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Subtle changes in TCRα CDR1 profoundly increase the sensitivity of CD4 T cells

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ABSTRACT

Changes in the peptide and MHC molecules have been extensively examined for how they alter T cell activation, but many fewer studies have examined the TCR. Structural studies of how TCR differences alter T cell specificity have focused on broad variation in the CDR3 loops. However, changes in the CDR1 and 2 loops can also alter TCR recognition of pMHC. In this study we focus on two mutations in the CDR1α loop of the TCR that increased the affinity of a TCR for agonist Hb(64-76)/I-E^k by increasing the on-rate of the reaction. These same mutations also conferred broader recognition of altered peptide ligands. TCR transgenic mice expressing the CDR1α mutations had altered thymic selection, as most of the T cells were negatively selected compared to T cells expressing the wildtype TCR. The few T cells that escaped negative selection and were found in the periphery were rendered anergic, thereby avoiding autoimmunity. T cells with the CDR1α mutations were completely deleted in the presence of Hb(64-76) as an endogenous peptide. Interestingly, the wildtype T cells were not eliminated, identifying a threshold affinity for negative selection where a 3-fold increase in affinity is the difference between incomplete and complete deletion. Overall, these studies highlight how small changes in the TCR can increase the affinity of TCR:pMHC but with the consequences of skewing selection and producing an unresponsive T cell.

1. Introduction

TCR:pMHC complex formation needs to exceed a specific binding energy to achieve a T cell response (Davis-Harrison et al., 2007; Gakamsky et al., 2004; Garcia et al., 2001; Holler and Kranz, 2003; Krogsgaard et al., 2003). Structural changes in the TCR are often required to form a stable TCR:pMHC complex, but it is not clear if such conformational changes are necessary for productive signaling through the TCR (Borg et al., 2005; Burrows et al., 2010; Davis et al., 1998; Holler and Kranz, 2004; Qi et al., 2006). It has long been known that the variable CDR loops form the binding footprint for TCR to contact the pMHC complex (Garcia et al., 1999; Huseby et al., 2006). More recently it has been proposed that the TCR maintains germline-encoded affinity for MHC by interactions between key residues on the MHC helices and CDRs 1 and 2 (Adams et al., 2011; Marrack et al., 2008). CDRs 1 and 2 differ in the V gene segments, and it has been shown that introduction of mutations in these CDR loops can result in generation of high affinity TCRs (Chlewicki et al., 2005; Manning et al., 1999), potentially as a consequence of an optimal binding conformation or enhanced TCR:pMHC stability (Adams et al., 2011; Burrows et al., 2010; Dai et al., 2008; Holler and Kranz, 2004; Willcox et al., 1999).

The TCR has inherent specificity for an agonist peptide but can retain binding to some variants of the agonist (Kersh and Allen, 1996). The strength of recognition of an altered peptide ligand (APL) regulates the level of T cell response (Evavold et al., 1992). Some TCR flexibility is critical during T cell development in the thymus as it ensures recognition of foreign antigens during an infection in addition to self peptides during selection. While flexible recognition of pMHC is advantageous, highly promiscuous T cells can inappropriately recognize self-peptides and cause autoimmune disease (Basu et al., 2001; Garcia et al., 2001). Therefore, it remains important to understand the process by which a T cell discriminates between peptides to generate a productive and appropriate immune response.

There is an affinity continuum of a TCR for endogenous pMHC that regulates selection of T cells in the thymus (Daniels et al., 2006). For T cells to be selected, they must have sufficient affinity for endogenous pMHC above the threshold for positive selection but below the threshold for negative selection (Hogquist and Bevan, 1996; Kosmrlj et al., 2008). A complex set of distinct signals regulates positive and negative selection (Alberola-Ila et al., 1996; Gascoigne and Palmer, 2011), tuning T cell responsiveness in the periphery. Negative selection results in apoptosis of T cells that

Abbreviations: APL, altered peptide ligand; CAb, clonotypic Ab for n3.L2 TCR; DN, double negative thymocytes; DP, double positive thymocytes; Hb, hemoglobin; k_{off}, off-rate constant; k_{on}, on-rate constant; MHCII, MHC class II; pMHC, peptide MHC complex; scTCR, single chain TCR; SP, single positive thymocytes; $t_{1/2}$, half-life; T_{reg}, regulatory T cell.

