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Tolerance induction in HLA disparate living donor kidney transplantation by facilitating cell-enriched donor stem cell Infusion: The importance of durable chimerism

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ABSTRACT

Successful solid organ transplantation currently requires the life-long use of medications to suppress the immune system in order to prevent transplant rejection. Drug-based immunosuppression significantly increases the risk of infection and cancer, as well as being very costly. Development of new therapies to minimize or eliminate entirely the need for anti-rejection drugs is of great interest to the transplant community. Therapeutic cell transfer for the control of the human immune system represents a compelling approach to reduce or eliminate the need for anti-rejection drugs. Establishment of durable hematopoietic chimerism through hematopoietic stem cell transplantation (HSCT) has been shown in preclinical models and patients to lead to donor specific tolerance. However, the application HSCT is limited by the potential toxicity of conditioning regimens, the risk of graft versus host disease (GVHD) and the challenge of HLA mismatching. In this review we describe the clinical outcomes and science behind a $CD8^+/TCR^-$ facilitating cell-based hematopoietic stem cell transplant approach (termed FCRx) to induce tolerance to mismatched renal allografts while minimizing the risk of graft-versus-host GVHD and achieving avoidance of long-term immunosuppressant drugs in living donor kidney transplant recipients.

1. Introduction

Kidney transplantation is the treatment of choice for most causes of end stage renal disease. Prevention of organ transplant rejection requires the routine use immunosuppressive drugs (IS) for life, generally a combination of agents including a calcineurin inhibitor (CNI), an antiproliferative agent, and corticosteroids [1–3]. Dependence on IS tempers the substantial benefit obtained from transplantation. Specifically, CNIs are nephrotoxic, a side effect of significant concern in renal transplantation. Antiproliferative agents such as mycophenolate have gastrointestinal toxicities and may lead to bone marrow suppression. Steroids exacerbate osteoporosis and hyperlipidemia, and cause avascular osteonecrosis. IS drugs may worsen glucose tolerance and hypertension, and are associated with unpleasant cosmetic effects, many times leading to non-compliance. Although short term results are good, these toxicities take a toll, contributing to a 50% patient mortality at 10 years. Development of alternate therapies that help to minimize the need for lifelong immunosuppression, or eliminate entirely the need for drugs through the induction of tolerance, are therefore of great interest.

It has been known since the early reports of Owen in 1945 [4] and Billingham et al. in 1953 [5] that chimerism induces tolerance to organ and tissue transplants. Owen observed that genetically disparate "freemartin" cattle twins sharing a common placenta were red blood cell chimeras, suggesting that each was reciprocally tolerant to the other sibling as evidenced by persistent chimerism after birth. Billingham, Brent and Medawar extended these findings to demonstrate that infusion of hematopoietic-derived cells into newborn mice resulted in chimerism and was associated with acceptance of donor skin grafts. In the ensuing years, significant effort was focused on overcoming specific obstacles preventing successful translation of a HSC-based approach to tolerance induction to the clinic, including the toxicity of myeloablative conditioning, the requirement for stringent HLA matching of bone marrow donors and recipients, and the complication of graft-versus-host disease (GVHD). Safe and effective non-myeloablative approaches to HSCT have been developed and widely adopted in clinical practice for the treatment of malignant and non-malignant diseases [6]. Insights into the composition of bone marrow has led to identification of cell populations which promote GVHD, versus those which facilitate stem cell engraftment and help reduce the development of GVHD [7]. As discussed below, leveraging the

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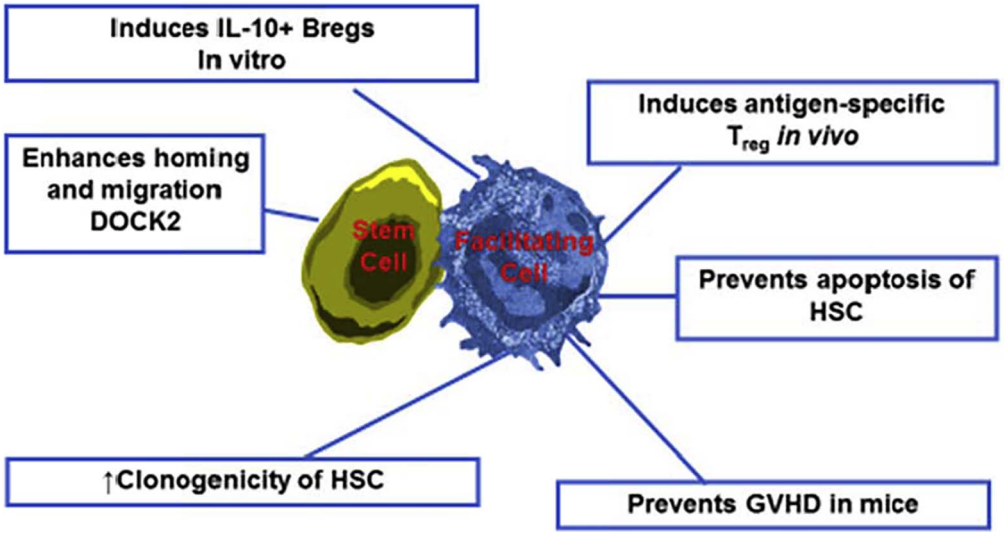

Fig. 1. Facilitating cells have multiple immunomodulatory and trophic effects on hematopoietic stem and progenitor cells to promote chimerism and tolerance.

development of nonmyeloablative conditioning and the ability to "engineer" the donor HSC graft has allowed for the clinical translation of combined donor stem cell/solid organ transplant approach to achieve durable chimerism and robust donor specific tolerance in renal allograft recipients.

