HLA alloreactivity by human viral specific memory T-cells

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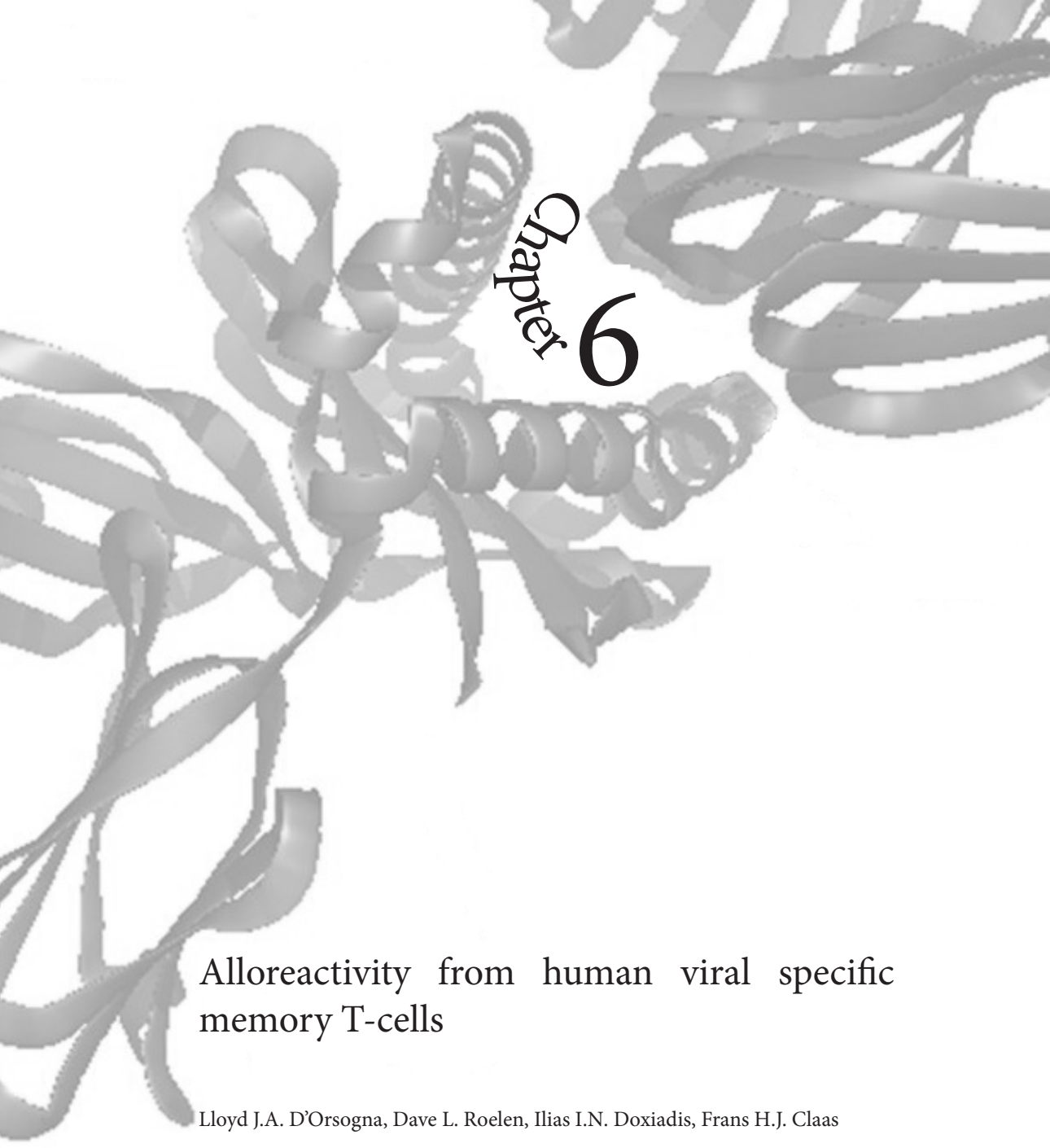

Chapter 6

Alloreactivity from human viral specific memory T-cells

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ABSTRACT

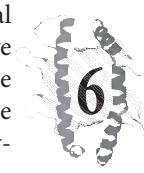
The mechanisms by which alloreactive memory T-cells are generated in non-sensitized individuals have begun to be elucidated. It is generally accepted that a very high level of crossreactivity is an essential feature of the T-cell receptor. Indeed it has recently been shown that alloreactivity from viral specific memory T-cells is far more common than predicted, 45% of viral specific T-cell clones were found to be allo-HLA crossreactive. In this overview the evidence for crossreactive alloresponses from human viral specific memory T-cells is discussed with special emphasis on the unexpected high frequency of these crossreactive responses, the peptide and tissue specificity of the responses, and the mechanistic insights gleaned from the elucidation of the crystal structure of an allo-HLA crossreactive viral specific TCR. The possible implications for clinical solid organ and bone marrow transplantation and tolerance induction will be discussed.

