Heterologous immunity in organ transplantation

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CHAPTER

8

SUMMARY & GENERAL DISCUSSION

Viral infections are an important health issue for transplant recipients. In healthy individuals, viral infections can be controlled by virus-specific T cells, but in immunosuppressed transplant recipients the response to viral infection is less efficient. As recently outlined by D'Orsogna et al, infectious pathogens can affect alloreactivity at all levels of the immune system (241). Therefore, common viruses that do not pose a significant health risk to non-immunosuppressed individuals, can become major threats to the health of transplant recipients and can severely affect transplantation outcome (242). Especially cytomegalovirus (CMV), a common virus with a prevalence of around 70% in the general population, is of great concern in kidney transplantation (243-246).

Current immunosuppressive drugs are not specific: they not only hamper anti-donor immune responses but also anti-viral immune responses. Ideally, these drugs should specifically target the immune response to the donor and only to a minor extent the anti-viral immune responses. For this purpose, the differences between these immune responses need to be investigated. One way to make this distinction is by looking at their composition: in theory, the anti-viral immune repertoire largely consists of memory cells that are primed by previous viral infections, whereas the donor-specific immune repertoire consists of naïve cells that are yet to be primed upon transplantation. Consequently, this implies that immunosuppressive drugs directed at the naïve T-cell compartment (such as co-stimulatory inhibitors) prevent the priming of naïve donor-reactive T cells, while they render anti-virus T cells with a memory phenotype unaffected. Thereby the host is protected from anti-donor immunity and life-threatening viral infections.

However, recent clinical trials in which calcineurin inhibitor-based immunosuppression against the memory repertoire was tapered or withdrawn, repeatedly reported impaired tolerance and acute graft rejection (75, 139, 247-249), which may (partly) be explained by heterologous immunity. Some of the virus-specific memory T cells that are so urgently needed for viral control also respond (cross-react) to donor HLA in addition to their cognate viral epitope – a "mistake" that occurs frequently (43). As a consequence, these virus-specific memory T cells can elicit potentially harmful immune responses against the donor allograft. Indeed, studies in mice have shown that cross-reactive virus-specific memory T cells can induce solid organ rejection (35, 250), but the impact of cross-reactive virus-specific memory T cells on allograft survival in humans remains a matter of debate. Clinical studies do not, as yet, show a significant impact on transplantation outcome (38, 40, 41). The number of studies and cross-reactivity models studied are however too limited to draw any generalizable conclusions. Besides, if the cross-reactive T cells are hampered by immunosuppression, they may become a serious issue in the future when alternatives are sought to replace or taper immunosuppression. Therefore,

it remains important to strive towards understanding the mechanisms and clinical impact of virus-specific TCR cross-reactivity. The role of T-cell alloreactivity, and in particular of cross-reactive virus-specific T cells, in transplantation outcome is discussed in **Chapter 2.**

There are several ways in which TCR cross-reactivity of virus-specific T cells can be detected in an experimental setting, and their potencies and flaws are discussed in **Chapter 3** (226). Although these methods do enable accurate detection, it is necessary to keep their limitations in mind. Importantly, one should be aware that current methods based on cross-reactivity detection with viral tetramers rely heavily on known public viral epitopes: and therefore only address the tip of the iceberg regarding the scope of all possible cross-reactivities mediated by virus-specific T cells.

Regardless of this limitation, we were able to show that infection with a single virus can induce many different allo-HLA cross-reactivities **(Chapter 4)**. For example, a large percentage of CMV-specific memory $CD8^+$ T cells are cross-reactive with allogeneic HLA, and we identified a cross-reactivity of CMV A2/NLV-specific T cells specifically recognizing the complex of allo-HLA-A*02:05 presenting a peptide derived from allo-HLA-B*50:01. Interestingly, a recent paper describes how CMV peptides often mimic human peptides and thereby induce alloreactivity (251), and therefore it is not unlikely that HLA-derived peptides could be among those as well.

Furthermore, we found identical allo-HLA cross-reactivity of virus-specific T cells in multiple individuals (public cross-reactivity), as described in **Chapter 6** (140). Partly because dominant TCR usage was considered uncommon, public cross-reactivity was believed to be extremely rare, despite the fact that a public allo-HLA response of EBV-directed T cells had already been documented. Our studies show that public alloresponses are more common than anticipated, which could potentially hold promise for the prediction of alloreactivity in transplant recipients and risk estimation for specific donor-recipient combinations. However, with the exception of the unlikely event of having a surplus of equally suitable donors, we stress that increased knowledge concerning public cross-reactivities should not lead to the discouragement of donor-recipient combinations. In the current climate of organ scarcity, it is not recommended to complicate donor selection or further restrict organ allocation. Rather, the identification of public cross-reactivities could provide a tool to monitor patients at risk for generating memory-like alloreactive T-cell responses, and ultimately to support clinical decision making. Such knowledge may for instance affect the composition of immunosuppressive regimens and the decision whether or not to taper immunosuppression (252). In addition, insights into public cross-reactivity could be used to selectively tailor the composition of pre-transplant vaccines

and adoptive anti-viral T-cell grafts (253). Yet, there is still a long way to go to determine whether public cross-reactivities could have clinical implications.

