# Heterologous immunity in organ transplantation

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CHAPTER

# 2

# T-CELL ALLOREACTIVITY AND TRANSPLANTATION OUTCOME - A BUDDING ROLE FOR HETEROLOGOUS IMMUNITY?

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*Curr Opin Organ Transplant 2015 Aug;20(4):454-60*

## ABSTRACT

### Purpose of the review

Despite the association between alloreactive T cells and poor graft survival, the mechanisms behind T-cell-mediated rejection are still under investigation. In this review, we will discuss the latest insights into the impact of T-cell alloreactivity on solid organ transplantation and hematopoietic stem cell transplantation (HSCT), with special emphasis on the potential impact of heterologous immunity.

### Recent findings

A large part of the memory T-cell repertoire is induced upon viral infections, and evidence for a role of T-cell receptor cross-reactivity of virus-induced memory T cells against allogeneic human leukocyte antigen (HLA) is accumulating in experimental and clinical solid organ transplantation studies. In HSCT, strong alloreactive potential of naïve T cells causes concerns for graft-versus-host disease while additional HLA-DP matching is suggested to prevent $CD4^+$ alloreactivity. Furthermore, virus-induced memory T cells hamper mixed chimerism induction, pointing once more towards a role for heterologous immunity.

### Summary

Both memory and naïve T cells contribute to the alloimmune response after transplantation. Monitoring for T-cell phenotypes could help predict rejection episodes and/or graft-versus-host disease, allowing timely intervention. Tailoring donor lymphocyte infusions and additional HLA matching could prevent strong alloreactivity in HSCT. Furthermore, the potential role of heterologous immunity in T-cell alloreactivity and transplantation is gaining interest.

## INTRODUCTION

T-cell-mediated alloreactivity is often involved in clinical complications after solid organ transplantation (SOT) and hematopoietic stem cell transplantation (HSCT). Whereas in SOT alloreactivity by T cells other than regulatory T cells (Tregs) is considered detrimental, the outlook on HSCT is more delicate. HSCT is frequently applied to cure hematological cancers, and a certain extent of T-cell alloreactivity is desired in order to attack residual malignant cells. This is known as the graft-versus-leukemia (GVL) effect. However, vigorous T-cell alloreactivity poses a risk for developing graft-versus-host disease (GVHD).

Both naïve and memory T cells have alloreactive potential. In HSCT, both are indispensable for proper functioning of the donor-derived immune system, although alloreactivity mediated by either subset can contribute to GVHD (57). In SOT, patients are treated with immunosuppressive drugs. Standard maintenance immunosuppressive therapy in SOT consists of a triad of calcineurin inhibitors (CNI; most commonly low-dose tacrolimus), mycophenolate mofetil (MMF) and corticosteroids. Together with induction therapy by CD25-blocking antibodies, this regimen is most potent compared to other current immunosuppressive regimens (45). Although effective in terms of patient survival and allograft rejection, treatment with CNI is associated with severe side effects such as nephrotoxicity (46), neurotoxicity (47), new-onset diabetes (48), metabolic syndrome (49) and increased susceptibility to viral infections (50, 58), which necessitate the identification of novel immunosuppressive drugs that specifically target alloreactive cells without hampering anti-viral responses. Hereto, costimulation blockade is suggested to be a promising strategy, and several studies have investigated the therapeutic potential of belatacept (a cytotoxic T lymphocyte-associated antigen 4 (CTLA-4)-specific fusion protein blocking CD28-B7 costimulation) as an alternative to CNI (59). Immunosuppression by costimulation blockade has the advantage of selectively targeting naïve T cells, leaving memory T cells largely unaffected as they are less dependent on costimulation to exert effector functions. Virus-specific memory T cells are therefore only minimally hampered by costimulation blockade, however, the same applies to alloreactive memory T cells. Furthermore, memory T cells have the capacity to exert more vigorous immune responses upon antigen recognition compared to naïve T cells, and therefore alloreactive memory T cells are considered more prone to impact SOT outcome compared to naïve T cells (60).

Interestingly, alloreactive memory T-cell responses can be mounted without prior encounter of alloantigen. This is explained by heterologous immunity of virus-induced memory T cells, in which cross-reactivity of the T-cell receptor (TCR) enables the recognition of allogeneic human

leukocyte antigen (HLA) (Figure 1). The cross-reacting TCR has never been trained to recognize allogeneic HLA by positive and negative selection in the thymus, which suggests that cross-reactive T cells simply mistake unknown allogeneic cells for virus-infected autologous cells. As cross-reactivity is an intrinsic feature of TCRs (24), one could envision that a substantial part of virus-induced memory T cells are cross-reactive. Indeed, 80% of all virus-induced T-cell lines and 45% of all virus-induced T-cell clones are reported to exert cross-reactivity against allogeneic HLA (43). Given the myriad of viral infections that are encountered throughout life, it is therefore likely to assume that all individuals harbor a large repertoire of virus-induced memory T cells with cross-reactive potential to allogeneic antigens. In vivo animal models have shown that virus-induced cross-reactive memory T cells hamper successful transplantation (35, 36, 61), but their impact on clinical transplantation remains elusive.