1. NON-SENSITIZED TRANSPLANTATION RECIPIENTS HAVE STRONG "MEMORY" RESPONSES FOR ALLO-HLA

Transplantation recipients can be sensitized against alloantigen by pregnancy, blood transfusion or previous transplantation. B-cell sensitization is revealed by the presence of HLA specific antibodies, which are not detectable in non-sensitized individuals. However, even in non-sensitized individuals a substantial portion of the pre-existing memory T-cell repertoire is already alloreactive (1-4), which is far greater than the proportion of T-cells that respond to any individual pathogen. The origin of these high-frequency pre-existing alloreactive memory T-cells in non-sensitized individuals was previously unclear, but has been hypothesized to relate to crossreactive allo-HLA responses from viral specific memory T-cells (5-7).

In humans, acute rejection has been associated with varying viral infections, and CMV prophylaxis with oral ganciclovir is associated with improved long-term renal graft survival (8). Mismatched donor HLA antigens have differential impact on graft survival depending on the HLA phenotype of the recipient (9), and one possible explanation for the occurrence of these harmful HLA combinations may be that patients have had previous immunological contact with pathogens that elicit T-cell responses which crossreact against the HLA mismatches (6-7,9). The fact that cord blood T-cells are less able to mediate graft vs. host disease (GvHD) than marrow derived T-cells because of their naïve status supports this theory (10-11).

In-vivo, the presence of virally induced alloreactive T-cell memory is a potent barrier to transplantation tolerance in mice (12-17). Many strategies have been used to successfully induce tolerance to transplanted tissue in mice, most of which primarily block the CD80/CD86/CD28 and/or CD40/CD154 co-stimulatory pathways. For example, donor specific transfusion and anti-CD154 antibody readily induce tolerance to solid organ grafts in pathogen free mice; however, all these protocols fail in pathogen exposed mice as viral infections induce alloreactivity and abrogate the induction of transplant tolerance (18-22). Furthermore, Adams clearly demonstrated a viral dose effect whereby mice previously exposed to multiple viral infections were refractory to tolerance induction and rejected their allografts, whereas naïve mice or single pathogen exposed mice were susceptible to tolerance induction (15). Evidence for virally induced alloreactive T-cell memory in mice is already extensively reviewed in the literature (12-15), therefore this review will focus on the evidence for allo-HLA crossreactivity by human T-cell clones.



Once generated, viral specific memory T-cells persist in high frequency and have lower activation requirements with novel co-stimulatory pathways that may be constitutively expressed (12, 23). Upon activation, memory T-cells produce a wide variety of cytokines including IL-2, IL-4, IFNγ, TNFα and are capable of rapid up-regulation of cytolytic effector function without the need for CD4 T-cell help (24). Taken together these factors provide strong support for the ability of viral specific memory T-cells to directly elicit acute rejection, and for viral memory having a negative influence on graft survival and/or tolerance induction.

2. EBV SPECIFIC CLONES ARE CROSSREACTIVE AGAINST ALLO HLA-B*4402 VIA MOLECULAR MIMICRY

Early work suggested that the explanation for the presence of alloreactive memory T-cells in non-sensitized individuals could be crossreactivity from viral specific memory T-cells against allo-HLA molecules (5-6). Burrows and colleagues demonstrated the dual specificity of EBV EBNA3A specific T-cell clones for the immunodominant peptide FLRGRAYGL presented on HLA-B*0801 and the alloantigen HLA-B*4402, to which the individual had never been exposed (6). This data also showed that the T-cell alloresponse can be dominated by a cross-reactive CTL induced by a single viral epitope.

In fact the HLA-B8/FLR restricted response in a HLA-$B8^+$ $B44^-$ individual gives rise to a public BV6S2 TCR which always cross-reacts against allogeneic HLA-B*4402 (25). This finding has been reproducibly found in different individuals from different genetic backgrounds using different techniques (2, 26-27). For example, we confirmed the alloreactivity of the EBV EBNA3A specific T-cell against HLA-B*4402 using single antigen expressing cell lines (single HLA transfected K562 cells) (26). In theory, viral infections that give rise to public TCR responses could therefore be used to determine unacceptable mismatches based solely on immunological history. Indeed HLA-B44 mismatching has been identified as higher risk among HLA-$B8^+$ renal transplant recipients (28).

