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HLA alloreactivity by human viral specific memory T-cells

D'Orsogna, L.J.A.

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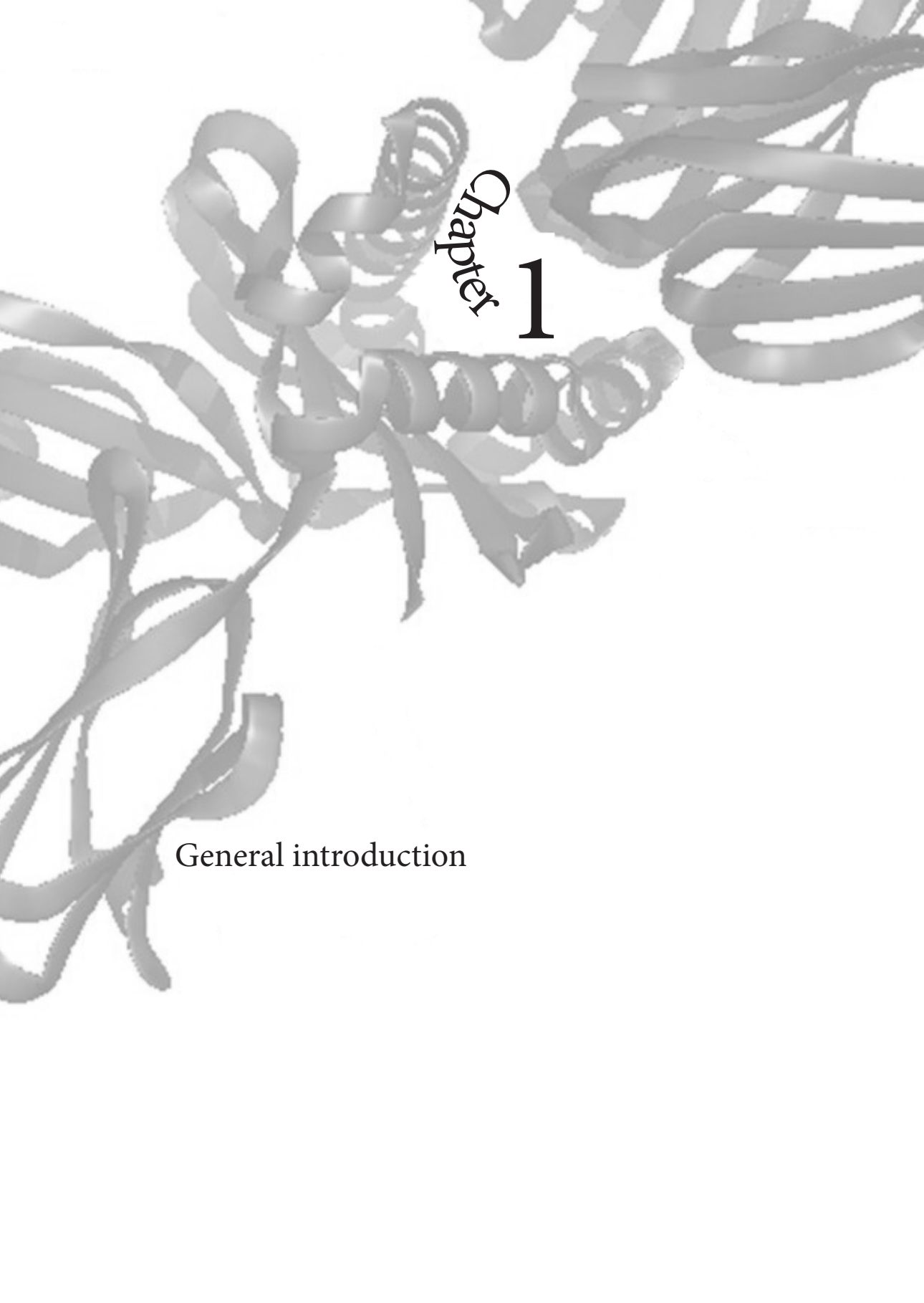
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Chapter 1

