Bone Marrow Transplantation (2019) 54:815–821
https://doi.org/10.1038/s41409-019-0620-3

PERSPECTIVE

Transplantation tolerance in nonhuman primates and humans

Megan Sykes[1,2,3,4] · Adam D. Griesemer[1,4]

Abstract

This review focuses on our recent studies involving nonmyeloablative bone marrow transplantation as an approach to inducing organ allograft tolerance across MHC barriers in nonhuman primates and in patients. The clinical studies are focused on mechanisms of tolerance involved in a protocol carried out at Massachusetts General Hospital in HLA-mismatched haploidentical combinations for the induction of renal allograft tolerance. These studies, in which chimerism was only transient and GVHD did not occur, suggest an early role for donor-specific regulatory T cells in tolerance induction, followed by partial and gradual deletion of donor-reactive T cells. We utilized high-throughput sequencing methodologies in a novel way to identify and track large numbers of alloreactive T cell receptors (TCRs). This method has been shown to identify biologically significant alloreactive TCRs in transplant patients and pointed to clonal deletion as a major mechanism of long-term tolerance in these patients. More recently, we adapted this sequencing method to optimally identify the donor-specific regulatory T cell (Treg) repertoire. Interrogation of the early posttransplant repertoire demonstrated expansion of donor-specific Tregs in association with tolerance. Our studies suggest a role for the kidney graft in tolerance by these mechanisms in patients who had only transient chimerism. Nonhuman primate studies indicate that other organs, including the heart, the lungs and the liver, are less readily tolerated following a period of transient mixed chimerism. Our efforts to extend the reach of mixed chimerism for tolerance induction beyond the kidney are therefore focused on the addition of recipient Tregs to the protocol. This approach has the potential to enhance chimerism while further reducing the risk of GVHD.

Introduction

Based on murine models, studies in nonhuman primates and trials involving initial mixed chimerism induction in patients with hematological malignancies, we carried out studies of combined kidney/bone marrow transplantation (CKBMT) across human leukocyte antigen (HLA) barriers in patients without malignant disease at MGH [1, 2]. Studies in the murine model of mixed chimerism induction aimed to achieve allograft tolerance [3] and required treatment with T cell-depleting mAbs, low-dose (3 Gy) TBI and thymic irradiation. The latter was needed to eliminate intrathymic alloreactive T cells that otherwise would eliminate donor cells entering the recipient thymus, preventing central deletional tolerance to the donor of newly-developing T cells [4–6]. A modification of this model for the treatment of hematologic malignancies in mice also involved mixed chimerism induction, this time as a platform for delayed donor lymphocyte infusion (DLI), which under these conditions have potent graft-vs-leukemia (GVL) effects without causing graft-vs-host disease (GVHD). This separation of GVL and GVHD is due to the lack of conditioning-induced inflammation by the time of DLI administration [7–12]. Our studies had demonstrated that inflammation is a critical "checkpoint" for the trafficking of GVH-reactive donor T cells to the recipient's epithelial GVHD target tissues, where they cause disease [11, 12]. We were able to apply these observations from murine models in clinical studies in patients with refractory hematologic malignancies who had exhausted all other treatment options. DLI in this setting led to dramatic tumor responses

✉ Megan Sykes
megan.sykes@columbia.edu

1 Columbia Center for Translational Immunology, Columbia University Medical Center, New York, NY, USA

2 Department of Medicine, Columbia University Medical Center, New York, NY, USA

3 Department of Microbiology & Immunology, Columbia University Medical Center, New York, NY, USA

4 Department of Surgery, Columbia University Medical Center, New York, NY, USA

and even some cures in patients with bulky, refractory lymphomas, and myelomas [13–19]. Our murine data demonstrated that maximal antitumor effects relied upon the ability of recipient-derived professional antigen-presenting cells (APCs) to activate GVH-reactive donor T cells, as markedly greater GVL effects of DLI were observed in mixed chimeras compared with full chimeras that lacked recipient APCs [8, 16, 17, 20–23]. Thus, mixed chimerism provides an excellent platform for achievement of potent GVL effects without GVHD. We translated these observations into the clinic, both in the HLA-identical and HLA-mismatched settings and obtained seminal proofs of principle that: (1) durable mixed chimerism can be achieved, even across HLA barriers, with nonmyeloablative conditioning in humans [13–19]. However this outcome was not achieved reliably; (2) both durable and transient mixed chimerism achieved under these conditions can develop without GVHD [13–19]; (3) transient chimerism across HLA barriers that developed with one of these regimens was reliably free of GVHD. This observation represented a key safety outcome that justified evaluation of the same basic regimen for CKBMT in patients who had no other indication for BMT i.e. no malignant disease; and (4) delayed DLI was shown to convert mixed chimerism toward full chimerism and this conversion was not necessarily associated with GVHD [13–19], even when HLA barriers were transgressed.

