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The Bottom Line

Selective Allodepletion: Have We Finally Found the Holy Grail?

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Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment option for patients with hematologic malignancies and nonmalignant disorders of hematopoiesis. Outcomes of allogeneic HSCT have improved steadily over the decades [1]. However, significant challenges remain. One of these challenges is the so-called Holy Grail of stem cell transplantation immunology: to preserve the beneficial effects of donor T cells against the tumor and against pathogens while avoiding allogeneic graft-versus-host disease (GVHD) [2]. To find the Holy Grail, we must develop methods or treatments that selectively deplete T cells that react against host alloantigens, yet preserve tumor-specific and pathogen-reactive T cells. Experiments published by Ross et al. in this issue of *Biology of Blood and Marrow Transplantation* [3] suggest that post-transplantation cyclophosphamide (PTC), a novel form of GVHD prophylaxis, may do just that.

PTC is celebrating its golden anniversary. In October 1963, Berenbaum and Brown reported that cyclophosphamide (Cy) treatment of rats prolonged the survival of skin allografts, and that graft survival was longest when the drug was given 1 to 3 days after, rather than before, transplantation [4]. The road to the use of PTC in allogeneic HSCT is long, but significant credit must be given to Hisanori Mayumi et al. in the laboratory of Kikuo Nomoto [5]. These investigators developed a method for establishing hematopoietic microchimerism and robust tolerance to minor histocompatibility antigens in mice by i.v. infusion of allogeneic spleen cells followed in 2 or 3 days by intraperitoneal administration of high-dose Cy [6]. They showed that a high dose of cells was required for tolerance induction, and that the dose and timing of Cy were critical to the outcome [7]. For example, tolerance and chimerism were induced if the drug were given on day 2 or 3 after cell infusion, but not if the drug were given on day 1 or on day 4. Interestingly, Cy-induced tolerance was blocked by peritransplantation treatment of the recipient with cyclosporine A [8]. Finally, they showed that tolerance of the microchimeric donor cells was mediated initially by Cy-induced destruction of alloreactive host T cells, then by intrathymic clonal deletion of donor-reactive host cells, and later by the breakdown of clonal deletion and emergence of donor-specific suppressor T cells in the periphery [9].

These initial studies aimed at generating tolerance to solid organ allografts and focused primarily on the effects of Cy on alloreactive T cells. In contrast, Ross et al. studied the differential effects of the drug on alloreactive versus non-alloreactive T cells infused into lethally conditioned recipients of syngeneic or allogeneic HSCT. By labeling donor T cells with the intravital dye carboxyfluorescein succinimidyl ester, they were able to study the toxicity of the drug according to the number of divisions a cell had undergone *in vivo*. After allogeneic HSCT, a dose of Cy that prevented GVHD (33 mg/kg each on days 3 and 4) killed the vast majority of cells that had divided more than 2 times, and only 2 to 3 generations of early dividing cells remained. In contrast, after syngeneic HSCT, donor cells underwent slow, lymphopenia-induced proliferation (LIP), and most of these cells survived the same dose of Cy. The authors then tracked the fate of ovalbumin-specific (OT-1) or melanoma antigen gp100-specific transgenic T cells undergoing lymphopenia- or antigen-induced proliferation. Cells responding to lymphopenia proliferated slowly and were largely resistant to Cy, whereas the same cells responding to antigen proliferated rapidly and were killed by Cy given on day 3 and 4. Finally, OT-1 cells were coinfused with alloreactive cells into lethally irradiated allogeneic recipients. PTC abrogated GVHD and enhanced reconstitution of the OT-1 cells, suggesting that a cytotoxic and immunosuppressive drug can actually improve immune reconstitution after allogeneic HSCT.

The work by Ross et al. is important for several reasons. First, it raises the possibility that a T cell's sensitivity to being killed by Cy is determined by its rate of proliferation. Second, it shows that LIP does not render a T cell susceptible to Cy-induced death. Finally, and perhaps most importantly, the work provides the first demonstration of selective *in vivo* allodepletion, placing the Holy Grail of stem cell transplantation immunology firmly in sight of clinicians. Indeed, after establishing its utility in preventing GVHD after allogeneic HSCT in humans [10,11], PTC is returning to the field of solid organ transplantation, helping to promote immunosuppression-free tolerance to combined kidney and bone marrow allografts [12].

Still, important questions remain unanswered. When transplant donors are primed to host alloantigens 1 week before allogeneic HSCT, the donor T cells resist PTC and induce lethal GVHD [13]. Are alloantigen-primed donor T cells able to resist PTC because they proliferate more slowly, or have they acquired additional mechanisms of drug resistance? Does LIP protect cells from Cy-induced tolerance, or would non-alloreactive cells resist Cy-induced killing in immunocompetent, i.e. non-lymphopenic, hosts? Finally, what is the fate of leukemia-specific, as opposed to alloreactive, T cells that are infused into an animal with minimal

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residual disease and then exposed to PTC? These and other questions should provide a hearty agenda for future research.

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Many are Called but Few are Chosen: Under-utilization of Unrelated Donor Transplantation

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Many within the transplantation community perceive that allogeneic hematopoietic cell transplantation (HCT) is an underutilized approach among appropriate adult patients who potentially may benefit from this procedure. This perception is particularly true for patients who do not have an HLA-identical sibling donor and for whom the graft must come from unrelated donors or umbilical cord blood (UCB) units (henceforth, both are referred to as URD). In this issue, Yao et al. [1] present a study that quantifies this view; after accounting for comorbidities, pretransplantation treatment mortality, and patient preferences, only one quarter of patients in whom URD HCT is indicated actually receive it. The utilization for specific diseases ranged from 11% for myeloma to 54% for chronic myeloid leukemia, 2 diseases for which indications for HCT have dropped. In the present era, when it appears that nearly all patients can find a suitable donor for transplantation, including haploidentical HCT [2], this low percentage is a staggering statistic. Further, Yao et al. [1] show that among acute leukemia patients, two thirds underwent transplantation only in late stage disease, a point in time when survival outcomes are inferior when compared with earlier use of HCT [3,4].

The National Marrow Donor Program (NMDP) has access to 21 million potential donors worldwide, including 11 million through its Be The Match Registry. This potential graft inventory also includes more than 600,000 cryopreserved UCB units. The likelihood of identifying an adult donor, unfortunately, reflects race, as white patients have a 93% likelihood of finding a match, whereas for minority ethnic and racial groups, the chance is less; the lowest likelihood is for blacks, at 66%. On the other hand, a suitable UCB unit can be found for transplantation for most patients in whom a matched adult donor cannot be found. The NMDP estimates that approximately 12,000 patients in the United States need an URD transplantation each year. An early step in this process is the initiation of a "formal search", which usually indicates a definitive intent on behalf of the transplantation center to move ahead with an URD HCT. In the 2012 fiscal year, a formal search was requested for only 7400 patients, just 62% of the need. Furthermore, only 60% of patients for whom a formal search was initiated actually proceeded to transplantation.

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