HLA alloreactivity by human viral specific memory T-cells

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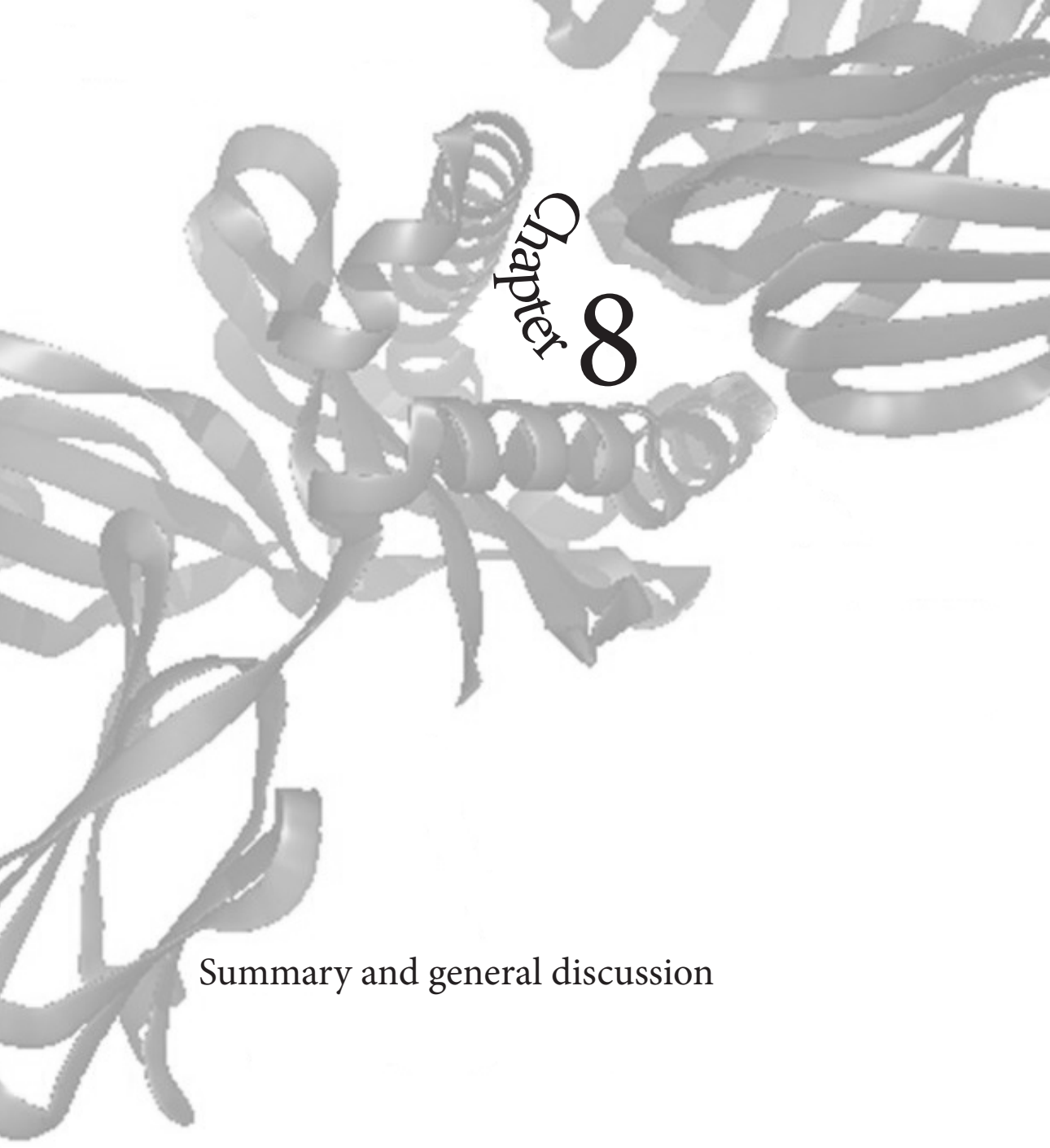

Chapter 8

Summary and general discussion

SUMMARY AND GENERAL DISCUSSION

T-cell memory is a hallmark of the adaptive immune response and is critical for protective immunity against pathogens. However, many studies reveal that pre-existing memory T-cells also pose a potent barrier to transplantation tolerance, even in non-sensitized individuals (1-12). It was previously unclear how these alloreactive memory T-cells arose in non-sensitized organ recipients.

