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Comprehensive Review

Advances in Direct T-Cell Alloreactivity: Function, Avidity, Biophysics and Structure

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Although T-cell-based adaptive immunity plays a crucial role in protection against infectious pathogens and uncontrolled outgrowth of malignant cells, a large portion of these T cells are also capable of responding to allogeneic HLA molecules, violating the paradigm of self-major histocompatibility complex (MHC) restriction. Recent studies have provided insights into the mechanisms by which these T cells recognize allogeneic targets. The role of antiviral T cells in direct alloreactivity through peptide-dependent molecular mimicry and alternate peptide-MHC docking modes has emerged as major models for the human alloresponse. Here, we review in depth recent advances in this field and discuss how molecular interactions between T cells and HLA molecules drive the activation of these effector cells and its potential implications for alloreactivity in human transplantation.

Key words: Alloreactivity, alloreactive T cells, allorecognition, bone marrow, HLA antigens, memory T cells, transplant, transplant immunobiology, viral immunity, viral infection

Abbreviations: NFAT, nuclear factor of activated T cells; SLP-76, SH2 domain containing leukocyte protein-76; TCR, T-cell receptor; ZAP-70, zeta chain associated protein kinase-70.

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T Cells in Adaptive Immunity

The ability of the adaptive immune system to distinguish self from nonself and establish immunological memory provides crucial defense against invading pathogens (1–3). Immunological memory allows the host to mount a more vigorous response against reinfection or challenge with related antigens. T lymphocytes play several roles in adaptive immunity which includes direct killing of virus-infected and malignant cells and also contribute to the generation and maturation of humoral immune responses. T cells recognize peptide epitopes bound to major histocompatibility complex (MHC) class I (MHC-I) or class II (MHC-II) molecules. These peptide epitopes are generated by the components of the antigen processing machinery which are specifically dedicated to generate epitopes for MHC-I and MHC-II molecules that are recognized by $CD8^+$ and $CD4^+$ T cells, respectively (4).

T cells express heterodimeric T-cell receptors (TCR), which are composed of either the $\alpha\beta$ or the $\gamma\delta$ chains. These chains are encoded by multiple variable (V), diversity (D; for β and δ chains) and joining (J) segments. Similar to immunoglobulin gene rearrangement, TCR diversity results from V(D)J recombination which allows T cells to recognize a diverse array of MHC-peptide complexes. Each T cell undergoes highly stringent thymic processes of positive and negative selection (2). This selection process is determined by the strength of interaction between TCR and self-peptide (p) MHC complexes and the cells that fail to interact strongly enough with self-pMHC complexes are eliminated by apoptosis (referred to as death by neglect). During the positive selection process, T cells which interact with MHC-I complexes mature to become $CD8^+$ T cells, whereas cells selected through interaction with MHC class II molecules emerge as $CD4^+$ T cells. T cells, which display high levels of avidity with self-pMHC complexes are eliminated to ensure that potentially self-reactive T cells do not survive into the periphery.

After exposure to an antigen, naïve T cells (both $CD4^+$ and $CD8^+$) undergo rapid clonal expansion and a small proportion of these cells survive the expansion phase and persist as memory T cells (1). Depending on the cytokine and costimulatory environment during antigen exposure, T cells acquire unique phenotypic and functional characteristics which include the expression of chemokine receptors that allow them to migrate to sites of inflammation or infection (5,6). In addition, these cells also acquire the ability to express effector cytokines such as IFN-γ, TNFα and/or IL-2 and direct cell contact-dependent cytolytic function (especially $CD8^+$ T cells; Refs. 7,8). $CD4^+$ T cells play

a crucial role in the maturation of the $CD8^+$ T-cell memory response by multiple mechanisms, including through the maturation of dendritic cells and the production of IL-2 (9–11). Memory $CD8^+$ T cells are broadly divided into central and effector memory cells, which reside in lymphoid and nonlymphoid organs, respectively (12). Whereas the central memory cells do not have immediate lytic function, effector cells generally express immediate lytic function on restimulation (13). Extensive studies in humans and murine models have shown that in addition to helping in the maturation and maintenance of $CD8^+$ T-cell memory responses (9–11), $CD4^+$ T cells also play a crucial role in B-cell-mediated immune regulation, including both high-affinity antibody production and class switching (14–16), and also directly mediate clearance of virus-infected cells. Historically, $CD4^+$ T cells were divided into two major subsets, Th1 and Th2 which could be distinguished by the secretion of specific cytokines (17). More recent studies have defined additional subsets including Th17, Th22, regulatory T cells and follicular helper T cells (Tfh), each distinguished by unique cytokine profiles and transcription factor expression (reviewed in Ref. 18). These T-cell subsets express distinct immunological functions to effectively mediate the response toward the antigen/pathogen to which they have been previously primed.