Efforts were made to translate from the initial murine nonmyeloablative mixed chimerism/tolerance protocol to a large animal, nonhuman primate model [24]. In our view, intermediate large animal models, which model human transplantation with considerable fidelity, are needed for ethical reasons to justify clinical trials that will deny patients the standard of antirejection therapy by withdrawing immunosuppressive therapy early posttransplant. Importantly, these early studies achieved kidney allograft tolerance in about two thirds of nonhuman primates receiving nonmyeloablative CKBMT even though chimerism was only transient [24, 25]. While these monkey studies were ongoing, we were simultaneously pursuing the nonmyeloablative mixed chimerism/DLI approach in patients with hematologic malignancies who had renal failure due to multiple myeloma and received HLA-identical sibling donor CKBMT. These were the first studies to demonstrate that both transient and durable mixed chimerism were associated with donor renal allograft tolerance. Unexpectedly, transient chimerism in this trial and in the lymphoma trials (without kidney transplantation) discussed above was often associated with remarkable tumor responses [18, 26–28]. These clinical observations suggested that initial donor hematopoietic engraftment followed by rejection might elicit antitumor immune responses. In an example of iterative translational research, we developed a murine model in which this hypothesis was tested and shown to be correct [29–32].

In sum, clinical trials of mixed chimerism in patients with hematological malignancies provided safety and efficacy data that were critical in allowing us to proceed with a trial of HLA-mismatched related donor CKBMT solely for the purpose of kidney allograft tolerance induction in patients who did not have a malignant disease [1, 2, 33]. These CKBMT trials were the first to intentionally achieve kidney allograft tolerance across HLA barriers in a majority of patients: of ten patients, seven were successfully removed from immunosuppression for periods of more than 5 to now more than 15 years without rejection. Three patients in the first iteration of the protocol returned to immunosuppressive therapy at 5 to 8 years for low-grade chronic rejection in two cases and recurrent membranoproliferative glomerulonephritis in a third. The three patients who were not successfully removed from immunosuppression included one early kidney graft loss due to preformed donor-specific antibodies (such patients were subsequently excluded from the trial and rituximab was added to the conditioning protocol), an early thrombomicroangiopathy and one acute cellular rejection shortly after immunosuppression withdrawal [1, 2, 33].

Mechanisms of tolerance in CKBMT recipients

In patients without malignancy who received HLA-mismatched CKBMT, in vitro assays (mixed lymphocyte reaction [MLR] and cell-mediated lympholysis [CML] assays) demonstrated that systemic donor-specific unresponsiveness or hyporesponsiveness had developed following the transplant and persisted following immunosuppression withdrawal [1, 34, 35]. Limiting dilution assays (LDA) provide quantitative measures of alloreactivity and may point to suppressive mechanisms of tolerance if they produce a “sawtooth” pattern upon responder cell dilution [36, 37]. The absence of a sawtooth pattern in LDAs performed on long-term HLA-mismatched CKBMT recipients [35] did not support a suppressive mechanism of tolerance in this late maintenance phase. Consistent with these results, some antidonor responsiveness could be revealed by Treg depletion in the first year posttransplant in some of these patients, but similar enhancement of MLR and CML responses was not observed upon depletion of Tregs in longer-term (>1 year) recipients [34]. These results implicated other mechanisms in the long-term tolerance seen in these patients. In contrast to the persistent donor-specific unresponsiveness observed in CKBMT recipients with transient chimerism, antidonor reactivity in vitro reappeared after loss of chimerism in recipients of hematopoietic cell transplants alone with similar regimens [38], pointing to a role for the kidney allograft itself in maintaining tolerance in HLA-mismatched CKBMT recipients.

As mentioned above, 1 of 10 patients in our haploidentical CKBMT trial had acute cellular rejection after immunosuppression was withdrawn and eventually lost his allograft. Nevertheless, this patient resembled tolerant patients in demonstrating donor-specific unresponsiveness in MLR and CML assays before and following graft rejection [35]. This result suggests that these functional assays have low specificity for identifying a tolerant state.

