Combining Treg therapy with mixed chimerism

Getting the best of both worlds

Nina Pilat and Thomas Wekerle*

Division of Transplantation; Department of Surgery; Vienna General Hospital; Medical University of Vienna; Vienna, Austria

Deliberate establishment of donor-specific immunologic tolerance is considered to be the “Holy Grail” in transplantation medicine, but clinical tolerance protocols for routine organ transplantation are still an unmet need. Mixed hematopoietic chimerism is an attractive tolerance strategy with considerable potential. Recent pilot trials provide proof-of-principle that mixed chimerism can induce tolerance in renal transplant recipients. Routine clinical translation, however, is impeded by the side effects of the cytotoxic recipient conditioning necessary for the transient engraftment of HLA-mismatched BM. In murine studies recently published in *The American Journal of Transplantation*, we demonstrated that the therapeutic application of polyclonal recipient regulatory T cells (Tregs) leads to engraftment of practicable doses of fully allogeneic BM and to donor-specific tolerance without any cytotoxic conditioning, thereby eliminating a major impediment for the clinical translation of the mixed chimerism strategy in the experimental setting. The background and the implications of these findings are discussed.

Limitations of Current Mixed Chimerism Protocols

Mixed hematopoietic chimerism is achieved through transplantation of donor hematopoietic stem cells (HSC) after appropriate recipient conditioning. The robustness of this approach in the experimental setting¹ and its effectiveness in recent clinical pilot trials²⁻⁴ underscore its potential. In one of these studies, operational tolerance

(i.e., long-term stable graft function without chronic immunosuppression) was achieved in four of five patients simultaneously transplanted with renal and bone marrow (BM) grafts from a haplo-identical living donor.² While the intentional establishment of clinical tolerance across HLA barriers is arguably a groundbreaking success, safety concerns preclude routine application of the employed BM transplantation (BMT) protocol. Capillary leak syndrome and profound leukopenia due to the extensive cytotoxic conditioning (which involves T-cell and B-cell depletion on top of myelosuppressive drug treatment) are toxicities widely regarded as unacceptable in organ transplant recipients. Thus, despite its proven effectiveness, the mixed chimerism approach has not made it into routine clinical practice due to unresolved safety concerns. Non-cytotoxic mixed chimerism regimens as a potential solution to this problem are therefore an important research goal.

Feasible non-cytotoxic mixed chimerism protocols have, however, remained elusive so far. Numerous attempts by several groups—including our own—have previously failed to achieve engraftment of conventional doses of BM in a non-cytoreductive setting as neither extensive in vivo T cell depletion nor costimulation blockade were sufficiently effective.⁵⁻⁷ Instead the administration of unrealistic ‘mega’ doses of BM was required to achieve irradiation-free mixed chimerism.^{7,8}

Treg Therapy—Potential and Limitations

Recently, the therapeutic exploitation of regulatory T cells (Tregs) has attracted

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Correspondence to: Thomas Wekerle;
Email: Thomas.Wekerle@meduniwien.ac.at

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a lot of attention which is largely based on their well established importance in maintaining self tolerance.^{9,10} Treg therapy has potent effects in autoimmune models.^{11–14} With regard to transplantation, efficacy of Treg therapy has been demonstrated employing lymphopenic hosts,^{15,16} Tregs engineered to express a transgenic TCR^{17,18} and models crossing minor¹⁷ or single major¹⁸ histocompatibility barriers.¹⁹ Importantly, however, no reports have been published to date that would demonstrate that Tregs on their own are capable of inducing skin graft tolerance across full MHC barriers in otherwise unmanipulated recipients with a polyclonal T-cell repertoire. In view of the numerous tolerance models developed over the last decades that have worked in mice but have nevertheless failed in large animal/clinical studies, the extent of clinical hope invested in a tolerogenic therapy that has so far failed to induce robust tolerance in mice is somewhat surprising.

Combining Treg Therapy with the Mixed Chimerism Strategy

Thus, mixed chimerism leads to robust tolerance, but current protocols are too toxic for widespread translation. Treg therapy, on the other hand, is appealing, but insufficiently potent to establish tolerance on its own. We recently joined these two strategies with the aim of developing a tolerance protocol that is both effective and safe.