In this review, we will discuss the latest insights into T-cell alloreactivity and its impact on solid organ and HSCT outcome. Special emphasis will be given to the role of heterologous immunity of virus-induced T cells.

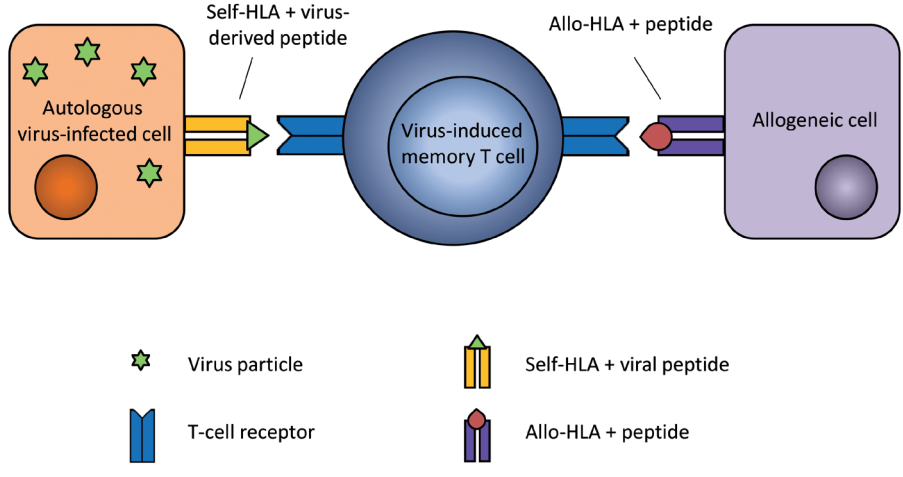


**Figure 1. Virus-induced memory T cells can recognize allogeneic HLA + peptide by means of heterologous immunity.** Through TCR cross-reactivity, a single TCR can recognize multiple antigens. In this schematic overview, virus-induced memory T cells recognize both self-HLA + viral peptide and allo-HLA + peptide using the same TCR. HLA, human leukocyte antigen; TCR, T-cell receptor.

## SOLID ORGAN TRANSPLANTATION

Transplantation is the most desired treatment for end-stage organ failure. Despite drastically improved graft outcomes over time, rejection remains a major threat to successful transplantation. Alloreactive T cells play a pivotal role in SOT rejection, although their mode of action is not always fully understood. However, recent experimental and human studies have generated new and relevant data on this topic.



### Solid organ transplantation: experimental studies

$CD4^+$ T cells play a prominent role in both humoral and cellular alloreactivity, by providing help to alloreactive B cells and $CD8^+$ cytotoxic T cells respectively. The latter requires that alloantigens are recognized by both $CD4^+$ and $CD8^+$ T cells: both as processed peptides in self-HLA as well as intact allogeneic HLA class I molecules. Accordingly, Sivaganesh et al. (62) showed that unprimed alloreactive $CD8^+$ T cells directly recognized intact allogeneic HLA class I antigens taken up and presented by recipient dendritic cells, provided that alloantigen peptides were also presented in self-HLA-class II. The authors therefore hypothesized that direct pathway $CD8^+$ alloreactive T cells depend on indirect pathway $CD4^+$ alloreactive T-helper cells for their activation (62). According to this model, matching for HLA class I alone would be sufficient to prevent $CD8^+$ T-cell activation. Yet, a recent study by Ishii et al. (63) suggested that $CD8^+$ T-cell responses could also be elicited upon sole HLA class II mismatching. In a kidney transplant model, $CD8^+$ T cells recognized peptides from mismatched MHC class II molecules presented by matched MHC class I, which led to allograft rejection (63). Thus, MHC class II peptides may serve as minor histocompatibility antigens (mHAg) that could trigger $CD8^+$ T-cell alloreactivity. This implies that matching for HLA class II may not only prevent $CD4^+$, but also $CD8^+$ T-cell allorecognition.