Structural Insights Into TCR and pMHC Interactions

During T-cell recognition of target cells, the highly flexible complementarity determining regions (CDRs) of TCR contact the surface of the MHC antigen-binding cleft as well as the peptide cradled by this molecule (Figure 1; Ref. 19). MHC-I and MHC-II molecules present the peptide epitopes in an extended conformation, within the peptide-binding groove which is formed by two flanking α-helices and a floor composed of antiparallel β-strands. Although the peptide-binding groove of MHC-I molecules is closed at either end, this groove is open in MHC-II molecules, which allows longer peptides to hang outside the groove. The first two turns of the MHC-I α1-helix are replaced in MHC-II by a β-strand. Over the last decade and a half, a consensus has emerged on the docking mode of TCR with the pMHC complex. These studies have shown that the TCR heterodimer is generally oriented, diagonally relative to the MHC-peptide-binding groove. The Vα domain of the TCR contacts the N-terminus of the peptide and is also in close proximity to the MHC α2 helix, whereas the Vβ domain more often contacts the C-terminal section of the peptide epitope and MHC α1 helix. The TCR contacts with the peptide epitope are primarily made through the CDR3 loops, which form the most diverse region of the TCR, although significant CDR1 loop contact has been observed. Generally, the germline-encoded CDR1 and CDR2 loops of the TCR drive the MHC specificity of T-cell recognition. It is important to note that there are many other examples of TCR-pMHC structures which do not display these conserved interactions, indicating that additional forces may guide the interaction of the pMHC complex with the TCR. Furthermore, not every interaction of pMHC complex with cognate TCR results in T-cell activation. Indeed, previous studies have shown that altered peptide ligands can either completely or partially inhibit T-cell activation, however, structural analysis have failed to provide a detailed insight into the mechanism of altered peptide ligand action. The structural analysis of pMHC and TCR interaction defines the point of the docking process from where the TCR signaling initiates.

T-Cell Activation

TCR and pMHC affinity/avidity

T-cell activation involves a series of signaling events that commence after engagement of the TCR with the pMHC complex. Paramount in the context of successful T-cell activation are a number of factors that dictate a successful signaling cascade including the affinity of the TCR for the pMHC complex, the number of pMHC complexes on the surface of the antigen-presenting cells (APCs), the influence of costimulatory signals delivered after ligation of coreceptors on the surface of both the T cell and APC and finally a complex set of signaling molecules which dictate T-cell activation.

The affinity of a TCR for a given pMHC complex can be defined by the rate at which the TCR associates and then dissociates with the pMHC complex (20). Although it is well established that TCR typically have low affinity for pMHC complexes, studies of a broad range of human and mouse TCR-binding affinities suggests that the generation of a strong agonistic signal can be influenced by the rate of association and is dependent upon a dissociation rate that is significantly long enough to allow complete intracellular signaling to occur. Both human and murine studies using peptide epitopes mutated at a single amino acid residue complexed to MHC have shown that reducing the TCR on-rate can result in a weak agonistic or even an antagonistic response (21,22). Conversely, TCR with rapid dissociation rates can also function as weak agonists or antagonists (23–25). Despite these observations, other studies have demonstrated that TCR with rapid dissociation rates can still function as agonists (26). Consequently, the binding parameters, which dictate the likely generation of agonistic signals, have not yet been fully defined. It is also evident that T-cell activation is not simply a consequence of pMHC and TCR affinity but can be influenced by other factors (e.g. expression of costimulatory molecules and levels of surface TCR expression) intrinsic to the T cell itself which may alter the avidity of the T cell.

As opposed to measurements of TCR affinity that define the rate of association and dissociation between the TCR and pMHC, measurements of T-cell avidity are based upon the more complex interaction of TCRs on the surface of

