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SLAP deficiency increases TCR avidity leading to altered repertoire and negative selection of cognate antigen-specific CD8+ T cells

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Abstract

How T cell receptor (TCR) avidity influences CD8+ T cell development and repertoire selection is not yet fully understood. To fill this gap, we utilized Src-like adaptor protein (SLAP)-deficient mice as a tool to increase TCR avidity on double positive (DP) thymocytes. We generated SLAP$^{-/-}$ mice with the transgenic MHC class I-restricted TCR (OT-1) and SLAP$^{-/-}$ Vβ5 mice, expressing only the β-chain of the TCR OT-1 transgene, to examine the effects of increased TCR surface levels on CD8+ T cell development and repertoire selection. In comparing SLAP$^{-/-}$ OT-1 and Vβ5 mice with wild-type controls, we performed compositional analysis and assessed thymocyte signaling by measuring CD5 levels. In addition, we performed tetramer and compositional staining to measure affinity for the cognate antigen, ovalbumin (OVA) peptide, presented by MHC. Furthermore, we quantified differences in α-chain repertoire in SLAP$^{-/-}$ Vβ5 mice. We have found that SLAP$^{-/-}$ OT-1 mice have fewer CD8+ thymocytes but have increased CD5 expression. SLAP$^{-/-}$ OT-1 mice have fewer DP thymocytes expressing Vα2, signifying increased endogenous α-chain rearrangement, and more non-OVA-specific CD8+ splenocytes upon tetramer staining. Our data demonstrate that SLAP$^{-/-}$ Vβ5 mice also have fewer OVA-specific cells and increased Vα2 usage in the peripheral Vβ5 CD8+ T cells that were non-OVA-specific, demonstrating differences in α-chain repertoire. These studies provide direct evidence that increased TCR avidity in DP thymocytes enhances CD8+ T cell negative selection deleting thymocytes with specificity for cognate antigen, an antigen the mature T cells may never encounter. Collectively, these studies provide new insights into how TCR avidity during CD8+ T cell development influences repertoire selection.

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Keywords

TCR avidity; SLAP; CD8$^+$ T cells; TCR α-chain repertoire

Introduction

Thymic selection shapes the peripheral T cell repertoire [1,2]. The fate of a developing thymocyte depends on the intensity of T cell receptor (TCR) complex-mediated signaling upon TCR recognition of a self-antigen peptide presented by major histocompatibility complex (MHC). A minimum signaling threshold is required to prevent death by neglect, such that weak signals through the TCR complex permit thymocyte survival or positive selection. However, strong signals trigger thymocyte deletion or negative selection to prevent the release of potentially self-reactive T cells [3].

A key determinant of signal strength through the TCR complex is the avidity of the TCR for MHC and the selecting peptide (MHC–peptide) [4]. TCR avidity is a result of both the intrinsic affinity of the TCR for MHC–peptide in addition to TCR density on the developing thymocyte [5]. Affinity of the TCR is governed by the specific repertoire generated through the rearrangement of the TCRα and TCRβ chains [6]. In contrast, a key regulator of the level and thus avidity of TCR on developing double positive (DP) thymocytes is the Src-like adaptor protein (SLAP) [7–10]. SLAP regulates TCR levels through its interaction with Casitas B-lineage lymphoma protein (c-Cbl), an E3 ubiquitin ligase that targets components of the TCR signaling complex for degradation limiting their expression and surface TCR levels [9, 10]. Previously, we have shown that SLAP deficiency has profound affects on CD4$^+$ thymocyte development leading to enhanced positive selection [7]. In addition, we have shown in the SKG mouse model of inflammatory arthritis that SLAP deficiency leads to the increased development of regulatory CD4$^+$ T cells, which have enhanced function and suppress the development of inflammatory arthritis [11]. These studies show that SLAP deficiency is a critical regulator of CD4$^+$ thymocyte development and function, but its importance in regulating CD8$^+$ T cell development is currently unknown. Further investigation is required to understand how increased TCR avidity affects CD8$^+$ T cell repertoire selection during development, as previous studies have either lowered avidity or altered affinity through the use of altered peptide ligands [12, 13]. Thus, SLAP-deficient mice provide us with a unique opportunity to test the contribution of elevated TCR levels leading to increased TCR avidity during CD8$^+$ thymocyte development and repertoire selection. As SLAP deficiency strengthens signaling during thymocyte development [7, 11], we hypothesized that SLAP deficiency would augment CD8$^+$ T cell negative selection and skew the TCR repertoire by either reducing the number of antigen-specific cells or reducing TCR affinity for MHC–peptide. To test this hypothesis, we generated both SLAP-deficient (SLAP$^{-/-}$) mice with a fixed MHC class I-restricted TCR (OT-1) [14], as well as SLAP$^{-/-}$ mice expressing only the TCRβ chain of the OT-1 transgene (Vβ5). The Vβ5 chain pairs with endogenous TCRα chains to create a TCR repertoire with a broad range of specificities, including reactivity with the cognate antigen, a peptide of ovalbumin (OVA) [15, 16]. These mouse models facilitate the examination of how TCR avidity during CD8$^+$ T cell development influences repertoire selection for cognate antigen.