A possible explanation is that alloreactive memory T-cells arise via exposure to environmental antigens (13-19). Recipients might have had immunological contact with pathogens that lead to crossreactive immune responses with the HLA mismatches. Limited evidence for this phenomenon exists in both mouse and human models, but was thought to be a rare occurrence. 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 (20-24). 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 molecular mechanisms that might underlie such crossreactivity were also unexplained.

In order to study the effect of environmental exposure on the alloreactive T-cell repertoire viral specific T-cell clones were single cell sorted based on viral peptide/HLA tetrameric complex staining, from healthy (non-sensitized) individuals. This technique proved to be the basis of an effective system for detection of allo-HLA crossreactivity (heterologous immunity) by viral specific memory T-cells. Results presented in this thesis indicate that heterologous immunity is much more common than anticipated. Furthermore, it has been confirmed that the virus specificity and alloreactivity are mediated by the same TCR and that the allo-HLA crossreactivity can not be predicted based solely on immunological history and HLA mismatch alone. Future clinical studies are now clearly warranted.

Several novel and important discoveries for the field of transplantation have been made in this thesis and are summarized below:

Alloreactivity from viral specific memory T-cells is common
This thesis confirms that allo-HLA responses from viral specific memory T-cells are in fact far more common than anticipated (25) (Chapter 3). 80% of virus specific T-cell lines and 45% of virus specific T-cell clones crossreacted against individual allo-HLA molecules. Allo-HLA crossreactivity was shown from EBV, CMV, VZV and influenza specific T-cell clones. 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 (25).

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 (25-26) (Chapters 2 and 3). Vaccination with live attenuated virus can also induce alloreactive memory T-cells, as shown in chapter 5 of this thesis for varicella vaccination.

The importance of these findings are reinforced by functional studies showing that the various viral specific CD8 T-cell clones can lyse multiple different target cells expressing the target HLA molecule, in a 4 hour cytotoxicity assay (25-26) (Chapters 2, 3 and 5). Further examination of MHC class II restricted pathogen specific CD4 T-cells is required, as it is likely that this T-cell population also plays a dominant role in allograft rejection (10,27-28). 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 these in-vitro crossreactive allo-HLA responses.

Human viral specific memory T-cells reported to give allo-HLA crossreactivity are summarized in table 1 of chapter 6 of this thesis (29).

HLA alloreactivity by viral specific memory T-cells is (self) peptide dependent
It is now generally accepted that alloreactive T-cells recognize allo-HLA molecules presenting self-peptides (25,30-33). 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*44:02 (31). The EBV EBNA3A specific T-cell crossreactivity against allogeneic HLA-B*44:02 is dependent on presentation of EEYLQAFTY peptide (derived from the ABCD3 protein) by the target tissue.

In this thesis, the peptide dependence of the allo-HLA crossreactivity from 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*57:01 expressing EBV LCLs, PHA Blasts and monocyte derived DCs, but does not recognize HLA-B*57:01 expressing B-cells, T-cells, monocytes nor fibroblasts (25) (Chapter 3). 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*44:02$^+$ EBV LCLs and SALs, allogeneic HLA-B*44:02$^+$ proximal tubular epithelial cells (PTECs) are poor targets for EBV EBNA3A specific CD8 T cells (Chapter 4). However the specific lysis of HLA-B*44:02 expressing PTECs was greatly increased by exogenous EEY peptide loading. HLA-B*44:02 expressing HUVECs were only killed by an EBV EBNA3A clone when loaded with exogenous EEY peptide. This confirms that kidney specificity of the alloresponse from the EBV EBNA3A specific T-cell is dependent on endogenous self-peptide processing and presentation.

Peptide dependent alloreactivity suggests that immunomodulating techniques could be used to inhibit these harmful T-cell clonotypes, as suggested by Burrows (30). Further studies are required.

HLA alloreactivity by viral specific memory T-cells can be tissue specific
Tissue specific alloresponses by viral specific memory T-cells are described in chapters 3,4 and 5. Differences in peptide antigen processing and presentation could account for this tissue specific alloreactivity. For example, EBV LCLs, PHA Blasts and K562 cells constitutively express the immunoproteosome which may generate novel antigenic allopeptides.

To further investigate tissue specificity by EBV EBNA3A specific T-cells, long peptides from the ABCD3 protein containing the EEYLQAFTY epitope were generated. Cleavage products from these long peptides were compared following immunoproteosome vs constitutive proteosome digestion, using mass spectrometry analysis. Results of the proteosomal digestion are shown in tables 1a and b.

