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Ask the Expert

Post-Transplant High-Dose Cyclophosphamide for the Prevention of Graft-versus-Host Disease

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Cyclophosphamide's lack of hematopoietic stem cell toxicity and its unique effects on the immune system have prompted several investigators to explore its potential for the prevention of graft-versus-host disease (GVHD). In haploidentical hematopoietic stem cell transplants, post-transplant cyclophosphamide together with standard prophylaxis reduces the incidence of GVHD to acceptable rates without the need for T cell depletion. In matched related and unrelated donor settings, cyclophosphamide alone has produced encouraging results. In particular, the low incidence of chronic GVHD is noteworthy. Here, we present a review of the current understanding of the mechanism of action of post-transplant cyclophosphamide and summarize the clinical data on its use for the prevention of GVHD.

INTRODUCTION

Although considerable advances have been made in HLA matching and donor selection, graft-versus-host disease (GVHD) remains a major impediment to the development and widespread applicability of allogeneic blood and marrow transplantation [1-3]. Despite the routine use of prophylactic therapies, acute GVHD complicates 40% to 50% of transplants, whereas chronic GVHD is encountered in 10% to 80% of cases [1-3]. The detrimental effect of current prophylactic regimens on immune reconstitution and the potential to abolish graft-versus-disease (GVD) effect have provided additional impetus to develop alternative regimens.

In 1963, based on personal work and that of others, Berenbaum [4] reported that skin allografts in mice have better survival after the administration of a single dose of cyclophosphamide 1 to 3 days after grafting. Subsequent animal experiments and clinical trials enhanced our understanding of the immune modulatory effects of cyclophosphamide and established a platform for innovation and progress in the field of transplantation. Here, we review the data on the use of post-transplant high-dose cyclophosphamide for the prevention of GVHD.

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RATIONALE AND MECHANISM OF ACTION

Cyclophosphamide is an alkylating agent of the nitrogen mustard category. It has been in use for many years and has an excellent toxicity profile [5]. Cyclophosphamide is oxidatively metabolized by the hepatic cytochrome P450 into 2 powerful metabolites, phosphoramide mustard and acrolein, and prevents cell division by crosslinking DNA strands. Although the alkylating effect of the active metabolites occurs throughout the cell cycle, it is most pronounced during the G1 and S phases of cell division [6]. Rapidly proliferating cells subjected to DNA crosslinking are more susceptible to cyclophosphamide because of their reduced ability to replicate damaged DNA. Conversely, hematopoietic stem cells that are rich in aldehyde dehydrogenase, the enzyme required for the conversion of phosphoramide mustard into the inactive metabolite carboxycyclophosphamide, are resistant to cyclophosphamide. Consequently, cyclophosphamide can be administered after allogeneic hematopoietic cell transplant without impairing engraftment [7].

A fundamental difference of the effect of cyclophosphamide on T cells in contrast to other immunosuppressive agents is its ability to induce apoptosis. Comparing the effects of different drugs on human peripheral blood T cells, cytotoxic cell lines, and Jurkat T cells, Strauss et al. [8] found that only cyclophosphamide (and methotrexate) triggered cell death. All other drugs, including steroids, calcineurin inhibitors, sirolimus, and mycophenolate mofetil (MMF)

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inhibited T cells without causing apoptosis. In addition, cyclophosphamide up-regulated Fas (CD95) expression, triggering activation-induced cell death within 6 days of activation. None of the other drugs affected CD95 expression.

Early animal experiments identified cyclophosphamide as the most effective drug to suppress antibody production in response to bacterial vaccines [9]. In addition, unlike other antimitotics, cyclophosphamide did not need to be used at low doses over prolonged periods of time [4,9]. In organ transplantation, pivotal experiments showed that cyclophosphamide administered intraperitoneally in single high dose early after skin allografts in mice was sufficient to delay graft rejection across MHC mismatches. The timing of cyclophosphamide administration was critical [4,10]. Although administration of cyclophosphamide to mice before or simultaneously with allogeneic stimuli suppressed antibody production, tolerance was not induced. The maximal effect in improving graft survival was observed when cyclophosphamide was given between grafting and day 4 afterward [4,10]. Furthermore, although cyclophosphamide only delayed graft rejection in MCH mismatches, it induced definitive tolerance across minor histocompatibility mismatches [11,12].

Based on earlier experiments performed by Nirmul et al. [11,12], Mayumi et al. [13] established a "cells-followed-by-cyclophosphamide" scheme in which administration of allogeneic spleen cells followed by cyclophosphamide administration 2 days thereafter induced tolerance to allografts in different mouse models. Several studies have examined the mechanism of action of cyclophosphamide. $V_{\beta 6}$-bearing T cells directed to minor lymphocyte stimulating-1^a (Mls-1^a) were examined in a skin allograft experiment in which BALB/c mouse recipient (Mls-1^b) were rendered tolerant to DBA/2 mouse donor (Mls-1^a) with donor spleen cells and cyclophosphamide [14]. In the early phase, the donor-reactive proliferative $CD4^+$-$V_{\beta 6}$ T cells in the lymph nodes were destroyed (clonal destruction), whereas the resting $CD8^+$-$V_{\beta 6+}$ T cells were spared. Furthermore, neither immature $CD4^+$-$V_{\beta 6}$ nor immature $CD8^+$-$V_{\beta 6}$ present in the thymus on day 14 were detectable by day 35 (clonal deletion). In contrast to clonal destruction, clonal deletion was long-lasting. These findings were supported by similar experiments based on different models [15-17]. In one similar study, the complete disappearance of $CD4^+$ cells in the periphery was delayed until thymus depletion occurred [18].