In this report, we show that SLAP deficiency in OT-1 mice enhances signaling in DP thymocytes and reduces the number of CD8$^+$ single positive (SP) thymocytes. SLAP$^{-/-}$ OT-1 mice also have both fewer DP thymocytes expressing Vα2, suggestive of increased endogenous α-chain rearrangement, as well as increased numbers of peripheral non-OVA-specific CD8$^+$ T cells. We further demonstrate that SLAP$^{-/-}$ Vβ5 mice also have fewer peripheral CD8$^+$ T cells specific for OVA, suggesting that SLAP deficiency enhances

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negative selection of cognate antigen-specific CD8$^+$ T cells. Finally, we find that both OVA-specific and non-OVA-specific peripheral SLAP$^{-/-}$ Vβ5 CD8$^+$ T cells have alterations in the α-chain repertoire. Thus, increasing TCR avidity and consequentially signal strength through the TCR complex in DP thymocytes enhanced the negative selection of CD8$^+$ thymocytes specific for cognate antigen and altered the repertoire of the resulting pool of peripheral T cells.

Research design and methods

Experimental animals

SLAP$^{-/-}$ mice have been described previously and have been backcrossed over nine generations onto a C57BL/6 background [8, 10, 17, 18]. C57BL/6 OT-1 [wild-type (WT) OT-1] mice were bred in house, and C57BL/6Vβ5 (WT Vβ5) mice were kindly provided by Pamela Fink [15, 16]. SLAP$^{-/-}$ mice were crossed into both the OT-1 mouse line and the Vβ5 to generate SLAP$^{-/-}$ OT-1 and SLAP$^{-/-}$ Vβ5 mice. All mice were maintained in specific pathogen-free conditions according to the guidelines of the National Jewish Health Institutional Animal Care and Use Committee.

Antibodies and tetramer

Monoclonal antibodies against the following antigens were purchased from eBioscience: CD24 (30-F1), CD44 (IM7), Vα2 (B20.1), Vα3.2 (RR3-16), Vα11 (RR8-1) conjugated with FITC, PE, PE-Cy7, PB, or APC. The monoclonal antibody against Vβ5.1/5.2 (MR9-4) conjugated with FITC was purchased from BD Biosciences. Monoclonal antibodies against the following antigens were purchased from BioLegend: B220 (RA3-6B2), CD4 (RM4-5), CD5 (53-7.3), CD8α (53-6.7), TCRβ (H57-97), Vα8.3 (B21.14) conjugated with FITC, PE, PerCP, PE-Cy7, PB, or APC, or APC–Cy7. PE-conjugated H-2K^b OVA$_{257–264}$ (SIINFEKL) tetramer was prepared as previously described [19].

Flow cytometry

Single-cell suspensions were prepared from mouse thymus and spleen. RBCs were lysed using a hypotonic ammonium chloride buffer followed by washing. Cells were stained in FACS buffer (PBS supplemented with 2 % Cosmic Calf Serum (Hyclone), 2 mM L-glutamine (Cellgro), 100 IU/ml penicillin/streptomycin (Cellgro), 2 mM EDTA (Amresco) with monoclonal antibodies for 30 min on ice or tetramer for 1 h at 37 °C before washing and fixing in 1.6 % paraformaldehyde (w/v) in PBS. Data were collected on a Cyan flow cytometer (Dako) and analyzed using FlowJo software (Tree Star). Surface expression levels were calculated as geometric mean fluorescence intensity.

Statistical analysis

Unpaired two-tailed Student's *t* tests were performed using Prism 5.0a (GraphPad Software). Differences were considered statistically significant for p values <0.05.