These results are unexpected given the tissue specificity reported in chapter 4 of this thesis. EBV LCL and PHA blasts were efficiently targeted by EBV EBNA3A specific T-cells, whereas endothelial and epithelial cells were poor targets. If these differences were attributable to proteosome peptide processing then theoretically the immunoproteosome (present in EBV LCLs and PHA Blasts) should generate relatively more EEYLQAFTY peptide, as compared to the constitutive proteosome. In fact the epitope is a target of proteosomal cleavage and there may therefore be little EEYLQAFTY peptide available for presentation on the cell surface in all cell lines. Alternatively the EEYLQAFTY peptide may not be the natural ligand. Nonetheless, these results nicely demonstrate how proteosomal digestion could alter the self-peptide (allopeptide) repertoire presented on allo-HLA molecules. Further antigenic processing studies are clearly warranted.

Alternatively, differences in expression of a protein that contains a peptide capable of competing with an antigenic peptide for the peptide-binding groove of the allogeneic molecule could also cause the tissue specific alloreactivity reported in chapter 4. HLA-B*44:02 is a highly tapasin-dependent HLA molecule (34-35) and therefore limited tapasin expression in PTECs and/or HUVECs could decrease EEY peptide presentation in these cell lines. However tapasin mRNA is strongly induced in endothelial cells following IFNγ treatment (36), and IFNγ treatment did not increase the targeting of HUVECs in our assays despite inducing elevated HLA-B44 expression.

Table 1a. EEYLQAFTYYKMGN peptide digestion

Res	i-prot(%)	c-prot(%)	Peptide
1--14	50.1	44.2	E E Y L Q A F T Y Y K M G N
1--4	13.5	16.0	E E Y L
1--6	4.1	5.1	E E Y L Q A
1--9	4.5	3.0	E E Y L Q A F T Y
1--10	8.2	8.9	E E Y L Q A F T Y Y
2--11	3.7	3.9	E Y L Q A F T Y Y K
5--10	6.9	7.1	Q A F T Y Y
6--10	4.7	5.3	A F T Y Y
7--10	4.0	5.3	F T Y Y

Table 1a & 1b: Immunoproteosome vs. constitutive proteosome digestion of ABCD3 protein.
Long peptides from the ABCD3 protein, containing the EEYLQAFTY epitope, were generated and then digested using the two different proteosomes. Peptide products were analysed using mass spectrometry, after 4 hours incubation. (Table 1a) Long peptide EEYLQAFTYYKMGN - The EEYLQAFTY epitope was generated by both proteosomes however accounted for less than 5% of the long peptide cleavage product. Multiple cleavage points were found within the epitope. (Table 1b) Long peptide TKYLYEEYLQAFTYYKMGN – The long peptide was completely degraded and the EEYLQAFTY epitope was not generated. The EEYLQAFTY epitope was a cleavage target for both proteosomes with all peptide products generated from cleavage of the long peptide within the epitope (Residues 6-14). Res= Residues. i-prot=Immunoproteosome. c-prot=constitutive proteosome. %= Results expressed as percentage of total detected peptide products.

Table 1b: TKYLYEEYLQAFTYYKMGN peptide digestion

Res	i-prot(%)	c-prot(%)	Peptide
1--19	0	0	T K Y L Y E E Y L Q A F T Y Y K M G N
1--8	15.3	10.2	T K Y L Y E E Y
1--9	22.2	26.8	T K Y L Y E E Y L
1--11	0	5	T K Y L Y E E Y L Q A
2--6	2.8	4.6	K Y L Y E
2--8	3.9	0	K Y L Y E E Y
2--9	0	3.8	K Y L Y E E Y L
2--11	1.9	0	K Y L Y E E Y L Q A
3--10	3.9	0	Y L Y E E Y L Q
6--14	0	0	E E Y L Q A F T Y
7--15	2.1	0	E Y L Q A F T Y Y
8--14	5.2	6.2	Y L Q A F T Y
9--19	12.7	5.5	L Q A F T Y Y K M G N
10--14	2.2	1.8	Q A F T Y
10--15	5.1	10.4	Q A F T Y Y
10--18	6.2	4.2	Q A F T Y Y K M G
10--18	9.0	6.0	Q A F T Y Y K M G
11--16	5.1	10.4	A F T Y Y K
12--15	2.1	4.8	F T Y Y

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. Several examples of differing alloresponses from T-cell clones with the same viral peptide/HLA restriction are reported in this thesis (25) (Chapters 3, 5 and 7).