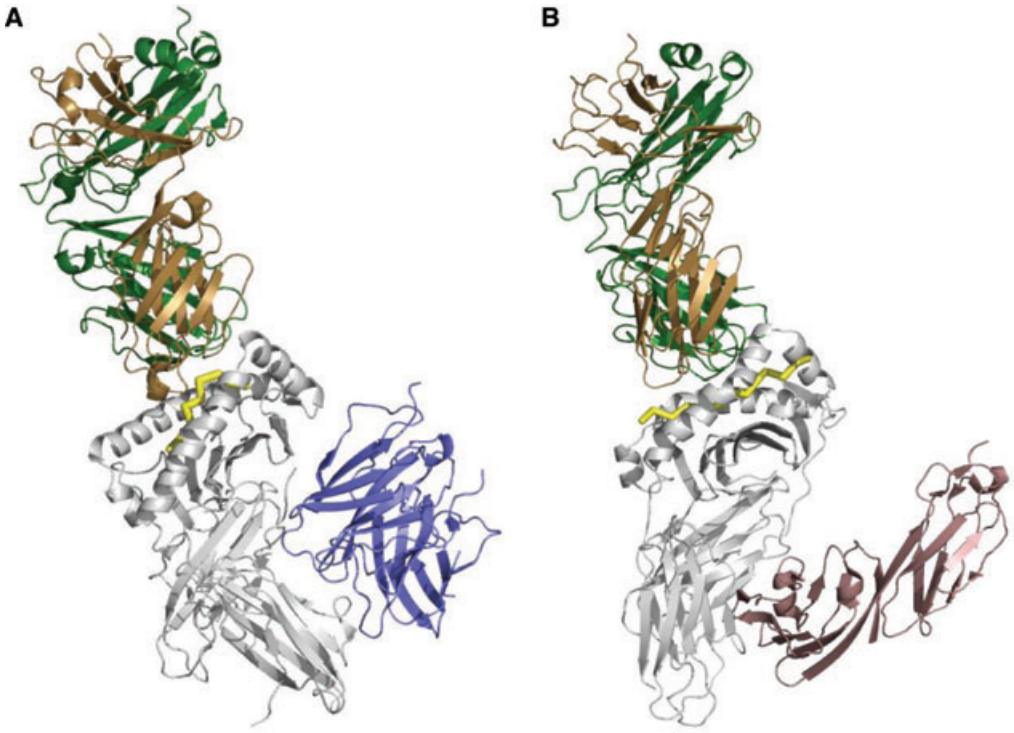

Figure 1: Examples of TCR and accessory molecules engaging pMHC-I and pMHC-II. The LC13 TCR engages its syngeneic pMHC-I target with CD8$\alpha\alpha$ superimposed engaging a conserved region of the MHC-I α3 domain (A). The YAe62 TCR engages its pMHC-II target with CD4 superimposed engaging conserved regions of MHC-II α1/β1 domains (B). The TCR α-chain (gold) and β-chain (green) are shown with cartoon representation. The MHC (gray) is shown with cartoon representation and the presented peptide (yellow) shown in ribbon. CD8 (blue) and CD4 (violet) are shown with cartoon representation. The figure was created using coordinates from PDB files 1AKJ, 1JL4, 1MI5 and 3C60.

T cells and pMHC complexes. Strategies to define T-cell avidity have generally been based upon the stability of binding of pMHC tetramers or through sensitivity to cognate peptide. T-cell avidity can be influenced by a number of factors other than the affinity of the pMHC/TCR interaction, including coreceptor binding and T-cell differentiation status.

The role of costimulation in T-cell activation

Costimulatory molecules have been shown to influence the activation of T cells after TCR engagement and can function, either after ligation directly with the MHC or after ligation with coreceptors on the surface of the APC. The primary coreceptor on T cells is CD3 which is found on all T cells, and contains immunoreceptor tyrosine-based activation motifs which are phosphorylated after TCR-pMHC ligation and are absolutely critical for recruitment of ZAP70 and the signaling cascade which follows (reviewed in Ref. 27). T cells also express either CD4 or CD8 which bind MHC-II and MHC-I, "respectively," and function differently with regard to their impact on TCR-binding kinetics and T-cell activation. CD4 seems to have minimal effect on the binding kinetics of TCR (28), although there has been some suggestion that CD4 reduces the number of pMHC complexes required for T-cell signaling to occur (29). CD4 may function to enhance signaling by increasing TCR cross-linking and promoting the formation of heterodimeric complexes of MHC with agonist or endogenous self-ligands (30,31). CD8 has been shown to have some influence on the binding kinetics of TCR for MHC-I molecules. Although high-avidity T cells can signal after TCR engagement in the absence of CD8 (32,33), lower avidity T cells are critically dependent upon CD8 for signaling (32). Studies using MHC molecules engineered to reduce the affinity of CD8 demonstrated that CD8 cobinding stabilizes the interaction of the pMHC-TCR complex, reducing the threshold for T-cell activation in low-avidity T cells (34) and functional studies have shown that CD8 can "tune" the number of pMHC ligands, which a TCR can recognize (35). Nevertheless, recent observations demonstrating allogeneic recognition of pMHC-II complexes by $CD8^+$ T cells further emphasize that T-cell activation is not critically dependent upon costimulation by CD4 or CD8 (36).

In addition to the coreceptors which bind MHC, T cells also express a range of receptors which bind other ligands on the surface of the APC and can enhance T-cell activation. Members of the CD28 receptor family signal after interaction with the family of B7 molecules on the surface of APCs, which can promote either stimulatory

of inhibitory signals to T cells (reviewed in Refs. 37,38). During an inflammatory response, licensing of APCs, in particularly dendritic cells, either by helper T cells or by inflammatory stimuli such as IFN-γ, induces the upregulation of a number of costimulatory ligands, including the B7 family members, CD80 and CD86. During TCR engagement with surface pMHC complexes, CD28, which is constitutively expressed on naïve $CD8^+$ T cells, interacts with CD80 or CD86 on the surface the APC and promotes the expression of IL-2 by NF-κB signaling (39). Although TCR signaling and the priming of the cytotoxic T lymphocyte (CTL) response against viral antigens can occur in the absence of CD28 (40,41), costimulation by CD28 has been shown to lower the activation threshold required for TCR signaling (42), and is important in the recall of memory responses against some pathogens (43,44). Despite this, it is well established that some memory CTL populations in humans, particularly those directed against human cytomegalovirus (HCMV), express low levels of CD28 (6,45) and are therefore either less dependent upon costimulation for TCR signaling or are dependent upon other costimulatory molecules to enhance TCR signaling. Other immunoreceptors, including ICOS and 4–1BB (CD137) that are up regulated after activation can function to replace the costimulatory signals delivered by CD28 (46,47), and potentially lower the threshold required for T-cell activation (48).

