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# Current Status of Alloimmunity

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## Abstract

**Purpose of review—**The present review aims to highlight the major recent advances in transplantation with regards to basic, translational and clinical research.

**Recent findings—**We describe new concepts in understanding allorecognition and allospecificity of T cells, and discuss current challenges in targeting memory T cells, including the limitation of rodent disease models. From a clinical perspective, we highlight the advances in molecular biopsy characterization, which have expanded our knowledge of potential drivers of injury and may provide better parameters for patient risk stratification. We also highlight the dual role of innate immunity in both stimulating and regulating adaptive immunity as well as novel insights into environmental exposures that may affect immune regulation, such as high-salt diet. Lastly, we discuss advances in understanding humoral response and novel technologies such as chimeric antigen receptors (CAR) engineered T cells, microparticle-based drug delivery and CRISPR/Cas9 gene editing that may provide intriguing and promising approaches to restrain alloimmunity.

**Summary—**Current advances in our understanding of the basic mechanisms of alloimmunity and their potential translation to clinical applications will permit the development of novel diagnostic and therapeutic strategies to improve long-term graft survival.

### Keywords

Allorecognition; memory response; transplantation; humoral immunity; immune regulation

## Introduction

This article provides an up-to-date review on our current understanding of alloimmunity and their translation on to clinical management of organ transplant. We discuss the current efforts in understanding the basic mechanisms of T cell allospecificity, memory response, antibody-mediated allograft injury and immune regulation. Furthermore, we highlight the recent technical advances in gene editing technologies and microparticle-based drug delivery, which are causing great excitement as novel potential therapeutic strategies.

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## Understanding the mechanisms of allorecognition and T-cell allospecificity

The immune response following transplantation is initiated upon recognition of foreign MHC molecules by the recipient's immune system via either the ‘direct’ or ‘indirect’ pathway. While donor MHC-peptide complexes is directly recognized by recipients' T cell in the former, allo-MHC is processed and re-presented by recipient antigen-presenting cells (APCs) in the indirect pathway, which plays a dominant role in the long term based on the continuous shedding of alloantigen from the graft. By using an adoptive transfer model of transgenic T cells in a murine chronic cardiac transplant model, Ali *et al.* elegantly showed that the direct-pathway CD4 T cells are short lived, mostly due to the quick elimination of donor-derived APCs, while the indirect-pathway CD4 T cell responses are persistent and heterogeneous in terms of duration and sites, depending on the targeted alloantigen (1): donor-MHC I-specific responses taking place in allograft parenchyma last long, whereas anti-MHC II responses decay earlier due to donor APCs eradication. These findings highlight the importance of restraining the indirect allorecognition, in particular against donor MHC class I molecules, to prevent chronic cellular-mediated rejection.

The specificity of the T-cell receptor (TCR), especially in terms of the cross-reactivity to different peptide-MHC complexes, is an important topic in transplantation since it may explain the presence of memory response in the absence of prior donor MHC exposure. Nelson *et al.* (2) recently demonstrated that antigenic peptides binding a same MHC II molecule (I-$A^b$) only require five amino acids in common to trigger cross-reactivity to the same TCR (2). They showed in mice that a naïve T cell population specific to self-tissue-restricted antigen that was incompletely deleted could be engaged by bacterial-derived peptides sharing contact amino acids with the self-peptides, thus triggering autoimmunity. This similar cross-reactivity phenomenon could explain why some patients can develop anti-HLA antibodies despite no prior sensitizing events (i.e. pregnancy, transplantation or blood transfusion). The ability to identify allospecific T cells against the donor organ has been a major challenge in transplantation. Morris *et al.* established an assay to identify donor-specific T cells clones prior to transplant and track their deletion in subjects who underwent combined kidney and bone marrow transplantation (CKBMT) for tolerance induction (3). In this study, CFSE-labeled recipient's peripheral blood mononuclear cells (PBMCs) were cultured with irradiated donor PBMCs, proliferating T cell subsets were subsequently isolated, and TCR clones characterized by deep sequencing in order to determine the donor-specific TCR clones. Clonal deletion of donor-reactive T cells was present in tolerant, but not in non-tolerant, CKBMT patients, supporting the monitoring of donor-specific clones as a potential biomarker of the tolerance development post-transplantation.

