Cell Reports
Article

Low-Affinity T Cells Are Programmed to Maintain Normal Primary Responses but Are Impaired in Their Recall to Low-Affinity Ligands

Karin M. Knudson,[1] Nicholas P. Goplen,[1] Cody A. Cunningham,[1] Mark A. Daniels,[1,*] and Emma Teixeiro[1,*]
[1]Department of Molecular Microbiology and Immunology, School of Medicine, University of Missouri, Columbia, MO 65212, USA
*Correspondence: danielsma@missouri.edu (M.A.D.), teixeiropernase@missouri.edu (E.T.)
http://dx.doi.org/10.1016/j.celrep.2013.07.008

SUMMARY

T cell responses to low-affinity T cell receptor (TCR) ligands occur in the context of infection, tumors, and autoimmunity despite diminished TCR signal strength. The processes that enable such responses remain unclear. We show that distinct mechanisms drive effector/memory development in high- and low-affinity T cells. Low-affinity cells preferentially differentiate into memory precursors of a central memory phenotype that are interleukin (IL)-12R^{lo}, IL-7R^{hi}, and Eomes^{hi}. Strikingly, in contrast to naive cells, low-affinity memory cells were impaired in the response to low- but not high-affinity ligands, indicating that low-affinity cells are programmed to generate diverse immune responses while avoiding autoreactivity. Affinity and antigen dose directly correlated with IL-12R signal input and T-bet but not with Eomes expression because low- affinity signals were more potent inducers of Eomes at a high antigen dose. Our studies explain how weak antigenic signals induce complete primary immune responses and provide a framework for therapeutic intervention.

INTRODUCTION

CD8 T cell memory contributes to the health of an individual by providing protection against re-exposure to intracellular pathogens and tumors. High-affinity interactions between the T cell receptor (TCR) and peptide-MHC ligands lead to acquisition of effector function and generation of long-term memory (Corse et al., 2011; Gourley et al., 2004). Interactions with low-affinity self-peptide MHC molecules are necessary for the survival of naive peripheral T cells (Jameson, 2005; Sprent et al., 2008). Strikingly, these low-affinity interactions are sufficient for the acquisition of effector function and memory differentiation (Zehn et al., 2009). This challenges the notion that central tolerance leads to complete nonresponsiveness to self and suggests an important role for low-affinity T cell interactions in the peripheral lymphoid compartment.

The biological significance of generating low-affinity memory T cells becomes evident when considering the following: first, low-affinity T cells are significant contributors to the effector response against autoantigens, tumors, and pathogens (Pardoll, 2002; Sabatino et al., 2011). Second, low-affinity T cells can participate in the maintenance of a diverse memory repertoire (Intlekofer et al., 2006). This may be especially relevant with age considering the drop in thymic output upon puberty (Ahmed et al., 2009; den Braber et al., 2012; Hale et al., 2006; Naylor et al., 2005). Additionally, this may be advantageous to control microbes that mutate T cell epitopes in an effort to evade the immune response (van Gisbergen et al., 2011). Therefore, understanding a T cells' ability to respond to low-affinity ligands is a critical aspect of T cell immunity.

How low-affinity TCR-peptide-MHC interactions lead to T cell effector and memory differentiation is not known. Studies of TCR signaling in response to low-affinity ligands have demonstrated that changes in potency are quantitatively and qualitatively unique and cannot be explained by simple antigen dose effects. Seminal studies with altered peptide ligands have shown that the TCR has the ability to trigger specific signaling events that support some functions but not others depending on the nature of the TCR-peptide-MHC interaction (Daniels et al., 2006; Davis et al., 1998; Jameson and Bevan, 1995; Jameson et al., 1993; Koniaras et al., 1999; Madrenas et al., 1995; Sloan-Lancaster et al., 1994a, 1994b). Therefore, low-affinity TCR interactions do not necessarily imply an overall dampening of TCR signal but rather may lead to qualitative changes in signal output that are sufficient to support unique T cell responses. Additionally, in the context of an immune response, TCR and proinflammatory signals cooperate to regulate T cell differentiation (Mescher et al., 2006). However, whether this is dependent on TCR affinity is poorly defined.

The T-box transcription factors T-bet and Eomes are crucial for effector and memory differentiation in T cell immune responses (Intlekofer et al., 2005). Both are induced by high-affinity TCR ligands and modulated by the proinflammatory cytokine interleukin (IL)-12 (Takemoto et al., 2006). Studies with T-bet and Eomes-deficient mice suggest that both can act redundantly to induce effector functions. However, although

T-bet suppresses Eomes expression and promotes terminal short-lived effector differentiation, Eomes supports self-renewal of antigen-specific memory cells, without affecting T-bet expression (Banerjee et al., 2010; Intlekofer et al., 2007). Thus, T-bet and Eomes expression profiles may be useful to predict the efficiency of generating a memory pool. Curiously, there is little insight into how TCR affinity impacts T-bet and Eomes expression profiles for effector/memory differentiation.

Central and peripheral tolerance mechanisms regulate the delicate balance between protective immunity and autoimmunity during an immune response. Central tolerance is regulated by a sharp border that defines the difference between positive and negative selection (Daniels et al., 2006; Naeher et al., 2007). This is an imperfect process, and there is considerable evidence for self-reactive clones that escape negative selection and can cause autoimmunity (Enouz et al., 2012; Hogquist et al., 2005; Liston et al., 2005). These cells, then, are kept in check by peripheral tolerance mechanisms to avoid autoimmunity. Among these mechanisms, transforming growth factor (TGF)-β signaling is critically important to control low-affinity T cell responses (Gorelik and Flavell, 2002; O'Sullivan et al., 2011; Zhang and Bevan, 2012). Therefore, for low-affinity T cells to develop effector function and memory without causing autoimmunity, one could envision a scenario where the quantity and/or quality of TCR signals regulate susceptibility to inflammation and tolerance, shaping memory programming in a way that maximizes diversity of the memory pool and limits induction of autoimmunity.

We tested this hypothesis with the OT-I TCR transgenic system. We compared immune responses to the strongest negative selecting peptide, N4 (agonist), and the strongest positive selecting ligand, Q4H7 (pseudo-self), and studied how differences in TCR affinity influence T cell memory programming and the susceptibility of T cells to inflammatory and regulatory signals. We show that high- and low-affinity T cells are different in their effector/memory programming. We provide mechanistic data about how TCR affinity, IL-12, and TGF-β signals interplay to modify this distinct program. We propose that quantity (antigen dose) and quality of the signal triggered by the stimulating TCR ligand not only determine the nature of the primary immune response but also have a profound impact on programming the sensitivity to low-affinity ligands during the secondary immune response.

RESULTS

T Cells Responding to High- and Low-Affinity Ligands Are Phenotypically Different

Numerous studies have led to the general conclusion that activation of a T cell is proportional to TCR ligand affinity (Gottschalk et al., 2010). Contrary to this, low-affinity T cells significantly contribute to the effector response against autoantigens and pathogens by developing into effector and memory cells (Zehn et al., 2009), which may pose a risk for autoimmunity. Thus, we sought to understand how very-low-affinity TCR ligands (in the range of positive selectors) are able to support these T cell fates. For this, we compared the responses against the cognate antigen for the OT-1 TCR, N4 (SIINFEKL), and the strongest positive selecting ligand Q4H7 (SIIQFEHL). Q4H7 selection potential was determined by analysis of a large panel of N4 variants in fetal thymic organ culture. It was found to have the highest affinity and potency and still induce positive selection of OT-1 T cells (Daniels et al., 2006). We deliberately chose Q4H7 because it would potentially present the highest risk for a positive selecting ligand to induce dangerous autoreactivity. As expected, *Listeria monocytogenes* (LM) expressing Q4H7 induced a much weaker response than LM-N4. Both LM-N4 and Q4H7 T cell responses gave rise to stable T cell memory pools. However, the ratio of effector to memory in response to LM-N4 and LM-Q4H7 were different (Figure 1A). As expected, 5%–10% of the cells present at the peak of the LM-N4 response survived contraction and established the memory pool (Williams and Bevan, 2007). Interestingly, in LM-Q4H7 responses, an approximately 6-fold higher ratio of T cells present at the peak ended up in the memory pool. This suggests that although low-affinity effector cell numbers were diminished, the percentage of low-affinity memory cells generated was comparatively increased. Looking further, we measured IL-7Rα expression as a surrogate marker for memory precursors during the immune response. T cells responding to LM-Q4H7 (low affinity) exhibited a substantially higher percentage of memory precursors than their LM-N4 (high affinity) counterparts (Figure 1B, left) (King et al., 2012). Furthermore, low-affinity responders expressed higher levels of IL-7Rα than naive or high-affinity responders at the peak of the response (Figure 1B, right), supporting the idea that antigen controls IL-7Rα levels (Hammerbeck and Mescher, 2008). IL-7R^{hi} cells responding to LM-Q4H7 exhibited an activated phenotype (CD44^{hi}, Granzyme B^{hi}, etc), discarding the possibility they were unstimulated naive T cells (data not shown). The percentage of CD62L^{hi} cells was significantly higher in response to LM-Q4H7 than to LM-N4, suggesting a correlation between TCR affinity and central memory phenotype (Figure 1C). Yet, the number of cells with IL-7R^{hi} and CD62L^{hi} phenotypes was significantly lower in low-affinity T cells (Figure S1). Low-affinity IL-7R^{hi} CD62L^{hi} T cells were present at similar frequencies in spleen, lymph nodes, and bone marrow, indicating they do not preferentially accumulate in one of these organs. Interestingly, a higher frequency of low-affinity cells remained CD27^{hi} (Figure 1D), correlating with the idea that its expression is required for low-affinity T cells to develop into memory (van Gisbergen et al., 2011). Collectively, these data show that T cells responding to high- and low-affinity TCR ligands are phenotypically different during the course of listeria infection and low-affinity T cells primarily develop into memory precursors with a central memory phenotype.