The EBV EBNA3A T-cell allo-HLA-B*4402 crossreactivity is dependent on presentation of the EEYLQAFTY self-peptide derived from the ABCD3 gene (29). Molecular mimicry, as revealed by crystallography studies, is the mechanism for this human T-cell alloreactivity from a viral specific memory T-cell (e.g. see figure 1). Despite extensive amino acid differences between HLA-B*0801 and HLA-B*4402, and the disparate sequences of their bound viral and self peptides respectively, the HLA-B8/FLR restricted TCR engages these peptide-HLA complexes identically. The viral and allopeptides adopted similar conformations after TCR ligation, revealing that molecular mimicry is associated with TCR specificity. This paper highlights the exquisite specificity of the TCR and the self peptide dependence of the T-cell alloreactivity.

It is also suggested that molecular mimicry operates in other alloreactions (30-33). Nonetheless, more definitive data on the mechanisms of T-cell allo-HLA crossreactivity from other clonotypes are still required.

3. ALLOREACTIVITY FROM VIRAL SPECIFIC MEMORY T-CELLS IS COMMON

Recently we reported that allo-HLA responses from viral specific memory T-cells are in fact far more common than anticipated (27). To analyze allo-HLA crossreactivity from viral specific T-cells, T-cell clones were tested against a panel of HLA typed target cells, and target cells transduced with single HLA molecules. These studies showed that 80% of virus specific T-cell lines and 45% of virus specific T-cell clones crossreact against certain allo-HLA mol-

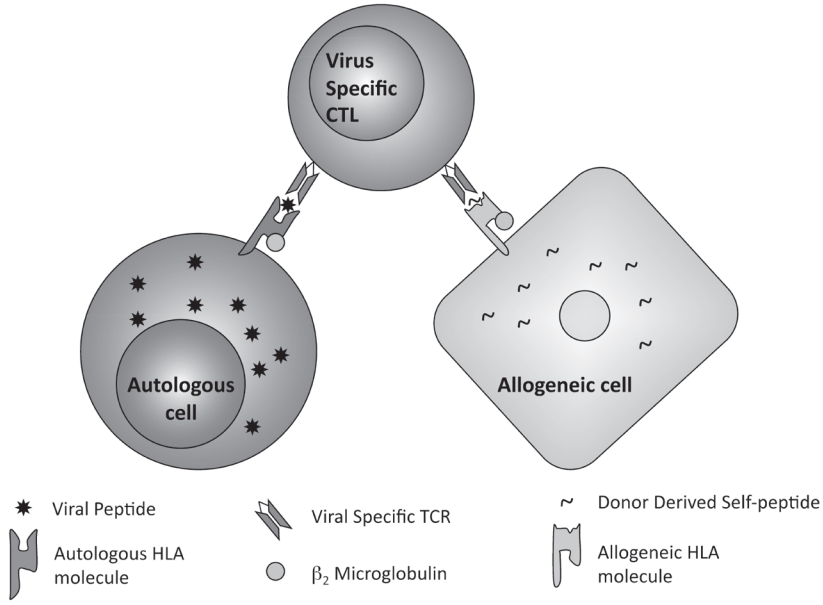


Figure 1. Allo-HLA crossreactivity by viral specific memory T-cells.
Viral specific memory T-cells target virus infected autologous cells presenting viral peptides in a self-HLA restricted fashion. The same viral specific TCR may crossreact against an allogeneic HLA molecule presenting a self-peptide. CTL=Cytotoxic T Lymphocyte.

ecules. Allo-HLA crossreactivity was shown from EBV, CMV, VZV and influenza specific T-cell clones (27). Multiple viral specific CD8 T-cell clones were shown to be alloreactive against allogeneic class I molecules, and likewise several viral specific CD4 T-cell clones were shown to crossreact against allogeneic class II molecules. Surprisingly, two separate CMV specific, class I restricted T-cell clones recognized allogeneic class II molecules (27), as has also been reported by others (34).

Additionally others have demonstrated allo-HLA crossreactivity by viral specific T-cell clones, although the target HLA molecule was not always clearly defined. HLA-A*0201 restricted HSV-2 specific T-cell clones have been shown to crossreact against the HLA-B44 family (35), and CMV specific CD8 T-cells have been shown to crossreact against undefined class I alloantigens by another group (36). EBV and tetanus toxoid specific CD4 T-cell clones have also been shown to exhibit allo-HLA class II responses (37-38). Table 1 lists human viral specific memory T-cells reported to give allo-HLA crossreactivity while table 2 compares the methods used for detection of the allo-HLA crossreactivity.