General introduction

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INTRODUCTION

Kidney transplantation is the treatment of choice for patients with end stage renal disease. However adaptive immune responses to donor HLA antigens are a potent barrier to successful transplantation and/or tolerance. Allograft rejection is initiated, and in many cases, executed by T-cells recruited into the graft (1). With current immunosuppressive regimens T-cell mediated rejection is less common than with previous regimens, but remains the dominant early rejection phenotype and is also associated with chronic allograft nephropathy. B-cells can make donor specific HLA antibodies which are associated with antibody mediated rejection.



The possible induction of specific tolerance towards the graft is the ultimate goal in clinical transplantation. The successful blockade of co-stimulatory pathways to induce prolonged graft survival in mice raised hopes for the successful transfer of tolerance inducing regimens into the clinic. However all these protocols rapidly failed in pathogen exposed mice (2-6).

Accumulating evidence suggests that graft rejection is a result of allo-HLA crossreactivity by self-HLA restricted T-cells. Furthermore memory T-cells that are generated as a result of previous infections may cross-react against allogeneic HLA molecules (2,7). These pre-existing memory T-cells may provide a potent barrier to transplantation tolerance because of their higher activation state, cytokine production, cytotoxicity and lower requirements for T-cell help and/or co-stimulation.

The aim of this thesis was therefore to determine if the high frequency of pre-existing alloreactive memory T-cells in non-sensitized individuals could be accounted for by allo-HLA crossreactivity by viral specific memory T-cells. Prior to discussing the current knowledge of mechanisms underlying T-cell alloreactivity, a review of the normal immune response against antigens is warranted.

1. GENERAL IMMUNOLOGY: THE HUMAN IMMUNE SYSTEM

Pathogens, such as viruses, represent a major threat to the human body and the immune system is the body's natural defence against these infections. The immune system can be divided into innate and acquired immunity. The innate immune system consists of physical barriers and a number of non-specific molecules, receptors and cells which provide immediate protection against invading organisms and initiate an adaptive acquired immune response.

The adaptive immune system comprises a repertoire of T-cells and B-cells that is generated upon antigenic challenge and thus depends on the individual's exposure to pathogens. These cells bear receptors on their surface that provide specificity. T-cells that have not yet encountered their cognate antigen are naïve T-cells. Upon encounter with their specific antigen these cells will expand and mature into effector and memory T-cells. The acquired immune system is specific and retains memory for pathogens that have been previously encountered.

Antigen presentation by the Major Histocompatibility Complex (MHC) initiates an antigen specific immune response by T-lymphocytes. In humans the MHC molecules are known as the human leukocyte antigens (HLA).

1.1 HUMAN LEUKOCYTE ANTIGENS

T-cells constantly survey tissue cells for the presence of pathogens. The T-cell receptor (TCR) recognizes foreign antigens in the form of peptides only when they are presented by specific molecules of the HLA complex. HLA molecules are expressed on all nucleated human cells and the phenomenon whereby T-cells recognize an antigenic peptide presented only by one self-HLA molecule is termed HLA restriction. There are two classes of HLA molecules, both with similar, yet distinct functions (Figure 1).

HLA Class I

The classical HLA class I molecules, HLA-A, HLA-B and HLA-C are constitutively expressed on all nucleated cells. HLA class I molecules consist of a transmembrane α -chain, a non-covalently associated light chain β 2-Microglobulin and the peptide presented in the peptide binding groove of the α -chain. The α -chain is encoded on chromosome 6 and contains three extracellular domains (α 1, α 2, and α 3). The α 1 and α 2 domains form the peptide binding groove, are the sites of most polymorphisms within the HLA class I molecule and are also the sites of TCR contact with the HLA molecule. The α 3 domain contains a CD8 binding site which is necessary for presentation of intracellular peptides to CD8 T-cells. Peptides presented by HLA class I molecules are generally 8-13 amino acids in length.