## Challenges in targeting the memory response

Targeting memory T cells pose a great obstacle to transplantation since they may trigger robust alloimmune responses with lower activation thresholds, and are less dependent on co-stimulatory signals or cytokines for full activation, thus, leading to greater resistance to current available immunosuppressive drugs. When compared to mice, humans carry a much more diverse immune repertoire as a result of an outbred nature and multiple immunological challenges such as infections and vaccinations exposed during a lifetime. Recently, Masopust's group pointed to certain limitations of the use of laboratory mice to study

immune responses, based on the lack of memory T cells and significant differences in immune phenotype when compared to an adult human immune system (4). Specifically, laboratory mice had an immune system very similar to a human neonate, while microbial-experienced mice—such as those found in pet stores—presented a similar immune cell composition to adult humans. By co-housing laboratory mice with pet store mice, the authors could successfully expand the memory phenotype of laboratory mice, indicating that this attractive strategy may allow performing murine immunology research with greater similarity to adult humans.

The role of tissue resident memory T cells in target organ protection is also being increasingly recognized. Steinert *et al.* observed that memory T cells stay local and survey and patrol non-lymphoid solid organs (5). After re-stimulation, local immune responses are carried by tissue-resident memory T cells, instead of recirculating T cells. These findings may explain why anti-donor memory T cells are so difficult to be targeted once they have been generated, since T cells may not need to recirculate to secondary lymphoid organs to be fully activated prior to causing graft injury.

## Crosstalk between innate cells and adaptive immunity

Innate immunity is primarily a non-antigen specific immune response driven by tissue injury such as ischemia or infection and it is capable of triggering and enhancing adaptive immunity. Among innate immune cells, dendritic cells (DCs) are the major group of APCs during the alloimmune responses and are crucial for T cell activation. Recent reports highlight the importance of innate immune cells in shaping the alloimmune response: Batal *et al.* demonstrated the clinical significance of intragraft DC-SIGN$^{+}$ dendritic cells in 105 “for-cause” allograft biopsies. DC-SIGN is involved in DC/T cell clustering, and is required for DC-initiated proliferation of resting T cells (6, 7). The authors observed that DC-SIGN$^{+}$ cell density (3.8>hpf) was adversely associated with allograft function and survival (2-yr after biopsy, r=-0.36, p<0.001), and with increased proliferation of T cells (Ki67$^{+}$) and higher inflammation scores (ti 2-3). Interestingly, DC-SIGN$^{+}$ cells clustered with lymphocytes were predominantly of recipient origin, especially at the later time points (8). This observation implies a possibly harmful effect of *in situ* DC-SIGN$^{+}$ DCs on the alloimmune response and provides a rationale for targeting DCs to halt allograft inflammation.

While the detrimental role of the innate immune cells is increasingly recognized in transplantation, recent studies have demonstrated that myeloid cells may have immunomodulatory properties and inhibit alloreactive T cell responses in both mice (9, 10) and humans (11). Conde *et al.* showed that a population of graft-infiltrating suppressive macrophages was expanded under a CD40L-CD40 blockade protocol in murine cardiac transplantation (12). Those cells were CD11b$^{+}$CSF1R$^{+}$Ly6C$^{low}$Ly6G$^{-}$CD169$^{+}$ and when isolated from tolerized transplanted mice could suppress CD8 T cells proliferation and expand Tregs *in vitro* in an IL-10-dependent manner.

## Molecular signature of acute rejection and chronic allograft injury

Delineating molecular signatures of acute and chronic rejection may improve classification and provide hints to the development of more effective treatments (13, 14). By incorporating a “molecular T cell-mediated rejection (TCMR) score” based on transcriptional signature captured by microarray to conventional histological analyses in 703 for cause biopsies, Reeve *et al.* suggested a new diagnosis algorithm to better categorize TCMR. Interstitial inflammation and tubulitis were the key histologic features compared to arteritis (15) in TCMR. Similarly, Modena *et al.* applied microarray platform to a collection of 234 allograft biopsy samples, looking specifically at chronic changes in comparison to acute rejection (16). The pathway analyses of this “molecular biopsy signature” revealed that, by comparing the transcriptional features of interstitial fibrosis/tubular atrophy (IFTA) to acute rejection (AR), there was a surprising overlap in gene expression patterns among those histologically distinct pathological processes [commonly upregulated genes included: immunoglobulin heavy and light chains, lactotransferrin (LTF), SERPINA3, and CXCL9 etc.]. Interestingly, even IFTA biopsy samples without histological evidence of inflammation, the expression of AR-associated genes correlated with a higher risk of graft loss at 5 or more years, suggesting most IFTA samples have subclinical and possibly ongoing immune-mediated injury. Whether targeting this inflammation would slow down progression remains to be determined.