These studies revealed that the therapeutic application of Tregs leads to engraftment of conventional doses of fully allogeneic BM and donor-specific transplantation tolerance in a murine protocol devoid of cytotoxic recipient treatment (i.e., no irradiation, cytotoxic drugs/mAbs).²⁰ Polyclonal recipient Tregs ($\sim 4 \times 10^6$ B6 Tregs/mouse) were co-transplanted with fully mismatched allogeneic donor BM ($\sim 20 \times 10^6$ unseparated Balb/c BM cells) into recipients conditioned solely with short-course costimulation blockade (CTLA4Ig, anti-CD40L) and rapamycin (thus employing a relatively costimulation blockade-resistant²¹ strain combination crossing major and minor histocompatibility barriers). Durable multi-lineage macrochimerism and long-term acceptance of

donor (but not 3rd party) skin was achieved with this ‘Treg-chimerism’ protocol. The three tested (polyclonal) Treg populations (FoxP3-transduced Tregs [Foxp3-Tregs], *in vitro* activated natural CD4⁺CD25⁺ Tregs[nTregs] and TGF β -induced Tregs [iTregs]) turned out to be similarly effective in this model, indicating that this is a robust effect.

Chimerism developed in most recipients of the Treg-chimerism protocol while recipients of the same regimen without Treg administration universally failed to show chimerism (41/51 long-term multi-lineage chimeras with Tregs vs. 0/34 without Tregs, pooled data, $p < 0.001$). Chimerism levels—albeit rather low—were similarly high as in a previous model transplanting ten times the dose of allogeneic BM (200×10^6) under costimulation blockade alone.^{7,22} Notably, secondary BMTs transferring BM harvested from Treg-chimeras revealed that donor HSC had engrafted in the primary recipients.

Treg-chimeras accepted donor skin permanently, while promptly rejecting third-party grafts. Immunohistochemical analysis revealed high frequencies of mast cells and FoxP3 positive cells in the tolerated donor grafts. Of note, these intra-graft Tregs did not originate from the therapeutically applied Tregs, which we found to have a limited life-span. Thus, the transferred Tregs seem to act in an ‘infectious tolerance-like’ fashion.^{23,24} Moreover, peripheral and central deletion of donor-reactive T cells, as assessed by following superantigen-reactive T-cell populations, was evident in Treg-chimeras. Deletion was not complete, however, even late after BMT, providing further evidence for an essential role of non-deletional mechanisms in this protocol.

Introduced about twelve years ago, costimulation blockade allowed the elimination of global T-cell depletion from BMT regimens.^{25–27} As mentioned above, it did not allow chimerism without irradiation, however, as long as conventional doses of BM were transplanted.^{7,8} Regulation is an important tolerance mechanism in costimulation blockade-based mixed chimerism protocols,^{28,29} with the extent of contribution depending on the specifics of the protocol.³⁰ Rapamycin had previously been shown to promote BM engraftment.^{31,32}

Rapamycin, however, again did not allow engraftment of conventional doses of BM without recipient irradiation (together with costimulation blockade).³³ The present studies revealed that Treg therapy (together with costimulation blockade) needs to be combined with short course rapamycin in order to achieve chimerism without cytoreduction.²⁰ Treg application alone failed to induce chimerism. Whether rapamycin affects Tregs directly,³⁴ or acts through other immunomodulating mechanisms³⁵ is currently unclear.

Although tolerance towards the renal allograft was achieved in the aforementioned clinical pilot trial,² three of four tolerant patients developed *de novo* donor-specific antibodies with or without intra-graft C4d deposition.³⁶ To date, no evidence for chronic rejection was observed in these patients, but the long-term clinical significance of these findings is unclear. In this regard it is of particular interest that Treg-chimeras completely lacked donor-specific antibodies even after skin grafting.²⁰ In contrast, recipients treated with the same regimen without Tregs developed substantial levels of donor-specific antibodies despite costimulation blockade and rapamycin treatment (Fig. 1).

Conclusion

Application of polyclonal recipient Tregs leads to engraftment of conventional doses of fully allogeneic BM in recipients conditioned solely with costimulation blockade and rapamycin. Robust donor-specific transplantation tolerance ensues. Thus, Treg therapy is uniquely effective in allowing the induction of lasting chimerism and tolerance in a stringent mouse model without cytotoxic recipient treatment. This non-cytotoxic mixed chimerism protocol offers hope that the development of non-toxic clinical tolerance protocols will eventually be achievable.

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Figure 1. Non-cytotoxic Treg-chimerism protocol leads to humoral tolerance. Serum was analyzed for the presence of anti-donor antibodies by flow cytometry. Treg-chimeras (nTregs, iTregs, FoxP3 Tregs, ~3 months post-BMT, i.e., ~1–2 months post skin grafting) failed to develop detectable levels of anti-donor antibodies, whereas BMT recipients without Treg treatment (but receiving BM, costimulation blockade and rapamycin) and naïve control mice transplanted with either Balb/c BM or skin (without any treatment) developed substantial antibody levels (6 weeks post transplantation). The reactivity of sera with syngeneic (B6; dotted black line) and donor (Balb/c; red thick line) thymocytes was analyzed by flow cytometry through indirect staining with anti-mouse IgG. Histograms for representative mice are shown.

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