In case of intracellular infection, e.g. virus infection, HLA class I molecules present pathogen derived peptides to CD8 cytotoxic T-lymphocytes (CTLs) which can then immediately and specifically eliminate the infected cell.

HLA Class II

HLA class II molecules are constitutively expressed on professional antigen presenting cells (APCs) such as dendritic cells (DCs), macrophages, B-cells and activated T-cells. However inflammatory cytokines, such as IFN γ , can induce HLA class II expression on most cell types. HLA class II molecules are encoded by the HLA-DR, HLA-DQ and HLA-DP genes on chromosome 6. HLA class II molecules consist of two transmembrane chains (α and β) that both contribute to the peptide binding site, and also contain a CD4 binding site. The β chain of the HLA-DR molecule is the most polymorphic of the class II molecules. Peptides presented in HLA class II molecules are typically 12-25 amino acids long.

The function of the HLA class II molecules is to present extra-cellular peptides to CD4 T-cells for the initiation of immune reactions and recruitment of other effector mechanisms.

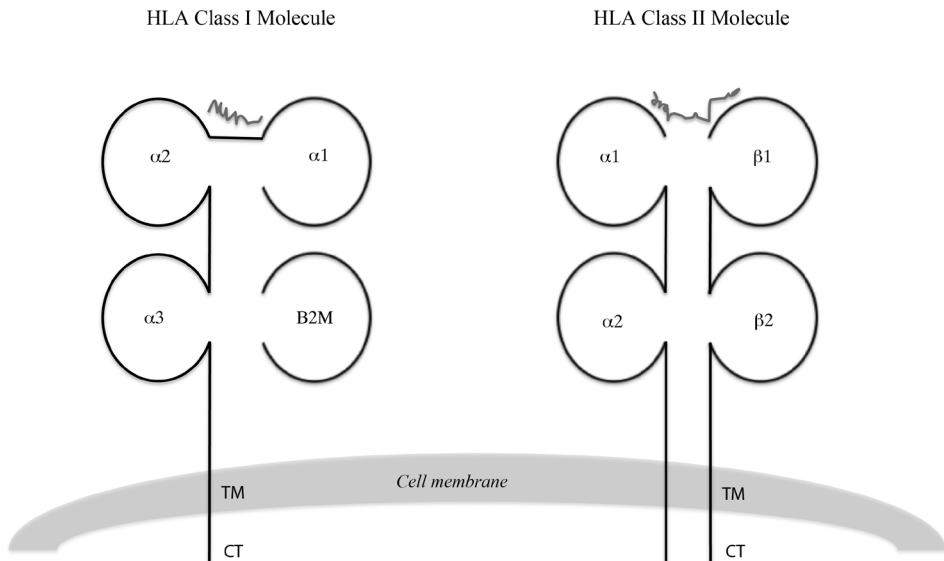


Figure 1. The structure of HLA class I and II molecules.

HLA class I consists of a heavy chain (α chain) and a non-covalently associated invariant light chain (β 2-Microglobulin). HLA class II is a heterodimer consisting of α and β chains. The peptide binding groove is formed solely by the α chain in HLA class I molecules and by both the α and β chains in HLA class II molecules. HLA class II molecules bind and present longer peptides than HLA class I molecules. TM=Transmembrane region. CT=Cytoplasmic tail. B2M= β 2-Microglobulin.

Antigen Processing and Peptide/HLA Restriction

In all cells proteasomes degrade cellular proteins that are poorly folded, damaged or unwanted. When a cell becomes infected, pathogen derived proteins in the cytosol are also degraded by the proteasome. Peptides are transported from the cytosol into the endoplasmic reticulum by a protein called transporter associated with antigen processing (TAP). Newly synthesized HLA class I molecules are also transported into the endoplasmic reticulum where they can now bind these peptides, before being transported to the cell surface in order to present these peptides to T-cells.