## Modulators of regulatory T cells in transplantation and allograft tolerance

Regulatory T cells have been explored as important players in immune regulation and as promising therapeutic targets. However, their exact suppressive mechanisms and interaction with other cells are not fully understood, especially in the transplant context. Bézie *et al.* demonstrated that IL-34 is both a $CD4^+$ and $CD8^+$ Treg-derived cytokine that can abrogate alloimmune responses (17). IL-34 can be expressed by rat $CD8^+CD45RC^{low}$ Tregs and both human $CD4^+CD45RC^{low}Foxp3^+$ and $CD8^+CD45RC^{low}Foxp3^+$ Tregs, and this cytokine was involved in their suppressive functions in inhibiting anti-donor immune responses *in vitro*. Overexpression of IL-34 *in vivo* inhibited the production of donor-specific antibodies and induced both CD4 and CD8 highly suppressive Tregs in a cardiac allograft rat model. Macrophages were required to induce these Tregs, demonstrating a cross-talk between these two cell populations. Overall, IL-34 appears to be an important regulatory cytokine and a potential therapeutic target to promote immune regulation.

Tolerance in small animals can be relatively easily achieved through multiple different strategies, while infection has been shown to precipitate rejection, suggesting the unstable nature of transplant tolerance. For example, Miller *et al.* showed that under a tolerance induction protocol with anti-CD40L (CD154) plus donor-specific transfusion (DST), tolerant mice recipients could lose their cardiac allograft after an infection with *Listeria monocytogenes* (18). Infection-triggered alloreactivity was characterized by an increase in anti-donor effector responses, but this response was not due to anti-bacterial T cells cross-reacting with donor antigens. Furthermore, re-transplantation of a second donor-matched allograft after resolution of the infection led to long-term tolerance without any additional immunosuppressive therapy and restoration of allo-specific tolerance was dependent on Tregs. (18). Interestingly, tolerant mice could mount a memory response to the bacterial

pathogen, demonstrating that the transient loss of the tolerance was specific to donor-antigens. Thus, immunological tolerance against the allograft is only transiently lost in the setting of infection but can be restored spontaneously once the infection is controlled.

In addition to infection, our group has recently demonstrated that high-salt diet is capable of accelerating rejection. Using a MHC class II mismatched cardiac transplant model, we showed that a high-salt diet (3.15% Na) when compared to normal diet (0.28%) alters immune regulation and precipitates allograft rejection (19). This phenomenon was related to a reduction in Tregs both in the periphery and in the allograft. Deleterious effect on Tregs occurred via activation of serum/glucocorticoid-regulated kinase 1 (SGK1) by salt, leading to downregulation of transcription factors Foxo1/Foxo3 critical for Treg homeostasis. Similarly, a high-lipid diet has been recently shown to precipitate rejection in preclinical models (20-22). Therefore, infection and environmental exposures such as high-salt or high-fat diet may negatively affect immune regulation by impacting Treg function and precipitate rejection.

## Targeting B cells in transplantation

Targeting B cells with rituximab in transplantation and autoimmunity has yielded mixed results. Using an allogeneic, syngeneic cardiac transplant model and TLR agonists, Laws *et al.* demonstrated that inflammation mitigates B cell depletion by altering the pharmacokinetics and pharmacodynamics (PK/PD) of anti-CD20 mAb therapy leading to accelerated reconstitution of the B cell pool (23). A single dose of anti-CD20 mAb at the time of transplant fails to maintain B cell depletion. Furthermore, IVIG co-administration with anti-CD20 mAb significantly shortened the half-life of the mAb and led to accelerated B cell recovery. Repeated dosing restored B cell depletion in secondary lymphoid organs and delayed graft rejection, independent of alloantibody production. Overall, these studies demonstrate the importance of further clinical studies of the PK/PD of monoclonal antibody treatment in inflammatory conditions and highlight the deleterious effect of IVIG when given with anti-CD20 and the relevance of re-dosing of anti-CD20 for effective B cell depletion in alloimmunity. Others have recently demonstrated negative effects of whole B cell depletion, which may be related depletion of regulatory B cell populations. Marino *et al.* reported a deleterious effect of anti-CD20-mediated B cell depletion in murine skin transplant model, by potentially enhancing allo-reactive memory T cell responses (24) and Chandraker *et al.* presented preliminary results of the CTOT11 study in heart transplant recipients, in which rituximab was given as induction therapy on days 0 and 12 post-transplantation and led to worsen coronary vasculopathy at 1 year (25). Further investigation is needed on how to optimally target B cells in transplantation.

## Alloantibody inhibition and complement-binding donor-specific antibody (DSA)

Despite the use of potent combination of immunosuppressive agents (calcineurin inhibitor, steroids, mycophenolate mofetil), around 15% of kidney transplant recipients develop donor-specific antibodies (DSA) at 4 years post-transplant and chronic antibody-mediated rejection is a major cause of long-term graft loss. Therefore, the 7-year follow-up of the BENEFIT trial was intriguing since it showed that patients treated with belatacept maintenance therapy developed less frequently *de novo* DSA post-transplant (3.1% and 1.4% on belatacept low

intensity and high intensity, respectively, compared with 11.6% on cyclosporine group) (26). There are still some caveats since only a subset of patients originally enrolled in this trial was included in the final analysis and the control group with cyclosporine was different than the current standard of care with tacrolimus. Whether the reduction in DSA development will impact long-term graft survival still remains to be determined but based on prior published data it is highly possible to be beneficial.