T-cell signaling in naïve and memory T-cell populations

In addition to the role affinity and costimulation play in defining the outcome of pMHC:TCR interactions, alterations in T-cell signaling pathways can also influence T-cell activation. This is particularly evident in memory T-cell populations, which respond more rapidly after activation (12,49,50), may be tissue resident (51), can be activated by a diverse range of APCs (52–54) and have a reduced antigen threshold required to induce TCR signaling (50,55,56). As outlined above, TCR engagement leads to the phosphorylation of CD3 molecules, followed by the recruitment and activation of zeta-chain-associated protein kinase 70 (ZAP-70). This is followed by the phosphorylation of key adaptor molecules, SH-2 domain containing leukocyte protein-76 (SLP-76) and linker for activation of T cells (LAT), inducing a signaling cascade that eventually leads to the translocation of nuclear factor of activated T cells (NFAT), the activation of NF-κB and the expression of genes associated with T-cell activation, including IL-2 (reviewed in Ref. 57). Memory $CD4^+$ T cells display increased expression of ZAP-70 and other associated downstream signaling, which is likely responsible for the rapid expression of effector functions (58). These observations suggest the presence of a distinct signaling pathway in memory T cells, not found in naïve T cells, which is likely responsible for the rapid expression of effector functions. Although memory T cells do require costimulation by CD28 to generate IL-2 for optimal proliferation *in vivo*, CD28 is not required for the rapid production of IFN-γ (59). It is therefore evident that memory T cells may have two distinct TCR signaling pathways, both dependent upon ZAP70 signaling, a CD28 independent pathway that leads to the rapid induction of effector functions and a CD28 dependent pathway that leads to later cytokine production and the induction of cell proliferation.

Although not completely evident from the current literature, it is possible to speculate that this CD28 independent memory specific activation pathway may have a lower activation threshold that allows the rapid generation of effector functions in the absence of cosimulation and potentially in the presence of lower levels of pMHC. This pathway may be particularly applicable in the context of memory CMV-specific T cells in humans which dominate the human T-cell repertoire (60,61), display a highly differentiated phenotype, including low levels of CD28 expression (6,61) and display immediate effector function after TCR engagement (62,63). These differential requirements for activation of effector memory T-cell populations, including less costimulatory requirements and a lower activation threshold could potentially facilitate a dominant role for these T cells in triggering alloreactivity after transplantation.

Direct Allorecognition by T Cells

The affinity of T-cell alloreactivity

Allorecognition occurs through three distinct mechanisms, that is, direct, indirect and semidirect. These pathways involve recognition of (1) intact allogeneic pMHC molecules on the surface of donor cells, (2) donor MHC molecules presented as peptide epitopes by the host APCs or (3) intact allogeneic pMHC complexes transferred through cell-to-cell contact (64). This review primarily focuses on the direct alloreactivity. Mapping of alloepitopes or "allotopes" recognized by these T cells has allowed the precise measurement of alloreactive TCR binding using surface plasmon resonance (SPR). These studies have revealed that an allospecific TCR can bind its MHC ligand with highly robust affinities of 1–49 μM (K_D; Refs. 65–67). These affinities are comparable to those seen for TCRs specific for viral epitopes (1–20 μM) and cancer antigens (20–200 μM) and are greater than those seen for autoimmune antigens (>250 μM; Refs. 19,68,69). Previous studies have also shown that in some cases the affinity of TCR for an allogeneic ligand can be greater than its syngeneic target. This is best exemplified by studies on the Epstein–Barr virus-specific LC13 TCR, which recognizes its syngeneic HLA-B*0801 bound to an EBV epitope (FLRGRAYGL; referred to as FLR) with affinity of 10 μM (70), whereas the allogeneic HLA B*4402 molecule bound to EEYLQAFTY peptide (referred to as EEY) is recognized with an affinity of 1 μM (65). These observations have also been confirmed with SPR analysis of the murine 2C TCR which binds its syngeneic H-$2K^b$ with an affinity of 83 μM and allogeneic H-$2L^d$ complex with an affinity of 2 μM (67). Recent studies have shown that alloreactive TCRs

are even capable of transcending MHC-I and MHC-II boundaries with minimal loss in affinity. The mouse YAe62 TCR binds its syngeneic MHC-II complex (IA^b) with an affinity of 9 μM and allogeneic MHC-I complex (K^b) with an affinity of 15 μM (66). Taken together, these studies demonstrate that the interaction between TCR and allo-pMHC complex can display strong affinity, which may be even higher than that seen for the syngeneic pMHC complex.