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are highly responsive to self pMHC in the process of central tolerance (Klein et al., 2009; Williams et al., 1999). While the majority of self-reactive T cells are deleted to generate central tolerance, it is thought that regulatory T cells can develop from these self-reactive, high affinity TCRs (Yu et al., 2008). T_{reg} development is not merely a consequence of a high affinity TCR, as specific environmental cues and TCR sequences may also be required (Bautista et al., 2009; Lathrop et al., 2008).

Negative selection is an incomplete process and some high affinity T cells escape to the periphery where they can cause autoimmune disease (Zehn and Bevan, 2006). While failure to eliminate these cells breaks mechanisms of central tolerance, a back up system is in place to prevent autoimmunity. Potentially autoreactive T cells can be rendered unresponsive to antigenic stimulation (De Boer et al., 2003) or deleted in the periphery to establish peripheral tolerance (Williams et al., 1999). Anergy, a state of hyporesponsiveness characterized by low IL-2 production and inhibited proliferation (De Boer et al., 2003), has been induced in naive T cells through lack of costimulation (Schwartz, 1996), and exposure to APLs (Evavold et al., 1993; Klein et al., 2009; Sloan-Lancaster et al., 1993). Anergized T cells downregulate TCR and costimulatory receptors to maintain the hyporeactive state. Because of the dual role of the TCR:pMHC interaction in selection and activation, the propensity for T cells to undergo tolerance may be set in the thymus by the affinity of the expressed TCR for self pMHC.

To understand how slight structural changes affect T cell development and sensitivity, we used a system that compares two TCRs recognizing the same cognate antigen, Hb(64-76)/I-E^k. In the mouse, the Hb protein exists in two naturally occurring allelic variants, Hb^d and Hb^s, such that T cells can develop normally in Hb^s strains and be reactive to cells from Hb^d mice. The n3.L2 TCR was generated against the Hb^d allele of the (64-76) peptide (Evavold et al., 1992). We previously generated a series of mutants of the n3.L2 TCR using a yeast display system (Weber et al., 2005). These mutants were selected for increased surface stability on yeast or increased binding to Hb(64-76)/I-E^k. Mutations selected for increased affinity to Hb(64-76)/I-E^k were generated in the CDR3 of the n3.L2 TCR. Surprisingly, one mutant, called M2, which had two mutations in the CDR1α loop (K25E and T28S), was selected only for increased surface levels on yeast and not for increased affinity, yet it exhibited an increase in affinity. Here we verify that the two CDR1α mutations in M2 resulted in a 3.7 stronger affinity due to a faster on-rate, as measured by surface plasmon resonance. We generated and used hybridomas and transgenic mice expressing the n3.L2 and M2 TCRs to determine how such a moderate change in the CDR1α loop could alter TCR specificity. The M2 TCR has a broader and more sensitive response to altered peptide ligands of the Hb(64-76) peptide. In addition, the M2 TCR has a stronger association with the I-E^k molecule, relying solely on contacts with the MHCIIβ chain for pMHC recognition. As a consequence of these changes, T cell selection in the thymus was also altered. M2 T cells were negatively selected at a higher rate and completely eliminated when exposed to Hb^d(64-76) as self antigen. Interestingly, the few peripheral M2 T cells that escaped negative selection were rendered anergic. These studies show how subtle changes in the TCR structure that modestly increase the TCR:pMHC affinity can have a profound effect on T cell development and activation.