To further understand the pathogenicity of alloantibodies, some studies have explored the biochemical features of anti-HLA antibodies, including IgG subclasses and complement binding capabilities. Lefaucheur *et al.* analyzed the relationship of IgG subclasses and AMR characteristics: IgG3 DSA was associated with acute AMR with shorter time to rejection, increased microcirculation injury and C4d deposition, while IgG4 DSA was associated with chronic allograft injury and IFTA, suggesting that DSA subclasses may help identify distinct pathological processes mediating AMR (27). In another study, identification of C1q-binding of DSA in 1016 kidney transplant recipients during the first year after transplant was associated with worse graft outcomes with a 4-fold higher risk of graft loss (hazard ratio, 4.78; 95% confidence interval [CI], 2.69 to 8.49) when adjusted for clinical, functional, histologic, and immunologic factors (28). Guidicelli *et al.* confirmed that 10-year death censored graft survival was lower in patients with C1q-binding *de novo* DSA detected at both 2 and 5 years ($p=0.002$) than in patients without *de novo* DSA (29). However, they also observed that long-term exposure to C1q-non-binding *de novo* DSA was associated with poor outcomes ($p<0.001$), in particular when persistent. Finally, Sicard *et al.* used another assay of complement binding in 69 biopsy proven AMR cases and demonstrated that C3d-binding DSA in a flow-bead-based assay predicted the graft outcomes at time of AMR, providing a potential risk stratification tool to decide on aggressiveness of therapeutic strategies to be employed upon AMR diagnosis (30).

## The benefits of desensitization

Transplantation of highly sensitized patients has been challenging. Orandi *et al.* reported a survival benefit of desensitization in HLA-incompatible live donor kidney transplants when compared to staying on the waitlist. This 22 multicenter study included 1025 highly sensitized kidney transplant recipients (either single-antigen screening positive, flow-cytometric crossmatch positive, or complement-dependent cytotoxicity (CDC) crossmatch positive) and showed a 5-year patient survival of 86% in the desensitized live donor group compared to 74% on the group that remained on the waiting list or was transplanted without desensitization and 59% on the waiting-list-only control group. (31). Despite those findings, not all sensitized patients can be successfully desensitized. Further exploring this, Yabu *et al.* compared immune profile of highly sensitized (cPRA 93-100%) ESRD patients who received a desensitization protocol (combination of monthly IVIg, rituximab, bortezomib and plasmaphresis) and categorized them into desensitization responders (cPRA decrease >5% pre- and post-desensitization) and non-responders, using CyTOF platform, phospho-signaling and gene array (32). They found that the combination of transitional B cells ($CD19^{+}CD24^{+}CD38^{+}$) and Tregs ($CD4^{+}CD25^{hi}CD127^{low}$) prior to desensitization could be a good predictor of response to desensitization. Furthermore, the combination of low HLA-$DR^{-}CD38^{+}CD4^{+}$ T cells and high expression levels of the tumor necrosis factor-associated

factor 4 interacting protein 3 (TRAF4IP3) gene may also separate responders from non-responders. Further validation of these findings is warranted in other sensitized cohorts.

## Bioengineering of T cell receptors and drug-loaded nanoparticles

Emerging data suggests that allospecific Tregs are significantly more powerful than polyclonal-expanded Tregs and with reduced risk of general immunosuppression if used in cell therapy. Chimeric antigen receptors (CARs) are engineered receptors that confer new specificity to T cells, opening the potential of creating allospecific Tregs in the lab (33). McDonald *et al.* generated human Tregs expressing second generation CARs specific to an antigen of the MHC I molecule: HLA-A2 (34). These cells displayed increased proliferation and antigen-triggered activation when stimulated via T cell receptor (TCR). These CAR-expressing Tregs maintained Treg phenotype: cell surface markers (Foxp3, CD25, Helios, cytotoxic T-lymphocyte associated protein 4 (CTLA4) and latency-associated peptide (LAP), epigenetic features (high degree of demethylation pattern in Treg-specific demethylation region (TSDR) of Foxp3 gene) and suppressive function both *in vitro* and *in vivo* graft-versus-host disease models (35). These antigen-specific Tregs had greater suppressive function than control Tregs *in vitro* cultures with either T cells and/or APCs expressing HLA-A2. Thus, the generation of Tregs that express alloantigen-specific CARs could be a promising therapeutic approach to manage allograft rejection, though the polyclonal nature of anti-HLA response post-transplant may pose some challenges.

