Revealing the TCR bias for MHC molecules
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Adaptive immunity has long been considered the hallmark trait associated with jawed vertebrates. Although unconventional forms of adaptive immunity, such as variable lymphocyte receptors in lampreys (1) and CRISPR/Cas in bacteria (2), have recently gained recognition, somatic recombination generating unique antigen receptors provides diversity unparalleled by other forms of immunity. On T cells, the T-cell antigen receptor (TCR) is one such receptor comprising α- and β-polypeptide chains (each of which is produced via DNA recombination), which together provide a combinatorial diversity estimated to exceed potentially 10^15 different receptors (3). As T cells develop and mature in the thymus, they undergo a process called selection, which grooms them for mounting appropriate immune responses upon future antigen exposures in peripheral tissues. Intriguingly, the vast majority of TCRs recognize linear peptides bound to MHC molecules during both development and immune responses (4, 5). Upon first identifying this phenomenon of MHC-restriction, investigators were immediately prompted to study and understand the molecular basis underlying this bias (4–6). Do TCRs bear regions in their protein structures to bias their interaction with MHC and make the selection process more efficient? Or are TCRs inherently polyspecific and it is the process of selection that forces their specificity for MHC? Despite years of work exploring reasons for this predisposition, a clear consensus remains elusive. In PNAS, Parrish et al., using a sensitive in vitro system, provide new evidence to suggest that TCRs possess an inherent bias to recognize MHC molecules (7).

Forty-five years have passed since Niels Jerne proposed the hypothesis that TCR molecules might have undergone coevolution with histocompatibility molecules (8). Since then, a number of studies have supported this idea. Recently, when the Bendelac group decided to pair unselected TCRs chains with random TCRβ chains in vivo, they observed that 15% of these TCR pairs were able to undergo selection and by extension, interact with MHC (9). Similarly, in an earlier study by the Raulet group, 30% of preselection T cells expressed TCRs that interacted with different MHC alleles in vitro, suggesting an intrinsic propensity for TCRs to react to MHC (10). A further implication from these studies was that TCRs likely have some conserved regions that promote interactions with MHC over other surface proteins. Pursuing this line of thought, certain germ line-encoded residues in some TCRα and TCRβ chains were found to be crucial for MHC recognition by both structural and functional analyses (5, 6, 11). By studying how a given TCR interacts with structurally-unrelated peptides presented by the same MHC molecules, the Garcia group recently illustrated the imprint of TCR–MHC coevolution at the molecular level. Their analysis of 12 independent TCR–pMHC crystal structures, involving the same TCR and the same MHC molecule, revealed a limited number of possible docking modes for a TCR interacting with diverse pMHC complexes (11). Importantly, the same germ line-encoded TCR residues were involved in three discrete binding solutions. If germ line-encoded TCR residues are indeed important in imposing interactions with MHC molecules, their experimental disruption should greatly affect the development and response of most T cells.

Indeed, when we mutated one TCRβ variable region (Vβ) residue implicated in conserved interaction with MHC molecules to alanine and used this mutant TCRβ chain to interrogate T-cell selection in vivo, over 90% of selection was abrogated (12). Surprisingly, this residue is conserved as far back evolutionarily as the shark (13). By replacing a murine TCRβ chain from a MHC-restricted TCR with the orthologous one from the shark, we continued to observe a response to murine MHC by the chimeric TCR, demonstrating that TCRs from divergent species possess a reactivity even to xeno-MHC molecules (13). Although evidence supporting the coevolutionary hypothesis continues to mount, alternative hypotheses have been put forward to explain MHC-restriction (14, 15). Thus, whether the TCR is inherently MHC-reactive remains a debated topic.