As an induction therapy prior to transplantation, T cells can be depleted by lymphocyte-depleting antibodies, such as antithymocyte globulin (ATG) or alemtuzumab. However, lymphocyte depletion can provoke a disturbed balance in T-cell subsets as a result of homeostatic proliferation in favor of memory T cells. To prevent this, Mai et al. (64) studied interleukin (IL)-7 receptor blockade following lymphodepletion. IL-7 is a central regulator of both $CD4^+$ and $CD8^+$ T-cell survival and homeostasis, and IL-7 receptor blockade indeed prevented memory T-cell proliferation and promoted allograft survival in both pancreatic island and skin transplantation mouse models (64). Alternatively, selective depletion of $CD2^{hi}CD8^+$ effector memory T cells by targeting CD2 has been shown to promote immunosuppression-free renal

allograft survival in nonhuman primates who were tolerized by a donor bone marrow transplant (65).

Apart from traditionally primed memory T cells (either by viral infection or alloantigen exposure), naturally occurring memory T cells with unknown priming history also exist. These may be primed by endogenous signals or even emerge spontaneously from naïve T cells without passing the effector phase (66, 67). Several studies on unprimed mice housed in pathogen-free conditions have indicated that these endogenous memory T cells could be alloreactive (57, 68). Recently, Su et al. (69) demonstrated that endogenous memory T cells could mediate cardiac allograft rejection. Graft infiltration, activation and enhanced effector function of these endogenous memory $CD8^+$ T cells were directly linked to prolonged cold ischemia times, suggesting that these cells may respond to danger signals, which has been suggested previously (70, 71).

Yet, not all memory T cells are detrimental, and distinctive memory T-cell phenotypes may in fact have antagonistic effects on transplantation outcome. Whereas effector memory $CD8^+$ T cells are mainly described to have a detrimental impact on transplant outcome, it appears that central memory $CD8^+$ T cells may instead promote allograft acceptance. Indeed, Krupnick et al. (72) showed that central memory $CD8^+$ T cells infiltrating into lung allografts were crucial for their acceptance. These cells had potent immunoregulatory properties, and were able to down-regulate $CD4^+$ and $CD8^+$ T-cell alloresponses (72). The observed regulation was attributable to nitric oxygen (NO) production by graft-infiltrating central memory $CD8^+$ T cells that were abundantly present in accepted lung allografts. In contradiction to this study, central memory T cells have also been described to mediate graft rejection in experimental skin transplantation (35). This discrepancy may be explained by the unique physiology of the lungs to limit pulmonary inflammation (73), or because nonvascularized skin allografts are less susceptible to tolerogenesis (74). The impact of central memory T cells on transplantation outcome may therefore vary depending on the transplanted organ.

A common feature of memory T-cell differentiation is the gradual loss of CD28, which can have direct implications for transplantation, since (alloreactive) memory T cells that have lost CD28 expression are less dependent on costimulatory signals for their activation. In addition, CD28 loss renders them insusceptible to costimulation blockade by belatacept (53). Therefore, costimulation blockade alone may be insufficient to suppress the activity of progressively matured alloreactive $CD28^{null}$ T cells. Indeed, a large phase III clinical trial (Belatacept Evaluation of Nephroprotection and Efficacy as First-line Immunosuppression Trial; BENEFIT) investigating

immunosuppression by belatacept as an alternative to cyclosporine, showed that allograft rejection was significantly higher in the belatacept versus the cyclosporine group (17-22% vs 7% respectively) (75). Surprisingly, a recent nonhuman primate renal transplantation model with belatacept in combination with the mammalian target of rapamycin (mTOR) inhibitor sirolimus, found no necessity for memory T-cell depletion to ensure allograft survival (76). A subsequent human study conducted by the same group supported these data (77), indicating that the combination of costimulation and mTOR inhibition could be sufficient to hamper memory T cell responses. The findings remain to be confirmed in larger human studies.

In addition to belatacept resistance, Demmers et al. (78) demonstrated that terminally differentiated $CD4^+CD28^{null}$ effector memory T cells can also be unresponsive to tacrolimus and everolimus. In addition, the authors showed that these terminally differentiated $CD4^+CD28^{null}$ effector memory T cells were able to proliferate in response to allogeneic renal tubular epithelial cells (TECs). Although terminally differentiated $CD4^+CD28^{null}$ effector memory cells are uncommon in healthy individuals, they are found during end-stage renal disease and are associated with cytomegalovirus (CMV) infection (79, 80).