Exploring targeted drug delivery, Azzi *et al.* utilized microparticles loaded with tacrolimus and coated with a monoclonal antibody (MECA79) specific to peripheral lymph node addressin (PNAd), which is expressed by high endothelial venules. The administration of MECA79-bearing particles prolonged cardiac allograft survival and showed significant preferential accumulation in the draining lymph nodes with negligible levels of systemic tacrolimus (36). Novel approaches to targeted delivery of immunosuppressive drugs are greatly needed to minimize systemic toxicity.

## Immune checkpoint inhibitors and allograft

Immune checkpoint inhibitors have been increasingly used in metastatic cancers as more efficacies are proven in different malignancy species. Since co-inhibitory signals play a critical role in the induction and maintenance of immune regulation post-transplant, concerns may exist in using theses therapies in transplant recipients. Recent case reports documented the successful treatment of metastatic melanoma with anti-CTLA4 (Ipilimumab) in two kidney transplant patients (37), while other reports described severe precipitation of allograft rejection when PD-1 blocking agent was used in kidney transplant recipients [anti-PD-1 alone (38, 39) or in tandem use of anti-CTLA4 and anti-PD-1: (40, 41)]. Enhancing cellular immunity by unleashing T effector cells can trigger both dormant auto-reactive T cells and alloreactive T cells at the same time, leading to immune related adverse events (irAE) (42) and allograft rejection, respectively. These limited reports suggest that sole CTLA4 blockade may be safe in selected transplant recipients compared to PD-1 blockade, though further investigation is still required.

### Xenotransplantation in the era of CRISPR/Cas9 technology

Xenotransplantation has been extensively explored as an option to concur organ shortage, however, limitations related to thrombotic complications and concern about transmission of retrovirus has limited its translational application. Recent advances in gene-editing technologies, such as CRISPR/Cas9 system, have made this option more realistic. CRISPR/Cas9 system allows simultaneous and precise editing of multiple genes in eukaryotic cells. Using this technology, Muhammad *et al.* (43) performed a pig-to-primate cardiac transplant model utilizing alpha 1-3 galactosyltransferase gene knockout- and human complement regulatory protein CD46 and human thrombomodulin knock-in-pigs in combination with a potent immunosuppressive regimen (induction with anti-thymocyte globulin (ATG) and anti-CD20 antibody, maintenance with mycophenolate mofetil and intensive dose of anti-CD40). Primate recipients achieved a successful long-term allograft survival (max 945 days) without thrombotic complications or humoral rejection. The safety concern of xenotransplant in regards to infections is also becoming within reach. Yang *et al.* (44) successfully disrupted over 60 porcine endogenous retrovirus (PERV) pol genes using CRISPR/Cas9, demonstrating the feasibility of this approach to eliminate potentially dangerous retroviruses prior to xenotransplantation.

## Conclusion

In this review, we highlighted the new advances in understanding and measurement of donor-reactive T cell responses as well as the role of indirect allorecognition in chronic rejection. Further studies reinforced the importance of different players in the alloimmune response, including innate cells, environmental exposures and infections in tolerance development. From a clinical perspective, we noted how elucidating molecular signatures of alloimmune response can further lead to new insights onto diagnostic and therapeutic approaches by redefining mechanisms of allograft injury. Lastly, we described how novel finding and approaches including the identification of IL-34, and the use of CRISPR/Cas9 gene editing technologies and CAR T cells are yielding promising preclinical results in the management of transplant rejection. The efficacy and safety of these strategies in humans will need to be further explored in order to translate from bench to the clinic and confirm their promise.

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## References

1*. Ali JM, Negus MC, Conlon TM, Harper IG, Qureshi MS, Motallebzadeh R, et al. Diversity of the CD4 T Cell Alloresponse: The Short and the Long of It. Cell Rep. 2016; 14(5):1232–45. Epub 2016/01/26. This study describes the differences between the alloreactive responses triggered by direct or indirect pathway. This article shows that chronic cellular-mediated rejection responses

are induced by indirect allorecognition due to the persistent presentation of donor MHC class I antigens. [PubMed: 26804905]

2. Nelson RW, Beisang D, Tubo NJ, Dileepan T, Wiesner DL, Nielsen K, et al. T cell receptor cross-reactivity between similar foreign and self peptides influences naive cell population size and autoimmunity. Immunity. 2015; 42(1):95–107. Epub 2015/01/21. [PubMed: 25601203]
3. Morris H, DeWolf S, Robins H, Sprangers B, LoCascio SA, Shonts BA, et al. Tracking donor-reactive T cells: Evidence for clonal deletion in tolerant kidney transplant patients. Sci Transl Med. 2015; 7(272):272ra10. Epub 2015/01/30.