HLA class II molecules are prevented from binding peptides in the endoplasmic reticulum by the presence of the invariant chain bound in the groove. The invariant chain also targets class II molecules to endocytic vesicles where they bind proteins derived only from the extracellular space. When the HLA class II molecule has lost its invariant chain and has a tightly bound peptide it is carried to the cell surface.

It is known that HLA molecules can present both self and non-self peptides on the cell surface. The T-cell receptor specifically recognizes both the presented peptide and the HLA molecule.

1.2 THE T-CELL RECEPTOR AND THYMIC EDITING

Antigen recognition by T-cells is central to the generation and regulation of an effective immune response. The TCR recognizes antigen fragments (peptides) which are bound and presented by HLA molecules. T-cells do not recognize free antigen.

The T-cell Receptor (TCR)

The TCR is the highly variable recognition molecule used by T-cells. A typical TCR consists of an α and β chain, both embedded in the membrane. The diversity of the TCR is generated by gene rearrangement (Figure 2). The variable parts of the TCR are encoded by separate gene segments called V, D and J segments, each of which is present in the genome as a tandem array of polymorphic forms. For a functional TCR to be made one each of the different gene segments must be brought together by gene rearrangement with elimination of the intervening regions. The numerous combinations of V, D and J segments that can be brought together are the principal source of variable region diversity of the TCR. Each lymphocyte is clonal; a single TCR is expressed in each lymphocyte. An adaptive immune response is initiated when a naïve T-cell recognizes a pathogen specific peptide presented by an APC on a self-HLA molecule.

Antibodies are the receptors for antigen specific B-cells and are formed by very similar gene rearrangements to that used in formation of the TCR. Adaptive B-cell responses are not discussed in this thesis.

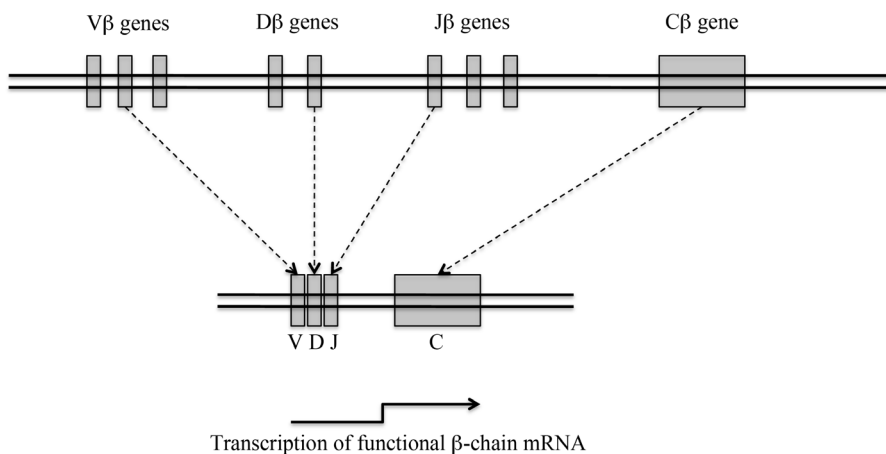


Figure 2. Synthesis of T-cell receptor β -chain.

Rearrangements of different V, D and J segments result in the formation of a unique β -chain. Productive β -chain gene rearrangement commits the T-cell to the α : β lineage. The T-cell receptor α -chain genes then commence comparable gene rearrangements except that they do not have D segments. Following successful α : β TCR generation the double-positive cell is signaled to survive and can proceed to positive and negative selection. V=Variable gene segment. D=Diversity gene segment. J=Joining gene segment. C=Constant region. TCR= T-cell receptor.

Thymic Selection

The first phase of T-cell development is the production of a functional TCR, irrespective of antigen specificity. The TCR repertoire that actually exits the thymus is then the product of “positive” and “negative” selection based on self-peptide/HLA recognition in the thymus. Only a small percentage of the T-cells with successful TCR gene rearrangements have a TCR that can interact with one of the HLA class I or II isoforms expressed by the individual, these T-cells are positively selected for further development. T-cells that are positively selected by HLA class I molecules become CD8 T-cells and T-cells that are positively selected by HLA class II molecules become CD4 T-cells. Thus both CD4 and CD8 T-cells develop from a common precursor in the thymus.



