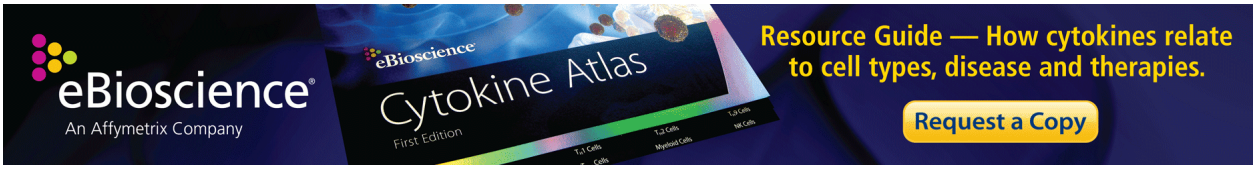

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Cutting Edge

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Cutting Edge: Allergen-Specific CD4 T Cells Respond Indirectly to Thymic Stromal Lymphopoietin To Promote Allergic Responses in the Skin

Ryan P. Larson,[*,†,1] Michael R. Comeau,[‡] and Steven F. Ziegler[*,†]

Thymic stromal lymphopoietin (TSLP) is an epithelial-derived cytokine that has been implicated in the initiation of allergic responses. CD4 T cells and dendritic cells are able to respond to TSLP in vitro; however, there has not been a careful dissection of the spatiotemporal response to TSLP by CD4 T cells in vivo during an allergic response. Previous work has suggested a requirement for TSLP in amplifying Th2 responses during allergen challenge by direct action on CD4 T cells; however, these studies did not determine whether there is an effect of TSLP on CD4 T cells during allergen sensitization. In this study we demonstrate an indirect role for TSLP on CD4 T cells during sensitization and challenge phases of an allergic response. This indirect effect of TSLP on CD4 T cells is due in part to the presence of TSLP exclusively in the allergen-sensitized and -challenged skin, rather than the draining lymph nodes. ***The Journal of Immunology*****, 2013, 190: 000–000.**

Thymic stromal lymphopoietin (TSLP) is a proallergic cytokine produced by epithelial cells at barrier surfaces in response to several stimuli, including tissue damage, inflammatory cytokines, allergens, or pattern recognition receptor ligation (1). Our group and others have demonstrated that TSLP is both necessary and sufficient for the initiation of allergic responses in several mouse models of atopic dermatitis (AD) and asthma. Conditional overexpression or administration of exogenous TSLP in the skin or lung results in diseases that mirror human AD and asthma, respectively (2–4). Additionally, the OVA-induced model of acute airway inflammation and contact hypersensitivity (CHS) models that have been used to study human asthma and AD, respectively, are both TSLP dependent (4, 5).

Although several cell types, including dendritic cells (DCs) and CD4 T cells, are able to respond directly to TSLP in vitro (4, 6), the identity of TSLP-responsive cells in vivo remains unclear. We have previously shown that TSLP is required at the sensitization phase of CHS induced by the hapten FITC in combination with the sensitizing agent dibutyl phthalate (DBP). TSLPR-deficient ($TSLPR^{-/-}$) mice experience a defective response after FITC/DBP sensitization characterized by reduced DC migration and T cell priming ability, demonstrating an important role for TSLP during allergen sensitization (5). Recent studies in the mouse have suggested that TSLP acts directly on CD4 T cells to amplify Th2 differentiation during chronic allergen exposure in the gut, lung, and skin (7–9). Studies using human cells have demonstrated that TSLP primarily acts on DCs to promote CD4 T cell expansion and Th2 differentiation (10). It remains controversial whether CD4 T cells require direct TSLP signals during allergen sensitization and challenge for proliferation, activation, and Th2 differentiation in vivo.

In this study we provide evidence that TSLP expression is induced by allergen sensitization in the target tissue, but not the draining lymph node (LN), suggesting that TSLP mediates its proallergic effects on a skin-resident cell type. Additionally, using adoptive transfer of allergen-specific CD4 T cells and bone marrow chimera approaches, we demonstrate a CD4 T cell–extrinsic requirement for TSLP that drives proliferation and Th2 cytokine production in response to acute sensitization with a cutaneous allergen. Furthermore, we demonstrate that TSLPR expression by CD4 T cells during allergen challenge is not required for expansion or Th2 cytokine production.

Materials and Methods

Mice

Six- to 12-wk-old BALB/c mice were purchased from Taconic or Charles River Laboratories. $TSLPR^{-/-}$, $TCR\beta^{-/-}$, $CD45.1^{+/+}$, and $TSLPR^{-/-}$ × CD45.1, DO11.10 × CD45.1, and DO11.10 × $TSLPR^{-/-}$ mice were bred and maintained in the Benaroya Research Institute Animal Facility. KN2 mice on the BALB/c background were received from Richard Locksley (University of California at San Francisco). All animals were housed in the Benaroya Research Institute Vivarium in specific pathogen-free conditions, and all experiments were done with approval from the Benaroya Research Institute Institutional Animal Care and Use Committee.

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The online version of this article contains supplemental material.

Abbreviations used in this article: AD, atopic dermatitis; CHS, contact hypersensitivity; DBP, dibutyl phthalate; DC, dendritic cell; LN, lymph node; TSLP, thymic stromal lymphopoietin; WT, wild-type.

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Contact hypersensitivity

CHS was induced using the hapten FITC (Sigma-Aldrich) in combination with DBP (Sigma-Aldrich) as previously described (5).

Tissue lysate ELISA

Ear tissue was homogenized in T-PER tissue protein extraction reagent (Thermo Fisher Scientific, Waltham, MA). Protein was quantified using a BCA protein assay (Pierce). Fifty micrograms total protein per sample was loaded per well using a murine TSLP ELISA kit (R&D Systems, Minneapolis, MN) following the manufacturer's protocol.