The importance of these findings are reinforced by functional studies showing that the vari-

ous viral specific CD8 T-cell clones can lyse multiple different target cells expressing the target HLA molecule, in a 4 hour ^{51}Chromium release assay (6,26-27). The fact that the same TCR complex mediates both virus specificity and allo-HLA crossreactivity has been confirmed by TCR PCR, viral tetramer inhibition and TCR transfection assays (6,26-27).

The multiple mechanisms of T-cell receptor crossreactivity have been reviewed extensively by others (39-42). Despite peptide/HLA diversity and TCR plasticity, these T-cell responses always exhibit exquisite HLA and peptide specificity.

3.1 Peptide dependence of allo-HLA crossreactivity from viral specific T-cells

It is now generally accepted that alloreactive T-cells recognize allo-HLA molecules presenting self-peptides (7,27,29,43-44). Macdonald and colleagues have provided clear structural evidence that self-peptide dependent molecular mimicry underpins the alloreactivity of the EBV EBNA3A specific T-cell against allogeneic HLA-B*4402 (29).

Furthermore, the peptide dependence of the allo-HLA crossreactivity from other viral specific memory T-cells is reinforced by differing potency of the alloreactivity exerted by virus specific T-cells against different cell targets. For example, a VZV specific HLA-A2 restricted T-cell clone recognizes allogeneic HLA-B*5701 expressing EBV LCLs, PHA Blasts and monocyte derived DCs, but does not recognize HLA-B*5701 expressing B-cells, T-cells, monocytes nor fibroblasts (27). Therefore allo-HLA expression is not solely sufficient to elicit target killing. Presumably the cell types that are not recognized do not present the relevant self-peptide.

In contrast to allogeneic HLA-B*4402$^+$ EBV LCLs and SALs, allogeneic HLA-B*4402$^+$ human umbilical vein endothelial cells (HUVECs) and proximal tubular epithelial cells (PTECs) are poor targets for EBV EBNA3A specific CD8 T cells. HLA-B*4402 expressing PTECs are specifically lysed by an EBNA3A T-cell clone without peptide loading albeit at high effector/target ratio only. The specific lysis of HLA-B*4402 expressing PTECs is greatly increased by exogenous EEY peptide loading. HLA-B*4402 expressing HUVECs are only targeted by an EBV EBNA3A clone when loaded with exogenous EEY peptide. The lack of recognition of endothelial and epithelial cells was not due to the lack of HLA-B*4402 expression. Thus organ (kidney) specificity of the alloresponse from the EBNA3A specific T-cell is dependent on endogenous self-peptide (EEY) processing and presentation.

3.2 Viral specific T-cell responses may not give predictable allo-HLA crossreactivity

Unlike the public BV6S2 TCR response against FLR peptide presented on HLA-B8, immune responses against other common pathogens are not so immunodominant and memory CD8 T-cells generated following viral infections often demonstrate a wide diversity of Vβ usage and therefore allo-HLA crossreactivity (Table 1). For example, Burrows showed that EBV EBNA3A specific T-cells that do not use the Vb6S2 TCR are alloreactive against HLA-B14 and -B35, but not HLA-B*4402 (45). Several other examples of differing alloresponses from T-cell clones with the same viral peptide/HLA restriction are also reported by Amir (27) and summarized in Table 1.

Table 1. Reported allo-HLA crossreactivity by human viral specific memory T-cells. # = Not determined.