Interestingly, the humoral arm of the immune system may also affect T-cell mediated alloimmunity, as was recently described by Jane-Wit et al. (81). They showed that alloantibody and complement deposition on the cell surface of allogeneic endothelial cells upregulated the transcription of pro-inflammatory genes, leading to recruitment and activation of alloreactive $CD4^+$ T cells and resulting in cardiac allograft vasculopathy in mice (81). Additionally, the group of Heeger described a direct interaction of complement and T cells. Naïve $CD4^+$ T cells were shown to express complement receptors C3aR and C5aR, which upon activation induced type 1 helper T cell (Th1) maturation (82). Recently, they found that blockade of the complement receptors also significantly enhanced the stability of alloantigen-induced Tregs (83). These findings create a therapeutic potential for C3aR and C5aR antagonists, as they could simultaneously hamper alloreactive T cells and promote alloantigen-induced Treg stability.

### Solid organ transplantation: clinical studies

Cytomegalovirus (CMV) infection is negatively associated with graft outcome, and recent clinical studies highlight the potential role of the anti-CMV response. Donckier et al. (84) found that strong effector memory $CD8^+$ T-cell expansion induced by lymphodepletion impeded early immunosuppression withdrawal in cadaver liver transplant recipients. The expansion mainly involved CMV- and Epstein-Barr virus (EBV)-directed T cells, and correlated with the occurrence of acute rejection. The authors therefore suggested a model in which virus-induced effector memory $CD8^+$ T cells homeostatically proliferated upon lymphodepletion, and

subsequently cross-reacted to the donor tissue: creating a causal link between graft rejection and heterologous immunity. Likewise, a role for heterologous immunity in graft rejection was suggested by Roux et al. (85), who performed a longitudinal study on lung transplant recipients. It was shown that memory $CD8^+$ T-cell responses directed against CMV comprised a large proportion of the intra-graft immune infiltrate and could therefore be involved in transplant rejection (85). Moreover, Nguyen et al. (86) found cross-reactive CMV-directed memory $CD8^+$ T cells in a lung-transplant recipient. Interestingly, despite increased numbers of highly activated cross-reactive $CD8^+$ T-cells prior to and during CMV reactivation, there was no negative correlation with long-term transplantation outcome. This finding is in concordance with their previous findings on cross-reactive EBV-induced $CD8^+$ T cells, which were also detected in human lung allografts without exerting a negative effect on transplant outcome (40). The latter two studies may therefore seem to argue against a role for cross-reactive virus-induced T cells in transplant outcome. However, one should keep in mind that the donor used in the CMV study did not express the cross-reacting HLA-B27 subtype, which could explain for the lack of detrimental effect on transplantation outcome. Furthermore, the cross-reactive response described in the EBV study is shown to be highly peptide-dependent and as a consequence is tissue-specific (87). The cross-reactive T cells specifically recognize a peptide derived from the ATP-binding cassette sub-family D member 3 (ABCD3) protein, and although lung tissue cells can express ABCD3, this study also showed that ABCD3 expression does not, as per definition, lead to surface presentation of the appropriate cross-reacting peptide (87). This cross-reactive response should therefore ideally also be investigated in other human transplantation types.

## HEMATOPOIETIC STEM CELL TRANSPLANTATION

Hematopoietic stem cell transplantation (HSCT) can be employed for both malignant and nonmalignant hematological diseases. Whereas HSCT for malignant diseases aims at inducing GVL by moderate alloreactivity, HSCT for nonmalignant hematological diseases aims at full donor-specific tolerance. The latter can be achieved by inducing a state of mixed hematopoietic chimerism, in which there is a stable balance between immune cells of the donor and the recipient (88).

### Hematopoietic stem cell transplantation: experimental studies

Recent experimental studies on T-cell alloreactivity in HSCT mainly focus on alloreactivity directed against malignant cells. Although entering the field of tumor immunology is beyond the scope of this review, lessons can be learned regarding the alloreactive potential of donor-derived T cells. For instance, Binsfeld et al. (89) developed a murine model to investigate

the curative properties of allogeneic SCT in multiple myeloma, and revealed that especially alloreactive $CD8^+$ T cells are potent mediators of the graft-versus-myeloma (GVM) effect.

Westerhuis et al. (90) recently studied the effect of heterologous immunity on the induction of mixed chimerism. In an experimental mouse model, the persistence of mixed hematopoietic chimerism was hampered by recipient $CD8^+$ T cells induced upon vaccination with the vaccinia virus (90). The authors therefore hypothesize that heterologous immunity of vaccinia-induced $CD8^+$ T cells directed against allogeneic antigens interfered with the mixed chimerism induction. This study is one of the first describing heterologous immunity in HSCT. Since patients undergoing HSCT are highly immunocompromized, infections are a regular complication (91), potentially interfering with mixed chimerism induction or contributing to GVHD.