Results

SLAP deficiency enhances thymocyte signaling and negative selection of CD8$^+$ thymocytes in OT-1 mice

WT OT-1 transgenic mice express an MHC class I-restricted TCR specific for residues 257–264 of OVA (SIINFEKL) resulting in CD8$^+$ T cells with TCRs that have a fixed affinity for OVA, their cognate antigen [14]. We hypothesized that increasing levels of the TCR complex in OT-1 mice during thymocyte development would enhance CD8$^+$ T cell negative selection and skew the TCR repertoire. To increase TCR levels in developing thymocytes,

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we crossed OT-1 mice with SLAP$^{-/-}$ mice. We analyzed thymic composition using a published gating scheme with minor modifications [20, 21] and found that SLAP deficiency decreased both frequency and absolute number of CD8^{+} thymocytes by 38 % compared to WT OT-1 mice (Fig. 1a, b), indicating enhanced negative selection. SLAP$^{-/-}$ OT-1 mice also had a 63 % increase in the number of thymocytes in the DP bright population, as well as a 31 % decrease in the number of thymocytes in the DP dull population, which have undergone early stages of positive selection to down-regulate CD4 and CD8 co-receptors [20]. SLAP$^{-/-}$ DP bright thymocytes had almost a threefold increase in TCR levels (TCRβ) compared to pre-selection TCRβ low DP bright thymocytes in WT OT-1 mice, as well a 55 % increase in CD5 expression, a marker known to correlate with TCR signal strength [22], that indicates enhanced signaling in SLAP$^{-/-}$ OT-1 DP bright thymocytes compared to pre-selection WT OT-1 DP bright thymocytes (Fig. 1c–e). Together, these data suggest that SLAP deficiency increases TCR levels and enhances signaling in OT-1 DP bright thymocytes resulting in a reduction in the number of CD8^{+} thymocytes.

More SLAP$^{-/-}$ OT-1 DP thymocytes express low levels of Vα2

Since SLAP deficiency enhanced signaling and reduced the number of SP CD8^{+} thymocytes in SLAP$^{-/-}$ OT-1 mice, we assessed whether it could also alter repertoire in the resulting pool of CD8^{+} thymocytes. As OT-1 mice can down-regulate their transgenic TCRα chain (Vα2) for secondary rearrangement of endogenous TCRα chains [23,24], we analyzed Vα2 usage in DP thymocytes as a measure of receptor editing through secondary α-chain rearrangement. Interestingly, approximately twice as many DP bright thymocytes from SLAP$^{-/-}$ OT-1 mice down-regulate Vα2 (Fig. 2a, b), supporting a role for SLAP in altering T cell repertoire not only through a reduction in CD8^{+} thymocytes but also through regulating receptor editing.

SLAP$^{-/-}$ OT-1 mice have increased non-OVA-specific CD8^{+} T cells

Since SLAP deficiency reduced the number of CD8^{+} thymocytes and decreased Vα2 usage in SLAP$^{-/-}$ OT-1 thymocytes, we assessed whether it would also alter composition in the resulting pool of peripheral T cells. SLAP$^{-/-}$ OT-1 mice displayed peripheral CD8^{+} T cell frequencies and absolute numbers similar to those in WT OT-1 mice (data not shown). Staining peripheral T cells with SIINFEKL tetramer [19] revealed that SLAP deficiency increases the frequency and absolute number of CD8^{+} T cells with low tetramer binding by 2 to threefold in the spleens of SLAP$^{-/-}$ OT-1 compared to WT OT-1 mice (Fig. 3a, b). In total, these data demonstrate that increased TCR levels on SLAP$^{-/-}$ OT-1 thymocytes, as well as increased signaling during thymocyte development, result in fewer CD8^{+} T cells, fewer DP thymocytes expressing Vα2, and more non-OVA-specific CD8^{+} T cells in mice where the T cell repertoire is relatively fixed.

More SLAP$^{-/-}$ Vβ5 DP thymocytes express high levels of Vα2

In contrast to WT OT-1 transgenic mice, WT Vβ5 transgenic mice express only the TCRβ chain (Vβ5) from the OT-1 TCR transgene which pairs with multiple endogenous TCRα chains. The result is more physiologic thymocyte development and a diverse TCR repertoire that still has increased TCR specificity for OVA in comparison with non-transgenic T cells [15, 16]. Therefore, Vβ5 expressing mice develop a diverse T cell repertoire whose affinity for OVA can be measured using the SIINFEKL tetramer. SLAP$^{-/-}$ Vβ5 mice displayed CD4^{+}, CD8^{+}, and DP thymocyte frequencies similar to those in WT Vβ5 mice, but decreased compared to WT and SLAP$^{-/-}$ controls (Fig. 4a, b, data not shown). However, SLAP$^{-/-}$ Vβ5 mice had an 18 % reduction in thymic cellularity, which resulted in decreased absolute numbers of total, DP and CD8^{+} thymocytes (Fig. 4b). Similar to the results seen in the OT-1 model, SLAP$^{-/-}$ Vβ5 DP thymocytes expressed twofold higher levels of both