Reference	Virus	Viral antigen	HLA restriction	Viral peptide restriction	Vb	Allo-HLA crossreactivity	Target cell(s)
Viral specific CD8 T-cells recognizing allo-class I							
27	CMV	pp50	A*0101	VTEHDTLLY	#	#	EBV LCLs
27	CMV	pp50	A*0101	VTEHDTLLY	3	A*1101	EBV LCLs
27	CMV	pp65	A*0201	NLVPTMVATV	8	#	EBV LCLs
36	CMV	pp65	A*0201	NLVPTMVATV	#	#	Splenocytes, EBV LCLs
36	EBV	BMLF1	A*0201	GLCTLVAML	#	#	Splenocytes, EBV LCLs
27	H. Influenzae	IMP	A*0201	GILGFVFTL	17	B*6401	EBV LCLs
27	VZV	IE62	A*0201	ALWALPHAA	14	B*5501	EBV LCLs
27	VZV	IE62	A*0201	ALWALPHAA	#	B*5701	EBV LCLs
27	VZV	IE62	A*0201	ALWALPHAA	21.3	A*0205	EBV LCLs
35	HSV-2	VP13/14	A*0201	FLVDAIVRVA	#	B*4402, B*4403, B*4407	EBV LCLs
35	HSV-2	VP13/14	A*0201	GLADTVVAC	#	B*4404	EBV LCLs
27	EBV	EBNA3A	A*0301	RLRAEAQVK	#	A*3101	EBV LCLs
27	EBV	BRLF1	A*0301	RVRAYTYSK	7.1	#	EBV LCLs
27	EBV	BRLF1	A*0301	RVRAYTYSK	14	#	EBV LCLs
27	EBV	BRLF1	A*0301	RVRAYTYSK	17	#	EBV LCLs
27	EBV	BRLF1	A*0301	RVRAYTYSK	7.2	A*0201	EBV LCLs
5	EBV	#	B8	#	#	B35, B62, B12 (B44/B45)	EBV LCLs
5	EBV	#	B8/B14	#	#	A3, A11	EBV LCLs
6	EBV	EBNA3A	B*0801	FLRGRAYGL	BV6S2	B*4402	PHA Blasts
26	EBV	EBNA3A	B*0801	FLRGRAYGL	BV6S2	B*4402 & B*5501	PHA Blasts, SALs
27	EBV	EBNA3A	B*0801	FLRGRAYGL	BV6S2	B*4402 & B*5501	EBV LCLs
2	EBV	EBNA3A	B*0801	FLRGRAYGL	#	B*4402	PHA Blasts
45	EBV	EBNA3A	B*0801	FLRGRAYGL	BV7S1B	B14	PHA Blasts
45	EBV	EBNA3A	B*0801	FLRGRAYGL	BV7S5	B35	PHA Blasts
5	EBV	#	B62	#	#	B57	EBV LCLs
Viral specific CD4 T-cells recognizing allo-class II							
27	CMV	pp65	DRB1*0101	KYQEFFWDANDIYRI	8	DRB1*0901	EBV LCLs
27	CMV	pp65	DRB1*0101	KYQEFFWDANDIYRI	#	DRB3*0101	EBV LCLs
37	EBV	#	DRB1*0101	#	#	DRB1*0404	EBV LCLs
27	H. Influenzae	HA	DRB1*0401	PKYVKQNTLKLAT	3	DRB1*1301	EBV LCLs
37	EBV	LMP2	DRB1*1001	ICLTWRIEDPPFNSILFALL	#	DRB1*0406	EBV LCLs
37	EBV	EBNA3C	DRB1*16	PHDITYPYTARNIR	#	DRB1*0101	EBV LCLs
38	Tetanus Toxoid	#	DR3	#	#	DR4	EBV LCLs
37	EBV	EBNA2	DRB3*0202	PRSTVFYNIPPMPLPPSQL	#	DRB1*0101	EBV LCLs
37	EBV	EBNA1	DPB1*1001	NPKFENIAEGLRALLARSHV	#	DRB1*0102	EBV LCLs
37	EBV	LMP2	DQB1*0601	TYGPVFMSLGGLLTMVA	#	DRB1*0901	EBV LCLs
Viral specific CD8 T-cells recognizing allo-class II							
27	CMV	pp65	B*0702	RPHERNGFTVL	7.2	DRB1*0801	EBV LCLS
27	CMV	pp65	B*3501	IPSINVHHY	5.1	DRB1*0401	EBV LCLs
34	CMV	pp65	Cw*0602	TRATKMQVI	13	DRB1*0401	EBV LCLs, B-cells

Variable allo-HLA crossreactivity by T-cell clones sorted from the same individual with the same specificity, but different TCR Vβ usage, was also reported. Single cell sorting of VZV IE62 specific T-cells from an individual with VZV infection generated three different clones with usage of Vβ 21.3, Vβ 14 and an undetermined Vβ (27). These T-cell clones cross-reacted against allo HLA-A*0205, HLA-B*5501 and HLA-B*5701 respectively. Demonstrating how a single viral peptide/HLA restricted immune response can generate different clonotypes with differing allo-HLA crossreactivity within the same individual.

Furthermore, a single EBV EBNA3A specific memory T-cell clone was able to recognize both allogeneic HLA-B*4402 and B*5501, in addition to the viral peptide presented on HLA-B8 against which it was originally selected (26).

