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A single peptide-MHC complex positively selects a diverse and specific CD8 T cell repertoire

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Abstract

Pathogen recognition by T cells is dependent upon their exquisite specificity for self major histocompatibility complex (MHC) molecules presenting a bound peptide. Although this specificity results from positive and negative selection of developing T cells in the thymus, the relative contribution of these two processes remains controversial. To address the relationship between the selecting peptide-MHC complex and the specificity of mature T cells, we generated transgenic mice that express a single peptide-MHC class I (MHCI) complex. We demonstrate that positive selection of CD8 T cells in these mice results in an MHC-specific repertoire. While selection on a single complex is peptide promiscuous, mature T cells are highly peptide-specific. Thus, positive selection imparts MHC and peptide specificity on the peripheral CD8 T cell repertoire.

In the thymus, through the processes of positive and negative selection, T cells develop the ability to respond to pathogen or tumor-derived peptides presented by MHC but remain tolerant to MHC presenting self peptides. Positive selection results from low avidity interactions between the T cell receptor (TCR) and peptide-MHC during thymic development, producing T cells that can recognize self MHC-presented foreign peptide. Negative selection deletes T cells with high avidity for self peptide-MHC. Although peptide was implicated in positive selection of CD8 T cells in vitro (1–6), the extent to which it contributes to the specificity of mature CD8 T cells and the structural relationship between the selecting and activating peptides has not been defined in vivo. To address these issues we generated transgenic mice that express a single peptide-MHCI complex.

MHCI molecules were engineered as single-chain trimers (SCTs) in which peptide, β_2 -microglobulin (β_2m), and the MHCI alpha chain are covalently attached through flexible linkers (fig. S1). SCTs are efficiently expressed, exceptionally stable at the cell surface, resistant to peptide exchange, and retain their native structure required to potently stimulate T cells (7–9). To study CD8 T cell development, we generated transgenic mice with a MHCI promoter driving the expression of SCTs based on the H2-K^b MHCI alpha-chain, and peptides derived from chicken ovalbumin (SIINFEKL, OVA_p) or Vesicular stomatitis virus (RGYVYQGL, VSV_p). We bred these mice to B6 mice deficient in β_2m and MHCI molecules K^b and D^b (3KO) to obtain mice that express only the SCT and not other MHCI molecules (SCT 3KO) (fig. S2A). With this system we analyzed the in vivo development of CD8 T cells in the presence of a single peptide-MHCI complex.

In vivo positive selection on a single peptide/MHC complex results in a diverse and highly specific CD8 T cell repertoire.

To assess CD8 T cell development in SCT 3KO mice, CD8 and CD4 expression on thymocytes and splenocytes was analyzed (fig. S3). In the control 3KO mouse there were very few splenic CD8 T cells ($0.26 \pm 0.14\%$), consistent with their deficiency of expression of MHCI. The number of splenic CD8 T cells in both OVAp/K^b and VSVp/K^b SCT 3KO mice was strikingly reduced ($4.2 \pm 0.9\%$) relative to wild type mice ($36.1 \pm 3.5\%$). These results differ from studies of CD4 T cell development in MHC class II single complex mice, where CD4 T cells are 20–40% of wild type (10–12). The reduced frequency of CD8 T cells in SCT 3KO mice is consistent with positive selection being peptide-specific because SCTs are expressed at levels comparable to native K^b on splenocytes and cortical thymic epithelial cells in wild type mice (fig. S2B and S2C). If positive selection required only the presence of peptide and not a specific peptide, then normal numbers of CD8 T cells would be expected. Despite the significant reduction in CD8 T cells, TCR diversity (TCR V β usage) did not differ from wild type mice (fig. S4), similar to MHC class II single complex mice as well as mice deficient in β_2m , MHCI, or transporter associated with antigen processing (TAP) (11–15).

Remarkably, the CD8 T cells selected by SCT 3KO mice were highly reactive when stimulated with wild type B6 (H2-K^bD^b) cells, as measured by CTL-mediated lysis of labeled target cells (Fig. 1A). This robust response permitted us to test rigorously the MHC and peptide specificity of SCT-selected CD8 T cells. It is possible that the absence of negative selection on multiple MHCI alleles would result in highly MHC cross-reactive CD8 T cells. If so, then a large percentage would be reactive with both K^b and D^b antigens. However, SCT-selected CD8 T cells are almost exclusively K^b-reactive as demonstrated by antibody blocking (Fig. 1A) and selective lysis of K^b expressing targets (Fig. 1B). The preferential K^b reactivity of SCT-selected CD8 T cells supports the notion that MHC restriction is based on self recognition during positive selection (16). Importantly, negative selection occurs normally in SCT 3KO mice because T cells from these mice did not react to self peptide-MHCI complexes (fig. S5).