Recipients of HLA-mismatched hematopoietic cell transplantation using anti-CD2 (siplizumab) in a nonmyeloablative regimen without a kidney transplant demonstrated enrichment of FOXP3 + CD25 + CTLA4 + cells among circulating CD4+ T cells early posttransplant [38]. CKBMT recipients with the siplizumab-based regimen produced similar findings and these were confirmed to be Tregs by their low expression of CD127 and by correlation of their percentages with Treg-specific demethylated region (TSDR) demethylation levels [39]. Up to 80% of circulating CD4 T cells were Tregs in the first week posttransplant and their percentages gradually declined to baseline over about one year. The absolute concentrations of circulating Tregs recovered to baseline levels within weeks, whereas non-Treg CD4 counts took >1 year to recover to baseline [39]. In one patient but not the others, a TCR sequencing analysis suggested that posttransplant Treg enrichment reflected, in part, conversion to Tregs from non-Tregs present prior to transplant [39]. High levels of Treg proliferation were evident early posttransplant, consistent with lymphopenia-driven expansion. Initially, a significant fraction of these Tregs had a "resting" [40] CD31+ phenotype and it is unclear whether this reflects early emigration from the thymus of newly developed Tregs or reflects selective resistance of this subset to T cell-depleting conditioning. After a few weeks, most Tregs showed an activated phenotype [39].

As mentioned above, antidonor reactivity could sometimes be revealed by Treg depletion within the first year posttransplant [34]. However, at later timepoints, depletion of Tregs did not increase antidonor reactivity in vitro, pointing to either deletion or anergy of donor-reactive T cells as a mechanism of long-term tolerance. Notably, some protocol renal allograft biopsies in these patients contained nondestructive lymphoid clusters that were greatly enriched for FoxP3 expression [1], suggesting that Tregs may play an important local role within the graft in the pathway to tolerance.

A new method of assessing alloreactivity, allowing assessment of clonal deletion in tolerant patients

Recently, platforms for high-throughput sequencing of human TCRβ hypervariable (CDR3) regions have become available. We hypothesized that a platform of this type could be used in conjunction with an MLR to identify large numbers of donor-reactive T-cell clones prior to transplant, and that tracking these clones post transplant would allow assessment of clonal deletion as a mechanism of long-term tolerance in CKBMT recipients. Host-vs-Graft (HvG) i.e. donor-reactive clones were identified as those that expanded in a pretransplant CFSE-MLR compared to unstimulated CD4 or CD8 T cells from the same blood sample. By testing for these HvG sequences in posttransplant blood samples against a uniform detection frequency (based on the smallest sample size, to normalize for sample size variation), we compared the frequency of circulating donor-reactive T cells prior to and at various times following transplantation. All tolerant patients showed significant reductions in the number of circulating HvG CD4 and CD8 clones following the transplant. In contrast, the one patient who did not achieve tolerance following the same CKBMT regimen showed no significant reduction in the number of circulating HvG T-cell clones [35], despite donor-specific unresponsiveness in MLR, CML, and LDA assays. These results suggested that a reduction in circulating HvG TCRs was more specific in identifying tolerance than the functional assays [35]. Furthermore, kidney transplantation with conventional immunosuppression (i.e. without a tolerance protocol) was associated with significant increases in the number of circulating HvG CD4 T-cell clones following transplantation, demonstrating the biological significance of HvG clones identified in MLRs [35].

The kinetics of loss of circulating HvG sequences varied between tolerant patients and some patients showed an initial increase in such clones before they gradually declined. Since the CKBMT regimen included potent T cell-depleting agents (siplizumab and cyclophosphamide), it was not surprising that considerable repertoire turnover occurred between pretransplant and posttransplant timepoints [35]. However, patients transplanted with conventional immunosuppression also showed considerable repertoire turnover within a year of transplant and nevertheless showed expansion of preexisting donor-reactive TCRs. Therefore, while reductions in HvG clones in tolerant patients might be part of a global T-cell depletion process, their failure to expand in the presence of T-cell lymphopenia and donor antigen, as seen in conventional transplant recipients, is a consequence of the tolerization process and occurs gradually over time. Of note, protocol biopsies did not reveal enrichment of donor-reactive TCR sequences in the grafts [39], which showed no evidence of rejection.

As discussed, the studies described above demonstrate the biological relevance of HvG clones identified with our TCR sequencing-based approach. Studies in intestinal transplant recipients have further validated the biological relevance of this method, as TCRs identified as donor-

reactive were shown to dominate among recipient-mappable TCRs in the allograft itself during rejection episodes [41]. Furthermore, GVH-reactive TCR sequences, also identified with pretransplant lymphocytes, were found to expand in the graft mucosa in association with replacement of donor APCs with those of the recipient [41]. The use of this technique has provided a novel window into events within the graft itself and the interplay between HvG and GVH alloreactivity in intestinal transplant recipients [41]. The approach will be interesting to explore in both additional types of solid organ transplants and in hematopoietic cell transplantation, where the presence of GVH clones in GVHD target tissues could enhance the tissue diagnosis and provide insight into disease pathogenesis.