Low-Affinity TCR Ligands Trigger a Distinct Effector/Memory Program

These results suggest that low-affinity T cells preferentially differentiate into memory at the expense of effectors or, alternatively, they are less efficiently recruited into the effector pool whereas memory development remains intact (or both). To distinguish between these possibilities, we investigated how low-affinity TCR ligands support effector and memory programming by examining the expression of T-bet and Eomes (Kallies, 2008; Rutishauser and Kaech, 2010). Naive OT-1 T cells were stimulated with 20 nM of N4 or Q4H7 peptide pulsed antigen-presenting cells (APCs) and T-bet and Eomes expression was

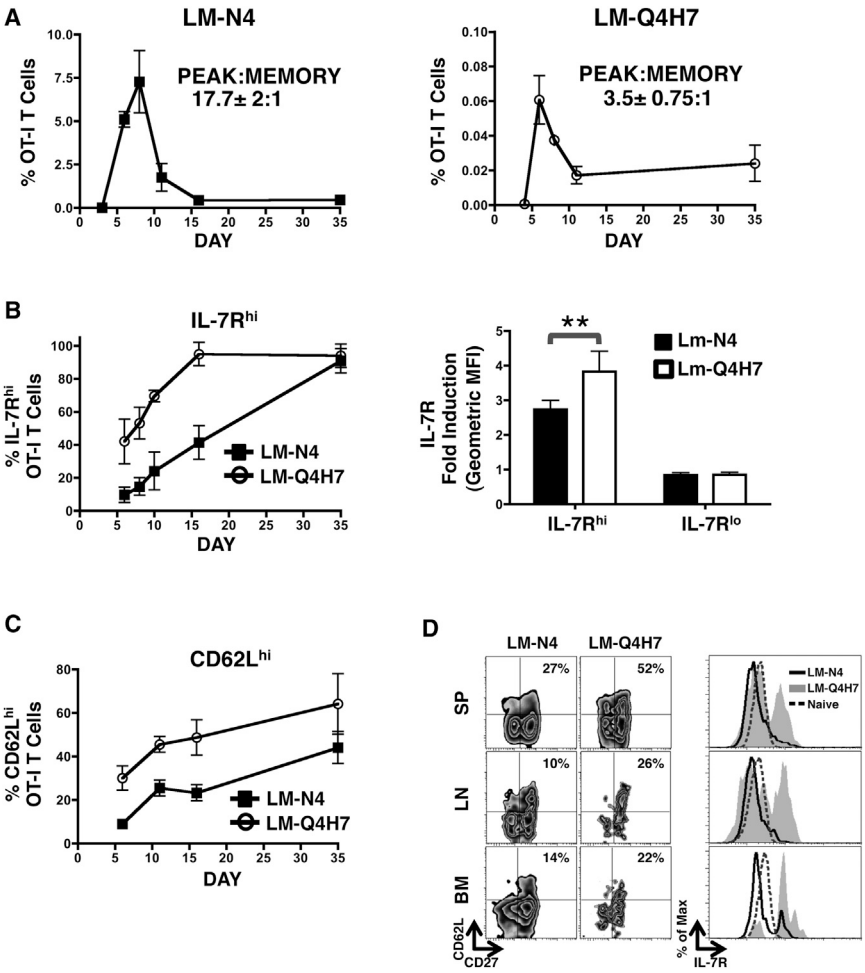


Figure 1. Low-Affinity Cells Preferentially Differentiate into Central Memory Precursors

(A–D) OT-I T cells (1×10^3) were transferred into congenic B6 hosts and challenged with 1×10^3 colony-forming units (cfu) LM-N4 or LM-Q4H7.

(A) Frequency of cells during the immune response to LM-N4 (left) or LM-Q4H7 (right) in blood. Ratio shown as mean ± SD.

(B) Frequency of cells expressing IL-7R^{hi} in blood (left). For all data points except day 35, $p < 0.0001$. Bar graph represents expression of IL-7R (mean fluorescence intensity, MFI) in IL-7R^{hi} and IL-7R^{lo} populations in lymph nodes at day 6 (right). Geometric MFIs were normalized to a naive control.

(C) Frequency of cells expressing CD62L^{hi} in blood. For all data points except day 35, $p < 0.0007$. Day 35, $p < 0.05$.

(D) CD27, CD62L, and IL-7R expression in lymph nodes, spleen, and bone marrow at day 6.

All graphs show mean ± SD and represent $n \geq 3$ independent experiments, with $n \geq 3$ mice per experiment (*$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$). See also Figure S1.

measured from days 1–4. We found that T cells responding to Q4H7 expressed very low levels of T-bet (Figures 2A). In contrast, low-affinity T cells expressed Eomes at much higher levels than high-affinity cells (Figure 2B). This was not exclusive to Q4H7, because we observed similar results with a lower affinity pseudo-self TCR ligand (Q7) that we have previously described in (Daniels et al., 2006) (Figure S2). Similar frequencies of CD25$^+$ and CD44$^+$ cells were observed for Q4H7 (or Q7) and N4 stimulation, ruling out that the differences in T-bet and Eomes expression resulted from unstimulated naive OT-1 T cells (Figure S2). Thus, as TCR affinity decreased, T-bet expression diminished, whereas the induction of the memory-associated factor Eomes increased.

Because inflammation, and more specifically IL-12, is required for CD8 effector and memory development (Xiao et al., 2009), we also tested whether high levels of exogenous IL-12 differentially affected the induction of T-bet and Eomes depending on TCR affinity. IL-12 increased the expression of T-bet for T cells responding to low- and high-affinity TCR ligands. However, IL-12 did not enable Q4H7-stimulated cells to reach the T-bet levels induced by N4 (Figures 2C and S2). More importantly, IL-12 did not alter Eomes expression for either ligand (Figures 2D and S2).

Next, we compared Eomes and T-bet expression levels in T cells responding to high- and low-affinity TCR ligands in the context of infection. At the peak of both immune responses, low-affinity cells exhibited a 6-fold greater frequency of cells expressing high levels of IL-7R and Eomes than their high-affinity counterparts (Figure 2E). Furthermore, on a per-cell basis, low-affinity T cells expressed higher Eomes levels than high-affinity cells, confirming the in vitro results. We also observed a higher frequency of low-affinity T cells expressing high levels of Bcl-6, a transcription factor linked to memory development (Crotty et al., 2010; Ichii et al., 2002). Interestingly, T-bet expression in vivo was comparable between high- and low-affinity cells (Figure 2F). Overall, these data show that, in the context of infection, low-affinity TCR ligands are able to induce T-bet levels comparable to high-affinity ligands. More importantly, they are uniquely capable of inducing very high levels of memory transcription factors Eomes and Bcl-6. This suggests that low-affinity TCR ligands induce a transcriptional program that favors memory development.

TCR Signals Differentially Regulate Eomes and T-Bet Expression

It seemed counterintuitive that weak TCR signals could induce T-bet and Eomes equal to or better than strong cognate antigen. Because antigen dose and/or inflammation shape a T cell response, we examined the ability of OT-1 T cells to induce T-bet and Eomes depending on antigen dose, in the absence or presence of excess exogenous IL-12. T-bet expression was directly proportional to TCR ligand affinity and antigen dose. Although N4 was generally better at the induction of T-bet, there

