REVIEW

Facilitating cells: Translation of hematopoietic chimerism to achieve clinical tolerance

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ABSTRACT

For over 50 y the association between hematopoietic chimerism and tolerance has been recognized. This originated with the brilliant observation by Dr. Ray Owen that freemartin cattle twins that shared a common placental blood supply were red blood cell chimeras, which led to the discovery that hematopoietic chimerism resulted in actively acquired tolerance. This was first confirmed in neonatal mice by Medawar et al. and subsequently in adult rodents. Fifty years later this concept has been successfully translated to solid organ transplant recipients in the clinic. The field is new, but cell-based therapies are being used with increasing frequency to induce tolerance and immunomodulation. The future is bright. This review focuses on chimerism and tolerance: past, present and prospects for the future.

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In 1945, a creative observation resulted in the inception of a new field of scientific pursuit: tolerance.¹ Dr. Ray Owen, then a young faculty member at the University of Wisconsin, made the observation that genetically disparate freemartin cattle twins that shared a common placenta were tolerant to each other. Although freemartin cattle twins are not rare, his critical observation focused attention on understanding how and why this occurred. Owen observed that freemartin cattle twins exhibited “phenotypic identity of blood type” or red blood cell mixed chimerism. Moreover, subsequent progeny from the twins did not pass on this mixed phenotype blood type. He speculated that freemartin cattle twins shared hematopoietic cells in the common placental vascular anastomosis. Subsequent studies in neonatal mice by Billingham et al. demonstrated that homogenized cellular suspensions transferred tolerance to donor resulted in acceptance of skin grafts from the same donor.² Hence the origin of the concept of chimerism and tolerance.

The corresponding author for this manuscript, Dr. Suzanne Ildstad, first became acquainted with

Dr. Owen as a pen pal. Dr. Ildstad explains, “Although we had never met, he sent me a post card thanking me for citing his work in one of my early manuscripts. Subsequent post cards from him followed with additional publications from our group. As a young assistant professor just starting my own laboratory, those kind gestures had a tremendous impact. They were truly motivating. I finally had the opportunity to meet Professor Owen at the celebration of his career on his 85th birthday in Madison Wisconsin in 2000. It was clear at the celebration that he had generously shared his mentorship with many young scientists.” We dedicate this review to the pioneering work of Dr. Owen. It has truly impacted the field of transplantation. Organ transplant recipients are now benefitting from his seminal contribution where chimerism-induced tolerance has become a clinical reality.

There are a number of types of tolerance. The two most mature applications to solid organ transplantation are operational tolerance and deletional tolerance. Operational tolerance refers to stable graft function off immunosuppression for at least one year in the absence of chimerism.^{3–5} Deletional tolerance refers to

the type of tolerance described by Medawar et al. and Owen, which requires the persistence of donor hematopoietic cells as a source of antigen.⁶

Operational tolerance – is it really robust?

According to the published literature, less than 1% of all recipients of renal transplants ever performed have maintained stable renal function after discontinuing immunosuppressant therapy.³ In a summary of the world experience in discontinuing immunosuppression in renal transplant recipients, the vast majority of subjects experienced rejection and early graft loss. In planned immunosuppression minimization, 80% of renal transplant recipients experienced rejection and failed weaning.⁷ The A recent publication by Hoshino in *Transplantation* reported subjects who were gradually weaned from immunosuppression following renal transplantation in order to minimize immunosuppression.⁸ The rate of production of donor-specific antibody (DSA) was inversely correlated with level of immunosuppression. In subjects tapered to low-dose immunosuppression, 53% had DSA by 3 months, 74% by 6 months, and 80% by one year. DSA is directly correlated with reduced allograft survival.⁸ Even with higher-dose immunosuppression, 55% of their subjects had developed DSA by 1 y. A similar trend has become apparent in the majority of 10 transiently chimeric subjects enrolled in the Massachusetts General Hospital protocol utilizing nonmyeloablative conditioning and infusion of mobilized unmodified hematopoietic stem cell product donor bone marrow following renal transplantation.^{9,10} This pioneering study showed the safety of cell based therapies using hematopoietic stem cell transplantation (HSCT) to induce tolerance and demonstrated a tolerogenic effect for transient chimerism. 7 out of 10 patients were immunosuppression free for 5 y while 3 had resumption of immunosuppression 7, 8 and 10 y after transplantation due to recurrence of disease or chronic rejection. Only one patient out of 5 developed DSA within one year post-transplant in the first cohort. Moreover, in a second set of 5 patients treated with rituximab, none of the recipients has developed DSA. However Currently, in long term follow-up, only 4 subjects currently remain immunosuppression-free from 4.5 – 11.4 y.⁹ A number of subjects who were initially tapered off immunosuppression developed donor-specific antibody and were reinstated on immunosuppression. Three kidneys were lost

to either rejection or recurrent disease. Importantly, subjects who are immunosuppression-free had less diabetes, hypertension and other metabolic complications related to immunosuppression, further supporting the importance of tolerance induction in improving the quality of life for organ transplant recipients. In other pioneering studies by the Stanford team a total lymphoid irradiation-based approach has been utilized.¹¹ The safety of the approach is confirmed but to date durable chimerism has only been achieved in HLA-matched related recipients.^{10,11} Taken together, these findings would suggest that while transient chimerism has been demonstrated to induce operational tolerance in nonhuman primates^{12,13} and humans,^{9,11} it is not sufficient to establish durable, robust donor-specific tolerance and that transient chimerism does not prevent recurrence of autoimmune disease. Therefore, operational tolerance after immunosuppression removal has not been reliably or predictably achieved by any approach and is currently not a viable clinical option.