2. Materials and methods

2.1. Mice and cells

In addition to the new mouse line described below, n3.L2/B6.K TCR transgenic mice, B6.K, Rag1$^{-/-}$, and Hb^d congenic mice were used in these studies. The n3.L2/B6.K mouse was previously generated in the laboratory (Kersh et al., 1998) and was crossed to the Rag1$^{-/-}$ and separately to the Hb^d mice for the studies presented in this manuscript. All mice were bred and maintained in a pathogen-free barrier facility within Washington University in St Louis following a protocol approved by and in accordance with guidelines from the Washington University Division of Comparative Medicine.

In addition to the new hybridoma cell lines described below, CH27 B cells were used to as antigen presentation cells in some of the hybridoma response experiments. CH27 cells were maintained in RPMI + 10% FCS + 1% L-glutamine + 5×10^{-5} M β-2-mercaptoethanol + 0.5% gentamicin at 37 °C and 5% CO_2.

2.2. Generation of the M2 TCR transgenic mouse line

The M2 mouse was generated using the method described for the n3.L2 mouse (Kersh et al., 1998). The n3.L2 V-Jα plasmid was mutated by PCR to express the two amino acid changes in the M2 CDR1α chain. The M2α chain was cloned into the TCR shuttle vector. TCRα and β minigene constructs were coinjected into C57Bl/6 pronuclei in the Washington University Department of Pathology and Immunology's Transgenic Core Facility. Transgenic mice were identified by PCR amplification of the M2α and β transgenes from tail DNA. Expression of the M2α chain was confirmed by sequencing the founders' genomic DNA. One founder expressed both the M2α and β transgenes and was bred to the B6.K strain to provide the selecting MHC. Peripheral CD4 T cells in the M2/B6.K mouse stained with the clonotypic antibody, CAb. M2 and n3L.2 mice were further crossed to a Rag-1$^{-/-}$ background producing n3.L2/B.6K/Rag1$^{-/-}$ and M2/B6.K/Rag1$^{-/-}$ mice. M2/B6.K mice were also crossed to Hb^d congenic mice. Mice were used at 4–8 weeks of age in these studies, unless otherwise noted. All mice were bred and maintained in a pathogen-free barrier facility within Washington University in St Louis following a protocol approved by and in accordance with guidelines from the Washington University Division of Comparative Medicine.

2.3. Flow cytometry

Antibodies used in flow cytometry are commercially available except for the CAb antibody, which was previously generated in the laboratory. CAb is a clonotypic antibody for the n3.L2 TCR. CAb was conjugated with AlexaFluor-647 using an antibody conjugation kit (Invitrogen). FITC-, PE-, PE-Cy7-, PerCP-, APC-, APC-Cy7-, Pacific Blue-, and Pacific Orange-labeled antibodies were used in various combinations. Intracellular labeling of FoxP3 was performed using a kit from eBioscience. Cells were permeabilized and fixed for 30 min followed by washing with permeabilization buffer and antibody labeling with PE- anti-FoxP3. Data collection was performed using a BD FACSCalibur, a FACSCanto, and a customized FACSLSR II. Data analysis was performed using FlowJo software.

2.4. Generation of n3.L2 and M2 hybridomas

n3.L2 and M2 TCRα and β chains were cloned into a p2A retroviral vector with an IRES-GFP tag (pMIIG) developed by the Vignali lab (Holst et al., 2006), which places the α and β chains as a single polypeptide linked by the p2A peptide. No stability mutations were added into the sequence. The 2A peptide (p2A) is cleaved post-translationally, ensuring equal expression of the α and β chains and resulting in efficient expression of transduced αβ TCRs. PlatE cells were transformed with lipofectamine + 30 μg plasmid DNA. Supernatants from PlatE cells containing packaged retrovirus were used to spinfect the 58 $\alpha^-\beta^-$ $CD4^+$ hybridoma cell line. M2 and n3.L2 expressing hybridomas were generated simultaneously, sorted for comparable high GFP expression, and equal expression of the n3.L2