2. The facilitating cell (FC)

FC were first described by Ildstad et al. as bone marrow derived $CD8^+TCR^-$ cell population that enables engraftment of hematopoietic stem cells (HSC) across major histocompatibility complex (MHC) barriers without causing GVHD [7]. Further cell surface marker characterization and functional assays revealed that murine FC are comprised of plasmacytoid-precursor dendritic cells (p-preDCs), B cells (B220 and/or CD19 positive), NK cells (NK1.1 positive), granulocytes (Gr-1 positive) and monocytes (CD14) [8]. FC express a unique FCp33-TCRβ heterodimer in place of αβ TCR [9]. This $CD8^+TCR^-$ BM-derived mixed cell population promotes HSC engraftment without causing GVHD and exerts its engraftment-promoting function through autocrine or paracrine interactions with HSC that enable their engraftment across major histocompatibility complex (MHC) barriers [8,10] (Fig. 1).

In pre-clinical and clinical studies conducted to date, the FC population has been shown to be instrumental in establishing donor-specific allogeneic tolerance in kidney and other solid organ and hematological transplants [7–13]. The FC population is a tolerogenic cell population that has been shown to induce a number of immunomodulatory effects, including $CD4^+/CD25^+/FoxP3^+$ antigen-specific T_{reg} *in vivo* and *in vitro* as well as to induce IL-10-producing Trl cells [14], both recognized as important components in the establishment of immunological tolerance [15,16].

The human FC population is composed of two equally divided phenotypic subpopulations: $CD56^{bright}$ and $CD56^{dim}$ FC [11,17]. The $CD56^{bright}$ FC subpopulation's main role appears to be enhancing durable donor HSC engraftment, chimerism, tolerance and production of factors that prime the efficiency of HSC migration to the hematopoietic niche [16,17]. The majority of $CD56^{bright}$ FC are $CD11c^+/CD11b^+/CXCR4^+$ and exhibit a dendritic morphology after stimulation with CPG-ODN. This phenotype and response to CPG-ODN is consistent with the regulatory cell-inducing effects of FC on T and B cells, as tolerogenic p-preDC also exert such an effect.

The role of the $CD56^{dim}$ FC subpopulation is to promote early HSC homing *in vivo* and enhance clonogenicity of HSC *in vitro* and *in vivo*. The majority of $CD56^{dim}$ express $CXCR4^+$ and CD3ε and exhibit a lymphoid morphology. Co-culture of both subpopulations with HSC results in upregulation of a number of factors that prime homing and migration of HSC. $CD56^{dim}$ FC enhance homing of human HSC to the BM in NOD-SCID gamma recipients and promote HSC clonogenicity. Recipients of HSC plus $CD56^{dim}$ or $CD56^{bright}$ FC showed durable donor human chimerism in peripheral blood, BM and spleen [17]. Taken together, one could hypothesize that FC exert multiple trophic and regulatory effects on HSC to promote chimerism and tolerance *in vivo*.

Based upon the phenotypic and functional characterization of FC, Ildstad et al. developed an approach to manufacture a clinical stem cell product enriched for FC, termed the FCRx. This proprietary immunomagnetic selection process allows one to deplete GVHD-causing cells yet retain HSC, progenitors and the subpopulations of FC. As detailed below, combining the FCRx with a living donor kidney transplant, in conjunction with reduced-intensity nonmyeloablative conditioning, has allowed establishment of high levels of donor chimerism in mismatched related and unrelated recipients [11–13].

3. Phase 2 trial of FCRx in kidney transplantation

Persistent multilineage chimerism achieved through the adoptive transfer of donor HSC has served as a surrogate biomarker for long-term graft survival and transplantation tolerance [18]. Over the past decade, therapeutic cell transfer of donor HSC in combination with a living donor kidney transplant has been used with some success to achieve transplantation tolerance. Work by Strober and colleagues has demonstrated the ability to achieve persistent HSCT engraftment in HLA-identical living related donor kidney transplant recipients, allowing for tolerance and full withdrawal of immunosuppression [19]. Other groups have suggested that donor HSCT serve an immunomodulatory function, allowing for, at best, transient chimerism yet still permitting the withdrawal of immunosuppression in well-matched organ transplant recipients [20,21]. The advantage of approaches leading to persistent chimerism is that chimerism is an easily measurable, validated, "real-time" assay that strongly correlates with transplantation tolerance. So-called operational tolerance, absent detectable donor chimerism, is currently difficult to detect and monitor as to its existence and stability with currently available tools.

Preclinical studies using BM enriched for FC have indicated the ability to safely achieve persistent donor chimerism without GVHD, despite marked MHC donor/recipient disparity. In 2009 our group initiated an ongoing Phase 2 trial to induce tolerance in mismatched and unrelated recipients of living donor renal allografts using donor HSCT engineered to be enriched for FC (FCRx). Recipients were treated with nonmyeloablative conditioning that included fludarabine (30 mg/m2,