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TCRβ and CD5, indicating enhanced signaling (Fig. 4c, d). Since Vβ5 paired with Vα2 can confer specificity for SIINFEKL in the OT-1 model, we analyzed Vβ2 usage by thymocytes in Vβ5 transgenic mice. Interestingly, SLAP$^{-/-}$ Vβ5 mice had almost a twofold increase in the absolute number of DP thymocytes that expressed Vα2 compared to WT Vβ5 mice (Fig. 5a, b). Thus, SLAP deficiency leads to the favored pairing of the Vβ5 transgene with Vα2, altering the selected TCR repertoire.

SLAP deficiency reduces the number of peripheral OVA-specific CD8$^+$ T cells and alters the peripheral CD8$^+$ α-chain repertoire in Vβ5 mice

As we were intrigued by the increased usage of Vα2 in developing SLAP$^{-/-}$ Vβ5 thymocytes, we next sought to determine whether this increase altered the number of peripheral OVA-specific T cells. Staining of splenic T cells with SIINFEKL tetramer demonstrated that SLAP deficiency decreased both the frequency and number of peripheral OVA-specific Vβ5 CD8$^+$ T cells by 24 % (Fig. 6a, b).

SLAP$^{-/-}$ Vβ5 mice exhibited an increased frequency and number of Vα2 expressing thymocytes and yet a decreased number of OVA-specific T cells in the periphery. Thus, we hypothesized that increased TCR levels during thymocyte development would select for a distinct TCRα-chain repertoire with a lower avidity for the original cognate ligand. Therefore, we characterized the cognate antigen-specific splenic CD8$^+$ T cells using commercially available Vα specific monoclonal antibodies. Further analysis was performed on both OVA-specific and non-OVA-specific T cells to examine their TCRα chains. SLAP$^{-/-}$ Vβ5 mice had 33 % fewer OVA-specific cells expressing Vα2 and 65 % fewer OVA-specific cells expressing Vα2, while non-OVA-specific peripheral Vβ5 CD8$^+$ T cells expressed 64 % more Vα2 but also 39 % less Vα2 (Fig. 7a, b). Collectively, these data demonstrate that increased TCR levels on SLAP$^{-/-}$ thymocytes and increased signaling during thymocyte development results in altered T cell repertoire in terms of antigen specificity and α-chain selection in Vβ5 mice.

Discussion

In this study, we show that SLAP deficiency increases TCR avidity leading to altered repertoire selection and negative selection of cognate antigen-specific CD8$^+$ T cells. SLAP is part of a critical signaling pathway in pre-selection DP thymocytes, which includes lymphocytic-specific protein tyrosine kinase (Lck), c-Cbl, the proline-rich sequence of CD3ε, noncatalytic region of tyrosine kinase protein (Nck), and lysosomal-associated transmembrane protein 5 (Laptm5) [25–34]. SLAP regulates TCR levels during thymocyte development through its direct association with c-Cbl, an E3 ubiquitin ligase that targets the TCRζ chain for ubiquitin-mediated degradation [8–10]. As TCRζ is the rate limiting chain for TCR complex assembly and surface expression, SLAP is a critical regulator of TCR levels and signaling in DP thymocytes. Despite previous studies demonstrating that SLAP deficiency alters positive thymocyte selection and agonist selection of thymic regulatory T cells (Tregs) [11, 35], there has been a significant gap in our understanding of how SLAP regulates CD8$^+$ T cell development and CD8$^+$ TCRα-chain repertoire selection.

We hypothesize that SLAP-dependent regulation of TCR levels in DP thymocytes allows for finer control of signaling at a time when selection decisions are made [8]. SLAP-dependent downmodulation of TCR levels lowers the amount of TCR available to interact with selecting MHC–peptide, thereby limiting TCR avidity and the intensity of TCR-generated signal. Reduced TCR avidity resulting from SLAP sufficiency may therefore allow for selection of a broader range of TCRs and a more diverse T cell repertoire. Diversity of TCR specificity is essential for the generation of a dynamic and plastic immune response to pathogens. SLAP may therefore be necessary to generate an optimal TCR repertoire. In

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contrast, by limiting the capacity of the developing thymocyte to downmodulate TCR levels, SLAP deficiency increases strength of signaling through the TCR complex during development, enhancing negative selection of thymocytes that would normally be positively selected on MHC-self peptide and limiting TCR repertoire. Therefore, SLAP deficiency could lead to the selection of a similarly sized T cell pool but a reduced TCR repertoire.