Tissue-specific proteins are expressed in the thymus and T-cells that bind self-peptides presented on self-HLA molecules are removed in the thymus by negative selection. For example the TCR that uses the VB6 gene segment is specific for the EBV FLRGRAYGL peptide presented by HLA-B*08:01 (7,8). This TCR also binds the EEYLQAFTY self-peptide from the ABCD3 gene presented on HLA-B*44:02 (9). In HLA-B8 B44 heterozygous individuals this TCR is negatively selected in the thymus to avoid auto-immunity (10).

Thus during T-cell development any T-cells having receptors that respond to complexes of self-peptide and MHC class I and II molecules of healthy cells are eliminated. However this quality control mechanism encompasses only HLA isoforms expressed by that individual (autologous HLA), and not other HLA isoforms (allogeneic HLA). Accordingly T-cells that can respond to complexes of self-peptide and allogeneic HLA class I and II molecules are theoretically able to exit the thymus as they are not negatively selected. T-cells that have survived positive and negative selection leave the thymus and enter the circulation as mature naïve T-cells. Mature naïve T-cells exhibit a high frequency (10%) of crossreactivity against allogeneic HLA to which they have not been previously exposed (11,12).

1.3 T-CELL EFFECTOR MECHANISMS

T-cell mediated immunity is critical to the control and eradication of infectious agents. The first part of an adaptive immune response occurs when a naïve T-cell encounters its specific antigen and undergoes T-cell activation in a germinal centre reaction, and is stimulated to differentiate into an effector T-cell. Effector CD8 T-cells are long lived and travel to the sites of infection where they can kill any type of cell whose HLA class I molecule are presenting antigens to which the T cells are specific. Effector CD4 T-cells recognize their specific antigen presented via HLA class II molecules and via cell-cell contact and cytokine production can make macrophages more proficient at killing pathogens and can activate B-cells to make antigen specific antibodies.

Naïve T-cells and T-cell activation

Naïve T-cells have not yet encountered their specific antigen and are characterized by surface expression of CD45Ra, the lymph node homing receptor CCR7 and the presence of costimulatory molecule CD28 (Table 1).

Dendritic cells are adept at capturing and processing antigens from pathogens. Dendritic cells travel to the afferent lymph node that drains from the site of infection, where naïve T-cells first encounter their specific antigen presented by the dendritic cells. The intracellular signal generated by ligation of the T-cell receptor with a specific peptide/HLA complex is necessary to activate a naïve T-cell, but is not sufficient. Participation of the CD4 or CD8 co-receptor is essential for effective naïve T-cell activation. Activation of naïve T-cells also requires a co-stimulatory signal delivered by an APC. The co-stimulatory signal is delivered by the CD80/CD86/CD28 and CD40/CD40L co-stimulatory molecules delivered only by the professional APCs – dendritic cells, macrophages and B-cells.

In the absence of infection the APCs do not express co-stimulatory signals and thus the capacity of APCs to activate naïve T-cells is acquired only during infection.

Memory T-cells

Immunological memory is the result of clonal selection of antigen specific T-cells. When naïve T-cells are activated by antigen and co-stimulatory signals they are driven to proliferate and differentiate into memory T-cells, a process driven by the cytokine interleukin-2. The activation of naïve CD8 T-cells generally requires stronger co-stimulatory signals than is needed to activate naïve CD4 T-cells. Memory T-cells express the marker CD45Ro and thereby the cells gain an increased survival potential.