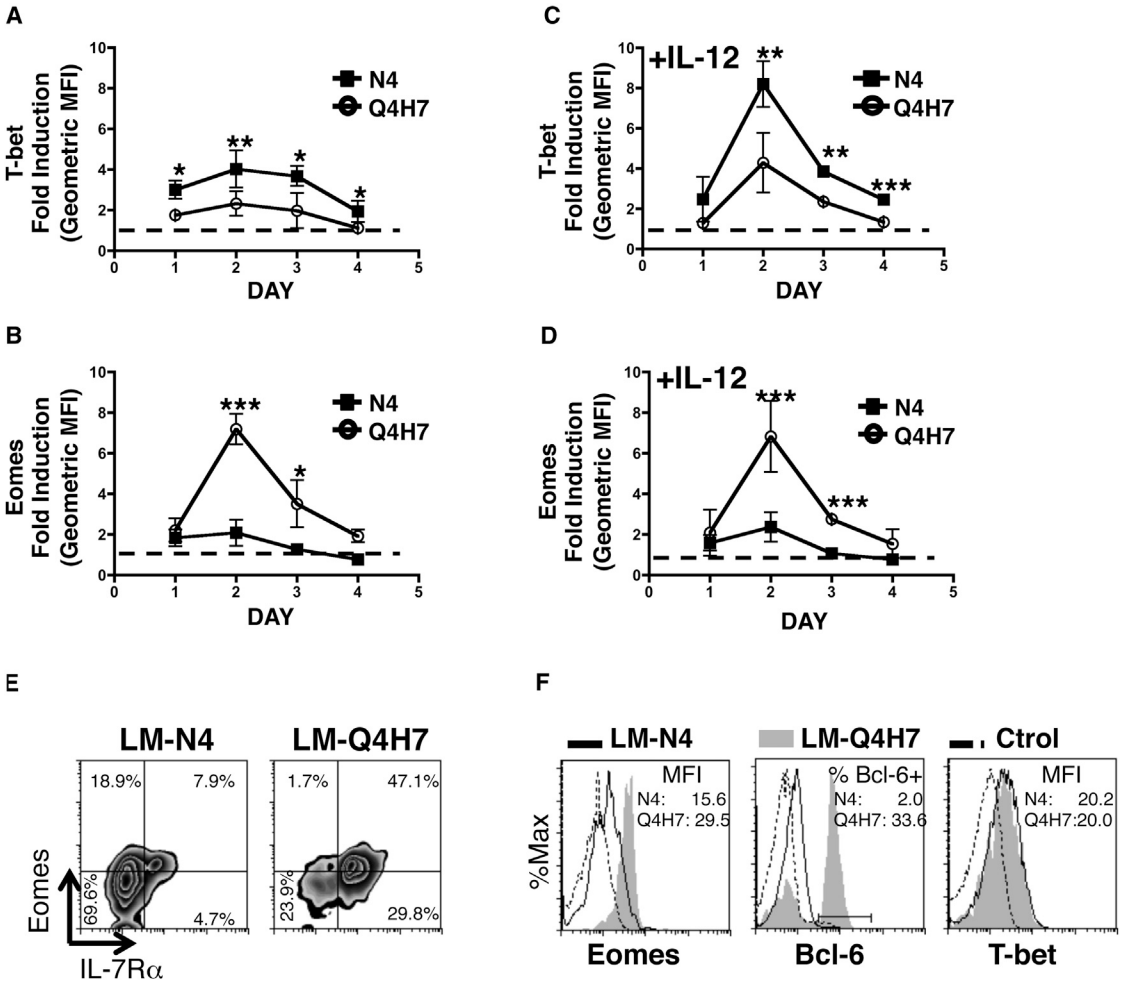


Figure 2. Induction of Eomes, T-Bet, and Bcl-6 Expression in Low-Affinity Cells

(A–D) Naive OT-I splenocytes were stimulated with 20 nM N4 or Q4H7 peptide-pulsed APCs in the absence (A and B) or presence (C and D) of 2 ng/ml IL-12. Expression of T-bet (A and C) and Eomes (B and D) were determined by flow cytometry on OT-1 T cells. Naive control (dashed line). Graphs show mean ± SD and represent $n \geq 5$ independent experiments (*$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$). OT-I naive T cells were transferred into congenic B6 hosts and challenged as in Figure 1.

(E) Frequency of cells expressing IL-7Rα^{hi} Eomes^{hi} at the peak of the LM-N4 and LM-Q4H7 immune responses (days 6–8) in blood. Dot plots are representative of five independent experiments, $n \geq 3$ mice per condition.

(F) Eomes, T-bet, and Bcl-6 expression determined as in (A) on OT-1 donors at the peak of the LM-N4 and LM-Q4H7 immune responses (days 6–8). Isotype or naive control, dashed line. Histograms and values are representative of three independent experiments, $n \geq 3$ mice per condition. Values show MFI (median) for Eomes and T-bet and percentage of Bcl-6^{+} cells.

See also Figure S2.

was no difference in T-bet expression between high- and low-affinity ligands at the highest antigen doses (Figure 3A). On the other hand, there was not a direct correlation between antigen dose and TCR affinity for Eomes expression. At low antigen doses ($\leq 10^{-10}$M), Q4H7 induced lower levels of Eomes than the high-affinity ligand N4. For N4, increase in antigen dose led to more Eomes expression; however, at the highest doses ($\geq 10^{-9}$ M), Eomes expression was repressed. Strikingly, at these highest doses ($\geq 10^{-9}$ M), Q4H7 induced much higher Eomes expression than N4. Importantly, high-affinity ligands were unable to reach these levels of Eomes expression at extremely low antigen doses (Figure 3B). This cannot be explained by simple differences in TCR occupancy as it only takes 5-fold higher concentration of low-affinity ligands to reach the same level of occupancy of their high-affinity counterparts (Daniels et al., 2006). Taken together, these data suggest that the quality of the TCR signal has a greater affect on Eomes expression than the quantity.

Next, we evaluated the effect of IL-12 on the pattern of T-bet and Eomes expression. T-bet expression was increased in a linear fashion across all doses for both TCR ligands with no change in sensitivity. Furthermore, similar to results in the absence of exogenous IL-12, low-affinity ligands only reached T-bet levels comparable to the high-affinity ligands at the highest

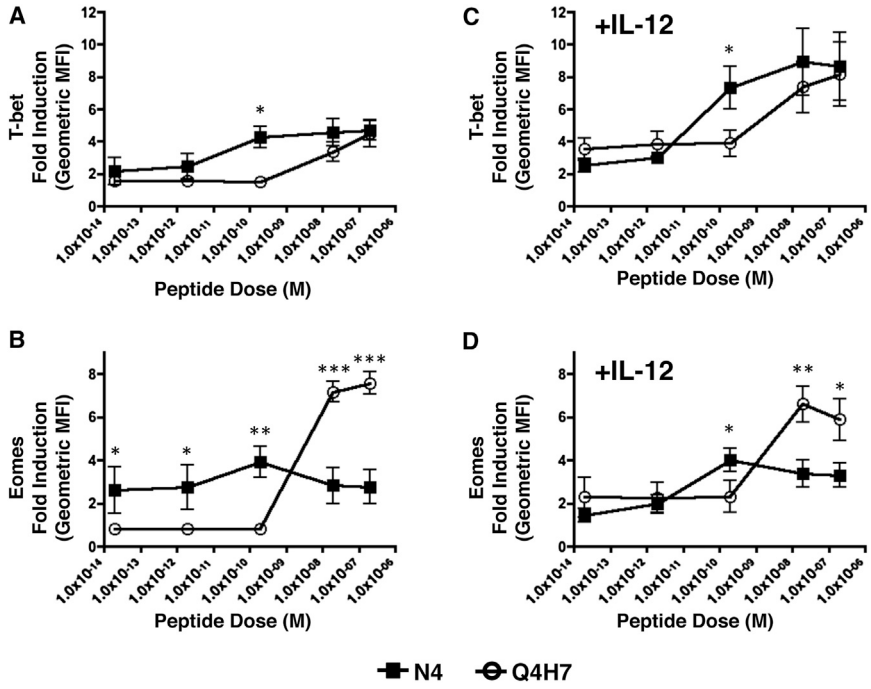


Figure 3. TCR Signal Strength Regulates T-Bet and Eomes in Distinct Ways

(A–D) Naive OT-I splenocytes were stimulated with APCs pulsed with varying concentrations of N4 or Q4H7 peptides in the absence (A and B) or presence (C and D) of 2 ng/ml IL-12. Expression of T-bet (A and C) and Eomes (B and D) was determined by flow cytometry as in Figure 2.

Graphs show mean ± SEM and are representative of $n \geq 4$ independent experiments. *$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$. See also Figure S3.

antigen doses (Figure 3C). In contrast, we observed two unique patterns of Eomes expression in the presence of exogenous IL-12. At the lowest antigen doses, IL-12 decreased Eomes expression for N4, whereas it increased Eomes levels for the low-affinity ligand Q4H7. At high antigen doses, IL-12 did not have any significant effect on Eomes expression for either ligand. Yet, Eomes levels were much higher for low- than high-affinity ligands (Figure 3D). Importantly, we found similar results with other high- (negative selectors) and low-affinity (positive selectors) ligands, indicating that these results are not restricted to N4 and Q4H7 peptides (Figure S3). Of note, even at very low antigen doses (2×10^{-10}M), low- and high-affinity cells were similarly activated (Figure S3). Yet, Eomes and T-bet expression were significantly different between high- and low-affinity cells, reinforcing the idea that both TCR affinity and antigen dose regulate Eomes and T-bet expression in distinct ways. Additionally, these results predict that the T-bet and Eomes expression profile observed in low-affinity cells in vivo (Figure 2) may correspond to T cells that have encountered high levels of low-affinity antigens.

TCR Signals Regulate the Susceptibility to IL-12

Given the effect of IL-12 on T-bet and Eomes was dependent on TCR affinity and antigen dose, we hypothesized that TCR signals regulate the ability of T cells to receive IL-12 signals. To test this, we evaluated whether TCR affinity and antigen dose could regulate the expression of IL-12R. Naive OT-1 T cells were stimulated with 20 nM N4 or Q4H7 peptide-pulsed APCs in the presence or absence of exogenous IL-12. In the absence of exogenous IL-12, N4 stimulation upregulated IL-12R expression by day 3. In contrast, IL-12R expression was not induced upon stimulation with the low-affinity ligand Q4H7 (Figure 4A). The addition of exogenous IL-12 increased the expression of IL-12R for both TCR ligands over naive levels by day 2 (Figure 4B). Yet, IL-12R expression induced by the low-affinity ligand Q4H7 did not reach the levels induced by the high-affinity TCR ligand N4. This was also observed upon infection (Figure 4C). Importantly, low IL-12R levels led to low STAT-4 phosphorylation upon IL-12 stimulation of low-affinity T cells (Figure 4D). Because antigen dose had a profound effect on T-bet and Eomes expression for low-affinity ligands (Figure 3), we next tested whether this also applied to IL-12R expression. The low-affinity TCR ligand Q4H7 was only able to induce levels of IL-12R comparable to the high-affinity ligand N4 at the highest antigen doses in both the presence and absence of exogenous IL-12 (Figures 4E and 4F). Collectively, these data clearly show that TCR signal strength (affinity and antigen dose) regulates the ability of T cells to respond to IL-12. Because IL-12 regulates T-bet and Eomes expression, this suggests that TCR signals regulate the input of IL-12 signals to instruct effector/memory programming, and thereby, T cell fate.