As a means of testing our hypothesis and determining the importance of SLAP in regulating the development and the repertoire selection of $CD8^+$ T cells, we generated a series of $SLAP^{-/-}$ transgenic mice. As expected from our published studies, we found that TCR levels were uniformly elevated in $SLAP^{-/-}$ OT-1 DP thymocytes [7, 10, 11]. However, while $SLAP^{-/-}$ OT-1 T cells had decreased specificity for the cognate antigen, OVA, even in the absence of thymic OVA expression, the majority of $SLAP^{-/-}$ OVA-specific $CD8^+$ T cells escaped negative selection. This could result from different self-peptides with lower affinity for the OT-1 TCR transgene selecting $SLAP^{-/-}$ OT-1 T cells [36, 37]. As an alternative and more physiologic system to study $CD8^+$ thymocyte development and repertoire selection, we generated $SLAP^{-/-}$ mice expressing only the TCRβ chain of the OT-1 transgene (Vβ5) [15]. In contrast to $SLAP^{-/-}$ OT-1 thymocytes, $SLAP^{-/-}$ Vβ5 thymocytes had increased usage of the TCRα chain, Vα2, which was originally isolated with the OT-1 TCRβ chain (Fig. 5). Further characterization of the α-chain repertoire of both OVA-specific and non-OVA-specific peripheral T cells revealed that $SLAP^{-/-}$ Vβ5 $CD8^+$ T cells that were OVA-specific had decreased usage of TCRα chains containing the Vα2 gene segment (Fig. 7a). In contrast, the non-OVA-specific $SLAP^{-/-}$ splenic $CD8^+$ T cells had a significant increase in the usage of TCRα chains containing the Vα2 gene segment, consistent with the enhanced Vα2 usage seen in DP thymocytes (Fig. 7b). Thus, SLAP deficiency favors pairing of transgenic TCRβ chain, Vβ5, with a TCRα chain containing the Vα2 gene segment. This result is likely because with SLAP deficiency, cells with otherwise weak affinity for MHC–peptide undergo positive selection. Collectively, these studies support a role for SLAP in regulating thymocyte negative selection, and secondary α-chain rearrangement, favoring a peripheral T cell pool with decreased reactivity to OVA and decreased usage of the Vα2 gene segment in OVA-specific T cells.

As most T cells never see their cognate antigen, it is likely that the most important interaction that T cells experience in shaping TCR repertoire is through conserved interactions with MHC in the context of self-peptides [38, 39]. Previous studies on repertoire selection decreased TCR levels or signal strength, varied the concentration of agonist peptide, or manipulated TCR affinity through the use of altered peptide ligands to identify thresholds required for thymocyte positive and negative selection [12, 13, 36]. However, these earlier studies did not quantify changes in TCR repertoire to both cognate and non-cognate antigens when TCR avidity and signaling are increased. Our studies demonstrate that SLAP, which increases TCR levels, avidity, and signaling in DP thymocytes, also has a significant impact on the peripheral T cell repertoire. SLAP deficiency leads to enhanced Vα2 usage by non-cognate antigen-specific T cells as well as a deletion in cognate antigen-specific T cells. Our studies therefore begin to fill a gap in our understanding of how SLAP regulates $CD8^+$ T cell development and repertoire selection. A limitation of these studies is that few commercially available anti-Vα antibodies exist, thus restricting complete analysis of the Vα repertoire in the $SLAP^{-/-}$ Vβ5 mice. Future studies could employ deep sequencing techniques to obtain a complete picture of how SLAP deficiency alters the Vα repertoire in $CD8^+$ T cells. Further investigation is also required to define the functional consequences of SLAP deficiency on peripheral $CD8^+$ T cell responses to identified selecting self-peptides and the cognate antigen, OVA [14, 37]. In summary, these studies expand our understanding of the effects of SLAP deficiency on T cell development and repertoire selection. Defining the specific signaling events regulated by SLAP that govern

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these processes should lead to future opportunities to manipulate T cell development and possibly peripheral function to ultimately treat immune-mediated disease.