4**. Beura LK, Hamilton SE, Bi K, Schenkel JM, Odumade OA, Casey KA, et al. Normalizing the environment recapitulates adult human immune traits in laboratory mice. Nature. 2016; 532(7600):512–6. Epub 2016/04/21. Authors demonstrated that T cell responses from mice housed under pathogen-free conditions is similar to human neonates. This article highlights limitations on the use of laboratory mice to study immunity and suggests that pathogen-experienced animals have an immune system similar to human adults. [PubMed: 27096360]

5. Steinert EM, Schenkel JM, Fraser KA, Beura LK, Manlove LS, Igyarto BZ, et al. Quantifying Memory CD8 T Cells Reveals Regionalization of Immunosurveillance. Cell. 2015; 161(4):737–49. Epub 2015/05/11. [PubMed: 25957682]
6. Geijtenbeek TB, Torensma R, van Vliet SJ, van Duijnhoven GC, Adema GJ, van Kooyk Y, et al. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. Cell. 2000; 100(5):575–85. Epub 2000/03/18. [PubMed: 10721994]
7. Geijtenbeek TB, Engering A, Van Kooyk Y. DC-SIGN, a C-type lectin on dendritic cells that unveils many aspects of dendritic cell biology. J Leukoc Biol. 2002; 71(6):921–31. Epub 2002/06/07. [PubMed: 12050176]
8. Batal I, De Serres SA, Safa K, Bijol V, Ueno T, Onozato ML, et al. Dendritic Cells in Kidney Transplant Biopsy Samples Are Associated with T Cell Infiltration and Poor Allograft Survival. Journal of the American Society of Nephrology : JASN. 2015; 26(12):3102–13. Epub 2015/04/10. [PubMed: 25855773]
9. Dugast AS, Haudebourg T, Coulon F, Heslan M, Haspot F, Poirier N, et al. Myeloid-derived suppressor cells accumulate in kidney allograft tolerance and specifically suppress effector T cell expansion. J Immunol. 2008; 180(12):7898–906. Epub 2008/06/05. [PubMed: 18523253]
10. Garcia MR, Ledgerwood L, Yang Y, Xu J, Lal G, Burrell B, et al. Monocytic suppressive cells mediate cardiovascular transplantation tolerance in mice. J Clin Invest. 2010; 120(7):2486–96. Epub 2010/06/17. [PubMed: 20551515]
11. Zhang W, Liang S, Wu J, Horuzsko A. Human inhibitory receptor immunoglobulin-like transcript 2 amplifies CD11b+Gr1+ myeloid-derived suppressor cells that promote long-term survival of allografts. Transplantation. 2008; 86(8):1125–34. Epub 2008/10/24. [PubMed: 18946352]
12. Conde P, Rodriguez M, van der Touw W, Jimenez A, Burns M, Miller J, et al. DC-SIGN(+) Macrophages Control the Induction of Transplantation Tolerance. Immunity. 2015; 42(6):1143–58. Epub 2015/06/14. [PubMed: 26070485]
13. Famulski KS, de Freitas DG, Kreepala C, Chang J, Sellares J, Sis B, et al. Molecular phenotypes of acute kidney injury in kidney transplants. Journal of the American Society of Nephrology : JASN. 2012; 23(5):948–58. Epub 2012/02/22. [PubMed: 22343120]
14. Dorr C, Wu B, Guan W, Muthusamy A, Sanghavi K, Schladt DP, et al. Differentially expressed gene transcripts using RNA sequencing from the blood of immunosuppressed kidney allograft recipients. PLoS One. 2015; 10(5):e0125045. Epub 2015/05/07. [PubMed: 25946140]
15. Reeve J, Chang J, Salazar ID, Lopez MM, Halloran PF. Using Molecular Phenotyping to Guide Improvements in the Histologic Diagnosis of T Cell-Mediated Rejection. Am J Transplant. 2016; 16(4):1183–92. Epub 2016/01/06. [PubMed: 26730747]
16. Modena BD, Kurian SM, Gaber LW, Waalen J, Su AI, Gelbart T, et al. Gene Expression in Biopsies of Acute Rejection and Interstitial Fibrosis/Tubular Atrophy Reveals Highly Shared Mechanisms That Correlate With Worse Long-Term Outcomes. Am J Transplant. 2016 Epub 2016/03/19.