First of all, the functional characteristics of the cross-reactive virus-specific T cells should be thoroughly analysed. The newly identified public cross-reactive T cells were able to produce cytokines, show cytotoxicity towards alloantigen-expressing target cells derived from different tissues, and reach similar cytokine and cytotoxicity levels in response to alloantigen as to viral antigen in vitro. If they retain these capabilities in vivo, these T cells could contribute to the generation of an inflammatory environment in or around the allograft or even directly kill donor cells.

Important to keep in mind is that a functional alloreactive T-cell response depends on T-cell activation, which is determined by the interaction of the TCR with the alloepitope. Indeed, TCR affinity and avidity for the alloepitope play a crucial role in determining whether a T cell becomes activated upon allorecognition (214, 231). In **Chapter 7**, we have shown that TCR avidity depends on the expression of the (viral and allo-) peptide (144). In other words: the onset of alloreactivity (and whether it is persistent or transient) could depend on alloepitope expression. Defining parameters that affect this expression could provide novel insights into if and when potentially harmful alloreactive responses may emerge in a transplantation setting. For example, if the cross-reactive allopeptide is involved in specific cell processes (for instance damage repair or cell division) cross-reactive alloresponses may be transient, and if its expression is affected by genetic polymorphisms the immunogenicity of allografts could vary between donors. Preliminary experiments in which public cross-reactive CMV B35/IPS T-cell clones were stimulated by cells of first-degree related individuals indeed suggest a genetically determined stimulation capacity. Additional experiments are needed to make conclusive statements (FIGURE 1, unpublished data).

Moreover, we showed that, depending on epitope expression, TCR avidity for the alloepitope could surpass TCR avidity for the viral epitope - indicating that the alloresponse could have similar immune potential as the anti-virus response.

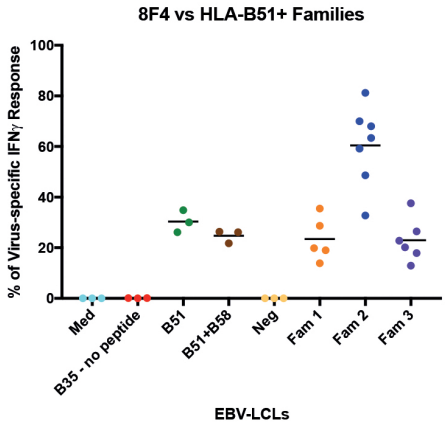

Figure 1. Family members may share immunogenicity. CMV B35/IPS T-cell clone 8F4, cross-reactive against allo-HLA-B*51:01, showed increased IFNγ production in ELISA towards HLA-B*51:01+ members of Family 2 (n = 7) compared to HLA-B*51:01+ members of Family 1 (n = 5) and Family 3 (n = 6). Depicted is the percentage of the virus-specific response (EBV-LCL B35 + IPS peptide) and experiments were performed in triplicate.

Two major determinants of TCR avidity are the interaction between the TCR and pMHC molecule (TCR affinity) and CD8 co-receptor binding to the side of the MHC molecule. TCR affinity is directly correlated with T-cell activation, however, most T-cell responses are mediated by T cells expressing intermediate TCR affinity to prevent overstimulation leading to rapid T-cell senescence and that could potentially pose a threat to the host (214). Unfortunately, TCR affinity for an alloepitope can only be accurately determined when the allopeptide is known, using methods as surface plasmon resonance (SPR) (223). When TCR affinity is sufficiently strong, CD8 co-receptor binding is not needed to elicit an immune response (237), but when TCR affinity is low, CD8 co-receptor binding is needed to strengthen the TCR-pMHC interaction. It thereby compensates for the low TCR affinity to ensure full T-cell activation (234, 254). Interestingly, CD8 co-receptor binding can also play an important role in allorecognition, and differences in CD8 expression on a cross-reactive T cell have been shown to alter its fine-specificity (255). Of note, such shaping would only occur for alloreactive T cells that are CD8 dependent. Although alloreactive T cells are generally considered to be CD8 dependent, recently alloreactive HCV-specific T cells have been documented that do not require CD8 binding for allorecognition (256).