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Biography

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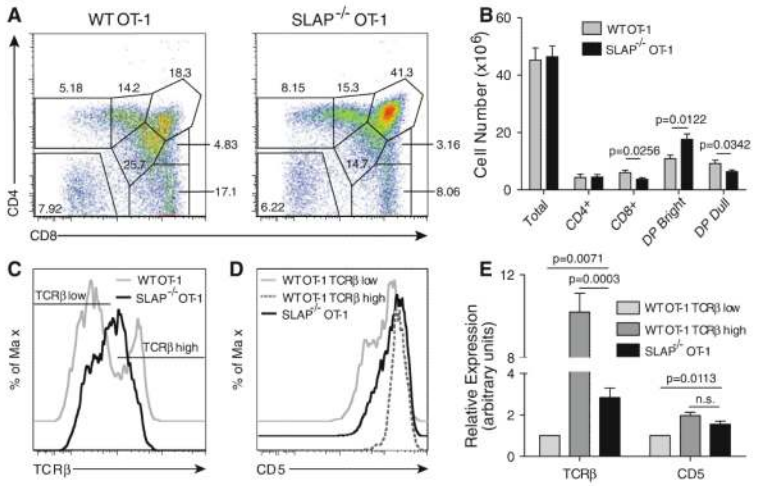

Fig. 1.

Fewer CD8$^+$ thymocytes and enhanced signaling in SLAP$^{-/-}$ OT-1 mice. **a** Thymocyte composition in WT and SLAP$^{-/-}$ OT-1 mice using a modified OT-1 gating scheme [20, 21]. **b** Absolute numbers of total thymocytes as well as CD4$^+$, CD8$^+$, DP bright, and DP dull thymocyte populations as shown in (**a**). **c** TCRβ expression on WT and SLAP$^{-/-}$ OT-1 DP bright thymocytes. **d** CD5 expression from WT OT-1 TCRβ low, WT OT-1 TCRβ high, and SLAP$^{-/-}$ OT-1 DP bright thymocytes. *Histograms* in **c** and **d** are offset for clarity. **e** Average TCRβ and CD5 expression from WT OT-1 TCRβ low, WT OT-1 TCRβ high, and SLAP$^{-/-}$ OT-1 DP bright thymocytes. Data from **e** are presented as relative fold change, compared with the WT OT-1 TCRβ low population ± SEM. Data represent the average of 8–11 mice per genotype (±SEM) from 4 independent experiments

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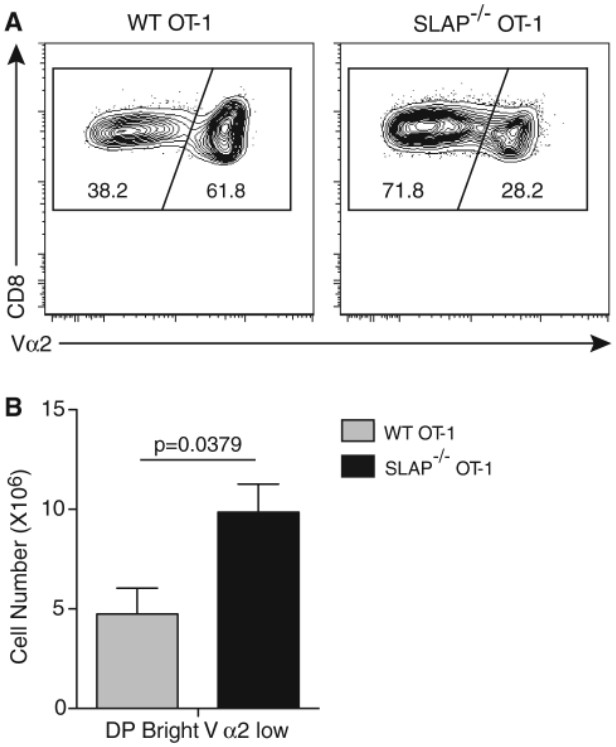

Fig. 2.

Increased numbers of DP Bright thymocytes with low levels of Vα2 expression in SLAP$^{-/-}$ OT-1 mice. **a** Vα2 expression in DP bright thymocytes from WT and SLAP$^{-/-}$ OT-1 mice. **b** Absolute numbers DP bright thymocytes expressing low levels of Vα2 as shown in (**a**). Data represent the average of 8–9 mice per genotype (±SEM) from 4 independent experiments

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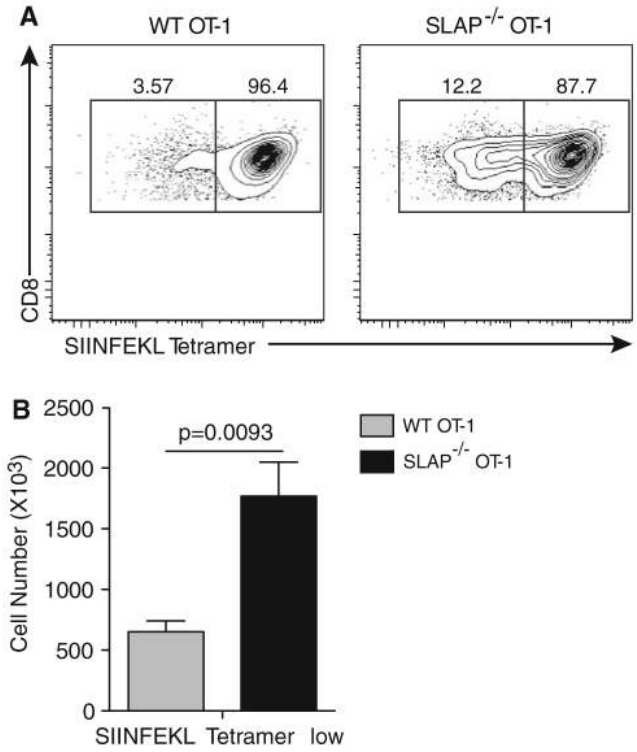