Table 2. Cellular Targets used for Detection of Allo-HLA Crossreactivity by Viral Specific Memory T-cells

Detection method	Advantages	Disadvantages
EBV LCLs	Cells easily generated and maintained	Does not allow conclusive definition of crossreactive HLA molecule
	Suitable for screening of alloreactivity against many common HLA molecules	Viral responder cells may recognize EBV peptides presented on HLA molecules
	High expression of HLA molecules	
Single HLA antigen cell lines	Allows definitive confirmation of crossreactive HLA molecule	Requires HLA molecule transfection for generation
	Once produced are relatively easily maintained and grown	Requires ML1 laboratory facilities
	K562 cell is an immortalized cell line	K562 cells possess many genetic variations which may give rise to recognition of tumour peptides
	Viral peptide free	Poor targets in 4 hour chromium release assay
PHA blasts	High expression of HLA molecules	Cannot be used in IFNγ based assays
	Easily generated	
	Viral peptide free[a]	
PBMC or splenocytes	Suitable for screening of alloreactivity against many common HLA molecules	Does not allow definitive definition of crossreactive HLA molecule without blocking by monoclonal antibodies
	Easily obtained	
	Viral peptide free[a]	
Tissue type specific cells e.g. PTEC/ HUVEC	Able to detect tissue specific alloresponses	Technically difficult and laborious to grow

* The authors have found that our EBV EBNA3A specific T-cell clone does not recognize HLA-B8^{+} EBV seropositive PBMCs or PHA Blasts without addition of exogenous FLR peptide

Pan HLA recognition is inherent in germline TCR sequences (46). T-cells can presumably exit the thymus due to their high crossreactivity, as they are "positively" selected by self-HLA molecules. These alloreactive T-cells are unable to discriminate between self and non-self peptides presented on allo-HLA molecules (29). Only T-cells with high affinity for self-peptides presented on self-HLA molecules are "negatively" selected from the pan HLA reactive T-cell repertoire. Indeed, in HLA-B8/B44 heterozygotes the public Vb6S2 TCR expressing EBV EBNA3A clonotype is deleted from the T-cell repertoire (47). CTLs from HLA-B8$^+$B44$^+$ individuals express different TCR gene combinations which maintain HLA-B8/FLR specificity, but do not possess HLA-B*4402 reactivity, thereby preventing auto-immunity. Self-tolerance shapes the TCR repertoire available to respond to any individual viral antigen (47-48), thereby also altering the allo-HLA crossreactivity of the viral specific TCR pool.

Therefore, alloreactive T-cells do escape thymic deletion and are subsequently activated by viral infection. However virus specific T-cells with the same antigen specificity, but with different TCR Vb usage, clearly exert alloreactivity against different HLA molecules. It is currently not known if viral specific T-cells from different individuals with the same specificity *and* the same Vβ usage will always demonstrate similar allo-HLA crossreactivity. This knowledge is essential in order to be able to predict (un)acceptable mismatches based on donor-recipient HLA mismatches and immunological history of the recipient.

4. PREVIOUS VIRAL (PATHOGEN) INFECTION IS CRITICAL TO INDUCTION OF THE ALLOREACTIVE T-CELLS

Memory T-cells demonstrate critical functional differences versus their naïve counterparts, such as immediate cytotoxicity without the need for co-stimulation nor CD4 T-cell help. For example, EBV EBNA3A specific memory T-cells demonstrate immediate cytolytic effector function against HLA-B*4402$^+$ PHA blasts in a 4 hour ^{51}chromium release assay (6,26). A CCR7$^+$ CD45Ra$^+$ naive T-cell with the same TCR (e.g. from a EBV seronegative individual), upon first contact with antigen, will secrete only IL2, is not cytolytic and requires CD4 T-cell and B-cell help within the germinal centre to initiate an immune response before expanding into the memory T-cell pool. Naïve T-cells recognizing an alloantigen without the appropriate co-stimulatory signals and T-cell help may gain regulatory function, be deleted or become anergic (49-50). This illustrates the critical importance of previous viral infection to the activation of alloreactive T-cells.

5. CLINICAL IMPLICATIONS OF STUDIES USING VIRAL SPECIFIC T-CELL CLONES

In humans, alloreactive memory T-cells are frequently generated by viral infection. This allo-HLA crossreactivity is likely peptide dependent but not predictable based on donor-recipient HLA mismatches alone. Allo-HLA crossreactivity from viral specific memory T-cells may have important clinical implications for the alloimmune response after transplantation

because memory T-cells have lower activation requirements, no need for CD4 T-cell help and can have immediate cytotoxic effector function as compared to their naïve counterparts. Therefore if truly alloreactive in-vivo, pre-existing memory T-cells may represent a common source of acute and/or chronic rejection and be a major obstacle to tolerance induction.

