Two mechanisms that account for major histocompatibility complex restriction of T cells
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Abstract
In recent studies, two distinct mechanisms have been proposed to account for major histocompatibility complex (MHC) restriction of T-cell activity: (a) evolution-driven interactions between T-cell receptor (TCR) variable regions and MHC, and (b) a requirement for CD4 or CD8 binding to MHC to initiate signalling through the TCR complex. Both mechanisms are likely to be essential, but for different reasons.

Introduction and context
Since the discovery that T cells are restricted by products of the major histocompatibility complex (MHC) [1], there has been considerable interest in understanding their molecular and structural basis. The process that yields a T-cell repertoire restricted to MHC ligands occurs in the thymus and is called positive selection [2]. Simply put, if the αβ T-cell receptor (TCR) does not have some basal binding affinity for MHC, then the T cell dies.

Despite two decades of study, the underlying mechanism that accounts for MHC restriction by T cells has remained unclear and, to some extent, controversial. In the last several years, two mechanisms that could account for MHC restriction and positive selection have made their way to the forefront. One mechanism involves observations that germline-encoded TCR variable (V) regions appear to have evolved residues that interact with the MHC. The other mechanism relies on a signalling requirement: the TCR/CD3 complex must be brought together with the co-receptors CD4 or CD8 upon co-binding of the MHC product. While in principle one could make the case that one of these proposed mechanisms is more important than the other, various considerations (including the low affinity threshold for positive selection and the diversity of TCRs) lead to the conclusion that both mechanisms are important.

Major recent advances
Germline-encoded TCR regions evolved to bind MHC products
Based on various studies, it was reasonable to expect that the structures of a collection of TCR:pepMHC complexes would reveal a clear mechanistic basis of MHC restriction [3-6]. For example, there could have been contacts between conserved TCR residues and conserved regions of MHC and these interactions could have accounted not only for MHC restriction, but for the ability of a TCR to distinguish class I from class II MHCs. To the surprise of many, there were few discernable atomic interactions that were conserved, although there was a conserved, roughly diagonal docking pattern of TCR on MHC [7-9].

In hindsight, these structural analyses were complicated by the diversity of TCRs and the variety of pepMHC ligands that were represented among the complexes. Once several TCR:pepMHC structures that shared TCR regions were compared, Garcia and colleagues [10-12] were able to identify a few conserved pairs of interactions (TCR:MHC) that represented germline-encoded recognition motifs. Several amino acid residues of the complementarity determining region 1 (CDR1) and CDR2 loops contacted the helices of the MHC and these interactions were important energetically. Accordingly, it was proposed that each V region might have evolved a few key
residues that contribute binding energy with the helix of an MHC molecule (Figure 1a,b). In this model, it has been suggested that the same V region might have evolved different contacts with a different allele, as in the case of the 2C TCR recognizing Kb compared with Ld [13]. To date, these TCR regions have focused mostly on the Vb, although there is some evidence that Va regions may also be involved [5,14]. Thus, the conserved diagonal orientation of TCRs on MHC could be a consequence of these evolution-driven V-region interactions with the corresponding helices of the class I or class II MHC ligands.

Kappler, Marrack, Gapin, and their colleagues soon verified this result at the structural level [15,16] and provided a comparative analysis of conserved amino acid residues among the repertoire of V-region genes [14]. In a key follow-up to the structural studies, they recently reported that the mutation of several of these conserved residues in Vβ8.2 (see ‘A case for both mechanisms’ section below) also had a significant quantitative impact on the positive selection of thymocytes [17], providing evidence of the physiological importance of these residues in interactions with MHC.

Finally, in a series of studies by Rossjohn, McCluskey, and colleagues [18], it is clear that the biased usage of TCR gene segments against a specific pepMHC is a product of many factors, including the antigen peptide itself and the MHC-restricting element. In a recent study comparing the structures of two Epstein-Barr virus (EBV)-specific TCRs, they showed that the process of negative selection (in their example, against an MHC allotype) had a significant impact on the ultimate repertoire of EBV-reactive TCRs that emerged [19]. A corollary of these findings is that evolution-driven TCR V-region binding to particular MHC alleles could be so strong as to lead to the deletion of T cells bearing these TCRs in the thymus. It is important to note that this same group has shown that at least some TCR:pepMHC interactions have energetic profiles that focus more on CDR3 interactions rather than those of CDR1 and CDR2 [20]. Thus, a strict evolution-based model of germline V-region interactions for all TCRs may be an overgeneralization. In these cases, the co-receptor mechanism described below may be more critical.

**CD4 and CD8 co-receptors ensure MHC restriction of TCRs**

An alternative mechanism that could drive the conserved docking orientation, and one that (in principle) is consistent with the absence of universally conserved atomic interactions, involves the requirement for co-receptors CD4 and CD8 [21,22]. Recently, Singer and colleagues [23] generated mice deficient in CD4, CD8, class I MHC, and class II MHC and showed that the mice contained a diverse repertoire of T cells that were not restricted by MHC. To account for this, they proposed that sequestration of the lymphocyte-specific tyrosine kinase (Lck) by CD4 and CD8 can dictate selection for TCR:MHC binding by preventing signalling by non-MHC ligands.

In this model, only TCRs that bind to an MHC molecule will be capable of bringing the CD4 or CD8 molecules into proximity with the TCR/CD3 complex, thereby allowing the associated Lck to initiate the signalling process through CD3 (Figure 2a). Those TCRs that bind to non-MHC molecules would not be capable of recruiting CD4/Lck or CD8/Lck to the TCR/CD3 complex (Figure 2b). If one assumes that co-receptor and/or CD3
must be positioned sterically to allow simultaneous binding of the TCR and co-receptor to the same MHC molecule, then one could imagine the need for a conserved diagonal imprint of TCR on the pepMHC.

