed at increasing the understanding of how PTCy works, and now the study of Wachsmuth provides critical information for further advances.

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