TCR Signals Regulate the Susceptibility to TGF-β Signals

TGF-β receptor signals negatively regulate the response of T cells against low-affinity TCR ligands (Johnson and Jameson, 2012; Zhang and Bevan, 2012). Thus, we tested whether the susceptibility to TGF-β signals was dependent on TCR signal strength by measuring TGF-βRII expression in experiments analogous to Figure 4. Interestingly, the low-affinity TCR ligand Q4H7 only induced the downregulation of TGF-βRII to the levels of the high-affinity TCR ligand N4 at the two highest antigen doses (Figure 5A). Furthermore, the difference in TGF-βRII expression between high- and low-affinity cells resulted in differences in TGF-βR signaling, as measured by phosphorylation of SMAD2/3 (Figure 5B). These data strongly suggest that TCR signal strength also dictates the susceptibility of T cells to TGF-β regulation.

Low-Affinity Memory T Cells Are Impaired in Their Effector Function in Response to Low-Affinity TCR Ligands

The previous findings indicate that OT-1 T cells responding to low-affinity TCR ligands in the presence of inflammation fully

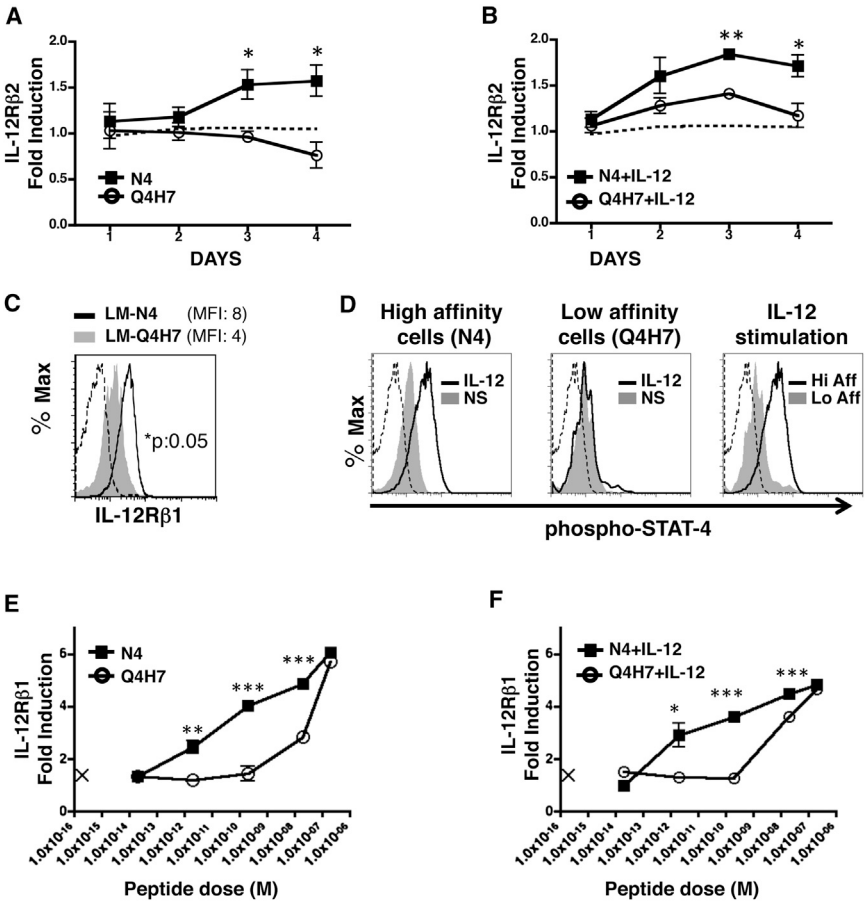


Figure 4. TCR Affinity Regulates IL-12R Expression and Signaling

(A and B) Naive OT-I splenocytes were stimulated with 20 nM N4 or Q4H7 peptide-pulsed APCs in the absence (A) or presence (B) of 2 ng/ml IL-12. Expression of IL-12Rβ2 (A and B) was determined by flow cytometry. Naive control (dashed line).

(C) IL-12Rβ1 expression on donor OT-1 cells challenged as in Figure 1 at day 5 of the immune response, in blood. Representative of n = 4.

(D) Phosphorylation of STAT-4 was determined on high- and low-affinity cells upon 30 min of 2 ng/ml IL-12 stimulation. Isotype control (dashed line). Histograms representative of two independent experiments.

(E and F) Naive OT-1 splenocytes were stimulated as in Figure 3 and IL-12Rβ1 expression was determined as in (A). X denotes IL-12Rβ1 expression in naive T cells.

All graphs show mean ± SEM and are representative of n ≥ 4 independent experiments (*p < 0.05, **p < 0.005, ***p < 0.0005).

support effector and memory programs. This could explain why, in vivo, naive T cells responding to low-affinity ligands are able to differentiate into effector and memory T cells (Zehn et al., 2009). However, memory T cells are expected to be higher in frequency and more sensitive to antigen than naive T cells (Williams and Bevan, 2007). Thus, we wondered whether low-affinity memory T cells would be more reactive to very-low-affinity antigens (pseudo-self) and, hence, a higher risk for autoimmunity. For this, we compared the response of naive T cells and memory OT-1 T cells generated against LM-Q4H7 (low-affinity memory). Low-affinity memory cells ($CD44^{hi}$, $IL\text{-}7R^{hi}$) were isolated from recipient mice. Then, equal numbers of naive or low-affinity memory cells were transferred into naive congenic hosts and their responses were compared upon infection with either LM-Q4H7 (low-affinity ligand) or LM-N4 (high-affinity ligand). As a control, high-affinity OT-1 memory cells (generated in response to LM-N4) were also rechallenged with high- or low-affinity ligands. In response to LM-N4, low-affinity memory cells expanded similarly to high-affinity memory cells (Figure 6A). Similar results were observed in competition experiments where equal numbers of high- and low-affinity memory cells were transferred into the same congenic recipient followed by challenge with LM-N4 (Figure 6B). For memory effector function, we observed that low-affinity memory cells expressed Granzyme B and interferon (IFN)-γ at levels comparable to their high-affinity counterparts. However, the percentage of low-affinity memory cells able to perform these effector functions was slightly but significantly lower (Figures 6C and 6D). This suggests that low-affinity memory T cells provide protection against high-affinity antigens, although not to the same extent as high-affinity memory T cells.

Next, we evaluated the response of low-affinity memory T cells to low-affinity ligands (LM-Q4H7 and LM-Q7). Low-affinity memory cell re-expansion in response to LM-Q4H7 or -Q7 was substantially better than naive T cells and similar to the re-expansion of high-affinity memory cells (Figure 7A; data not shown). Strikingly, we found that low-affinity memory T cells were impaired in the expression of Granzyme B, IFN-γ, T-bet, and Eomes (Figures 7B and 7C; data not shown). These results are in marked contrast to the response of naive T cells against LM-Q4H7, which is characterized by the expression of Granzyme B and IFN-γ at levels comparable to their high-affinity counterparts (Figures 7B, 7C, and S4) (Zehn et al., 2009). These data suggest, unexpectedly, that low-affinity memory T cells are less efficient effectors than naive T cells in response to low-affinity ligands. For high-affinity memory cells responding to low-affinity ligands, expression of IFN-γ was also lower, whereas Granzyme B was similar to naive cells (Figure S4). Collectively, these results suggest that although both high- and low-affinity memory T cells undergo normal secondary expansion, they are impaired in their effector function against ligands in the range of positive selectors. However, they remain reactive (although not equally) to high-affinity ligands.

DISCUSSION

In the context of infection, low-affinity cells are able to acquire effector function and develop into memory. However, the

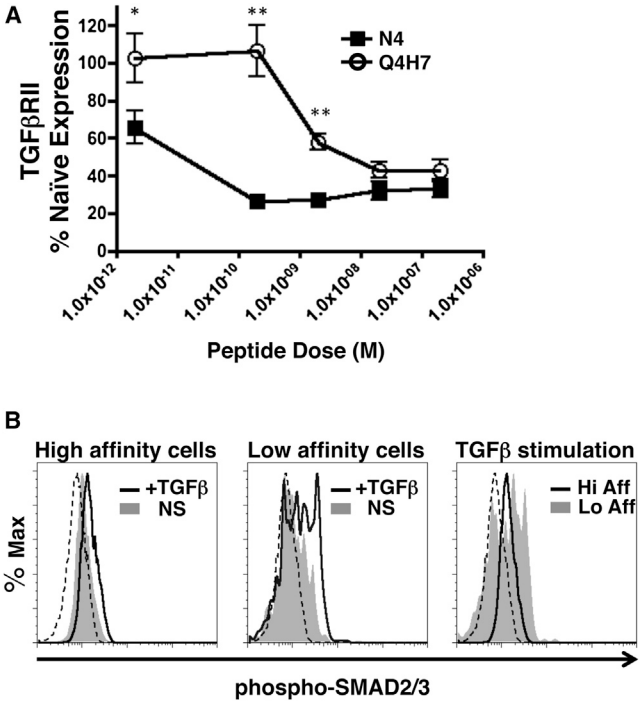


Figure 5. TCR Affinity Regulates TGF-βRII Expression and Signaling

(A) Naive OT-I splenocytes were stimulated as in Figure 3, in the presence of 2 ng/ml IL-12 for 3 days. Expression of TGF-βRII was determined by flow cytometry. Graph shows percentage of downregulation relative to naive T cells (mean ± SEM, n = 5 independent experiments) (*$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$).