Fig. 3.

Increased peripheral non-antigen-specific CD8$^+$ T cells in SLAP$^{-/-}$ OT-1 mice. **a** SIINFEKL tetramer binding in CD8$^+$ splenocytes from WT and SLAP$^{-/-}$ OT-1 mice. **b** Absolute numbers of CD8$^+$ splenocytes with lower levels of tetramer binding as shown in (**a**). Data represent the average of 4 mice per genotype (±SEM) from 2 independent experiments

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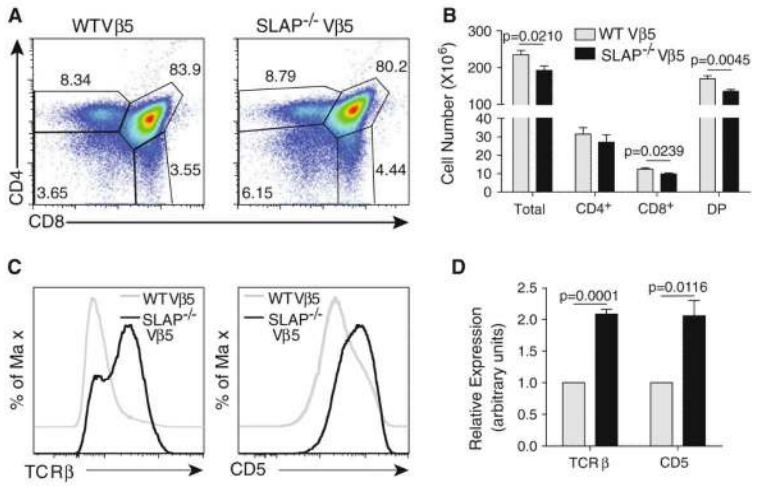

Fig. 4.

Normal thymic composition with decreased overall cellularity and enhanced signaling in SLAP$^{-/-}$ Vβ5 mice. **a** Thymocyte composition in WT and SLAP$^{-/-}$ Vβ5 mice. **b** Absolute numbers of total thymocytes as well as CD4^{+}, CD8^{+}, and DP thymocyte populations as shown in (**a**). **c** TCRβ and CD5 expression on WT and SLAP$^{-/-}$ Vβ5 DP thymocytes. Histograms are offset for clarity. **d** Average TCRβ and CD5 expression from WT and SLAP$^{-/-}$ Vβ5 DP thymocytes. Data from **d** are presented as relative fold change, compared with the WT Vβ5 DP population ± SEM. Data represent the average of 10–11 mice per genotype (±SEM) from 3 independent experiments

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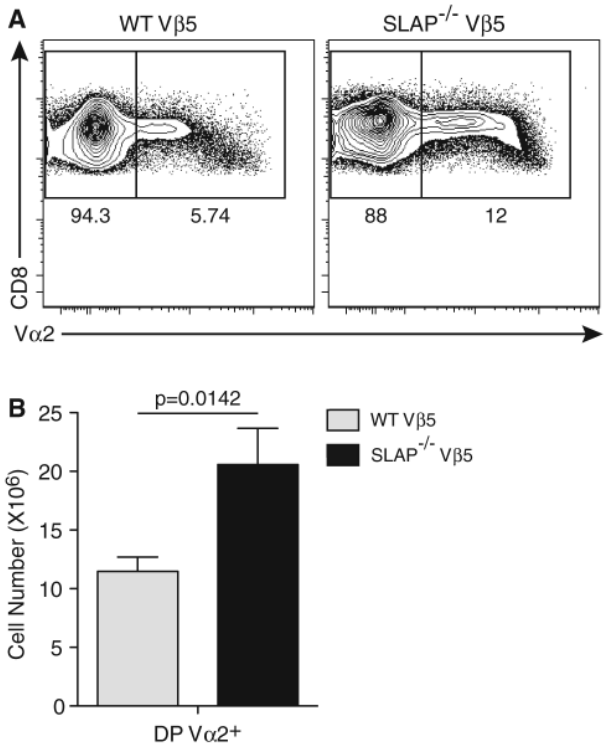

Fig. 5.