Naïve CD8 T-cells are activated to become cytotoxic effector memory CD8 T-cells. Effector memory CD8 T-cells lose expression of the CCR7 receptor and therefore leave the lymph node and enter the circulation where they can home to sites of inflammation. Effector function is turned on when the TCR bind to specific peptide/HLA complexes on a target cell, however effector T-cells have major functional differences versus their naïve counterparts as their responses to infection do not depend on co-stimulatory signals. Once generated, CD8 memory T-cells persist in high frequency and have lower activation requirements with novel co-stimulatory pathways that may be constitutively expressed (5,13). Upon activation, memory T-cells produce a wide variety of cytokines including IL-2, IL-4, IFN γ , TNF α and are capable of rapid up-regulation of cytolytic effector function without the need for CD4 T-cell help (14) (Table 1).

Effector memory CD8 T-cells are selective and specific serial killers of target cells at sites of infection. Therefore if viral specific memory T-cells do indeed crossreact against allogeneic HLA to which they have never been exposed they may be a major barrier to successful transplantation.

On activation CD4 T-cells acquire distinctive helper functions. Activated CD4 T-cells synthesize cell-surface molecules and cytokines that activate and help other types of cells, particularly macrophages and B-cells, to participate in the immune response. Antigen specific regulatory CD4 T-cells can limit the activities of effector CD4 and CD8 T-cells via production of inhibitory cytokines such as IL-4, IL-10 and TGF- β .



Table 1. Properties of CD8 T-cell subsets (14).

	Naïve	Effector memory
<i>Genes Expressed</i>		
CD45Ra	+	-
CD27	+	+/-
CD28	+	+/-
CD95	-	+
CCR7	+	-
Fas ligand	-	+
IL-2	+	+
IL-4	-	+/-
IFN γ	-	+
TNF α	-	+
Perforin	-	+
Granzyme	-	+
<i>Functions</i>		
Cytotoxicity	-	+
B-cell differentiation	-	+/-

2. ALLORECOGNITION

Alloreactive T-cells are recruited to the transplanted graft and initiate and execute organ rejection. A series of recent studies have characterized the frequency and cytokine profiles of T-cells responding to allogeneic grafts (12). Naïve and memory T-cells are capable of responding with similar frequency against allogeneic cells, even in non-sensitized transplantation recipients. Furthermore CD4 and CD8 memory populations mount similar proliferative responses and contain comparable frequencies of alloreactive precursors, even though effector molecule expression is significantly higher among CD8 T-cells. These alloreactive memory T-cells are a major barrier to successful transplantation because of their lower activation thresholds, absent requirements for T-cell help and immediate cytotoxic function.

2.1 DIRECT ALLORECOGNITION

Direct allorecognition occurs when recipient T-cells directly recognize donor cells expressing intact mismatched HLA molecules, and is usually associated with acute T-cell mediated rejection. It is generally accepted now that direct allorecognition is dependent on donor derived self-peptide presentation by the allogeneic HLA molecule (1). Direct allorecognition from pre-existing viral specific CD8 T-cells is the topic of this thesis.

2.2 INDIRECT ALLORECOGNITION

Indirect allorecognition involves donor antigen uptake by recipient APCs. Allopeptides can be derived from allogeneic HLA molecules or minor histocompatibility antigens that differ between donor and recipient. After processing and peptide presentation in the context of autologous HLA class II molecules, antigen specific CD4 T-cells are activated and can initiate an alloimmune response. The frequency of T-cell clones involved in indirect allorecognition is about 100 fold lower than in the direct pathway. Indirect allorecognition is not investigated as part of this thesis.

2.3 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 (12,15-17), 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 (7,18-19).

3. ALLOREACTIVITY BY VIRAL SPECIFIC MEMORY T-CELLS

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 (20). Mismatched donor HLA antigens have differential impact on graft survival depending on the HLA phenotype of the recipient (21), 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 (7,19,21). 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 (22-23).



In-vivo, the presence of virally induced alloreactive T-cell memory is a potent barrier to transplantation tolerance in mice (2-3,5,24-26). 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 associated with the development of memory cells, which abrogate the induction of transplant tolerance (1,6,27-29). 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 (2). Evidence for virally induced alloreactive T-cell memory in mice is already extensively documented in the literature (2-3,5,24).