In view of the above studies in human transplant recipients validating the biological significance of TCR sequences identified as alloreactive by our method, we have pursued this approach to obtain a deeper understanding of the human alloresponse [42]. While there have been many estimates of the number and proportion of the T-cell repertoire recognizing a given set of alloantigens, our method provided an opportunity to actually quantify this response. However, we observed that a large proportion of alloreactive TCRs are present in the circulation at very low frequency, suggesting that any given sample is likely to be of insufficient size to allow detection of most of these clones. We therefore developed an extrapolation methodology that took into account the "unseen species" and were able to estimate the cumulative frequency of alloreactive T cells against any extensively HLA-mismatched donor to be in approximately the 0.5–5% range for both CD4 and CD8 T cells [42]. Moreover, the alloreactive repertoire was highly specific for each unique allogeneic donor and its diversity correlated directly with the degree of HLA mismatching between the responder and the stimulator. The low frequency of most individual alloreactive clones in the circulation suggested that they included mainly naïve T cells. Indeed, very few sequences among a panel of "public" virus-specific TCRs were detected among the alloreactive sequences detected in our studies [42]. Collectively, these results argue against the notion that the alloresponse reflects predominantly a cross-reaction with heterologous pathogen-reactive T cells and instead suggest that almost any TCR is likely to show alloreactivity against some allogeneic donor if a sufficiently large panel could be tested.

More recently, we have adapted the TCR sequencing approach to the detection and fate tracking of donor-specific Tregs, an effort that was needed in order to determine whether the Treg expansions seen early posttransplant in CKBMT recipients were indeed donor-specific. This was a challenging question, as the low abundance of Tregs in peripheral blood made detection of donor-specific Tregs in a given sample likely to be inefficient. Indeed, when we interrogated CFSE-low CD4 cells proliferating in MLRs for the presence of sequences that mapped to the sorted Treg fraction of unstimulated T cells in the same sample, we found that only a very small fraction of proliferating cells were in fact Tregs [43]. However, by expanding Tregs with activated donor B cells, we were able to detect a much broader repertoire of Tregs that had high specificity for that donor and potent suppression of antidonor reactivity [43]. By subjecting CKBMT recipients' pretransplant Tregs to this method to identify a significant proportion of the donor-specific Treg repertoire, then interrogating posttransplant specimens for these sequences, we were able to demonstrate that donor-specific Tregs were indeed expanded in the circulation of tolerant patients, but not in that of the patient who failed tolerance induction, at 6 months post transplant [43]. These results strongly implicate donor-specific Tregs in the tolerance achieved in CKBMT recipients.

Extending allograft tolerance beyond the kidney: nonhuman primate studies

As noted above, the kidney plays an active role in promoting tolerance in CKBMT recipients with transient chimerism. The use of a regimen promoting transient chimerism across HLA barriers in these studies was supported by monkey studies demonstrating the efficacy of tolerance induction with this approach [24]. However, studies of lung, heart, and islet transplantation in this monkey model have shown that these organs and tissues are less able to promote tolerance in combination with BMT that leads to transient chimerism [44–48]. For this purpose, we believe that induction of durable mixed chimerism will be essential. The approach we have taken to achieve this goal involves infusion of polyclonal recipient Tregs that are expanded and cryopreserved prior to transplantation and infused shortly after the BMT. Polyclonal recipient Tregs had shown efficacy in promoting durable chimerism in a murine model [49] and this approach held the promise of enhancing the durability of chimerism without increasing the risk of GVHD, in contrast to other approaches to improving HLA-mismatched hematopoietic cell engraftment. Our initial studies with this approach demonstrated its potential in the monkey model, achieving more prolonged chimerism, including donor T cells, and, remarkably, allowing acceptance without immunosuppression of a donor kidney grafted 4 months after the bone marrow transplant [50]. This achievement demonstrated robust tolerance and contrasted with concurrent and historical controls not receiving Tregs, which uniformly reject donor kidneys grafted so long after BMT [50, 51]. However, success with this regimen was limited by cytomegalovirus reactivation, which occurred uniformly during the initial period when the animals were

treated with cyclosporine and which (along with its treatment) had a deleterious effect on donor marrow engraftment [50]. Switching the single agent immunosuppression given for this short initial period (2 months) from cyclosporine to rapamycin has helped to alleviate this problem [52]. While many challenges remain, some of which reflect particular challenges in the use of expanded cynomolgus monkey Tregs, studies in progress have confirmed the tolerance-promoting effect of Tregs in this setting and we are optimistic about the clinical potential of this approach.