In 1984, Ildstad and Sachs reported that actively acquired tolerance could be achieved in adult mice ablatively conditioned and transplanted with a mixture of T cell depleted syngeneic plus allogenic or xenogeneic rat bone marrow cells.¹⁴ The primary hurdles to applying chimerism to induce tolerance are the toxicity of conditioning, the need for perfect HLA-matching between donor and recipient, graft vs. host disease (GVHD), and securing durable engraftment (Fig. 1). Over time the morbidity of this procedure was substantially reduced by development of nonmyeloablative conditioning approaches, bringing it one step closer to clinical application.^{9,11,15-17} Nonmyeloablative conditioning is now widely used in the clinic resulting in a significant reduction in the morbidity and mortality of conditioning for hematopoietic progenitor cell (HPC) transplantation.

Conquering the need for HLA matching between donor and recipients

Two other significant challenges that had to be addressed before chimerism could be applied to induce tolerance were GVHD and the need for HLA matching between donor and recipients. Using a mouse model we phenotypically characterized which cells in bone marrow were essential for engraftment of purified stem cells. The result was the discovery of CD8⁺TCR⁻

Figure 1. The primary hurdles to applying chimerism to induce tolerance. (GVHD: graft versus host disease).

graft facilitating cells.^{18,19} Facilitating cells (FC) are a bone marrow derived $CD8^+TCR^-$ cell population that enable engraftment of hematopoietic stem cells (HSC) across major histocompatibility complex (MHC) barriers without causing GVHD.²⁰ In pre-clinical and clinical studies conducted to date, the FC cell population has been shown to be instrumental in establishing donor specific allogeneic tolerance in kidney and other solid organ and hematological transplants.^{21–25} The FC cell population is a tolerogenic cell population that has been shown to induce a number of immunomodulatory effects (Fig. 2), including $CD4^+CD25^+FoxP3^+$ antigen-specific regulatory T cells *in vivo* and *in vitro* as well as to induce IL-10-producing Tr1 cells, both recognized as important components in the establishment of immunological tolerance.

The human FC cell population is composed of 2 equally divided phenotypic sub-populations that are critical to overall FC function: $CD56^{bright}$ FC and

$CD56^{dim/-}$ FC.²⁶ The main role of the $CD56^{bright}$ FC sub-population 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. The majority of $CD56^{bright}$ FC are $CD11c^+CD11b^+CXCR4^+$ and exhibit a dendritic morphology after stimulation with CpG Oligodeoxynucleotide (ODN). This phenotype and response to CpG ODN is consistent with the regulatory cell-inducing effects of FC on T cells and B-cells, as tolerogenic precursor-plasmacytoid dendritic cells (pre-pDC) also exert such an effect.

The role of the $CD56^{dim/-}$ FC sub-population role is to promote early HSC homing *in vivo* and enhance clonogenicity of HSC *in vitro* and *in vivo*.²⁶ The majority of $CD56^{dim/-}$ FC express $CXCR4^+$ and $CD3\epsilon$ and exhibit a lymphoid morphology. Co-culture of both subpopulations with HSC results in upregulation of a number of factors that prime homing and

Figure 2. Facilitating cells have multiple immunomodulatory and trophic effects on haematopoietic stem and progenitor cells to promote chimerism and tolerance.

Figure 3. Algorithm for conditioning, kidney and FCRx transplant. Fludarabine is administered on days -4 , -3 , and -2 (30 mg/kg per dose) relative to the living donor kidney transplant (day 0). Dialysis is performed at least 6 hours after each dose before kidney transplant. Two doses of cyclophosphamide (Cy) (50 mg/kg) are given on days -3 and day $+3$. FCRx is administered on day $+1$.

migration of HSC. CD56^{dim/-} FC enhance homing of human HSC to the bone marrow in NOD-scid gamma (NSG) recipients and promote HSC clonogenicity. NSG recipients of HSC plus CD56^{dim/-} FC or CD56^{bright} FC exhibit durable donor human chimerism in PB, BM and spleen. Taken together, one could hypothesize that FC exert multiple trophic and regulatory effects on HSC to promote chimerism and tolerance *in vivo*.