The frequency of memory T-cells are highest for the chronically persistent viruses such as human herpes viruses EBV and CMV. It remains to be determined if the alloreactive memory T-cell pool consists of many responding memory T-cells each of different specificities and each of low precursor frequency, or of a single (or few) viral specific memory T-cells that individually account for a large portion of the alloresponse. If alloreactive T-cells are driven by reactivation of viral infection then anti-viral therapy may decrease the proportion of these allo-HLA crossreactive alloresponses. Supportive evidence is provided by the finding that CMV prophylaxis with oral ganciclovir is associated with less acute rejection and improved long-term renal graft survival (8). Alternatively vaccination could induce alloreactivity, as suggested by others (38,51).

Given the longevity of viral specific memory T-cells, it is likely that allo-HLA crossreactive memory T-cells generated after infection are maintained and able to elicit acute rejection, particularly when immunosuppression is tapered (12). Ex-vivo staining for the presence of viral specific T-cells within rejecting kidney or GvHD biopsy samples may help confirm the clinical relevance of crossreactive allo-HLA responses.

Long term antigen specific tolerance to engrafted tissue is the ultimate goal in transplantation but despite numerous successful rodent models clinical human tolerance has remained an elusive goal. The presence of viral specific memory T-cells may be responsible for the failure to induce tolerance in clinical settings (12,15), although it is unclear what role primary infection vs. reactivation may play. For example, mice work reveals that viral infection abrogates the tolerance induced by donor specific transfusion and anti-CD154 blocking (13). Similar effects from memory T-cells following viral infection are also reported in many other studies (12,14-15,18). This relates to decreased dependence of memory T-cells on co-stimulatory pathways. Humans are not immunologically naïve and we propose that memory T-cells generated after environmental exposures may account for the difficulty in transferring tolerance studies from mice into the human setting. Therefore, we suggest caution when interpreting tolerance protocols studied in pathogen free animals.

The self-peptide dependency of alloreactivity from viral specific memory T-cells, as confirmed by Macdonald and colleagues (29), is of interest and may present several therapeutic opportunities. Allo-HLA recognition from viral specific T-cells may exhibit different tissue specificities depending on household gene expression and self-peptide presentation. For example, preliminary work shows that PTEC and HUVEC cell lines are poor targets for EBV EBNA3A specific T-cells, likely due to decreased EEY peptide presentation. Therefore a HLA-B*4402 mismatch in a HLA-B8^{+} B44^{-} kidney recipient may not be associated with high risk of rejection if the EEY peptide is not presented on the donor cell surface.

Conversely, a HLA-B8 mismatch in a HLA-B8^{-} B*4402^{+} bone marrow recipient could theoret-

ically be associated with graft vs. leukaemia (GvL) effect but low risk of GvHD. Interestingly, haploidentical bone marrow transplantation may be associated with increased GvL effect. Exploitation of the differential peptide and tissue specificity of alloreactivity from viral specific T-cells for therapeutic benefit should become a major research focus.

Peptide dependent alloreactivity also implies that immunomodulating techniques could be used to inhibit these harmful T-cell clonotypes, as suggested by Burrows (52). While the alloreactivity of the EBV EBNA3A specific T-cell has been confirmed to be dependent on peptide dependent molecular mimicry, and not degenerate recognition, further structural studies on the mechanisms of allorecognition are clearly warranted.

Given the abundant crossreactivity contained within the T-cell repertoire deletion of any individual virus specific clonotype might not be associated with viral reactivation. While the CD8 memory T-cell pool created after a viral infection has a distinct immunodominant hierarchy, many clonotypes are capable of recognizing the viral peptide/HLA complex. Nonetheless, it can not yet be excluded that successful tolerance induction may occur at the expense of a T-cell clone that has an important role in controlling a chronic viral infection, possibly leading to viral reactivation.

Monitoring of alloreactive T-cells is also critical as this may allow individualization of immunosuppression (53). Currently in-vitro assay for renal transplantation monitoring does not have adequate sensitivity or specificity to enter routine clinical practice. However such assays routinely use donor PBMCS or spleen cells as stimulator, and it is unclear if these target cells present a comparable peptide pool to that presented by the relevant donor (kidney) cells. Perhaps future studies of transplantation monitoring could use a pool of tissue specific self-peptides which are known to be presented by the donor organ. At the current point in time HLA matching remains the best predictor of long-term renal graft survival.

DR-matching has beneficial effects on transplantation survival. Allo-HLA class II crossreactivity from class I restricted viral specific T-cells was previously unreported (27,34). We suggest that DR matching may, in part, be associated with improved graft survival due to the inability of viral specific T-cells to crossreact against allogeneic HLA class II. Further examination of MHC class II restricted pathogen specific CD4 T-cells is required, as it is likely that this T-cell population plays a dominant role in allograft rejection (2,54-55).