CD8 dependency varies between HLA class I molecules, depending on mutations in the a3 domain (primarily due to a negatively charged loop at residues 223-229) (257). Decreased CD8 binding capacity can hamper the recognition of certain allo-HLA antigens and shape

the fine specificity of the allorepertoire. All self-HLA molecules (HLA-B*35:01; HLA-A*02:01) and cross-reactive allo-HLA molecules (HLA-B*51:01, HLA-B*58:01, HLA-B*57:01; HLA-B*38:01; HLA-B*55:01) tested in this thesis were identical in amino acid composition in their CD8 binding region of the a3 domain (DQTQDTE), indicating that the binding potential of the CD8 co-receptor was comparable between the different HLA molecules. We evaluated CD8 co-receptor dependency in functional assays by CD8 blocking and observed that CD8 co-receptor dependency was generally stronger for allorecognition compared to viral recognition **(Chapter 7)**. Although one should keep in mind that other interactions at the cell surface, such as those mediated by adhesive molecules, contribute to TCR avidity as well, a potential explanation for the difference in CD8 co-receptor dependency may be lower TCR affinity for the alloepitope versus the viral epitope (especially since the CD8 binding potential was similar for all HLA molecules). For example, the FLU A2/GIL TCR is known to recognize its cognate antigen in an CD8-independent manner, plausibly due to its specific pMHC docking mode that results in sufficient TCR affinity that diminishes the need for CD8 co-receptor signaling (9, 258). Our TCR sequencing data suggest that the CDR3a region, in contrast to cognate docking, is vital for HLA-B*38:01 allorecognition, hence a potential explanation for the observed CD8 dependency for allorecognition could be an alternative TCR docking mode. It is however impossible to make any conclusive statements without generating crystal structures of this interaction, which was beyond the scope of this thesis.

Interestingly, TCR affinity of the EBV B8/FLR cross-reactivity model is lower for the alloepitope versus the viral epitope (44). However, it remains to be investigated whether this can be generalized for all virus-specific T cell cross-reactivities. Perhaps more importantly, one should keep in mind that partial T-cell activation as a result of low affinity T cell - antigen interaction could be detrimental as well (259). Interestingly, a recent study in mice shows that low-affinity priming with pathogen antigen induces a different differentiation program in cross-reactive T cells compared to high-affinity priming; yet these low-affinity primed T cells are equally potent in inducing graft rejection upon exposure to high-affinity alloantigen (260). This holds promise for the priming of HIV-cross-reactive allospecific T cells as suggested by Almeida et al (261): in **Chapter 5**, we showed that virus-specific T cells can indeed be induced by allostimulation (143), and if priming is independent of TCR affinity, this would suggest that TCR affinity for allo-HLA is no limiting factor for generating a potent HIV-reactive T-cell repertoire.

Finally, in the quest to determine the clinical relevance of (public) cross-reactivities, the most important element that is missing so far is the identification of the peptide presented by the cross-reactive HLA allo-antigen. If the origin of the peptide is known, this can provide

information on tissue-specificity, as well as on the (patho)physiological expression of the alloepitope. In addition, TCR affinity can be assessed using SPR and functional assays can be optimized: for example, knowing the allopeptide would allow for TCR avidity determination using the same levels of viral and alloepitope expression. Furthermore, the allopeptide can unravel the structural mechanisms behind the TCR cross-reactivities using crystallography. With regard to monitoring, tetramers of the allo-HLA and allopeptide could be used to determine the frequency of T cells recognizing this complex in the blood of patients, pre-transplant and at follow-up. When alloreactivity mediated by these cross-reactivities plays an important role in graft rejection, knowing the allopeptide may ultimately even hold therapeutic potential with regard to the administration of altered peptide ligands (205). Unfortunately, the identification of the allopeptide is a long and complicated process with no universal experimental strategy. The only allopeptide that has been described so far is the EEY allopeptide, and its identification took several years. It was finally indirectly identified using the baculovirus vector system in insect cells that happened to yield a peptide mimic in the human genome (44, 262).