Taken together this evidence provides 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.

3.1 HUMAN EBV SPECIFIC CLONES ARE CROSSREACTIVE AGAINST ALLO-HLA-B*44:02 VIA MOLECULAR MIMICRY

Burrows and colleagues demonstrated the dual specificity of EBV EBNA3A specific T-cell clones for the immunodominant peptide FLRGRAYGL presented on HLA-B*08:01 and the alloantigen HLA-B*44:02, to which the individual had never been exposed (7). 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*44:02 (8). This finding has been reproducibly found in different individuals from different genetic backgrounds using different techniques (7-8,12,18). HLA-B44 mismatching has been identified as higher risk among HLA-B8⁺ renal transplant recipients (30).

The EBV EBNA3A T-cell allo-HLA-B*44:02 crossreactivity is dependent on presentation of the EEYLQAFETY self-peptide derived from the ABCD3 gene (9). Molecular mimicry, as revealed by crystallography studies, is the mechanism for this human T-cell alloreactivity from

a viral specific memory T-cell (figure 3). Despite extensive amino acid differences between HLA-B*08:01 and HLA-B*44:02, 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. Structural studies confirm the exquisite specificity of the TCR and the self-peptide dependence of the T-cell alloreactivity.

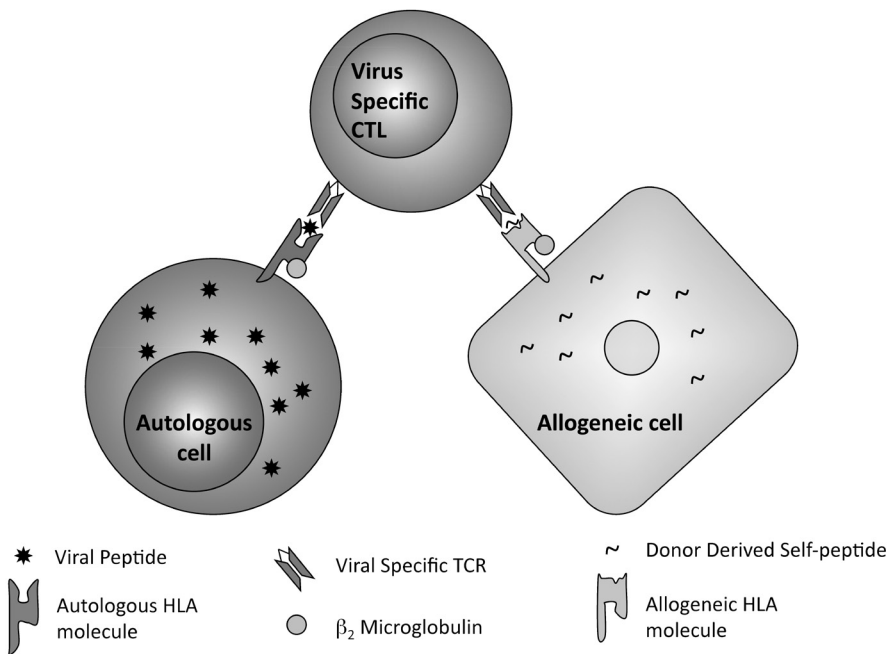


Figure 3. Allo-HLA crossreactivity by viral specific memory T-cells.

Viral specific memory T-cells target virus infected autologous cells presenting viral peptides in a self-HLA restricted fashion. Alternatively, the same viral specific TCR may crossreact against an allogeneic HLA molecule presenting a self-peptide.

3.2 MECHANISMS OF TCR CROSSREACTIVITY

A very high level of crossreactivity is an essential feature of the T-cell receptor (31). While the human immune system does generate a vast number of clonotypically unique T-cell receptors, it is not possible to generate a unique TCR for every immunogenic peptide. Crossreactivity of the TCR ensures that the number of T-cells that can recognize an individual pathogenic peptide presented on a HLA molecule is sufficiently large to elicit a rapid response, and that no pathogenic peptides go unrecognized.