One major obstacle preventing immunologists from directly measuring TCR–MHC interactions from preselection TCRs has been the low affinities these
TCRs have for peptide–MHC (pMHC) complexes. Even postselection TCRs tend to have low affinities, usually in the micromolar range, for their cognate pMHC ligands (16). These measurements also do not shed light on the strength of potentially conserved TCR–MHC interactions because the peptide contributions to the TCR–pMHC interaction are hard to uncouple from the TCR–MHC contributions. Thus, a sensitive system is vital to elucidate any conserved interactions. Some intricate tools have been developed to measure specific TCR–pMHC complex interactions, such as the biomembrane force probe (17) and optical tweezers (18), but have not yet been widely adopted. Instead, Parrish et al. use a cellular biomembrane force probe (17) and optical tweezers (18), but have not yet been widely adopted. Instead, Parrish et al. use a cellular approach to measure the readout of the interactions (7). The authors took advantage of the fact that having a high local concentration of Lck, a kinase responsible for transducing TCR-mediated signals, around the TCR helps boost the T-cell response (19). Parrish et al. (7) introduced a CD4–Lck fusion protein into a TCR− T-cell hybridoma because CD4 is in close proximity to the ITAMs. Parrish et al. (7) expressed Lck cells expressing these TCRs (D) into a TCR− MHC complexes on their surface were cultured in the presence of the various hybridomas, and a functional readout of hybridoma activation was measured by its production of the cytokine IL-2. In the cartoon, signaling is depicted by the lightning arrow and absence of signaling is depicted by the no-signal sign. Any combination of peptide–MHCII complexes at the surface of the antigen-presenting cells lead to the stimulation of the TCR-expressing hybridomas. This stimulation was MHC-dependent because it could be blocked by the addition of anti-MHC mAbs to the cultures.

Using the model TCRs SC.C7 [specific for a moth cytochrome c (MCC)-derived peptide presented by I-E^d] and OT-II (specific for an ovalbumin-derived peptide presented by I-A^b), Parrish et al. (7) found, as expected, that CD4–Lck cells expressing these TCRs responded far more sensitively to agonist ligands than WT cells expressing the same TCRs. Next, they set out to determine the extent of sensitivity by stimulating the cells with low-affinity ligands, which usually do not elicit strong responses in WT cells. Because SC.C7 is a widely studied TCR, its antigenic peptide was modified in numerous ways allowing for various altered peptide ligands, each of which stimulates WT SC.C7 T cells to a different degree. Not only did the CD4–Lck SC.C7 cells respond to a weak agonist ligand, they also responded to an antagonistic ligand, against which WT SC.C7 cells have repeatedly been shown to be unresponsive. Perhaps even more unexpectedly, the cells responded to an irrelevant peptide presented by I-E^d, suggesting that the response was peptide-independent. This phenomenon was reproduced when OT-II TCR− cells were cocultured with I-A^b-expressing antigen-presenting cells presenting noncognate peptides. Based on these results, Parrish et al. (7) predicted that their cells were sensitive enough to be stimulated by conserved TCR-MHC interactions and decided to test this further.

Not all of the residues in a peptide presented by MHC are TCR-exposed because some of them are required for proper anchoring to the MHC molecule itself. When Parrish et al. (7) replaced the TCR-exposed residues in the peptides with alanines, both the SC.C7 and OT-II CD4–Lck cells continued to be stimulated in a MHC-restricted manner. Interestingly, this response was not MHC-allele specific because OT-II hybridomas responded to I-E^d and SC.C7 hybridomas responded to I-A^b. The existence of alloreactivity has long been considered as evidence for TCR-MHC coevolution because some TCRs respond to multiple MHC alleles even though they were selected on one because of degenerate recognition of MHC molecules by conserved regions in the TCR (20). This experiment (7) further that claim by providing functional data as evidence.

Finally, in an experiment exemplifying the bias TCRs have for MHC, Parrish et al. (7) mixed and matched the TCRα and TCRβ chains from different TCRs. In doing so, they could test whether these chimeric TCRs could still react to MHC even though the subunits were from unique TCRs and a priori would create a new specificity. However, these in vitro-generated TCRs responded specifically to different MHC alleles as robustly as the original TCRs. Overall, this study lends substantial credence to the coevolutionary hypothesis because several experiments support the notion that TCR molecules are intrinsically drawn to MHC irrespective of the MHC allele or the TCRα/β chains involved (Fig. 1).