**A case for both mechanisms: affinity threshold of positive selection, TCR diversity, and binding energetics**

Which of these two mechanisms is more important for MHC restriction by T cells? The discussion to follow is based on two primary considerations. First, the TCR:MHC interaction affinity required for positive selection has been evolutionarily set to be quite low [24]. Second, the extensive diversity of TCRs, like antibodies, is likely to allow low-affinity interactions with many different ligands (sometimes referred to as polyspecificity [25]).

As indicated above, the affinity threshold for positive selection has been estimated to be quite low (in the range of 10^4/M) [26,27]. Thus, thymocytes have evolved an exquisite sensitive mechanism for guiding the selection process [28]. This low affinity threshold allows a set of minimal conserved interactions between TCR and MHC to be effective. In this regard, it is worth considering whether the proposed evolution-driven atomic interactions (for example, Y46 and Y48 of mouse Vβ8.2) contribute sufficient binding energy to drive positive selection. Alanine scans of the 2C TCR, which contains Vβ8.2 and has been studied in detail structurally [13,29,30], have been performed for binding to both a syngeneic ligand K^d^ and an allogeneic ligand L^d^ [31,32]. The binding energies associated with the two key CDR2β residues Y46 and Y48 (Y48 and Y50 in [31,32]) were each shown to be 1.5 kcal/mol or greater (that is, no binding was observed for the alanine mutants) (Figure 1c). Another residue in CDR2β of Vβ8.2 which has been shown to be important in class II binding, E54, was actually shown to have a negative effect on binding to QL9/L^d^ (residue E56 in [31,33]), and mutation of this residue to alanine had a diminished effect on positive selection of CD8^+^ cells in the recent work [17].

The net effect of the two residues Y46 and Y48 (assuming no cooperative effects) would be at least 3 kcal/mol. Residue N29β (N31β in [31]) also had an impact of 1.5 kcal/mol, and several Vα3.1 CDR1 and CDR2 residues had significant effects. Are the binding energies associated with only a few atomic interactions sufficient to make a substantial contribution, perhaps even the dominant contribution, to positive selection? Based purely on the TCR:MHC affinities reported to be responsible for positive selection, the answer is yes. Thus, at a K_A value of 10^4/M, the binding free energy of positive selection interactions would be in the range of 5.4 kcal/mol (calculated from ΔG = −RT ln K_A). Even the interactions associated with only Y46, Y48, and N29β would contribute the majority of this binding energy. Clearly, the basal binding energies associated with conserved interactions would be influenced, either negatively or positively, by adjacent CDRs (including CDR3s) or by the peptide bound to the MHC product [18,20,34,35].

What would be the probability that a TCR would have a basal binding affinity of 10^4/M for an MHC molecule if there were not an evolutionarily-based selection of V region:MHC interactions? While there are no data to provide guidance here, it would seem that this would be a highly infrequent event and that reliance on random chance would be a considerable waste in terms of the number of thymocytes necessary to yield such interactions. Furthermore, the conserved diagonal docking angle indicates that orientation is not random, further reducing the probability of a chance encounter with adequate affinity between TCR and MHC (that is, in just the right orientation).

Notwithstanding the arguments in favor of evolved V region:MHC binding interactions, the low affinity threshold of positive selection also requires that a mechanism be in place to ensure that TCR interactions with non-MHC ligands do not lead to positive selection. The very same TCR diversity that allows a broad response against foreign antigens would enable such interactions.
While we are unaware of any studies that have assessed the frequency of such reactivities of the TCR repertoire, it is useful to examine what we know about this with antibodies, since germline (non-mutated) antibodies are similar to TCRs in terms of diversity. It has been reported that a single germline antibody could react with up to 1% of a protein library derived from cDNA [36]. (Note: the affinities of these interactions were not reported, but it is reasonable to guess that they were at least 10^3/M or binding would not have been detected.) Furthermore, it has been shown that diversity in a single CDR3H (CDR of the antibody heavy chain) region (that is, identical germline regions) was capable of generating reactivity with virtually any antigen tested [37].

Accordingly, it is reasonable to predict that TCRs would have a high frequency of low affinity for other cell surface molecules (consistent with the observations in [23]). The low affinity threshold necessary for positive selection makes it even more likely that such interactions would occur. Thus, it is imperative that a precise mechanism such as CD4/CD8 co-receptor sequestration of Lck be in place to avoid positive selection of TCRs with such reactivities, even if the V-region repertoire has been evolutionarily selected for interactions with MHC.

Future directions
To further support the concept of evolution-driven V region:MHC interactions, it will be important to examine many more structures of TCR:pepMHC complexes, focusing on TCRs with the same V region (Vα and/or Vβ) in complexes with diverse ligands. These ligands should include different peptide bound to the same MHC, different MHC alleles, and both class I and class II MHCs. It would also be useful to examine TCRs isolated through in vitro selections of germline V-region libraries [38] for binding to MHC products, in the absence of co-receptors. This could reveal the role of CD4 or CD8 in guiding the docking orientation (for example, if the diagonal were not observed, one would predict a significant role of CD4 and CD8). Finally, additional thymic development studies with TCRs containing selected point mutations [17] would assess how broadly these findings can be extended to other V regions.

Abbreviations
CDR, complementarity determining region; EBV, Epstein-Barr virus; Lck, lymphocyte-specific tyrosine kinase; MHC, major histocompatibility complex; pepMHC, peptide complexed with an MHC product; TCR, T-cell receptor; V, variable.

Competing interests
The author declares that he has no competing interests.

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