(B) Naive OT-I splenocytes were stimulated with 0.2 nM N4 or Q4H7 peptide-pulsed APCs for 3 days and stimulated with 5 ng/ml TGF-β for 30 min. Phosphorylation of SMAD2/3 was determined by flow cytometry. Isotype control (dashed line). Histograms representative of n = 2 independent experiments.

mechanisms that enable such responses were unclear. We show that low- and high-affinity T cells are unexpectedly different in their effector/memory differentiation. Low-affinity cells expressed higher levels of Eomes and lower to equal levels of T-bet compared to high-affinity cells. This results in low-affinity cells primarily developing into memory precursors that rapidly acquire a central memory phenotype. TCR signal strength (affinity and antigen dose) distinctively regulated the expression of these transcription factors, in part through the control of IL-12R signals. Strikingly, and in contrast to naive T cells, both high- and low-affinity memory T cells are partially desensitized to low-affinity TCR ligands (in the range of positive selectors) in secondary responses.

Most of our knowledge about T cell memory is based on data generated in response to high-affinity ligands. From these studies, a Goldilocks model of antigenic signal strength was proposed, where "too little"stimulation would lead to poor memory development and "too much" would lead to CD8 memory exhaustion (Gourley et al., 2004). This model, however, cannot explain how, in lymphopenic conditions, naive T cells develop into functional memory in response to self peptide (low affinity) and low inflammatory signals. Furthermore, it cannot explain how, during infection, low-affinity TCR ligands can generate memory T cells (Zehn et al., 2009). The data presented here show that, transcriptionally, very-low-affinity TCR ligands have the full potential to support effector function and develop into memory (Cordaro et al., 2002; Turner et al., 2008; Zehn et al., 2009). More importantly, low-affinity TCR ligands favor memory by uniquely inducing high levels of Eomes and Bcl-6. How very weak TCR signals are inherently better at inducing Eomes is unclear. It may be related to a poor induction of T-bet. In fact, in T-bet-deficient animals, IL-7R expression is higher, the number of memory precursors is increased, Eomes expression levels are higher and central memory differentiation is enhanced upon infection (Intlekofer et al., 2007). Consistent with this, low-affinity T cells express higher levels of IL-7R, exhibit a higher frequency of memory precursors that persist throughout an immune response, and generate a higher percentage of cells with a central memory phenotype ($CD44^{hi}$, $CD62L^{hi}$) than high-affinity cells. Low-affinity T cells are also poor inducers of T-bet in vitro and require high doses of the low-affinity ligand to reach maximum T-bet levels. Although we did not observe low expression of T-bet in low-affinity cells in vivo, it may be that this is a sequential process and a T-bet deficiency manifested earlier leads to a later overexpression of Eomes. Alternatively, IL-12-independent signals, such as IL-7 or CD27, may play a role in T-bet or Eomes induction, respectively (Dong et al., 2012; Li et al., 2011). Curiously, we have made similar observations in polyclonal responses where B6 mice were challenged with LM-OVA. T cells that exhibited low binding to Kb-OVA tetramer had an activated phenotype were higher for Eomes but equal for T-bet expression. However, we cannot discard the fact that these T cells might have also crossreacted with a Listeria epitope.

Proinflammatory signals have a profound impact on T cell differentiation (Agarwal et al., 2009; Li et al., 2006; Zhang and Bevan, 2012). Thus, high levels of inflammation are required to drive effector function, but they also favor terminal effector differentiation (Harty and Badovinac, 2008; Joshi et al., 2007; Mescher et al., 2006; Pipkin et al., 2010). This is thought to be in part because IL-12 enhances T-bet expression, which, in turn, can repress Eomes levels (Intlekofer et al., 2007; Takemoto et al., 2006). Although this may be true for high-affinity ligands, our data provide an important insight into how Eomes expression is differentially regulated depending on affinity, antigen dose, and the level of IL-12R. We find that, contrary to what has been described for high-affinity ligands (Rao et al., 2010), high IL-12 does not repress Eomes for low-affinity ligands and, actually, has the opposite effect at low antigen doses. Strikingly, at high antigen doses, IL-12 does not repress Eomes for either TCR ligand. This indicates that IL-12-dependent regulation of Eomes depends on TCR signal strength. This may be in part linked to the regulation of IL-12R signals by TCR signal strength, because low-affinity TCR ligands are poor inducers of IL-12R. Alternatively, it may be that Eomes expression is negatively regulated by very strong TCR signals (independently of IL-12) and low-affinity ligands, even at the highest antigen doses, are not able to trigger the mechanisms that induce this repression. Another possibility could be that low-affinity TCR ligands are inherently better inducers of a specific signaling pathway that drives Eomes expression. Notably, TCR affinity does not

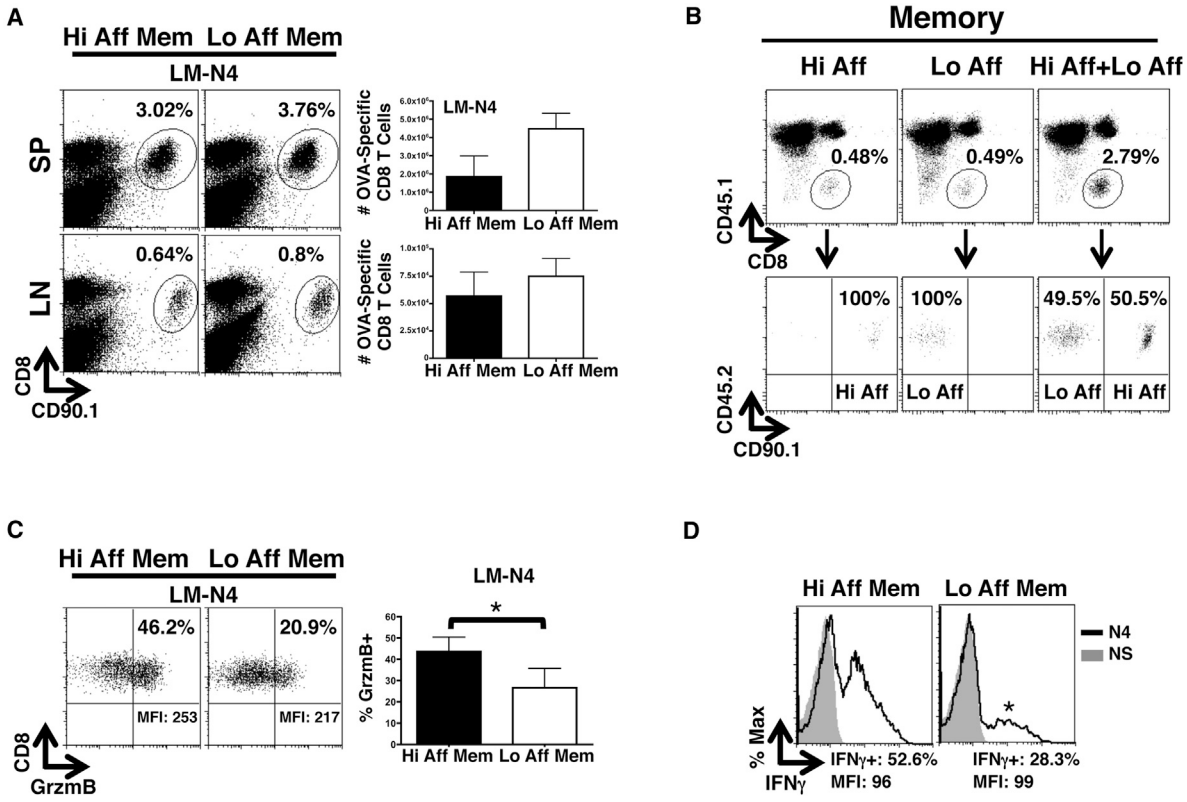


Figure 6. Low-Affinity Memory T Cells Respond to High-Affinity Ligands

OT-I CD8$^+$ T cells (2 × 10^5) were transferred into congenic B6 hosts and challenged with 1 × 10^4 cfu LM-N4 or LM-Q4H7, and N4-primed (high-affinity memory [Hi Aff. Mem]) or Q4H7-primed (low-affinity memory [Lo Aff. Mem]) memory cells were isolated after ≥30 days.

(A, C, and D) Hi Aff. Mem or Lo Aff. Mem cells (1 × 10^4) were transferred into naive hosts and rechallenged with 1 × 10^5 cfu LM-N4. Frequency and total number of responding cells (A), Granzyme B (C), and IFN-γ expression (D) were determined in Hi and Lo Aff. Mem cells at day 6 postinfection (p.i.). Average MFI and percentage of positive cells for Granzyme B and IFN-γ are indicated. Graphs show mean ± SD.