Structural overview of T-cell alloreactivity

Before the resolution of the stoichiometric structural landscape of TCR-allo-pMHC complexes, it was hypothesized that the docking of TCR with allo-MHC molecules may be heavily focused on the MHC molecule rather than the peptide epitope bound to this molecule. However, growing evidence based on structural analysis of allogeneic TCR:pMHC complexes has shown that the "peptide independent" hypothesis is incorrect. Allogeneic TCR dock with normal binding geometries, heavily engage the peptide epitope, and interact in a manner that is generally indistinguishable from syngeneic docking. Structural analysis of the murine 2C TCR revealed that this receptor docks with both syngeneic pMHC-I (K^b) and allogeneic pMHC-I (L^d) complexes centrally with a crossing angle of 15° and 30°, respectively (67). The buried surface area (or area contact footprint) of the syngeneic and allogeneic complexes was 1975 $Å^2$ and 1700 $Å^2$, respectively which are well within the range 1100–2400 $Å^2$ observed for other syngeneic TCR-pMHC complexes. In the syngeneic complex, MHC contact was roughly split between the TCR α- and β-chain, whereas the allogeneic complex, showed dominant interaction between pMHC and TCR β-chain. One of the important conclusions drawn from these studies was that the allorecognition of unrelated pMHC complexes could occur in the absence of molecular mimicry. However, recent studies using a human TCR (LC13) have shown that allorecognition can be driven by molecular mimicry (65). These studies showed that although the two peptide epitopes (FLR and EEY) recognized by the LC13 TCR display little sequence homology they seem remarkably 3D superimposable when comparing the syngeneic and allogeneic complexes (Figure 2). Across both syngeneic and allogeneic HLA molecules, the LC13 TCR was perched with the same superimposable 60° crossing angle and engaged the same C-terminal region of both peptides, verifying peptide-dependent molecular mimicry. Thus, both molecular mimicry and disparate docking modalities have been recognized as mechanisms of direct alloreactivity (Figure 2). More recently, these studies have been extended to another murine TCR YAe62, which displays a highly unusual cross-recognition of both pMHC-I and pMHC-II (66). These studies revealed that the YAe62 TCR adopts a similar orientation on the syngeneic pMHC-II (IA^b) and allogeneic pMHC-I (K^b) molecules, but slightly adjusts its CDR3 loops to accommodate the different peptides in each MHC groove (Figure 2). Interestingly, the TCR also "flexes" the region between the V and J genes when swapping between MHC-I and MHC-II ligands. It will be important to expand these analyses to other TCR-pMHC complexes to determine which mode of interaction, that is, disparate docking versus molecular mimicry, can best describe the phenomenon of T-cell alloreactivity.

Quantifying and mapping of T-cell alloreactivity

In vitro studies based on mixed lymphocyte reactions have shown that 1–10% of T cells in then peripheral blood display strong alloreactive responses (71,72). The degree of alloreactivity is influenced by a number of variables, including the number of mismatched MHC alleles (73) and taboo allele combinations, which together significantly enhance or decrease graft survival (74). Interestingly, taboo combinations include specific HLA-A/B and HLA-DR pairings, indicating that there is cross-talk across MHC-I and MHC-II alleles (74). Recent studies have shown that MHC class I-restricted CMV-specific T cells display strong cross-recognition of allogeneic MHC class II molecules bound to an as yet undefined allotope (36). This cross-MHC class I and class II recognition can also work in reverse with T cells specific for syngeneic MHC-II (IA^b) molecules and allogeneic MHC-I (K^b) alleles (66). These observations provide a new paradigm shift for T-cell recognition of allogeneic targets and potentially have new implications for clinical transplantation. For instance, the ability of $CD8^+$ and $CD4^+$ T cells to cross-recognize MHC-I and MHC-II alleles suggests that matching for both HLA class-I and class-II alleles may be equally important during allograft selection. This proposition contrasts with some historical studies, which suggest matching HLA-DR alleles imparts greater effect on graft and recipient survival than matching HLA-A, -B or -C alleles (75–77). Although an earlier study indicated, matching HLA class-I alleles can be as equally important as matching HLA-DR (78). These findings are supported by previous studies which have shown that HLA-B allele-restricted T-cell responses often dominate during host defense (79). Other factors influencing alloreactivity include gender, with females exhibiting generally higher frequencies of T-cell alloreactivity and allogeneic immunodominance, where clear alloreactivity hierarchies can be seen across different HLA alleles—more frequent responses toward HLA-B alleles than HLA-A, for example (80).