Crossreactivity by pathogen specific memory T-cells may help protect against subsequent unrelated infections, however, in the transplantation setting such crossreactivity may give rise to harmful alloresponses.

Induced Fit

Structural adjustments in the TCR binding site can allow a single receptor to recognize different peptide/MHC ligands. Usually such flexibility is observed in the CDR loops of the TCR. For example the TCR BM3.3 is able to recognize three distinct peptides bound to H-2Kb through changes in the conformation of the flexible complementarity-determining region loops, especially the CDR3 loop (32).

Differential TCR docking

Disparate docking orientations can allow the same TCR to engage different peptide-MHC ligands. The 2C TCR utilizes a different binding strategy to recognize its allogeneic ligand H-2Ld-QL9 and the self-ligand H-2Kb-dEV8 by which it was positively selected (33).

Structural Degeneracy

TCR cross-reactivity can also occur when there is a paucity of peptide-MHC interactions. The TCR 3A6 recognizes a self-peptide from myelin basic protein presented on HLA-DR2a, but is also able to recognize many other peptides presented on HLA-DR2a because of absence of hydrogen bonds between the TCR and the peptides (34).

Molecular Mimicry

Molecular mimicry, whereby the TCR engages the allogeneic ligands and viral ligands with the same overall docking topology, has long been proposed to explain TCR crossreactivity. This can occur despite disparate sequences of the allo and viral peptides (9). It is also suggested that molecular mimicry operates in other alloreaactions (35-39).

Antigen-Dependent Tuning of Peptide-MHC Flexibility

Conformational flexibility of peptide-MHC can also allow recognition of different ligands by the same TCR. Recognition of Tax-HLA-A2 antigen (from HTLV-1 virus) by TCR A6 proceeds without substantial adjustments in the ligand, whereas the same TCR recognizes the Tellp-HLA-A2 antigen (from *S. Cerivisiae*) only following large conformational changes in both the peptide and MHC (40).

4. AIM OF THIS THESIS

The aim of this thesis is to determine if the presence of alloreactive T-cells in non-sensitized individuals can be explained by allo-HLA crossreactivity by viral specific memory T-cells. If true, a further aim is to determine the frequency of allo-HLA crossreactivity by viral specific memory T-cells. The ability of viral specific T-cells to exert HLA alloreactivity could have especially serious consequences as memory T-cells lack the requirement for costimulation and therefore could be efficiently triggered by nonprofessional antigen-presenting cells after HLA-mismatched stem cell transplantation or solid organ transplantation. In order to detect allo-HLA crossreactivity from viral specific memory T-cells, viral specific T-cell clones were generated using single cell sorting based on viral peptide/HLA tetrameric complex staining. The viral specific T-cell clones were then tested for alloreactivity by stimulating with various tissue cells expressing allogeneic HLA molecules.

Chapter 2 of this thesis describes a new tool to detect allo-HLA crossreactivity from viral specific memory T-cell clones using K562 cells transfected with single HLA molecules. The appendix to chapter 2 extensively describes the methodology used in chapter 2. Chapter 3 uses multiple different viral specific T-cell clones to address the frequency of allo-HLA cross-reactivity from viral specific memory T-cells. An example of how self-peptide presentation can alter the tissue specificity of allo-HLA crossreactivity from viral specific T-cell clones is described in chapter 4. In chapter 5 it is shown that anti-viral vaccination, not just viral infection, can also induce alloreactive T-cells. The current evidence for alloreactivity by human viral specific memory T-cells is reviewed in chapter 6. In chapter 7 it is confirmed that allo-HLA stimulation of non-sensitized blood cells can conversely elicit a viral specific cytolytic T-cell response, and the possible clinical implications are discussed. Chapter 8 provides a general conclusion and discussion to summarize all findings and put them into clinical perspective. Included in the general discussion are unpublished results describing how proteosomal digestion could generate or destroy allopeptides.

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