(B) 1 × 10^4 Hi Aff Mem (CD45.2$^+$CD90.1$^+$), Lo Aff. Mem (CD45.2$^+$CD90.1$^-$) or a mixture of 1 × 10^4 Hi Aff. Mem and 1 × 10^4 Lo Aff. Mem cells were transferred into naive hosts and rechallenged with 1 × 10^5 cfu LM-N4. Frequency of responding cells was determined in the blood 5 days p.i.

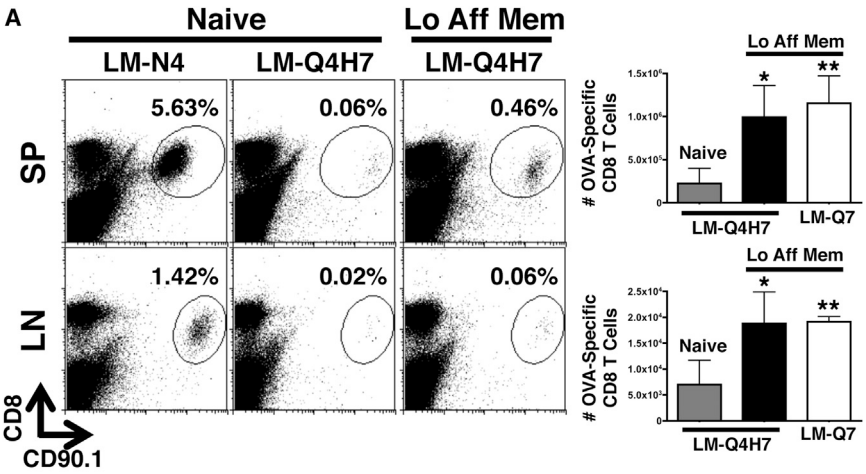
Dot plots, histograms, and graphs are representative of n ≥ 2 experiments, with three to six mice each. *p < 0.05, **p < 0.005, ***p < 0.0005.

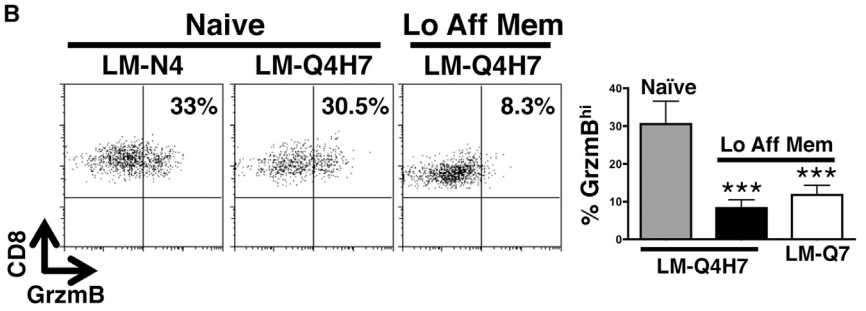
regulate T-bet expression in the same way as Eomes. T-bet levels increase proportionally to TCR affinity. The positive influence of IL-12 in T-bet expression equally applies to both high- and low-affinity ligands. Collectively, these findings provide evidence that TCR affinity can differentially regulate transcription factors (T-bet and Eomes) that are associated with different T cell fates. This also supports previous data indicating that the TCR signaling requirements for effector function and memory development are different (Smith-Garvin et al., 2010; Teixeiro et al., 2009) and raises the intriguing idea that TCR signals could be manipulated to direct a T cell toward a specific fate.

It has been reported that inflammation regulates TCR sensitivity to antigen (Richer et al., 2013). Remarkably, our data demonstrate that TCR signal strength also regulates the response of a T cell to inflammatory signals. Hence, because there is a mutual regulation and cooperation of both signals, neither inflammation nor antigenic signals can be considered alone to fully understand CD8 T cell differentiation.

For low-affinity T cells, negative regulation by TGF-β signals has an important impact in their immune response (Johnson and Jameson, 2012; Zhang and Bevan, 2012). We have observed that low-affinity T cells are unable to downregulate TGF-βRII (except at the highest antigen doses), even in the presence of IL-12. TGF-βR signals have been shown to regulate CD8 short-lived effector cell survival but do not affect memory cell development (Sanjabi et al., 2009). This adds credence to the idea that low-affinity TCR signals favor memory differentiation, maybe at the expense of effector differentiation. The fact that TCR signal strength regulates both IL-12R and TGF-βR signals suggests that low-affinity T cells need to be tightly regulated (low for IL-12R signals and high for TGF-βR signals) to limit overt activation in the context of infection. This may explain in part why low-affinity T cells are able to differentiate to a certain extent but do not cause stable autoimmunity (King et al., 2012).

In the thymus, a sharp border of TCR affinity defines the threshold between positive selection of tolerant T cells and the deletion of autoreactive T cells (Daniels et al., 2006). In the periphery, these positively selected T cells are expected to react against their cognate antigen and remain tolerant to self. Because of this, it is striking that weak TCR signals from positive





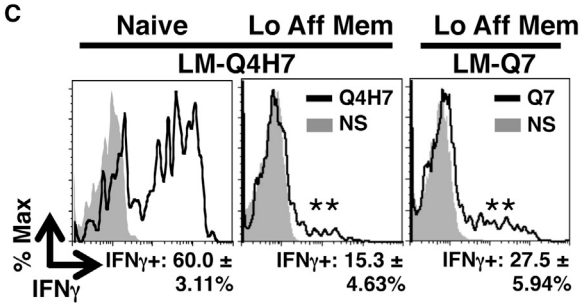


Figure 7. Low-Affinity Memory T Cells Are Impaired in Their Effector Function against Low-Affinity Ligands (Pseudo-self)

(A–C) Lo Aff. memory cells were obtained as in Figure 6. Lo Aff. Mem or naive OT-I cells (1×10^4) were transferred into naive hosts and challenged with 1×10^5 (memory) or 1×10^4 (naive) cfu LM-N4 or LM-Q4H7or LM-Q7. Frequency and total number of responding cells (A), expression of Granzyme B (B), and IFN-γ (C) were determined by flow cytometry in naive or Lo Aff Mem cells at day 6 p.i. Values shown in dot plots are averages. Histograms and graphs show mean ± SD. Data are representative of $n \geq 2$ experiments, with three to six mice each. Significance shown (*$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$) denotes comparison versus naive OT-I challenged with LM-Q4H7. See also Figure S4.

selecting ligands (low affinity) can induce complete immune responses (Zehn et al., 2009). Based on our data, we propose that the purpose of these responses is to maintain the diversity of the memory repertoire. This could be crucial to protect against viral escape variants and heterologous infections in secondary immune responses (van Gisbergen et al., 2011) or tumors. Intriguingly, low-affinity memory cells re-expanded in response to low-affinity challenge but in contrast to naive T cells responding to low-affinity ligands in the range of positive selectors, they were impaired in their effector function (IFN-γ and Granzyme B expression). This may be explained in part by the fact that low-affinity memory cells exhibit higher levels of TGF-βRII than their high-affinity counterparts (O'Sullivan et al., 2011). Alternatively, this could be a consequence of their memory programming during the primary immune response. Interestingly, low-affinity memory cells were able to respond against high-affinity TCR ligands but not to the same extent as high-affinity memory cells. This suggests that low- and high-affinity memory cell responses are not equal, an idea that is also inferred in studies comparing true memory T cells (generated against strong antigens) and HP-memory T cells (generated against self-antigens in lymphopenic conditions) (Cheung et al., 2009; Jellison et al., 2012).

Others have shown that monoclonal or polyclonal T cells with low functional avidity can escape negative selection and after, being primed and/or rechallenged with TCR ligands that are at or below the threshold of negative selection, can cause autoimmunity to tissue expressing self-antigen at or above this threshold (Enouz et al., 2012; von Herrath et al., 1994; Zehn and Bevan, 2006). Our data suggest that reactivity against ligands below the threshold of positive selection could exist if certain biochemical criterion is met. Thus, low-affinity T cells were able to integrate TCR and IL-12R signals to support an effector program (high levels of T-bet and Eomes) similar to high-affinity ligands only at the highest doses of antigen in the presence of inflammation. Interestingly, low-affinity primed-OT-1 memory T cells, which are, in principle, more reactive than naive T cells, were impaired in their ability to express effector molecules IFN-γ and Granzyme B but not in their ability to proliferate upon rechallenge with positive selecting ligands Q4H7 and Q7. This suggests that T cells mount restricted secondary responses to ligands in the range of positive selectors.

Is it possible that restriction could be overcome and lead to autoimmunity? Polyclonal T cells from RIP-OVA mice challenged or rechallenged with a pathogen that expresses OVA caused autoimmunity in 30% of the RIP-OVA mice (Enouz et al., 2012). Curiously, also in the RIP-OVA model, King et al. showed that, only at high doses of the very low-affinity ligand (Q4H7), autoimmunity was transiently induced (King et al., 2012), again suggesting there may be a specific tolerance mechanism to keep these responses in check below the threshold of negative selection. The differences between these two systems highlight the importance of understanding the mechanisms that underlie breaks in self-tolerance and indicate that the equilibrium between reactivity to foreign and self is more complex than originally thought.

In summary, we show that high- and low-affinity memory T cells are differentially programmed. Despite the fact that low-affinity T cells are recruited into the memory pool, they undergo normal secondary expansion, yet they are impaired in their effector response to positive selecting ligands. Hence, we propose that the sharp TCR affinity threshold that distinguishes positive and negative selection is relevant for memory responses. This information has important implications for vaccine development, tumor immunotherapies, and autoimmunity.