Although the study claims, with strong evidence, that the TCRs used here are predisposed to recognize MHC, how do these data fit with the previously aforementioned data (9, 10) demonstrating that although some TCRs might display a bias, the biased

### TCRα and TCRβ Shuffling

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**Fig. 1.** The vast majority of TCRs recognize peptides bound to proteins of the MHC. TCRs expressed by CD4+ T cells (blue) usually recognize peptides presented by MHCII molecules (green) (A), whereas TCRs expressed by CD8+ T cells (orange) recognize peptides presented by MHC molecules (pink) (B). The earliest biochemical modifications that occur upon ligation of the TCR in model T-cell lines, hybridoma, and cultured primary T cells are phosphorylation of the tyrosine residues on the TCR-associated cytoplasmic CD3 and ζ-chains by the nonreceptor tyrosine kinase Lck. The tyrosines are part of the ITAMs. ITAMs are contained as single copies within the CD3ε, CD3δ, and CD3γ chains and as three copies within the ζ-chain. The coreceptors CD4 and CD8 promote signaling by bringing Lck in close proximity to the ITAMs. Parrish et al. (7) expressed either MHCII-restricted TCRs (C) or MHCII-restricted TCRs (D) into a TCR− T-cell hybridoma. They also expressed TCRs of unknown specificities by shuffling the TCRα and TCRβ chains of various TCRs (E). Each hybridoma expressed a CD4–Lck fusion protein to increase the sensitivity of the cells to TCR stimulation. Antigen-presenting cells expressing various combinations of peptide–MHCII complexes on their surface were cultured in the presence of the various hybridomas, and a functional readout of hybridoma activation was measured by its production of the cytokine IL-2. In the cartoon, signaling is depicted by the lightning arrow and absence of signaling is depicted by the no-signal sign. Any combination of peptide–MHCII complexes at the surface of the antigen-presenting cells lead to the stimulation of the TCR-expressing hybridomas. This stimulation was MHC-dependent because it could be blocked by the addition of anti-MHC mAbs to the cultures.
TCRs do not represent the majority of the repertoire? It is true that as a consequence of the nature of the studies a sufficiently sensitive readout might have been unlikely because one of the studies was conducted in vivo (9), whereas the other did not attempt to boost sensitivity as Parrish et al. (7) did in vitro (10). However, in both of those studies, either one or both of the chains used in their TCRs were derived from the preselection repertoire, making their claims for an intrinsic TCR bias stronger. In the present study, Parrish et al. (7) exclusively use TCRs that have already been selected on MHC molecules and might contain within them the appropriate recognition motifs to maintain conserved contacts to numerous MHC molecules and alleles. Would they observe a similar response profile if they used TCRs from the preselection repertoire? Additionally, all of the observed responses by Parrish et al. were directed against MHCI. Although they use MHCI-restricted TCRs in their system and demonstrate MHCI reactivity of those TCRs, they do not conduct the analogous experiment of testing MHCI reactivity of MHCI-restricted TCRs. CD8 molecules do contribute more to MHCI–TCR interactions than do CD4 molecules to MHCI–TCR interactions (21). So perhaps MHCI-restricted TCRs interact much more strongly with MHCI than MHCI-restricted TCRs do with MHCI because of a lack of added scaffolding provided by the coreceptor. However, the MHCI-restricted TCRs Parrish et al. (7) used in this study generated as strong a response to MHCI (if not stronger) as MHCI-restricted TCRs, which does not align with the idea that these TCRs have a weaker bias for MHC. One explanation could be that the relatively weak CD4 interactions with MHCI are still sufficient for the covalently linked Lck to be brought into close enough proximity to phosphorylate the CD3 signaling immunoreceptor tyrosine-based activation motifs (ITAMs). In such a setting, even if the ectodomains of the TCR heterodimer are replaced with non-TCR proteins, a signal would continue to be propagated as long as the CD3 ITAMs remain. Thus, the results by Parrish et al. could represent an artifact of their system and be, perhaps, irrelevant to the co-evolutionary hypothesis. Finally, although the authors did not pursue this, it would be of great interest to identify the key residues on these TCRs mediating reactivity to MHC, and if they are the same residues that are important irrespective of the MHC being scanned. The TCR molecule has six loops, called complementarity determining regions (three contributed by TCRα and three contributed by TCRβ), which are the only parts of the protein that interact with the pMHC complex (4–6). The residues can be replaced in each of these loops and MHC reactivity can be measured. Such a potential study would lend insight into more conserved regions of TCRs and how different TCRs have devised unique strategies to interact with their ligands. The present study (7) provides further clues into how 500 million years of evolution has led to a recognition system that is paradoxically both specific and flexible. Although many questions linger and require additional investigation, whether the TCR has an inherent bias for MHC is becoming an answerable one.