We further analyzed for MHC cross-reactivity with H2^k and H2^d alleles. Polyclonal CD8 T cells from control (K^b-/-D^b+/+) and SCT 3KO mice stimulated with K^b+/+D^b-/- splenocytes exhibited low MHC cross-reactivity (Fig. 1C). Furthermore, K^b-reactive T cell clones from SCT 3KO mice were not more MHC cross-reactive on H2^d and H2^k alloantigens than clones generated from control (K^b-/-D^b+/+) mice (Fig. 1D). We conclude that SCT 3KO CD8 T cells are highly MHC specific.

The observation that single peptide-selected cells were highly reactive with K^b plus endogenous peptides demonstrated that SCT exclude thymic presentation of endogenous K^b ligands, and permitted assessment of the peptide specificity of SCT 3KO T cells. To this end, we identified an extensive panel of naturally processed, K^b-binding peptides by using HPLC fractionation and mass spectrometry (MS). Ninety peptides were selected for analysis based on their K^b binding motif and MS confidence score from a protein database search (Fig 2A). Polyclonal SCT 3KO CTLs were generated and tested by stimulating with all ninety peptides individually by using TAP^{-/-} splenocytes as antigen presenting cells and peptide-treated TAP^{-/-} tumor target cells. As shown in Fig. 2A, 47 peptides were recognized by both strains of SCT 3KO mice, 37 peptides were not recognized by either strain and 6 were recognized by one or the other (differentially recognized). We identified one peptide (RTYTYEKL) shown to be a selecting peptide with structural homology to the agonist peptide, OVA (17). However, computational analyses using Weblogo (18) identified no overall pattern of similarity between the peptide sequences within the groups, consistent with studies demonstrating that selecting and agonist peptides do not have to be structurally related (19,20). The striking lack of sequence homology between the selecting and antigenic peptides is consistent with the fact that positive selection is based on low avidity TCR/peptide-MHC interactions, and TCR cross-reactions are difficult to predict based on peptide sequences alone or known crystal structures of peptide-MHC complexes.

Several of the peptides stimulated CD8 T cells from both OVAp/K^b and VSVp/K^b SCT 3KO mice, raising the possibility that these T cells are degenerate in their peptide recognition. To test this, 6 individual peptides were selected that strongly activated CTLs from both strains of SCT 3KO mice. Primary CTLs were generated from OVAp/K^b SCT 3KO using these peptides, then tested for cross-reaction to 18 other strongly recognized peptides. Polyclonal T cells stimulated with each of the peptides were highly peptide-specific, with minimal cross-reaction (Fig. 3). Thus, CD8 T cells selected by the SCT are highly peptide-specific.

The question remained why a high percentage of the peptides are recognized by both or neither strain of SCT 3KO mice. If the recognition were of contaminating peptides, then responsiveness to the peptides would correlate with affinity of the peptide for K^b. However, the activating and non-activating peptides displayed a similar range of relative binding affinities (Fig. 2B). Furthermore, the evidence strongly indicates that the SCT efficiently excludes exogenous peptides (7). We conclude that the response to common peptides is the result of promiscuity during selection; i.e. a single peptide-MHC complex can select T cells specific for several different peptides, consistent with some (6,12,20,21) but not other (22) studies. Furthermore, two unrelated peptide-MHC complexes can select T cell repertoires with overlapping specificities.

The differentially recognized peptides (Fig. 2A) were further analyzed to determine if disparities in recognition were absolute or due to differences in TCR avidity. The SCT-selected T cells that recognized the peptides did so with high avidity, whereas the same peptide was not recognized by the other SCT-selected T cells, even at high peptide concentrations and at several effector to target ratios (Fig. 4). Thus the difference in peptide recognition between the two strains of mice is absolute. Accordingly, all six of the differentially recognized peptides were stimulatory to T cells from the (OVAp/K^b x VSVp/K^b) F1 SCT 3KO mouse (Fig. 4B and fig. S6), indicating that the response to these peptides was due to positive and not negative selection.