An additional variable to consider in alloreactivity is the past and current viral status of the host. The memory T-cell response to human viruses, especially to the human herpesviruses, can be strikingly high in frequency. In healthy individuals, EBV and CMV-specific tetramer frequencies can range from 10% to 40% of the total $CD8^+$ T-cell repertoire (81). In general, as the magnitude of the memory response increases, clonality decreases and these virus specific T-cell populations tend to exhibit highly focused TCR repertoires (82–86). There is now increasing evidence to indicate that these extremely large, virus-specific T-cell

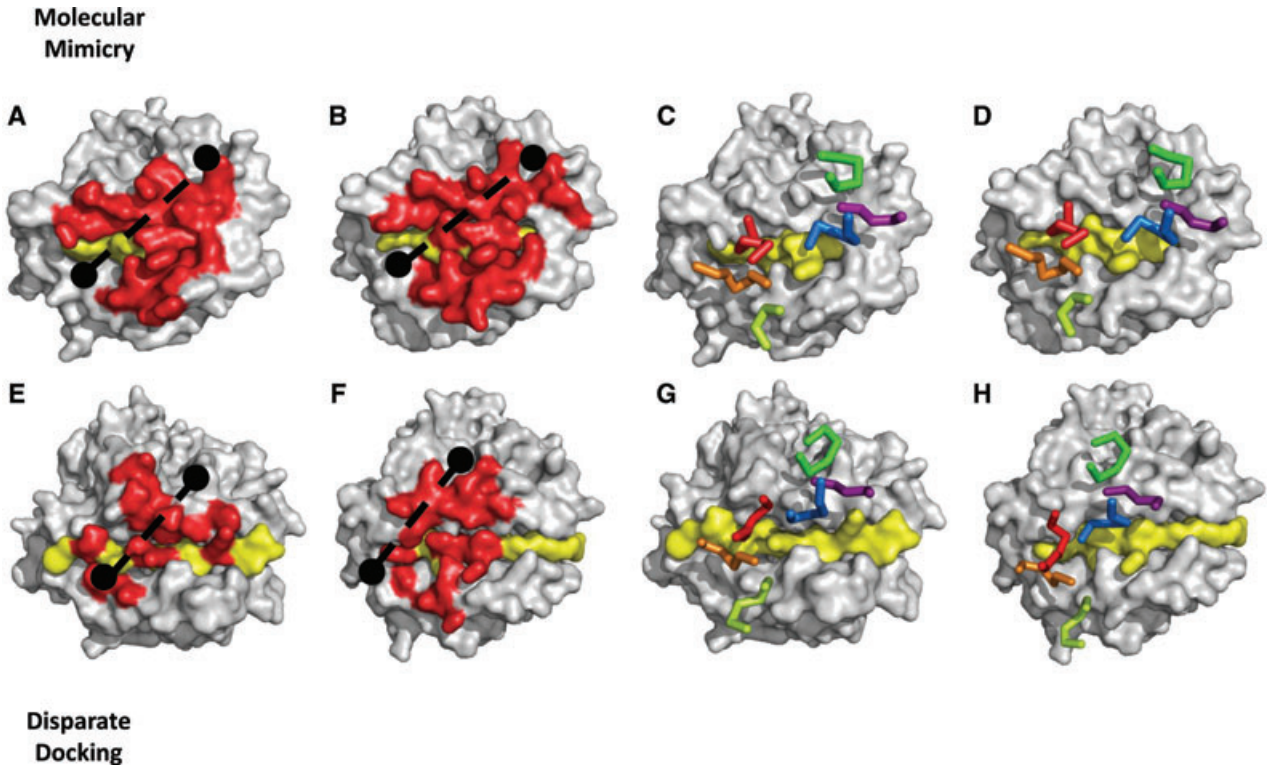

Figure 2: Representative examples of molecular mimicry and disparate docking. Molecular mimicry is shown by the contact footprint of the LC13 TCR (A and C) docked with the syngeneic HLA-B*0801-FLR complex and (B and D) the allogeneic HLA-B*4405-EEY complex. Disparate docking is shown by the contact footprint of the YAe62 TCR (E and G) docked with the syngeneic IA^b complex and (F and H) the allogeneic K^b complex. All molecules are shown with surface representation. The peptide is depicted in yellow, the MHC is depicted in gray and TCR contact areas are depicted in red. Contacts are calculated <4 Å. The TCR crossing angle is shown as a black line between the conserved disulfide-forming sulphur atoms in the TCR α- and β-chains as previously described (see Ref. 19). TCR CDR loops are depicted with ribbon representation, CDR1α (orange), CDR2α (lemon), CDR3α (red), CDR1β (purple), CDR2β (green) and CDR3β (blue) loops are shown.

populations have significant allospecific footprints. Indeed, $CD8^+$ T cells specific for EBV and CMV frequently demonstrate cross-recognition of MHC-I alloantigens (84,87–89). Similarly, $CD4^+$ T-cell clones specific for EBV and CMV antigens also display strong alloantigen-specific cross-recognition (90,91). In a recent study, Amir et al. reported a comprehensive analysis of alloreactivity by virus-specific memory T cell by scanning a large panel of T-cell clones against 37 unrelated MHC mismatched lines, which collectively expressed roughly 100 HLA class-I and class-II alleles (92). These analyses revealed that 45% of virus-specific T cells were alloreactive and recognized one or more HLA alleles. A summary of these analyses and other previously published alloreactivities display by virus-specific T cells is presented in Figure 3. It will be of great interest to gauge the alloreactivity rate of a TCR across the bulk of the ~6800 HLA alleles found in the *Homo sapiens* lineage.