Because rodent studies have indicated that the liver is a particularly tolerogenic organ [53–56], we hypothesized that the transient chimerism regimen that achieved tolerance in renal allograft recipients (without Treg infusion) would also be successful in promoting liver allograft tolerance. Before testing this in patients, however, we believed it was critical to test this approach directly in the monkey model and therefore took up the challenge of establishing a cynomolgus monkey liver transplant model [57]. Much to our surprise, despite the achievement of robust, transient multilineage chimerism, donor liver grafts were uniformly rejected [58]. However, when we modified the regimen to include robust in vivo depletion of donor and recipient T cells while excluding donor marrow infusion, we achieved transient lymphoid chimerism (from passenger cells in the liver) and long-term liver allograft survival with only a short (2 months) course of immunosuppression. Studies are in progress to refine this protocol and better understand the immune interactions at play while we are considering a clinical protocol based on this approach. These observations underscore the importance and utility of nonhuman primate studies before embarking on clinical trials of tolerance induction.

Conclusions

Ultimately, we aim to achieve durable mixed chimerism without GVHD to induce long-term deletional tolerance to all types of organs and to islet allografts. In view of the proof of principle that this can be achieved in a handful of patients with haploidentical donors [13, 19], this is a realistic goal. We are optimistic about the potential of expanded recipient Tregs to promote durable chimerism and look forward to further preclinical and clinical refinements of this approach.

Acknowledgements The authors thank Nicole Casio for assistance with the manuscript.

Funding Research reported in this publication was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award UM1 AI109565. The study was also supported by NIAID grants RO1 AI084074 and PO1 AI106697, the ITN grant NO1 AI015416, and by PO1 CA111519. NHP studies were supported by NIH grants RO1 OD017949 and R56 AI122332. The content is solely the responsibility of the author and does not necessarily represent the official views of the National Institutes of Health. Publication of this supplement was sponsored by Gilead Sciences Europe Ltd, Cell Source, Inc., The Chorafas Institute for Scientific Exchange of the Weizmann Institute of Science, Kiadis Pharma, Miltenyi Biotec, Celgene, Centro Servizi Congressuali, Almog Diagnostic.

Compliance with ethical standards

Conflict of interest MS is chairman of the scientific advisory board of ITB-MED AB. MS serves as Scientific Advisory Board member and owns equity in Magenta Therapeutics, and received grant support from United Therapeutics, Lung Biotechnology, ITB-Med AB. Additional co-author has declared that no conflict of interest exists.

References

1. Kawai T, Cosimi AB, Spitzer TR, Tolkoff-Rubin N, Suthanthiran M, Saidman SL, et al. HLA-mismatched renal transplantation without maintenance immunosuppression. New Engl J Med. 2008;358:353–61.
2. Kawai T, Sachs DH, Sykes M, Cosimi AB, Immune Tolerance N. HLA-mismatched renal transplantation without maintenance immunosuppression. New Engl J Med. 2013;368:1850–2.
3. Sharabi Y, Sachs DH. Mixed chimerism and permanent specific transplantation tolerance induced by a non-lethal preparative regimen. J Exp Med. 1989;169:493–502.
4. Tomita Y, Khan A, Sykes M. Mechanism by which additional monoclonal antibody injections overcome the requirement for thymic irradiation to achieve mixed chimerism in mice receiving bone marrow transplantation after conditioning with anti-T cell mAbs and 3 Gy whole body irradiation. Transplantation. 1996;61:477–85.
5. Tomita Y, Sachs DH, Khan A, Sykes M. Additional mAb injections can replace thymic irradiation to allow induction of mixed chimerism and tolerance in mice receiving bone marrow transplantation after conditioning with anti-T cell mAbs and 3 Gy whole body irradiation. Transplantation. 1996;61:469–77.
6. Nikolic B, Khan A, Sykes M. Induction of tolerance by mixed chimerism with nonmyeloblative host conditioning: the importance of overcoming intrathymic alloresistance. Biol Blood Marrow Transplant. 2001;7:144–53.
7. Sykes M, Sheard MA, Sachs DH. Graft-versus-host-related immunosuppression is induced in mixed chimeras by alloresponses against either host or donor lymphohematopoietic cells. J Exp Med. 1988;168:2391–6.
8. Mapara MY, Kim Y-M, Wang S-P, Bronson R, Sachs DH, Sykes M. Donor lymphocyte infusions mediate superior graft-versus-leukemia effects in mixed compared to fully allogeneic chimeras: a critical role for host antigen-presenting cells. Blood. 2002;100:1903–9.
9. Mapara MY, Kim Y-M, Marx J, Sykes M, DLI-mediated GVL. effects in mixed chimeras established with a non-myeloablative conditioning regimen: extinction of GVL effects coincides with loss of alloreactive cells following conversion to full donor chimerism. Transplantation. 2003;76:297–305.
10. Pelot MR, Pearson DA, Swenson K, Zhao G, Sachs J, Yang Y-G, et al. Lymphohematopoietic graft-vs-host reactions can be induced

