

# Host stem cells repopulate liver allografts

## Reverse chimerism

Zhaoli Sun\* and George Melville Williams

Department of Surgery; Johns Hopkins University School of Medicine; Baltimore, MD USA

**L**iver transplant has become life-saving therapy for thousands of patients with end stage liver disease in the United States, but chronic rejection and the toxicities of immunosuppression remain significant obstacles to the further expansion of this modality and “transplant tolerance” remains a central goal in the field. So we and others are looking for alternative post-transplant strategies. We set out to ‘engineer’ repopulation after transplantation in a strain combination [dark agouti (DA) to Lewis green fluorescent protein<sup>+</sup> (LEW-GFP<sup>+</sup>)] which rejects liver grafts strongly, a model that more closely resembles the situation in humans. Our central finding is purposeful manipulation of the immune response with low dose immunosuppression and liberation of stem cells for a very short period after transplantation results in long-term transplant acceptance by two mechanisms: transforming the liver (donor) to self (host) phenotype, and auto-suppression of the specific allograft response.

### Introduction

Current knowledge suggests that transplant rejection may be thwarted three different ways. Conventional immunosuppression (IS) is designed to keep the recipient in a “state of ignorance” of the graft by employing powerful agents inhibiting the immune response. This approach is responsible for the success currently enjoyed in clinical transplantation. However, when IS coverage is inadequate, rejection episodes occur which must be treated with increased amounts

of IS which leads to an increased risk of developing certain cancers and opportunistic infections. Lifelong treatment is needed to avoid acute and chronic rejection. The second paradigm of recipient-graft interaction is that in which the recipient takes on some of the donor’s properties, e.g., ‘mixed-chimerism.’ This scenario may be unintentional, i.e., when passenger leukocytes migrate from the donor graft,<sup>1,2</sup> or may be therapeutically facilitated by donor hematopoietic stem cell (HSC) transfer which may generate a state of donor specific unresponsiveness or “transplant tolerance.”<sup>3-5</sup> Approaches utilizing mixed chimerism strategies seek to generate a state of donor-specific unresponsiveness in the host while leaving the recipient immune system largely intact. The durability and mechanism of the tolerance depend on the regimen utilized. Disadvantages include: the toxicity of preparatory regimens and the ongoing battle between recipient and graft which can lead to loss of tolerance or graft vs. host disease (GVHD). A third possible donor-recipient interaction paradigm was considered but lacked experimental support and occurs if a recipient significantly repopulates a transplanted donor graft, the conceptual reverse of mixed chimerism. For the first time, studies<sup>6</sup> have revealed the conditions which enable this form of graft acceptance to occur.

### Reverse Chimerism and Liver Allograft Acceptance

The phenomenon of recipient repopulation of donor graft was first reported in an aortic allograft model over 40 y ago by

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\*Correspondence to: Zhaoli Sun;  
Email: zsun2@jhmi.edu

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George Melville Williams<sup>7,8</sup> and has been observed subsequently to a very limited extent in human liver, kidney and heart transplant recipients. Our interest in host repopulation was revived after studying a unique MHC-mismatched liver transplantation model in rats [Lewis into dark agouti (DA)]. In this model, leukocytes invade the portal areas on day 7–10, but animals survive this rejection episode without immunosuppressive agents and live until sacrifice a year or more later. We have shown that repopulation of liver allografts by recipient cells occurs in this model using three methods which discriminate between donor and recipient cells: MHC-I antigen, green fluorescent protein (GFP) and Y-chromosome. Repopulation was accelerated when a reduced-size (50%) liver graft was transplanted. We found that donor GFP<sup>+</sup> cells (sorted by FACS) did not have the Y-chromosome of the recipient (by PCR). Therefore, transdifferentiation rather than cell fusion appeared to be the most likely mechanism of repopulation in our animal model.<sup>9</sup> Recipient cells were initially smaller than hepatocytes and clustered in the central vein areas. Their cytoplasm was positive for albumen. We did not find bone marrow cells repopulating syngeneic grafts after strain concordant liver transplant. Likewise, the provision of exogenous immunosuppression (cyclosporine 5–10 mg/kg) inhibited the process of repopulation. Consequently low-grade rejection was a necessary factor allowing recipient stem cells to have the upper hands in replacing cells injured by rejection.

### Strategies to Promote Reverse Chimerism

To test this theory, we set out to ‘engineer’ repopulation after transplantation in a strain combination [DA to Lewis GFP<sup>+</sup> (LEW GFP<sup>+</sup>)] which rejects liver grafts strongly, a model that more closely resembles the situation in humans. We employed three strategies to promote long-term graft acceptance via stem cell mobilization: (1) transplantation of half-sized livers to stimulate regeneration; (2) provision of low dose tacrolimus during the first week to prevent outright acute rejection; and (3) provision of plerixafor which frees stem cells from their bone marrow niche.

The results enable us to report that plerixafor mobilizes an appropriate stem cell population; that the addition of low dose tacrolimus prevented outright fatal rejection; and that the combination of plerixafor and low dose tacrolimus provided for just the first week after transplantation resulted in long-term liver transplant survival without further drug treatment.

### Reverse Chimerism and Donor-Specific Immunosuppression

In addition to their capacity for multilineage differentiation and participation in reconstitution of the hematopoietic niche, bone marrow derived stem cells, especially mesenchymal stem cells (MSCs) have been shown to exert powerful immunomodulatory effects, including the inhibition of proliferation and effector function of T cells, B cells and natural killer cells.<sup>10-13</sup> These properties make MSCs of potential interest for clinical applications in tissue engineering and immunosuppression. It has recently been recognized that MSCs isolated from adult bone marrow are able to modify the alloimmune response in vitro and in vivo.<sup>14</sup> Interestingly, the CD133 positive cell fraction contains more MSCs with high proliferative potential.<sup>15</sup> We found in dual drug-treated animals, both CD4 Foxp3 and CD8 Foxp3 regulatory cells were abundant in the spleen and allograft while the CD4 phenotype predominated in the transplant. To test if the combination of Plerixafor and low-dose Tacrolimus treatment induced donor-specific immune suppression/tolerance, donor strain and third party skin allografts were performed at 1 mo, which was three weeks after cessation of drug therapy. In these animals, donor strain skin allografts survived for more than one month after which there was slow progressive loss of skin. Third party grafts were rejected aggressively with normal kinetics indicating that combination treatment induced self-perpetuating antigen specific immunosuppression but not tolerance to all donor alloantigens.

### Speculation and Future Work

We propose that controlled rejection not only creates injury signals resulting in

stem cell entrance but also prevents graft regeneration by donor intra-hepatic stem/progeny cells because they are under immunological attack. The combination of injury and limited donor ability to repair provides a significant advantage to recipient-derived stem cells in repopulating the rejecting liver. The process is facilitated by a “push” of stem cells from their niches via pharmacologic mobilization. A small liver provided better retention and differentiation of stem cells. We propose that combined treatment has two mechanisms leading to long-term graft survival without long-term drug treatment: gradual replacement of rejecting donor cells by functioning host stem cells and immunosuppression mediated by regulatory T cells.

Our long-term goal is to develop therapeutic strategies for direct clinical application. In so doing, we plan to test our approaches in large animal (Swine) pre-clinical transplantation models, and to further characterize the underlying biologic mechanisms resulting in this promising novel finding so that the approach can be utilized with confidence clinically.

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