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 in this thesis (25) (Chapter 3). 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. These T-cell clones cross-reacted against allo HLA-A*02:05, HLA-B*55:01 and HLA-B*57:01 respectively. Demonstrating how a single viral peptide/HLA restricted immune response can generate different clonotypes with differing allo-HLA crossreactivity within the same individual.

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. At the current point in time functional assays, such as those described in this thesis, are required to determine if a certain HLA mismatch is a target for memory T-cells in a given individual. HLA matching remains the best predictor of long-term renal graft survival.

HLA alloreactivity likely occurs via molecular mimicry
The multiple mechanisms of T-cell receptor crossreactivity have been reviewed extensively by others (37-40). Despite peptide/HLA diversity and TCR plasticity, these T-cell responses always exhibit exquisite HLA and peptide specificity.

Work presented here strongly supports molecular mimicry, but not structural degeneracy, as the mechanism of TCR crossreactivity from viral specific memory T-cells. Differing potency of the virus specific T-cells against different cell targets, as reported in chapters 3, 4 and 5, is consistent with a TCR specifically crossreacting against single (or limited) self-peptide(s) presented on an allo-HLA molecule. If a viral specific TCR was crossreactive against an allo-HLA molecule via structural degeneracy then the peptides presented via the allo-HLA molecule should be irrelevant and the T-cell would recognize all allo-HLA expressing tissue cells equally.

Previous viral (pathogen) infection is critical to induction of the alloreactive T-cells
In this thesis we show that virus specific memory T-cells can demonstrate immediate cytolytic effector function against allogeneic HLA molecules in cytotoxicity assays (25-26) (Chapter 2,

3 and 5). A CCR7^{+} CD45Ra^{+} naive T-cell with the same TCR (e.g. from a seronegative individual), upon first contact with alloantigen, will secrete only IL-2, is not cytolytic and requires CD4 T-cell and B-cell help within the germinal centre to initiate an immune response. In a pilot study we found that stimulation of HLA-B8^{+} B44^{-} cord blood T-cells with HLA-B*44:02^{+} irradiated blood cells does not result in an alloresponse by HLA-B8/FLR specific naïve T-cells. 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 (41-42). This illustrates the critical importance of previous viral infection to the activation of alloreactive T-cells.

Consistent with this theory cord blood T cells are less able to mediate GvHD than marrow derived T-cells because of their naïve status (41,43).

Selective therapies to inhibit alloreactive memory T-cells are required

Renal transplantation is a life saving procedure for end stage renal disease and generally short-term transplantation outcome is excellent. The introduction of calcineurin inhibitor therapy has been critical for the prevention of acute rejection and improved one-year graft survival, although any beneficial effect on long-term graft survival is small. "Memory" is a critical barrier to long-term transplantation outcome and tolerance induction (1), therefore, the effect of newer immunosuppressive drugs on alloresponses by viral specific memory T-cells may be critical to graft survival and/or tolerance induction and should be studied further.

Selective therapies at the time of transplantation may allow inhibition of allo-HLA crossreactivity from pre-existing 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 (2,44) 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 (27). Unfortunately leukocyte depleting therapies such as antithymocyte globulin and alemtuzumab are less able to diminish the memory T-cell pool (27).

Results presented in this thesis also suggest caution is warranted when interpreting tolerance protocols studied in pathogen free animals.

Adoptive transfer of pathogen specific T-cells could be complicated by GvHD disease

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 (45), 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 (46). 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 (47), which could also be alloreactive. Screening of adoptively transferred antigen or leukaemia specific T-cells for allo-HLA crossreactivity may help prevent GvHD.

HLA alloreactivity could be useful to conversely stimulate a human cytolytic viral specific T-cell responses using allogeneic cell therapy

Finally in chapter 7 of this thesis we provide evidence that allogeneic cell therapy may be useful to conversely stimulate a beneficial anti-viral cytolytic effector response for treatment of viral infection. We demonstrated that human viral specific memory T-cells gain cognate viral antigen specific cytolytic effector function following stimulation with allogeneic HLA molecules against which they are crossreactive. This proof-of-principle technique could provide important future options for the treatment of viral infections. This approach should be investigated further.

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. Experiments presented in this thesis 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 donor (antigen) specific transplantation tolerance.

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