without graft-vs-host disease in murine mixed chimeras established with a cyclophosphamide-based non-myeloablative conditioning regimen. Biol Blood Marrow Transplant. 1999;5:133–43.
11. Mapara MY, Leng C, Kim YM, Bronson R, Lokshin A, Luster A, et al. Expression of chemokines in GVHD target organs is influenced by conditioning and genetic factors and amplified by GVHR. Biol Blood Marrow Transplant. 2006;12:623–34.
12. Chakraverty R, Cote D, Buchli J, Cotter P, Hsu R, Zhao G, et al. An inflammatory checkpoint regulates recruitment of graft-versus-host-reactive T cells to peripheral tissues. J Exp Med. 2006; 203:2021–31.
13. Sykes M, Preffer F, McAffee S, Saidman SL, Colby C, Sackstein R, et al. Mixed lymphohematopoietic chimerism and graft-vs-lymphoma effects are achievable in adult humans following non-myeloablative therapy and HLA-mismatched donor bone marrow transplantation. Lancet. 1999;353:1755–9.
14. Toh HC, Spitzer TR, Preffer F, Alexander SI, MCafee S, Dombkowski D, et al. Fluctuating lymphocyte chimerism, tolerance and anti-tumor response in a patient with refractory lymphoma receiving non-myeloablative conditioning an a haploidentical related allogeneic bone marrow transplant. Cytokines Cell Mol Ther. 2002;7:43–7.
15. Spitzer TR, MCafee S, Sackstein R, Colby C, Toh HC, Multani P, et al. The intentional induction of mixed chimerism and achievement of anti-tumor responses following non-myeloablative conditioning therapy and HLA-matched and mismatched donor bone marrow transplantation for refractory hematologic malignancies. Biol Blood Marrow Transplant. 2000;6:309–20.
16. Dey BR, MCafee S, Sackstein R, Colby C, Saidman S, Weymouth D, et al. Successful allogeneic stem cell transplantation with nonmyeloablative conditioning in patients with relapsed hematologic malignancy following autologous stem cell transplantation. Biol Blood Marrow Transplant. 2001;7:604–12.
17. Dey BR, MCafee S, Colby C, Sackstein R, Saidman S, Tarbell N, et al. Impact of prophlactic donor leukocyte infusions on mixed chimerism, graft-vs-host disease and anti-tumor response in patients with advanced hematologic malignancies treated with nonmyeloablative conditioning and allogeneic bone marrow transplantation. Biol Blood Marrow Transplant. 2003;9:320–9.
18. Dey BR, MCafee S, Colby C, Cieply K, Caron M, Saidman S, et al. Anti-tumor response despite loss of donor chimerism in patients treated with nonmyeloablative conditioning and allogeneic stem cell transplantation. Br J Haematol. 2005;128:351–9.
19. Spitzer TR, MCafee S, Dey BR, Colby C, Hope J, Grossberg H, et al. Non-myeloablative haploidentical stem cell transplantation using anti-CD2 monoclonal antibody (MEDI-507)-based conditioning for refractory hematologic malignancies. Transplantation. 2003;75:1748–51.
20. Chakraverty R, Eom HS, Sachs J, Buchli J, Cotter P, Hsu R, et al. Host MHC Class II+antigen-presenting cells and CD4 cells are required for CD8-mediated graft-versus-leukemia responses following delayed donor leukocyte infusions. Blood. 2006;108:2106–13.
21. Chakraverty R, Flutter B, Fallah-Arani F, Eom HS, Means T, Andreola G, et al. The host environment regulates the function of CD8+ graft-versus-host-reactive effector cells. J Immunol. 2008; 181:6820–8.
22. Chakraverty R, Sykes M. The role of antigen-presenting cells in triggering GVHD and GVL. Blood. 2007;110:9–17.
23. Childs R, Clave E, Contenin N, Jayasekera D, Hensel N, Leitman S, et al. Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: full donor T-cell chimerism precedes alloimmune responses. Blood. 1999;94:3234–41.
24. Kawai T, Cosimi AB, Colvin RB, Powelson J, Eason J, Kozlowski T, et al. Mixed allogeneic chimerism and renal allograft tolerance in cynomologous monkeys. Transplantation. 1995;59:256–62.