In sum, we provide direct in vivo evidence that the MHC and peptide specificity of the CD8 T cell repertoire is imparted by positive selection. Studies of mice expressing a single class II/peptide complex (10,12,22–25) arrived at conflicting conclusions regarding the role of positive versus negative selection in determining the specificity of the CD4 T cell repertoire (22,24, 25). Our results contrast with the report of Huseby et al., which found that CD4 T cells selected on class II single complex mice are highly MHC and peptide degenerate and concluded that MHC and peptide specificity result from negative selection (25). It is unclear if these differences are due to the approaches used or disparities in how CD4 versus CD8 T cells are selected (26).

Studies using fetal thymic organ culture identified exogenous peptides capable of positive selection in vitro (1–6). This led to different theories regarding the nature of the selecting peptide and its relationship to the agonist ligand (2,4). In our in vivo repertoire analysis, positive selection of CD8 T cells is peptide promiscuous in that a single peptide/MHCI complex can select T cells with disparate peptide specificities, yet once selected the T cells are highly peptide-specific. Furthermore, we show that two different peptide/MHCI complexes select T cells with both overlapping and unique peptide specificities and that there is no structural homology between selecting and agonist peptides. In contrast to the promiscuity of peptide specificity, MHC specificity imparted by positive selection was striking as indicated by the predominance of K^b-restricted T cells after SCT selection. Importantly, once selected all mature CD8 T cells are highly MHC- and peptide-specific, consistent with both being inherent properties of TCR/peptide-MHC interactions (27,28).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

SCT 3KO primary CTLs are K^b-specific and non-MHC cross-reactive. (A) OVAp/K^b SCT 3KO splenocytes stimulated with B6 splenocytes were cultured with RMA (H2^b) target cells in the absence or presence of mAb. Data represent one experiment of 6. (B) OVAp/K^b SCT 3KO anti-B6 CTL clones were cultured with RMA (K^bD^b), RMA.S (*TAP*^{-/-}, H2^b) or R8.15 (K^bD^b) target cells. Data represent one experiment of 3. (C) K^b-reactive CTLs generated from control K^b-/-D^b+/+ splenocytes and SCT 3KO splenocytes, stimulated with K^b+/+D^b-/- splenocytes, were replated with indicated stimulator cells. Production of intracellular IFN- γ by CD8⁺ T cells was measured by flow cytometry. Data represent one experiment of 3. (D) CTL clones were generated from SCT 3KO mice and K^b-/-D^b+/+ mice stimulated with B6. The CTL clones were cultured with RMA (H2^b), P815 (H2^d), or R1.1 (H2^k) target cells. Data are one representative experiment. Clones were assayed 2 to 4 times.

Fig. 2. Recognition by SCT 3KO mice of K^b endogenous peptides complexed with H2-K^b. (A) The sequences of the 90 K^b-binding peptides are listed based on their ability to stimulate polyclonal T cells from the two strains of SCT 3KO mice. Peptides were assayed at least 3 times. (B) K^b binding was measured using an MHC class I surface induction assay as described (29). Binding is plotted as the concentration of peptide that resulted in a threefold increase in the expression level of K^b on RMA.S cells. The bar indicates the mean peptide concentration. Data represent one experiment of 3.

Fig. 3. SCT-selected cells are highly peptide-specific. Splenocytes from OVAp/K^b SCT 3KO mice were stimulated with 6 different peptides, indicated at the top of each panel, and tested with the stimulating peptide and 18 other peptides as indicated. Data represent one experiment of 3.

Fig. 4.

T cell responses to differentially recognized peptides. (A) Primary SCT 3KO CTLs, stimulated with peptides recognized exclusively by OVAp/K^b (SAMVFSAM) or VSV/K^b (YQYSFPEL), were tested for reactivity over a range of peptide concentrations. Data represent one experiment of 3. (B) The same peptides in (A) were used to generate CTLs from OVAp/K^b SCT 3KO, VSVp/K^b SCT 3KO and (OVAp/K^b × VSVp/K^b) F1 SCT 3KO mice and were tested for lysis of peptide-treated RMA.S target cells. Data represent one experiment of 3.