Ultimately not only HLA phenotype, but also immunological history, may be used to determine donor-recipient suitability. However major studies on the public nature of anti-viral responses in individuals of different HLA background are still required. Early work suggests that, unlike the HLA-B8/FLR restricted immune response, most viral specific T-cell responses do not give rise to a public TCR nor predictable allo-HLA crossreactivity. Nonetheless studies of viral peptide/HLA restricted T-cell responses, TCR Vb usage and allo-HLA crossreactivity are ongoing.

Even if immunological history can not be utilized to avoid alloreactivity, selective therapies at the time of transplantation may allow inhibition of allo-HLA crossreactivity from pre-ex-

isting memory T-cells while still allowing de-novo naïve responses against viral antigens. For example, selective blockade of ICOSL and CD86 which represent two major co-stimulatory signals for the activation of resting peripheral blood memory T-cells (12,56) may still allow immune responses via the CD40/CD154 and/or CD70/CD27 co-stimulatory pathways which are important for naïve T-cell activation. While the effect of immunosuppressive drugs on allo-HLA crossreactivity from viral specific T-cells has not been studied, the calcineurin inhibitors are able to inhibit proliferation and cytokine production from effector CD4 memory T cells (54). Unfortunately leukocyte depleting therapies such as antithymocyte globulin and alemtuzumab are less able to diminish the memory T-cell pool (54).

Adoptive transfer of virus or fungal specific T-cells offers an effective option for the management of specific immune defects in an immune compromised host (57), particularly following allogeneic BMT. However given the high frequency of allo-HLA crossreactivity from viral specific T-cells, it is not surprising that adoptive transfer has already been associated with GvHD. For example, adoptive transfer of CMV specific T-cells to nine recipients after allogeneic BMT resulted in three cases of GvHD, including one patient who died (58). Similarly, TCR gene transfer to induce anti-leukaemia reactivity is associated with α and β chain rearrangements and therefore the formation of mixed dimer TCRs (59), which could also be alloreactive. Screening of adoptively transferred antigen or leukaemia specific T-cells for allo-HLA crossreactivity may help prevent GvHD.

Consistent with this theory cord blood T cells are less able to mediate GvHD than marrow derived T-cells because of their naïve status (49,60).

Finally, some groups have suggested that allo-HLA crossreactivity by viral specific T-cells does not play a significant role in transplantation. Nickel and colleagues found no association between CMV specific memory T cells and alloreactivity (61). However this study only measured CMV specific responses against viral peptides loaded on autologous cells and did not specifically document if these responses were crossreactive against mismatched donor HLA molecules. While 45% of virus specific T-cells have demonstrable allo-HLA crossreactivity against one HLA molecule (27), the target HLA molecule may not have been present on the donor cell. All kidney recipients received anti-IL2R mAb, calcineurin inhibitor, mycophenolate mofitil and steroids as induction therapy, possibly suppressing allo-HLA crossreactive responses until the immunosuppressive drugs were tapered. Furthermore, recipients received pre-emptive ganciclovir therapy guided by asymptomatic CMV viraemia. While we agree that CMV specific T-cell responses that are not allo-HLA crossreactive are likely to benefit a recipient, this study does not exclude the role of allo-HLA crossreactivity from viral specific T-cells in kidney rejection.

Therefore, crossreactivity by viral specific memory T-cells or "heterologous immunity" is common. While this crossreactivity by pathogen specific memory T-cells may help protect against subsequent unrelated infections, in the transplantation setting such crossreactivity may give rise to harmful alloresponses.

6. CONCLUSION

An essential feature of the T-cell response is the ability to recognize a diverse array of potentially unlimited antigens, necessitating that the TCR be inherently crossreactive. The memory T-cells that are specific to previously encountered pathogens accumulate following repeated infectious exposure and have low activation thresholds. Mice in-vivo, and human in-vitro, experiments reveal that these viral specific memory T-cells are commonly crossreactive with allo-HLA molecules in a self-peptide specific manner. Thus, getting a certain infection in an individual with a certain HLA type might have significant adverse consequences in the event of organ or marrow transplantation. Human ex-vivo studies are clearly warranted. We suggest that current research objectives should focus on the human in-vivo relevance of allo-HLA crossreactivity from viral specific memory T-cells, and specifically how self-peptide dependent allorecognition from viral specific T-cells alters tissue specificity. Allo-HLA crossreactivity could also have serious adverse effects in the setting of adoptive transfer and TCR transfection of viral specific T-cells. New understandings of the origin of alloreactivity may lead to an era whereby donor suitability is defined not only by HLA typing but also using immunological history, and hopefully toward successful antigen-specific transplantation tolerance.

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