EXPERIMENTAL PROCEDURES

Mice and Reagents

C57BL/6, B6.SJL, and OT-I and OT-1.PL (Thy1.1$^+$) TCR transgenic mice were bred and maintained according to the University of Missouri OAR, ACUC. Peptides were purchased from New England Peptides and rmIL-12 from Peprotech. IL-2 (X63-IL-2 hybridoma) was used at 50 U/ml. TGF-β was from R&D Systems.

Bacteria

Listeria strains were generously provided by M. Bevan (University of Washington). Listeria strains were grown to an OD_{600} of 0.5 and were grown as described in Zehn et al. (2009).

Adoptive Transfer and Infections

Donor naive (1×10^3–1×10^4) or memory OT-1 T cells were purified from the lymph nodes of OT-1 or host mice and transferred intravenously into congenic mice. All infections were performed intravenously at least 1 day after adoptive transfer of transgenic T cells.

Flow Cytometry and Antibodies

Anti-CD8α (53–6.7), CD90.1 (OX-7), IL-7Rα (A7R34), CD62L (MEL-14), Vβ5 (MR9-4), IL-12Rβ1 (114), IL-12Rβ2 (HAM10B9), IFN-γ (XMG1.2), and phospho-STAT-4 from BD PharMingen. Anti-CD45.1 (A20), CD45.2 (I04), CD27 (LG.7F9), and Eomes (Dan11Mag) were from eBioscience. Anti-T-bet (4B10) and Bcl-6 (Clone 7D1) were from Santa Cruz Biotechnology, and secondary antibodies and Granzyme B (GB12) were from Invitrogen. Anti-phospho-SMAD2/3 was from Cell Signaling Technology. Flow cytometry was performed on a FACSCalibur flow cytometer (Becton Dickinson), and the data were analyzed with FlowJo FACS Analysis Software (Tree Star). For determining induction of Eomes and T-bet, geometric mean fluorescent intensity was normalized to a naive control. For determining induction of IL-12Rβ2 and IL-12Rβ1, geometric mean fluorescent intensity was normalized to an isotype control.

Intracellular Staining

Splenocytes were washed in FACS buffer (1% FCS, 0.02% sodium azide in PBS) and stained for CD8 and congenic markers (CD45.1 or CD45.2 or CD90.2 or CD90.1) to identify donor cells. Cells were washed in FACS buffer and then fixed and permeabilized using Cytofix/Cytoperm or Phosflow (Perm. Buffer III) kit according to the manufacturer's instructions (BD Biosciences). Next, cells were stained with specific antibodies. For restimulation and determining cytokine production, splenocytes were incubated in 96-well round-bottom plates with 1 μl/ml Golgistop (BD Biosciences) in the presence of 250 nM OVA or Q4H7 or Q7 peptides for 4 hr at 37°C. Data were analyzed by flow cytometry.

Statistical Analysis

For statistical analysis, unless otherwise stated in figures, two-tail unpaired Student's t test was applied using Prism software (GraphPad). Significance was set at $p < 0.05$. For T-bet and Eomes dose-response curves (Figure 3), data were analyzed using the multiple t tests platform (Holmes method correction for multiple comparisons, alpha = 5.000%), utilizing Prism software (GraphPad).

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2013.07.008.

ACKNOWLEDGMENTS

This work was supported by University of Missouri Life Sciences Fellowship (K.M.K.) and grants from University of Missouri Mission Enhancement Fund and University of Missouri Research Board Grant. We thank M. Bevan and D. Zehn for providing Listeria m strains and H. Mullen, S. McKarns, and B. Hahm for providing reagents.

Received: February 6, 2013
Revised: May 18, 2013
Accepted: July 8, 2013
Published: August 8, 2013

REFERENCES

Agarwal, P., Raghavan, A., Nandiwada, S.L., Curtsinger, J.M., Bohjanen, P.R., Mueller, D.L., and Mescher, M.F. (2009). Gene regulation and chromatin remodeling by IL-12 and type I IFN in programming for CD8 T cell effector function and memory. J. Immunol. *183*, 1695–1704.

Ahmed, M., Lanzer, K.G., Yager, E.J., Adams, P.S., Johnson, L.L., and Blackman, M.A. (2009). Clonal expansions and loss of receptor diversity in the naive CD8 T cell repertoire of aged mice. J. Immunol. *182*, 784–792.

Banerjee, A., Gordon, S.M., Intlekofer, A.M., Paley, M.A., Mooney, E.C., Lindsten, T., Wherry, E.J., and Reiner, S.L. (2010). Cutting edge: The transcription factor eomesodermin enables CD8+ T cells to compete for the memory cell niche. J. Immunol. *185*, 4988–4992.

Cheung, K.P., Yang, E., and Goldrath, A.W. (2009). Memory-like CD8+ T cells generated during homeostatic proliferation defer to antigen-experienced memory cells. J. Immunol. *183*, 3364–3372.

Cordaro, T.A., de Visser, K.E., Tirion, F.H., Schumacher, T.N.M., and Kruisbeek, A.M. (2002). Can the low-avidity self-specific T cell repertoire be exploited for tumor rejection? Immunology *168*, 651–660.

Corse, E., Gottschalk, R.A., and Allison, J.P. (2011). Strength of TCR-peptide/MHC interactions and in vivo T cell responses. Immunology *186*, 5039–5045.

Crotty, S., Johnston, R.J., and Schoenberger, S.P. (2010). Effectors and memories: Bcl-6 and Blimp-1 in T and B lymphocyte differentiation. Nat. Immunol. *11*, 114–120.

Daniels, M.A., Teixeiro, E., Gill, J., Hausmann, B., Roubaty, D., Holmberg, K., Werlen, G., Holländer, G.A., Gascoigne, N.R.J., and Palmer, E. (2006). Thymic selection threshold defined by compartmentalization of Ras/MAPK signalling. Nature *444*, 724–729.

Davis, M.M., Boniface, J.J., Reich, Z., Lyons, D., Hampl, J., Arden, B., and Chien, Y. (1998). Ligand recognition by alpha beta T cell receptors. Annu. Rev. Immunol. *16*, 523–544.

den Braber, I., Mugwagwa, T., Vrisekoop, N., Westera, L., Mögling, R., de Boer, A.B., Willems, N., Schrijver, E.H., Spierenburg, G., Gaiser, K., et al. (2012). Maintenance of peripheral naive T cells is sustained by thymus output in mice but not humans. Immunity *36*, 288–297.

Dong, H., Franklin, N.A., Roberts, D.J., Yagita, H., Glennie, M.J., and Bullock, T.N. (2012). CD27 stimulation promotes the frequency of IL-7 receptor-expressing memory precursors and prevents IL-12-mediated loss of CD8(+) T cell memory in the absence of CD4(+) T cell help. J. Immunol. *188*, 3829–3838.

Enouz, S., Carrié, L., Merkler, D., Bevan, M.J., and Zehn, D. (2012). Autoreactive T cells bypass negative selection and respond to self-antigen stimulation during infection. J. Exp. Med. *209*, 1769–1779.

Gorelik, L., and Flavell, R.A. (2002). Transforming growth factor-beta in T-cell biology. Nat. Rev. Immunol. *2*, 46–53.

Gottschalk, R.A., Corse, E., and Allison, J.P. (2010). TCR ligand density and affinity determine peripheral induction of Foxp3 in vivo. J. Exp. Med. *207*, 1701–1711.

Gourley, T.S., Wherry, E.J., Masopust, D., and Ahmed, R. (2004). Generation and maintenance of immunological memory. Semin. Immunol. *16*, 323–333.

Hale, J.S., Boursalian, T.E., Turk, G.L., and Fink, P.J. (2006). Thymic output in aged mice. Proc. Natl. Acad. Sci. USA *103*, 8447–8452.

Hammerbeck, C.D., and Mescher, M.F. (2008). Antigen controls IL-7R alpha expression levels on CD8 T cells during full activation or tolerance induction. J. Immunol. *180*, 2107–2116.

Harty, J.T., and Badovinac, V.P. (2008). Shaping and reshaping CD8+ T-cell memory. Nat. Rev. Immunol. *8*, 107–119.

Hogquist, K.A., Baldwin, T.A., and Jameson, S.C. (2005). Central tolerance: learning self-control in the thymus. Nat. Rev. Immunol. *5*, 772–782.

Ichii, H., Sakamoto, A., Hatano, M., Okada, S., Toyama, H., Taki, S., Arima, M., Kuroda, Y., and Tokuhisa, T. (2002). Role for Bcl-6 in the generation and maintenance of memory CD8+ T cells. Nat. Immunol. *3*, 558–563.

Intlekofer, A.M., Takemoto, N., Wherry, E.J., Longworth, S.A., Northrup, J.T., Palanivel, V.R., Mullen, A.C., Gasink, C.R., Kaech, S.M., Miller, J.D., et al. (2005). Effector and memory CD8+ T cell fate coupled by T-bet and eomesodermin. Nat. Immunol. *6*, 1236–1244.

Intlekofer, A.M., Wherry, E.J., and Reiner, S.L. (2006). Not-so-great expectations: re-assessing the essence of T-cell memory. Immunol. Rev. *211*, 203–213.