In hematopoietic stem cell transplantation, the same 2 mechanisms of action of cyclophosphamide were confirmed to prevent GVHD by affecting host-reactive cells. When AKR/J-derived Thy-1.1 cells expressing $V_{\beta 3}$ directed against Mls-2^a were examined in a model using a CH3/He mouse recipient (H-2^k, Mls-1^b, Mls-2^a) and AKR/J mouse donor (H-2k, Mls-1^a, Mls-2^b), cyclophosphamide similarly induced early clonal destruction of donor cells followed by clonal deletion of host-reactive cells [19]. These results were supported by the findings by Huyan et al. [20], who examined the effect of intraperitoneal administration of cyclophosphamide on peripheral blood lymphocyte counts in mice. Although 50 mg/kg produced no significant effect, higher doses induced a dose-dependent decrease in $CD4^+$ cells. The decrease began on day +1 and reached nadir on day +4. After a transient rebound, cell numbers decreased again after day +10. The same pattern was observed for B lymphocytes. In contrast, $CD8^+$ cells increased on day 4 [20]. Conceivably, the early decrease in $CD4^+$ but not $CD8^+$ counts was due to clonal destruction, whereas the later decrease in $CD4^+$ was secondary to thymocyte depletion.

Nirmual et al. [11,12] observed that timely administration of cyclophosphamide prevented sensitization of mice receiving large dose of donor spleen cells to skin allografts and prolonged the survival of the grafts. They postulated that cyclophosphamide acted on proliferating cells triggered into cycling after contact with antigen. Their hypothesis was recently confirmed by Ross et al. [21]. In a GVHD mouse model, donor cells were labeled with proliferation dyes and traced in syngeneic and allogeneic transplantation experiments. Mice received increasing doses of cyclophosphamide 3 to 4 days after transplantation. In the syngeneic model, lymphopenia-induced T cell proliferation was slow, sparing a large fraction of cells; the nondeleted cells retained functionality. In the allogeneic model, 22 mg/kg cyclophosphamide failed to prevent GVHD when antigen-stimulated T cells undergoing multiple divisions remained present. However, when the dose was increased to 66 mg/kg, GVHD was alleviated, and only the first 2 to 3 generations of the rapidly dividing alloreactive T cells remained. Cells undergoing more divisions did not survive, whereas slowly dividing cells were also preserved. In a different study published in abstract form, Cieri et al. [22] studied immune reconstitution during the first month in a series of patients undergoing T cell–replete peripheral blood haploidentical transplantation. GVHD prophylaxis consisted of post-transplant cyclophosphamide combined with MMF and sirolimus. Although proliferating alloreactive T lymphocytes were abated, memory cells, including stem memory cells ($CD45RA^+$, $CD62^+$, and $CD95^+$), were increased. The authors postulated that the increase in stem cell memory cells was due to differentiation from naïve T cells. These cells escaped the cyclophosphamide purging effect because of their delayed proliferation in comparison with the alloreactive T cells.

Taken together, these findings suggest that cyclophosphamide can prevent GVHD without compromising timely immune reconstitution. This selective action of cyclophosphamide on proliferating T cells has a number of practical implications. The first implication relates to its potential use in combination with other immunosuppressive agents. For instance, the concomitant use of drugs that induce T cell anergy and may prevent cells from cycling might be theoretically counterproductive [13]. The addition of such drugs must rather be delayed. Pan T cell antibodies, on the other hand, might be synergistic and enhance the deletion of the relevant T cell clones [13]. On the downside, the selective action of cyclophosphamide allows memory T cells induced by prior alloimmunization of the recipient to escape cyclophosphamide and increase the risk of graft failure.

Finally, the effect of cyclophosphamide on regulatory T cells (Tregs) has been the subject of intense investigation. Although early animal studies suggested a negative effect of cyclophosphamide on Tregs, different results were obtained in human studies. North [23] demonstrated that cyclophosphamide can facilitate adoptive immunotherapy of established tumors in mice. Although the administration of cyclophosphamide failed to cause tumor regression, the combination of cyclophosphamide and spleen cells permanently abolished the tumor. The authors postulated that "suppressor but not immune T cells" were sensitive to cyclophosphamide. Similarly, Ghiringhelli et al. [24] demonstrated that the administration of a single dose of cyclophosphamide to rats with established tumor depleted