Increased numbers of DP thymocytes expressing Vα2 in SLAP$^{-/-}$ Vβ5 mice. **a** Vα2 expression in DP thymocytes from WT and SLAP$^{-/-}$ Vβ5 mice. **b** Absolute numbers DP thymocytes expressing Vα2 as shown in (**a**). Data represent the average of 6–7 mice per genotype (±SEM) from 2 independent experiments

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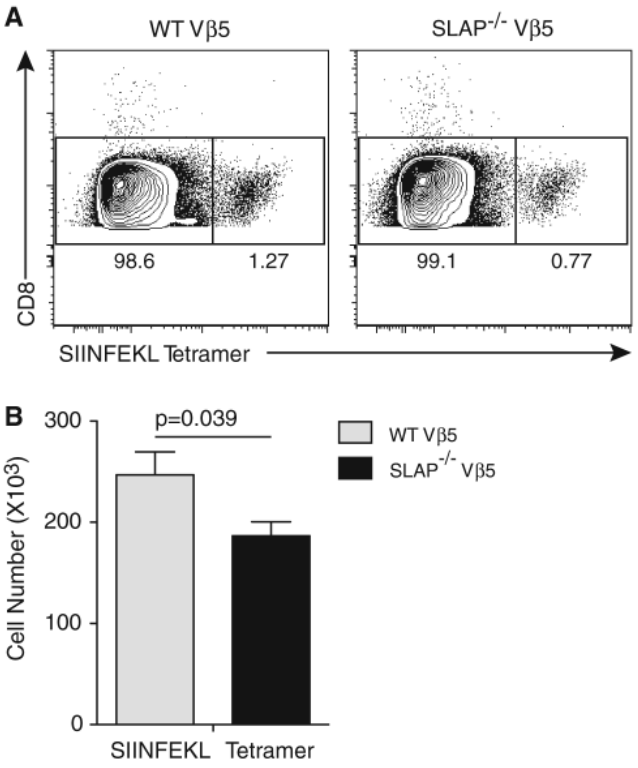

Fig. 6.

Fewer peripheral antigen-specific CD8$^+$ T cells in SLAP$^{-/-}$ Vβ5 mice. **a** SIINFEKL tetramer binding in CD8$^+$ splenocytes from WT and SLAP$^{-/-}$ Vβ5 mice. **b** Absolute numbers of CD8$^+$ splenocytes that bind tetramer as shown in (**a**). Data represent the average of 10–11 mice per genotype (±SEM) from 3 independent experiments

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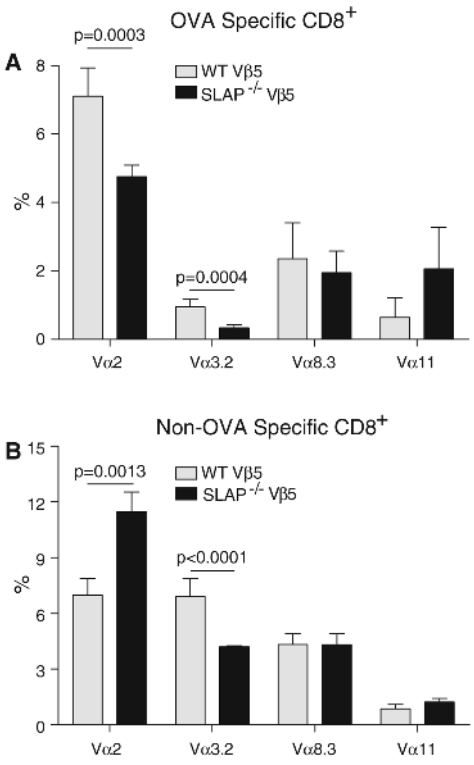

Fig. 7.

Altered peripheral CD8+ TCRα-chain repertoire in SLAP$^{-/-}$ Vβ5 mice. **a** Percentages of SIINFEKL tetramer positive CD8+ splenocytes expressing Vα2, Vα3.2, Vα8.3, or Vα11 in WT and SLAP$^{-/-}$ Vβ5 mice. **b** Percentages of SIINFEKL tetramer negative CD8+ splenocytes expressing Vα2, Vα3.2, Vα8.3, or Vα11 in WT and SLAP$^{-/-}$ Vβ5 mice. Data represent the average of 6–7 mice per genotype (±SEM) from 2 independent experiments