To benefit other cross-reactivity models as well, a more generalizable approach to identify allopeptides is therefore needed. A promising strategy for allopeptide identification combines functional T-cell assays with chromatography, mass spectrometry, biochemistry and crystallography, and is currently under development in the groups of Prof. Anthony Purcell and Prof. Jamie Rossjohn at Monash University (Melbourne, Australia) by Dr. Nicole Mifsud and Dr. Stephanie Gras, respectively. Using peptide elutions of the allogeneic target cells and HPLC fractionation of the resulting peptidome, functional screening of those HPLC fractions can reveal one (or more) positive fractions containing the allopeptide(s). Having joined their efforts for a year, we gathered preliminary results showing that a positive fraction for the CMV B35/IPS TCR cross-reactivity against HLA-B*51:01 can indeed be identified in CD69 assays (FIGURE 2, unpublished data).

Subsequently, the peptides present in the positive HPLC fraction are identified by mass spectrometry. A single HPLC fraction still contains hundreds to thousands of peptides - but comparing the positive and negative HPLC fractions can already rule out peptides that are unlikely to induce cross-reactivity. Furthermore, attempts are made to re-fractionate the positive fraction into smaller fractions, despite the requirement of extensive numbers of allogeneic cells for elution (>1 x 10^{10}). Another promising path aims to identify a peptide motif that the cross-reactive TCR favors for recognition, for comparison with the peptides in the positive HPLC fraction.

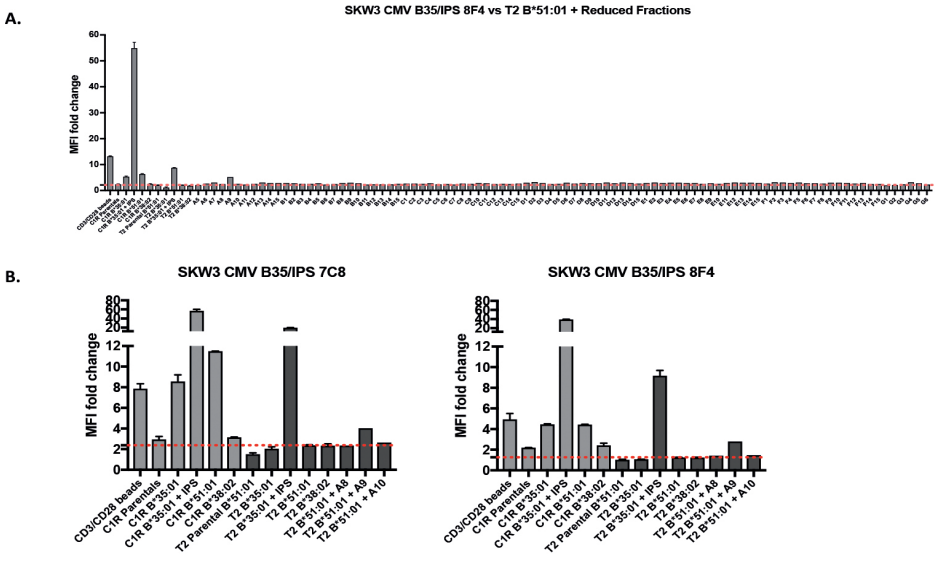

Figure 2. HPLC fractionation can be used to identify fractions containing allopeptides. A) A positive HPLC fraction (A9) was identified in CD69 assay for the CMV B35/IPS cross-reactivity model against HLA-B*51:01. B) Verification in additional CD69 assays showed both cross-reactive TCRs (7C8 and 8F4) were able to recognize peptides in the A9 faction. X-axis: Responder cells: retrovirally transduced SKW3s expressing the cross-reactive CMV B35/IPS TCRs; Stimulator cells: C1Rs and T2s retrovirally transduced with allo-HLA-B*51:01, of which three conditions HLA-B*51:01+ T2s loaded with HPLC fractions. Y-axis: MFI fold change of CD69 expression compared to media alone values.

The peptide motif can be achieved in an unbiased way using a combinatorial peptide ligand library (CPL), which covers the entire peptide spectrum divided into subgroups based on a single shared amino acid at a fixed position. Screening those subgroups in functional assays (the MIP-1b ELISA proves to be most sensitive) reveals an amino acid pattern, i.e. motif, that the TCR is most responsive to (238, 263, 264). Using this knowledge, peptides that are unlikely to cross-react based on the peptide motif can be discarded from the positive HPLC fraction. However, a downside of CPL is the requirement of cross-reactive T-cell clones, which are not always available, and it has only ever been used to identify cognate peptides rather than allopeptides. Currently, experiments are performed at Monash University to evaluate whether the above-mentioned approaches are suitable to identify allopeptides for different cross-reactivity models, including the public CMV B35/IPS and FLU A2/GIL cross-reactivity models described in this thesis. If successful, this would yield a generalizable strategy that can be implemented for the allopeptide identification of all (public and private) virus-specific TCR cross-reactivities, and their clinical relevance for transplantation can finally be resolved.