17. Bezie S, Picarda E, Ossart J, Tesson L, Usal C, Renaudin K, et al. IL-34 is a Treg-specific cytokine and mediates transplant tolerance. J Clin Invest. 2015; 125(10):3952–64. Epub 2015/09/22. [PubMed: 26389674]

18. Miller ML, Daniels MD, Wang T, Chen J, Young J, Xu J, et al. Spontaneous restoration of transplantation tolerance after acute rejection. Nat Commun. 2015; 6:7566. Epub 2015/07/08. [PubMed: 26151823]

19. Safa K, Ohori S, Borges TJ, Uehara M, Batal I, Shimizu T, et al. Salt Accelerates Allograft Rejection through Serum- and Glucocorticoid-Regulated Kinase-1-Dependent Inhibition of Regulatory T Cells. Journal of the American Society of Nephrology : JASN. 2015; 26(10):2341–7. Epub 2015/04/03. [PubMed: 25833841]

20. Molinero LL, Yin D, Lei YM, Chen L, Wang Y, Chong AS, et al. High-Fat Diet-Induced Obesity Enhances Allograft Rejection. Transplantation. 2016; 100(5):1015–21. Epub 2016/03/24. [PubMed: 27007226]

21. Bagley J, Yuan J, Chandrakar A, Iacomini J. Hyperlipidemia Alters Regulatory T Cell Function and Promotes Resistance to Tolerance Induction Through Costimulatory Molecule Blockade. Am J Transplant. 2015; 15(9):2324–35. Epub 2015/06/17. [PubMed: 26079467]

22. Yuan J, Bagley J, Iacomini J. Hyperlipidemia Promotes Anti-Donor Th17 Responses That Accelerate Allograft Rejection. Am J Transplant. 2015; 15(9):2336–45. Epub 2015/06/17. [PubMed: 26079335]

23. Laws LH, Parker CE, Cherala G, Koguchi Y, Waisman A, Slifka MK, et al. Inflammation causes resistance to anti-CD20 mediated B cell depletion. Am J Transplant. 2016 Epub 2016/06/07.

24**. Marino J, Paster JT, Trowell A, Maxwell L, Briggs KH, Crosby Bertorini P, et al. B Cell Depletion With an Anti-CD20 Antibody Enhances Alloreactive Memory T Cell Responses After Transplantation. Am J Transplant. 2016; 16(2):672–8. Epub 2015/11/10. This study reported the outcomes of long-term follow-up of BENEFIT trial. The frequency of *de novo* DSA development was significantly lower in Belatacept-treated group. [PubMed: 26552037]

25. Chandraker A, K J, Stehlik J, Givertz M, Pierson R, Pinney S, Joren M, Nissen S, Guleria I, Morrison Y, Armstrong B, Bridges N, Sayegh M, Starling R. Rituximab Induction in Cardiac Transplantation Is Associated with Accelerated Coronary Artery Vasculopathy: CTOT11. Am J Transplant. 2016; 16 abstract.

26. Vincenti F, Rostaing L, Grinyo J, Rice K, Steinberg S, Gaite L, et al. Belatacept and Long-Term Outcomes in Kidney Transplantation. N Engl J Med. 2016; 374(4):333–43. Epub 2016/01/28. [PubMed: 26816011]

27. Lefaucheur C, Viglietti D, Bentlejewski C, Duong van Huyen JP, Vernerey D, Aubert O, et al. IgG Donor-Specific Anti-Human HLA Antibody Subclasses and Kidney Allograft Antibody-Mediated Injury. Journal of the American Society of Nephrology : JASN. 2016; 27(1):293–304. Epub 2015/08/22. [PubMed: 26293822]

28. Loupy A, Lefaucheur C, Vernerey D, Prugger C, Duong van Huyen JP, Mooney N, et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. N Engl J Med. 2013; 369(13):1215–26. Epub 2013/09/27. [PubMed: 24066742]

29*. Guidicelli G, Guerville F, Lepreux S, Wiebe C, Thaunat O, Dubois V, et al. Non-Complement-Binding De Novo Donor-Specific Anti-HLA Antibodies and Kidney Allograft Survival. Journal of the American Society of Nephrology : JASN. 2016; 27(2):615–25. Epub 2015/06/07. This study highlighted the survival benefit of kidney transplant in moderately or highly sensitized patients following desensitization. [PubMed: 26047793]

30. Sicard A, Ducreux S, Rabeyrin M, Couzi L, McGregor B, Badet L, et al. Detection of C3d-binding donor-specific anti-HLA antibodies at diagnosis of humoral rejection predicts renal graft loss. Journal of the American Society of Nephrology : JASN. 2015; 26(2):457–67. Epub 2014/08/16. [PubMed: 25125383]