After the discovery of FC, a method to manufacture FCRx (a tolerance-promoting FC-based HSC graft) product by removing GVHD-producing cells yet retain HSC and FC was developed. In an FDA-regulated phase I/II study in mismatched related and unrelated recipients with hematologic malignancy it was confirmed that engraftment could be achieved while avoiding GVHD.²⁷ This approach to HSCT overcame the final 2 hurdles that had prevented the use of HSCT to induce tolerance to solid organ transplants (Fig. 1): GVHD and the requirement for HLA-matching.

The first successful application of FCRx to solid organ tolerance was for living donor related and unrelated kidney transplantation at Northwestern University in a phase I/II FDA-regulated study (IDE 13947, ClinicalTrials.gov Identifier: NCT00497926). Donors were mobilized with G-CSF $\pm$ prelixifor, apheresed, FCRx prepared, and the product cryopreserved. At least 2 weeks later the recipients were conditioned with 200 cGy TBI, 3 doses of fludarabine (30 mg/m²/

dose) and 2 doses of cyclophosphamide (50 mg/kg/dose) as depicted in Figure 3. MMF and tacrolimus were administered for 6 months. At 6 months, if chimerism, a normal protocol biopsy and stable renal function were present, the MMF was discontinued.²⁸⁻³⁰ At 9 months the tacrolimus is tapered to trough levels of 3. At 12 months if a repeat protocol biopsy and renal function are normal and if chimerism levels are $> 50\%$, the tacrolimus is discontinued.

A total of 30 31 subjects have been transplanted, with follow-up ranging from 1 month to 72 months. Sixteen subjects are completely off immunosuppression with stable renal function (manuscript in preparation). This review will focus on the first 20 subjects that have longer follow-up.²⁹ In the early stages of the study there was a learning curve with respect to sensitization status, post-transplant management, and identifying the minimum target FC and HPC cell dose.²⁸⁻³⁰ Two subjects with panel reactive antibody (PRA) $> 20\%$ did not durably engraft.²⁸ As a result, exclusion criteria were added to avoid sensitized subjects with a PRA $> 20\%$. Early post-transplant management was modified to avoid myelotoxic agents such as Gancyclovir and a hybrid approach to hematopoietic stem cell transplantation combined with solid organ transplant was developed for management of subjects early following transplant. Subjects are discharged on postoperative day 2 and managed as outpatients. With these modifications nearly 100% success in achieving high levels of donor

chimerism has been achieved in the most recent subjects (manuscript in preparation). All subjects experience an expected nadir lasting approximately one week which is managed by neutropenic precautions as an outpatient. Notably, by far the majority of severe adverse events occurred while the subjects were still on double drug immunosuppression. Chimeric subjects were immunocompetent to respond to vaccination with pneumococcal vaccine.²⁹ In fact, durably chimeric subjects off all immunosuppression had more robust immune responses than transiently chimeric subjects on maintenance immunosuppression. T-cell receptor excision circle (TREC) analysis revealed that 97% of T cells were distinct from donor or host, suggesting that the T cell repertoire was newly generated in the post-transplant environment. Early in the study we utilized the mixed lymphocyte reaction (MLR) was utilized as a metric to wean immunosuppression.²⁸ However, the recipients who exhibited only transient donor chimerism in peripheral blood also demonstrated donor specific hyporesponsiveness (DSH), even after loss of chimerism. Moreover, *in vitro* hyporesponsiveness did not predict histologic findings on protocol biopsy. Two transiently chimeric subjects exhibited DSH at the same time a protocol biopsy was positive for subclinical Banff 1A rejection.³⁰ As a result, we no longer perform MLR and rely upon donor T cell donor chimerism as a metric the primary endpoint for decisions regarding immunosuppression weaning and withdrawal. To date this has been directly correlated to with success.

The era of cell-based therapies has arrived and promising results have been generated.³¹ It is astounding to realize how far the 2 pivotal observations in the late 1940s and early 1950s by Owen and Medawar's groups have brought us. The real beneficiaries of over 50 y of translational research on chimerism and tolerance are our patients and their families. The future is bright as applications of these therapies expand to inherited metabolic disorders, hemoglobinopathies, autoimmune disorders and numerous other indications that can be treated by a safe form of HPC chimerism.

Abbreviations

DSA	donor specific antibody
DSH	donor specific hyporesponsiveness
FC	facilitating cells

FCRx	a tolerance-promoting facilitating cell-based hematopoietic stem and progenitor cell graft
GVHD	graft versus host disease
HPC	hematopoietic progenitor cells
HSC	hematopoietic stem cells
HSCT	hematopoietic stem cell transplantation
MHC	major histocompatibility complex
NSG	NOD scid gamma
ODN	oligodeoxynucleotide
PRA	panel reactive antibody
pre-pDC	precursor-plasmacytoid dendritic cells
TREC	T-cell receptor excision circle

Disclosure of potential conflicts of interest

Dr. Suzanne Ildstad has equity interest in Regenerex, LLC, a start-up biotech company. In 2013, the company entered into a global licensing and research collaboration agreement with Novartis for the development of FCRx.

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