Intlekofer, A.M., Takemoto, N., Kao, C., Banerjee, A., Schambach, F., Northrop, J.K., Shen, H., Wherry, E.J., and Reiner, S.L. (2007). Requirement for T-bet in the aberrant differentiation of unhelped memory CD8+ T cells. J. Exp. Med. *204*, 2015–2021.

Jameson, S.C. (2005). T cell homeostasis: keeping useful T cells alive and live T cells useful. Semin. Immunol. *17*, 231–237.

Jameson, S.C., and Bevan, M.J. (1995). T cell receptor antagonists and partial agonists. Immunity *2*, 1–11.

Jameson, S.C., Carbone, F.R., and Bevan, M.J. (1993). Clone-specific T cell receptor antagonists of major histocompatibility complex class I-restricted cytotoxic T cells. J. Exp. Med. *177*, 1541–1550.

Jellison, E.R., Turner, M.J., Blair, D.A., Lingenheld, E.G., Zu, L., Puddington, L., and Lefrançois, L. (2012). Distinct mechanisms mediate naive and memory CD8 T-cell tolerance. Proc. Natl. Acad. Sci. USA *109*, 21438–21443.

Johnson, L.D., and Jameson, S.C. (2012). TGF-β sensitivity restrains CD8+ T cell homeostatic proliferation by enforcing sensitivity to IL-7 and IL-15. PLoS ONE *7*, e42268.

Joshi, N.S., Cui, W., Chandele, A., Lee, H.K., Urso, D.R., Hagman, J., Gapin, L., and Kaech, S.M. (2007). Inflammation directs memory precursor and short-lived effector CD8(+) T cell fates via the graded expression of T-bet transcription factor. Immunity *27*, 281–295.

Kallies, A. (2008). Distinct regulation of effector and memory T-cell differentiation. Immunol. Cell Biol. *86*, 325–332.

King, C.G., Koehli, S., Hausmann, B., Schmaler, M., Zehn, D., and Palmer, E. (2012). T cell affinity regulates asymmetric division, effector cell differentiation, and tissue pathology. Immunity *37*, 709–720.

Koniaras, C., Carbone, F.R., Heath, W.R., and Lew, A.M. (1999). Inhibition of naïve class I-restricted T cells by altered peptide ligands. Immunol. Cell Biol. *77*, 318–323.

Li, M.O., Wan, Y.Y., Sanjabi, S., Robertson, A.-K.L., and Flavell, R.A. (2006). Transforming growth factor-beta regulation of immune responses. Annu. Rev. Immunol. *24*, 99–146.

Li, Q., Rao, R.R., Araki, K., Pollizzi, K., Odunsi, K., Powell, J.D., and Shrikant, P.A. (2011). A central role for mTOR kinase in homeostatic proliferation induced CD8+ T cell memory and tumor immunity. Immunity *34*, 541–553.

Liston, A., Lesage, S., Gray, D.H., Boyd, R.L., and Goodnow, C.C. (2005). Genetic lesions in T-cell tolerance and thresholds for autoimmunity. Immunol. Rev. *204*, 87–101.

Madrenas, J., Wange, R.L., Wang, J.L., Isakov, N., Samelson, L.E., and Germain, R.N. (1995). Zeta phosphorylation without ZAP-70 activation induced by TCR antagonists or partial agonists. Science *267*, 515–518.

Mescher, M.F., Curtsinger, J.M., Agarwal, P., Casey, K.A., Gerner, M., Hammerbeck, C.D., Popescu, F., and Xiao, Z. (2006). Signals required for programming effector and memory development by CD8+ T cells. Immunol. Rev. *211*, 81–92.

Naeher, D., Daniels, M.A., Hausmann, B., Guillaume, P., Luescher, I., and Palmer, E. (2007). A constant affinity threshold for T cell tolerance. J. Exp. Med. *204*, 2553–2559.

Naylor, K., Li, G., Vallejo, A.N., Lee, W.W., Koetz, K., Bryl, E., Witkowski, J., Fulbright, J., Weyand, C.M., and Goronzy, J.J. (2005). The influence of age on T cell generation and TCR diversity. J. Immunol. *174*, 7446–7452.

O'Sullivan, J.A., Zloza, A., Kohlhapp, F.J., Moore, T.V., Lacek, A.T., Dulin, N.O., and Guevara-Patiño, J.A. (2011). Priming with very low-affinity peptide ligands gives rise to CD8(+) T-cell effectors with enhanced function but with greater susceptibility to transforming growth factor (TGF)β-mediated suppression. Cancer Immunol. Immunother. *60*, 1543–1551.

Pardoll, D. (2002). T cells take aim at cancer. Proc. Natl. Acad. Sci. USA *99*, 15840–15842.

Pipkin, M.E., Sacks, J.A., Cruz-Guilloty, F., Lichtenheld, M.G., Bevan, M.J., and Rao, A. (2010). Interleukin-2 and inflammation induce distinct transcriptional programs that promote the differentiation of effector cytolytic T cells. Immunity *32*, 79–90.

Rao, R.R., Li, Q., Odunsi, K., and Shrikant, P.A. (2010). The mTOR kinase determines effector versus memory CD8+ T cell fate by regulating the expression of transcription factors T-bet and eomesodermin. Immunity *32*, 67–78.

Richer, M.J., Nolz, J.C., and Harty, J.T. (2013). Pathogen-specific inflammatory milieux tune the antigen sensitivity of CD8(+) T cells by enhancing T cell receptor signaling. Immunity *38*, 140–152.

Rutishauser, R.L., and Kaech, S.M. (2010). Generating diversity: transcriptional regulation of effector and memory CD8 T-cell differentiation. Immunol. Rev. *235*, 219–233.

Sabatino, J.J., Jr., Huang, J., Zhu, C., and Evavold, B.D. (2011). High prevalence of low affinity peptide-MHC II tetramer-negative effectors during polyclonal CD4+ T cell responses. J. Exp. Med. *208*, 81–90.

Sanjabi, S., Mosaheb, M.M., and Flavell, R.A. (2009). Opposing effects of TGF-beta and IL-15 cytokines control the number of short-lived effector CD8+ T cells. Immunity *31*, 131–144.

Sloan-Lancaster, J., Evavold, B.D., and Allen, P.M. (1994a). Th2 cell clonal anergy as a consequence of partial activation. J. Exp. Med. *180*, 1195–1205.

Sloan-Lancaster, J., Shaw, A.S., Rothbard, J.B., and Allen, P.M. (1994b). Partial T cell signaling: altered phospho-zeta and lack of zap70 recruitment in APL-induced T cell anergy. Cell *79*, 913–922.

Smith-Garvin, J.E., Burns, J.C., Gohil, M., Zou, T., Kim, J.S., Maltzman, J.S., Wherry, E.J., Koretzky, G.A., and Jordan, M.S. (2010). T-cell receptor signals direct the composition and function of the memory CD8+ T-cell pool. Blood *116*, 5548–5559.

Sprent, J., Cho, J.-H., Boyman, O., and Surh, C.D. (2008). T cell homeostasis. Immunol. Cell Biol. *86*, 312–319.

Takemoto, N., Intlekofer, A.M., Northrup, J.T., Wherry, E.J., and Reiner, S.L. (2006). Cutting Edge: IL-12 inversely regulates T-bet and eomesodermin expression during pathogen-induced CD8+ T cell differentiation. J. Immunol. *177*, 7515–7519.

Teixeiro, E., Daniels, M.A., Hamilton, S.E., Schrum, A.G., Bragado, R., Jameson, S.C., and Palmer, E. (2009). Different T cell receptor signals determine CD8+ memory versus effector development. Science *323*, 502–505.

Turner, M.J., Jellison, E.R., Lingenheld, E.G., Puddington, L., and Lefrançois, L. (2008). Avidity maturation of memory CD8 T cells is limited by self-antigen expression. J. Exp. Med. *205*, 1859–1868.

van Gisbergen, K.P.J.M., Klarenbeek, P.L., Kragten, N.A.M., Unger, P.-P.A., Nieuwenhuis, M.B.B., Wensveen, F.M., ten Brinke, A., Tak, P.P., Eldering, E., Nolte, M.A., and van Lier, R.A. (2011). The costimulatory molecule CD27 maintains clonally diverse CD8(+) T cell responses of low antigen affinity to protect against viral variants. Immunity *35*, 97–108.

von Herrath, M.G., Dockter, J., and Oldstone, M.B. (1994). How virus induces a rapid or slow onset insulin-dependent diabetes mellitus in a transgenic model. Immunity *1*, 231–242.

Williams, M.A., and Bevan, M.J. (2007). Effector and memory CTL differentiation. Annu. Rev. Immunol. *25*, 171–192.

Xiao, Z., Casey, K.A., Jameson, S.C., Curtsinger, J.M., and Mescher, M.F. (2009). Programming for CD8 T cell memory development requires IL-12 or type I IFN. J. Immunol. *182*, 2786–2794.

Zehn, D., and Bevan, M.J. (2006). T cells with low avidity for a tissue-restricted antigen routinely evade central and peripheral tolerance and cause autoimmunity. Immunity *25*, 261–270.

Zehn, D., Lee, S.Y., and Bevan, M.J. (2009). Complete but curtailed T-cell response to very low-affinity antigen. Nature *458*, 211–214.

Zhang, N., and Bevan, M.J. (2012). TGF-β signaling to T cells inhibits autoimmunity during lymphopenia-driven proliferation. Nat. Immunol. *13*, 667–673.