### Hematopoietic stem cell transplantation: clinical studies

In essence, replacing the recipient's immune system by that of a donor results in the presence of donor-derived naïve T cells in the recipient. Unfortunately, naïve $CD4^+$ T cells can be highly alloreactive and may predispose for developing acute GVHD, which was recently confirmed by Chérel et al. (92). Analyzing HSCT in HLA-identical twins of different sex, they found that naïve $CD4^+$ T cells exerted vigorous alloreactivity towards the H-Y minor antigen (92). Selective depletion of naïve $CD4^+$ T cells from HSCT allografts may therefore prevent GVHD. This is in line with data by Distler et al., who showed that CD45RA-negative T cells exert only limited alloreactivity (93), and therefore assessed the functional properties of CD45RA-depleted donor leukapheresis products. When naïve $CD4^+$ and $CD8^+$ T cells were depleted from leukapheresis products by using CD45RA, they indeed observed reduced alloreactive $CD8^+$ T cell numbers, without effecting responses against pathogens. Unexpectedly, alloreactive $CD4^+$ T-cell numbers were not reduced (94). The authors ascribe this to the completely HLA-mismatched setting and emphasize this is not representative for clinical HSCT. In addition, CD45RA targeting may deplete late-differentiated memory T cells and naïve Tregs. Depletion of late-differentiated memory T cells may hamper anti-virus responses, but could also be beneficial, given their high alloreactive potential as previously discussed (78). The depletion of Tregs, on the contrary, does raise concerns as they are potent tolerogenic cells and are even employed for tolerance induction (95). Inevitably, the forthcoming multicenter pilot trial using CD45RA-depleted donor lymphocyte infusions (DLI) will give more insights into the overall effects of CD45RA depletion.

Currently, HLA matching for HSCT does not include HLA-DP, and mismatching of HLA-DP has previously been suggested to selectively induce GVL (and not GVHD) in T-cell-depleted allo-HSCT patients (96). However, the immunogenicity of HLA-DP is still under investigation, and

recent studies call for awareness of $CD4^+$ reactivity against HLA-DP. For instance, Stevanovic et al. (97) described two patients who underwent severe acute GVHD after prophylactic $CD4^+$ DLI, induced by alloreactive $CD4^+$ T cells directed against mismatched HLA-DP on epithelial cells. Interestingly, both patients suffered from CMV reactivation shortly after HSCT. A likely explanation is therefore that anti-viral inflammatory responses induced HLA class II expression on colonic epithelial cells, enabling the recognition of HLA-DP by alloreactive $CD4^+$ donor T cells and the establishment of severe acute colonic GVHD. Whereas prophylactic $CD4^+$ T-cell DLI is an effective treatment of residual malignant hematopoietic cells, this study calls for caution of performing this procedure in the presence of concurrent viral infections. Interestingly, a great effort is put into the understanding of the molecular mechanisms behind anti-HLA-DP cross-reactivity (98, 99), which recently resulted in the development of a prediction algorithm (100, 101). The algorithm accurately predicted known and unknown allo-HLA-DP $CD4^+$ T-cell cross-reactivity *in vitro* (100), and thereby creates a valuable tool for risk prediction in unrelated HSCT.

Combining these data, it appears that there is an association between virus infection, T-cell cross-reactivity against allogeneic HLA and GVHD. Plausibly, the majority of these alloreactive cells are primed by direct alloantigen recognition, and inflammation induced by viral infection simply upregulates allogeneic HLA on the donor cells. Yet, as suggested by the experimental study by Westerhuis et al., virus-induced T cells may also affect HSCT outcome. Research into heterologous immune responses in HSCT is of special interest at present, as progress is being made into the employment of adoptive transfer of virus-induced T cells to treat viral infections in HSCT patients (102).

## CONCLUSION

Recent literature describes the alloreactive potential of both naïve and memory T-cells. Research on HSCT revealed that especially naïve $CD4^+$ T cells have high alloreactive potential. Tailoring donor-lymphocyte infusions by partial selection of naïve alloreactive T cells and additional HLA-DP matching to prevent strong $CD4^+$ alloreactive responses are therefore promising therapeutic strategies. Whereas in SOT, naïve T cells are efficiently hampered by immunosuppression, memory T cells could escape suppression and provide a barrier to successful transplantation. A large part of the memory T-cell compartment is induced by viral infection, and heterologous immunity of virus-induced memory T cells directed against donor-HLA demands attention in both solid organ transplantation and HSCT. However, much is still unknown with regard to their impact on clinical transplantation outcome, and additional research is strongly urged.