25. Kawai T, Sogawa H, Boskovic S, Abrahamian G, Smith RN, Wee SL, et al. CD154 blockade for induction of mixed chimerism and prolonged renal allograft survival in nonhuman primates. Am J Transplant. 2004;4:1391–8.
26. Buhler LH, Spitzer TR, Sykes M, Sachs DH, Delmonico FL, Rubin-Tolkoff N, et al. Induction of kidney allograft tolerance after transient lymphohematopoietic chimerism in patients with multiple myeloma and end-stage renal disease. Transplantation. 2002;74:1405–9.
27. Fudaba Y, Spitzer TR, Shaffer J, Kawai T, Fehr T, Delmonico F, et al. Myeloma responses and tolerance following combined kidney and nonmyeloablative marrow transplantation: in vivo and in vitro analyses. Am J Transplant. 2006;6:2121–33.
28. Spitzer TR, Sykes M, Tolkoff-Rubin N, Kawai T, McAfee SL, Dey BR, et al. Long-term follow-up of recipients of combined human leukocyte antigen-matched bone marrow and kidney transplantation for multiple myeloma with end-stage renal disease. Transplantation. 2011;91:672–6.
29. Rubio MT, Kim YM, Sachs T, Mapara M, Zhao G, Sykes M. Anti-tumor effect of donor marrow graft rejection induced by recipient leukocyte infusions in mixed chimeras prepared with nonmyeloablative conditioning: critical role for recipient-derived IFN-{gamma}. Blood. 2003;102:2300–7.
30. Rubio MT, Saito TI, Kattelman K, Zhao G, Buchli J, Sykes M. Mechanisms of the anti-tumor responses and host-versus graft reactions induced by recipient leukocyte infusions in mixed chimeras prepared with nonmyeloablative conditioning: a critical role for recipient CD4+ T cells and recipient leukocyte infusion-derived IFN-gamma-producing CD8+ T cells. J Immunol. 2005; 175:665–76.
31. Rubio MT, Zhao G, Buchli J, Chittenden M, Sykes M. Role of indirect allo- and autoreactivity in anti-tumor responses induced by recipient leukocyte infusions (RLI) in mixed chimeras prepared with nonmyeloablative conditioning. Clin Immunol. 2006;120:33–44.
32. Saito TI, Rubio MT, Sykes M. Clinical relevance of recipient leukocyte infusion as antitumor therapy following nonmyeloablative allogeneic hematopoietic cell transplantation. Exp Hematol. 2006; 34:1271–7.
33. Kawai T, Sachs DH, Sprangers B, Spitzer TR, Saidman SL, Zorn E, et al. Long-term results in recipients of combined HLA-mismatched kidney and bone marrow transplantation without maintenance immunosuppression. Am J Transplant. 2014;14:1599–611.
34. Andreola G, Chittenden M, Shaffer J, Cosimi AB, Kawai T, Cotter P, et al. Mechanisms of donor-specific tolerance in recipients of haploidentical combined bone marrow/kidney transplantation. Am J Transplant. 2011;11:1236–47.
35. Morris HDW, DeWolf S, Robins H, Sprangers B, Locascio SA, Shonts B, et al. Tracking donor-reactive T cells: evidence for clonal deletion in tolerant kidney transplant patients. Sci Transl Med. 2015. https://doi.org/10.1126/scitranslmed.3010760.
36. Bonnefoix T, Bonnefoix P, Mi JQ, Lawrence JJ, Sotto JJ, Leroux D. Detection of suppressor T lymphocytes and estimation of their frequency in limiting dilution assays by generalized linear regression modeling. J Immunol. 2003;170:2884–94.
37. Bonnefoix T, Bonnefoix P, Perron P, Mi JQ, Ng WF, Lechler R, et al. Quantitating effector and regulatory T lymphocytes in immune responses by limiting dilution analysis modeling. J Immunol. 2005;174:3421–31.
38. Shaffer J, Villard J, Means TK, Alexander S, Dombkowski D, Dey BR, et al. Regulatory T-cell recovery in recipients of haploidentical nonmyeloablative hematopoietic cell transplantation with a humanized anti-CD2 mAb, MEDI-507, with or without fludarabine. Exp Hematol. 2007;35:1140–52.
39. Sprangers B, DeWolf S, Savage TM, Morokata T, Obradovic A, LoCascio SA, et al. Origin of enriched regulatory T cells in patients receiving combined kidney-bone marrow transplantation