To explain the high frequency of alloreactive T cells, two divergent theories have been proposed. The "multiple binary complex" theory predicts that T cells engage a large number of different allo-pMHC complexes with the TCR predominantly recognizing processed antigen (93). Conversely, the "high-determinant density" theory predicts that TCR predominantly recognize the foreign MHC rather than processed antigen (94). Structural analyses of TCR-allo-pMHC complexes support the multiple binary complex theory as the processed peptide is heavily involved in TCR recognition (65,66), while MHC interaction is similar to the syngeneic complex. Indeed alloreactive TCRs with multiple peptide specificities have been identified (95). In addition, gene silencing of a self-protein encoding, a known allotope only partially reduced the allorecognition suggesting additional allotopes may be involved in allorecognition (65). However, the precise mechanisms underlying high-alloreactive T-cell precursor frequencies, including the role of large memory monoclonal T-cell expansions remain to be fully defined.

Another important factor to consider in alloreactivity is the indirect pathway, defined by allogeneic protein fragments, often of MHC origin, presented in the context of self-MHC. MHC-derived peptides constitute more than 30% of the MHC-bound repertoire on the cell surface (96,97)

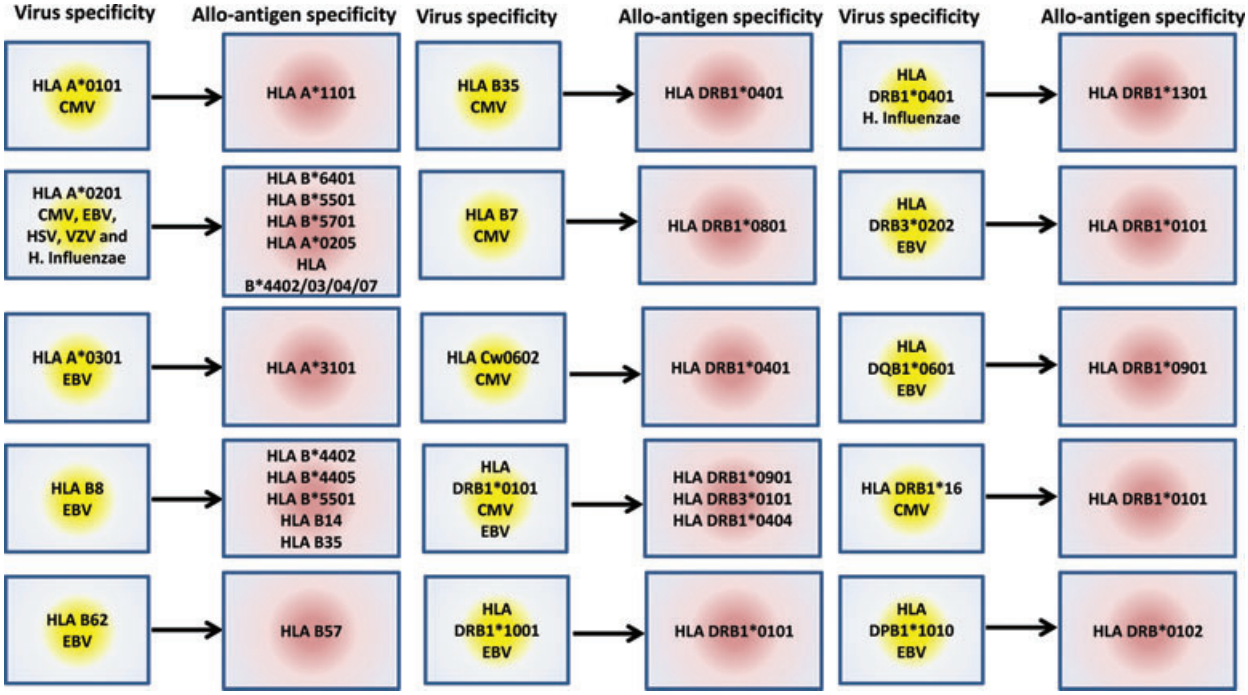

Figure 3: Schematic outline of cross-reactivity between HLA class-I and class-II-restricted antiviral T cells with allo-HLA alleles. This cross-recognition of allo-HLA alleles have been demonstrated both *ex vivo* and *in vitro* expanded virus-specific T cells (see Refs. 36, 82–87).

and may represent key targets for T-cell alloreactvity. A good example of MHC fragments being presented by other MHC molecules is the murine Y-Ae model (98). The Y-Ae-specific antibody detects a highly conserved peptide fragment from the H2-Eα chain presented by H2-Ab (99). Interestingly, this pMHC complex can be detected in mice expressing H2-Ab and H2-E but not in mice expressing H2-Ab alone or in mice expressing H2-E with other H-2A alleles. It is also known that T cells specific for a MHC fragment can be relevant in experimental pathology (100) and in a clinical setting (101,102).