31. Orandi BJ, Luo X, Massie AB, Garonzik-Wang JM, Lonze BE, Ahmed R, et al. Survival Benefit with Kidney Transplants from HLA-Incompatible Live Donors. N Engl J Med. 2016; 374(10): 940–50. Epub 2016/03/11. [PubMed: 26962729]

32**. Yabu JM, Siebert JC, Maecker HT. Immune Profiles to Predict Response to Desensitization Therapy in Highly HLA-Sensitized Kidney Transplant Candidates. PLoS One. 2016; 11(4):e0153355. Epub 2016/04/15. The article is the first study to describe human regulatory T

cells expressing chimeric antigen receptors (CARs). This study brings a novel approach to treat not only transplant rejection, but also autoimmunity and other inflammatory disorders. [PubMed: 27078882]

33. Gill S, June CH. Going viral: chimeric antigen receptor T-cell therapy for hematological malignancies. Immunol Rev. 2015; 263(1):68–89. Epub 2014/12/17. [PubMed: 25510272]
34. MacDonald KG, Hoeppli RE, Huang Q, Gillies J, Luciani DS, Orban PC, et al. Alloantigen-specific regulatory T cells generated with a chimeric antigen receptor. J Clin Invest. 2016; 126(4): 1413–24. Epub 2016/03/22. [PubMed: 26999600]
35. Toker A, Engelbert D, Garg G, Polansky JK, Floess S, Miyao T, et al. Active demethylation of the Foxp3 locus leads to the generation of stable regulatory T cells within the thymus. J Immunol. 2013; 190(7):3180–8. Epub 2013/02/20. [PubMed: 23420886]
36. Azzi J, Yin Q, Uehara M, Ohori S, Tang L, Cai K, et al. Targeted Delivery of Immunomodulators to Lymph Nodes. Cell Rep. 2016; 15(6):1202–13. Epub 2016/05/03. [PubMed: 27134176]
37. Lipson EJ, Bodell MA, Kraus ES, Sharfman WH. Successful administration of ipilimumab to two kidney transplantation patients with metastatic melanoma. J Clin Oncol. 2014; 32(19):e69–71. Epub 2014/02/05. [PubMed: 24493726]
38. Lipson EJ, Bagnasco SM, Moore J Jr, Jang S, Patel MJ, Zachary AA, et al. Tumor Regression and Allograft Rejection after Administration of Anti-PD-1. N Engl J Med. 2016; 374(9):896–8. Epub 2016/03/11.
39. Boils CL, Aljadir DN, Cantafio AW. Use of the PD-1 pathway inhibitor nivolumab in a renal transplant patient with malignancy. Am J Transplant. 2016 Epub 2016/03/19.
40. Alhamad T, Venkatachalam K, Linette GP, Brennan DC. Checkpoint Inhibitors in Kidney Transplant Recipients and the Potential Risk of Rejection. Am J Transplant. 2016; 16(4):1332–3. Epub 2016/01/12. [PubMed: 26752406]
41. Spain L, Higgins R, Gopalakrishnan K, Turajlic S, Gore M, Larkin J. Acute renal allograft rejection after immune checkpoint inhibitor therapy for metastatic melanoma. Ann Oncol. 2016 Epub 2016/03/10.
42. Murakami N, Borges TJ, Yamashita M, Riella LV. Severe acute interstitial nephritis after combination immune-checkpoint inhibitor therapy for metastatic melanoma. Clin Kidney J. 2016; 9(3):411–7. Epub 2016/06/09. [PubMed: 27274826]
43. Mohiuddin MM, Singh AK, Corcoran PC, Thomas ML Iii, Clark T, Lewis BG, et al. Chimeric 2C10R4 anti-CD40 antibody therapy is critical for long-term survival of GTKO.hCD46.hTBM pig-to-primate cardiac xenograft. Nat Commun. 2016; 7:11138. Epub 2016/04/06. [PubMed: 27045379]
44. Yang L, Guell M, Niu D, George H, Lesha E, Grishin D, et al. Genome-wide inactivation of porcine endogenous retroviruses (PERVs). Science. 2015; 350(6264):1101–4. Epub 2015/10/13. [PubMed: 26456528]

**Key points**

- Novel assay to identify and track donor-specific T cells clones may allow better monitoring of the allospecific immune response and tolerance development
- Co-housing of laboratory mice with pet store mice may better generate a memory compartment in mice, more closely resembling the adult human immune system
- Molecular monitoring of allograft injury will revolutionize approaches towards diagnosis and treatment.
- Environmental exposures such as a high-salt or infections may be deleterious to immune regulation and precipitate rejection
- Approaches includingtargeted drug delivery using microparticles, CAR T cells and theCRISPR/Cas9 systemhave emerged as novel therapies to improve graft outcomes