to induce transplantation tolerance. Am J Transplant. 2017;17: 2020–32.
40. Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, Niwa A, et al. Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. Immunity. 2009;30:899–911.
41. Zuber J, Shonts B, Lau SP, Obradovic A, Fu J, Yang S, et al. Bidirectional intragraft alloreactivity drives the repopulation of human intestinal allografts and correlates with clinical outcome. Sci Immunol. 2016. https://doi.org/10.1126/sciimmunol.aah3732.
42. DeWolf S, Grinshpun B, Savage T, Lau SP, Obradovic A, Shonts B, et al. Quantifying size and diversity of the human T cell alloresponse. JCI Insight. 2018. https://doi.org/10.1172/jci.insight.121256.
43. Savage TM, Shonts B, Obradovic A, Dewolf S, Lau S, Zuber J, et al. Early expansion of donor-specific Tregs in tolerant kidney transplant recipients. JCI Insight. 2018. https://doi.org/10.1172/jci.insight.124086.
44. Aoyama A, Ng CY, Millington TM, Boskovic S, Murakami T, Wain JC, et al. Comparison of lung and kidney allografts in induction of tolerance by a mixed-chimerism approach in cynomolgus monkeys. Transplant Proc. 2009;41:429–30.
45. Kawai T, Cosimi AB, Wee SL, Houser S, Andrews D, Sogawa H, et al. Effect of mixed hematopoietic chimerism on cardiac allograft survival in cynomolgus monkeys. Transplantation. 2002;73:1757–64.
46. Kawai T, Sachs DH, Cosimi AB. Tolerance to vascularized organ allografts in large animal models. Curr Opin Immunol. 1999;11:516–26.
47. Oura T, Ko DS, Boskovic S, O'Neil JJ, Chipashvili V, Koulmanda M, et al. Kidney versus islet allograft survival after induction of mixed chimerism with combined donor bone marrow transplantation. Cell Transpl. 2016;25:1331–41.
48. Tonsho M, Lee S, Aoyama A, Boskovic S, Nadazdin O, Capetta K, et al. Tolerance of lung allografts achieved in nonhuman primates via mixed hematopoietic chimerism. Am J Transplant. 2015;15:2231–9.
49. Pilat N, Baranyi U, Klaus C, Jaeckel E, Mpofu N, Wrba F, et al. Treg-therapy allows mixed chimerism and transplantation tolerance without cytoreductive conditioning. Am J Transplant. 2010;10:751–62.
50. Duran-Struuck R, Sondermeijer HP, Buhler L, Alonso-Guallart P, Zitsman J, Kato Y, et al. Effect of ex vivo-expanded recipient regulatory T cells on hematopoietic chimerism and kidney allograft tolerance across MHC barriers in cynomolgus macaques. Transplantation. 2017;101:274–83.
51. Kawai T, Poncelet A, Sachs DH, Mauiyyedi S, Boskovic S, Wee SL, et al. Long-term outcome and alloantibody production in a non-myeloablative regimen for induction of renal allograft tolerance. Transplantation. 1999;68:1767–75.
52. Alonso-Guallart P, Zitsman JS, Stern J, Kofman SB, Woodland D, Ho SH et al. Characterization, biology, and expansion of regulatory T cells in the Cynomolgus macaque for pre-clinical studies. Am J Transplant. 2019; https://doi.org/10.1111/ajt.15313.
53. Li W, Kuhr CS, Zheng XX, Carper K, Thomson AW, Reyes JD, et al. New insights into mechanisms of spontaneous liver transplant tolerance: the role of Foxp3-expressing CD25+CD4+ regulatory T cells. Am J Transplant. 2008;8:1639–51.
54. Li W, Lu L, Wang Z, Wang L, Fung JJ, Thomson AW, et al. Costimulation blockade promotes the apoptotic death of graft-infiltrating T cells and prolongs survival of hepatic allografts from FLT3L-treated donors. Transplantation. 2001;72:1423–32.
55. Starzl TE, Murase N, Thomson A, Demetris AJ. Liver transplants contribute to their own success. Nat Med. 1996;2:163–5.
56. Dangi A, Sumpter TL, Kimura S, Stolz DB, Murase N, Raimondi G, et al. Selective expansion of allogeneic regulatory T cells by hepatic stellate cells: role of endotoxin and implications for allograft tolerance. J Immunol. 2012;188:3667–77.
57. Kato Y, Griesemer AD, Wu A, Sondermeijer HP, Weiner JI, Duran-Struuck R, et al. Novel H-shunt venovenous bypass for liver transplantation in cynomolgus macaques. Comp Med. 2017;67:436–41.
58. Chaudhry S, Emond J, Griesemer A. Immune cell trafficking to the liver. Transplantation. 2019. https://doi.org/10.1097/TP.0000000000002690.