Given both the high intrinsic potential of alloreactivity and the immensity of the T-cell repertoire, it could be argued that T-cell alloreactivity may be difficult to delineate and more difficult to predict. However, extensive analysis of human T-cell repertoires has revealed that there are inherent biases in T-cell repertoire formation and common CDR3 sequences can be detected in different individuals. These "public" TCR sequences can be consistently found against both infectious pathogens and self-antigens and encompass an element of predictability in an otherwise complex TCR selection process (reviewed in Ref. 69). Restricted TCR repertoire profiles have also been observed during transplantation including clonal persistence at the graft site (103) and biased repertoire formation coinciding with clinical events (104). Predictable TCR bias has been observed in experimental alloreactive transplant models in the rat (105) and swine (106). Interestingly, in rats, prolonged allograft survival could be achieved by vaccinating animals with the pathologic TCR Vβ family gene (107) suggesting induction of tolerance. In a xenograft model, bona fide public T cells were identified in both the lymph nodes at the site of graft rejection (108). Across genetically heterogeneous human beings, the reality is that the alloreactive TCR repertoire is considerably more multifaceted, however Vβ gene bias and oligoclonal expansions have been observed in acute lesions of kidney allograft patients (109) and curiously in operationally-tolerant kidney allograft patients (110). A large study in a cohort of renal transplant patients found that alterations in T-cell repertoire correlated directly with Banff score (111), an observation in agreement with a similar study showing that the magnitude of T-cell repertoire distortion correlated with graft failure (112). Thus, mapping the alloreactivity footprints of a high frequency, herpesvirus-specific, public T-cell clonotype may produce a valuable alloreactivity "roadmap" for clinicians when deciding on appropriate host and graft matches. Overall, drafting alloreactive roadmaps across sets of commonly found public clonotypes may improve consequences for graft survival after transplantation and may delineate the mechanism underlying the "taboo concept". Such disease-predictive T-cell strategies could be complimented with other predictive tools such as HLA-Matchmaker, a quantitative algorithm used to determine the degree of a HLA mismatch and likelihood of alloantibody responses (reviewed in Ref. 113).

Role of Virus-Specific T Cells in Transplant Rejection and Graft-Versus-Host Disease

As outlined above, recent observations have demonstrated the high prevalence at which virus-specific T cells are capable of recognizing allo-MHC-I and/or MHC-II molecules (87,92). However, the precise role of these cross-reactive T cells in organ rejection remains to be elucidated. Recent studies by Macedo et al. have shown that T cells from both the naïve and memory T-cell compartments display similar levels of alloreactivity, suggesting that both these populations have the potential to initiate rejection (114). Furthermore, previous studies have shown that memory T-cell populations can rapidly infiltrate allografts (115) and can be found in human allografts (116–118). Experimental animal models have demonstrated that alloreactive T cells can be generated after viral infection (119,120) and can mediate transplant rejection (121). These studies indicated that strategies used to induce tolerance, such as the blockade of T-cell costimulation, were ineffectual in animals exposed to multiple rounds of infections (121). Similar observations using costimulation blockade in humans are also suggestive of a role for memory T cells in mediating acute transplant rejection. Clinical studies have commenced in humans investigating the use of costimulation blockade as an alternative approach to current immunosuppressive regimes (122,123). These studies indicated that while blockade of CD28 using belatacept was associated with reduced toxicity compared to cyclosporine it was also associated with an increased risk of acute graft rejection (122).

The clear association between CMV reactivation and an increased risk of allograft rejection in solid organ transplantation is also suggestive of a potential direct role for viral reactivation in initiating transplant rejection (124–126). Studies have begun to investigate the role of alloreactive virus-specific T cells in mediating transplant rejection. Although preliminary observations have thus far, found no direct evidence (127), it will be important to carefully design these studies as the probability of detecting cross-reactive virus-specific T cells would be much higher during the acute phase of viral reactivation. Furthermore, it is apparent that *in vitro* evidence for alloreactivity by virus-specific T cells will not necessarily translate to an *in vivo* setting, and that antigen context will play a significant role in determining if an alloresponse is generated. This was exemplified in a recent study demonstrating tissue specific alloreactivity (128). Although HLA-B*0801-restricted EBV-specific T cells efficiently recognized HLA-B*4402-restricted allotope EEY on human B cells, these effector cells were unable to recognize proximal tubular endothelial cells from kidney transplant biopsy specimens or human umbilical vein endothelia cells from B*4402 positive donors. Thus, tissue antigen specificity adds another level of complexity for detecting the role of cross-reactive virus-specific T cells play in transplant rejection. Comprehensive mapping of virus-specific alloreactive T cells, defining the public nature of TCR usage in the alloreactive populations and the prevalence of the alloantigen in different tissues, and finally validating a role of these alloreactivities *in vivo*, will be necessary to provide definitive evidence of a role for virus-specific T cells in transplant rejection and graft-versus-host disease.

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