Bone marrow chimeras

Host mice were given split dose lethal irradiation of 900 rad. Directly following the second dose of irradiation mice were reconstituted with at least 1×10^6 T cell–depleted bone marrow cells.

Adoptive transfer and intradermal immunization studies

CD4 T cells were purified from DO11.10 × CD45.1 and DO11.10 × TSLPR$^{-/-}$ mice using the MACS CD4 purification kit (Miltenyi Biotec). CFSE-labeled wild-type (WT) and TSLPR$^{-/-}$ CD4 T cells were mixed at a 1:1 ratio, and 1×10^6 CD4$^+$KJ126$^+$ T cells were transferred into WT and TSLPR$^{-/-}$ hosts. Host mice were sensitized and challenged intradermally with 5 μg OVA protein with or without 5 μg TSLP.

Flow cytometry

The following Abs were used for FACS analysis (BD Biosciences and eBioscience): anti-CD4, anti-BrdU, anti-CD45.1, anti-CD45.2, anti-CD44, KJ126 idiotypic Ab specific for the DO11.10 TCR, anti–IL-4, and anti-human CD2.

Results and Discussion

Epicutaneous allergen induces TSLP expression in the skin

We have previously shown that the sensitizing agent DBP induced TSLP expression in the skin and that TSLP responsiveness is required for the development of CHS (5). Additionally, there are a variety of other factors that are likely acting downstream of TSLP that are required for the development of the FITC CHS response, including CD4 T cells, IL-4, and STAT6 (11). In light of recent studies demonstrating that basophils and DCs are capable of expressing TSLP in lymphoid tissues (12, 13), we sought to determine whether TSLP is preferentially induced in the skin versus the skin-draining LNs after sensitization with FITC/DBP. Elevated TSLP mRNA and protein expression was detected in the skin 24 h after sensitization; however, we did not observe induction of TSLP mRNA or protein in skin-draining LNs after allergen sensitization (Supplemental Fig. 1). These findings suggest that TSLP acts on a skin-resident cell type, rather than cells residing in the skin-draining LNs, to promote allergen sensitization.

Defective CD4 T cell response in FITC-sensitized TSLPR$^{-/-}$ mice

TSLP plays a critical role in the sensitization phase of the Th2 CHS response to FITC/DBP, in which the absence of TSLP signaling results in a significant diminution of the allergic response (5). The defective CHS response observed in TSLPR$^{-/-}$ mice was in part due to a defect in DC function during allergen sensitization; however, it was unclear whether there was also an intrinsic defect in the CD4 T cell response in allergen-sensitized TSLPR$^{-/-}$ mice (5). Both CD4 T cells and DCs express the heterodimeric TSLP receptor complex (14); however, the spatiotemporal cellular response to TSLP in vivo has not been investigated. To determine whether TSLP plays a role in CD4 T cell activation and proliferation during allergen sensitization in vivo, we measured CD44 expression and BrdU incorporation, respectively, in FITC-sensitized WT and TSLPR$^{-/-}$ mice. We observed reduced BrdU incorporation (Fig. 1A, 1B) and CD44 upregulation (Fig. 1C) by CD4 T cells from FITC-sensitized TSLPR$^{-/-}$ mice. These findings demonstrate that CD4 T cells from TSLPR$^{-/-}$ mice are defective in proliferation and activation under acute inflammatory conditions during a TSLP-dependent response.

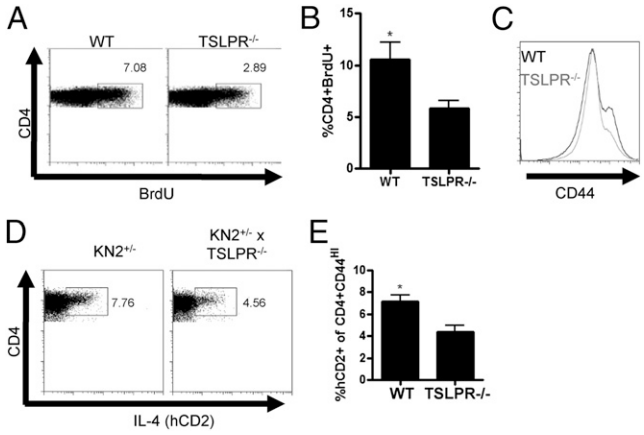

FIGURE 1. Defective CD4 T cell response after FITC sensitization of TSLPR$^{-/-}$ mice. WT and TSLPR$^{-/-}$ mice were sensitized epicutaneously with 100 μl 0.5% FITC and treated with BrdU for 5 d. Skin-draining inguinal and axillary LNs were harvested on day 6 after sensitization. Representative FACS plots (**A**) and graphs (**B**) depicting BrdU incorporation by CD4 T cells from FITC-sensitized WT and TSLPR$^{-/-}$ mice are shown. (**C**) Representative histogram depicting CD44 expression by CD4 T cells from FITC/DBP-sensitized WT and TSLPR$^{-/-}$ mice. Representative FACS plots (**D**) and graphs (**E**) depicting frequency of IL-4–expressing CD44$^+$ CD4 T cells from FITC-sensitized WT and TSLPR$^{-/-}$ mice on an IL-4 reporter background are shown. Statistical significance was calculated using a Student *t* test. Data are representative of three independent experiments with three or more mice per group. $^*p < 0.05$.

To determine whether TSLP plays a role in Th2 differentiation in vivo following FITC sensitization, we crossed TSLPR$^{-/-}$

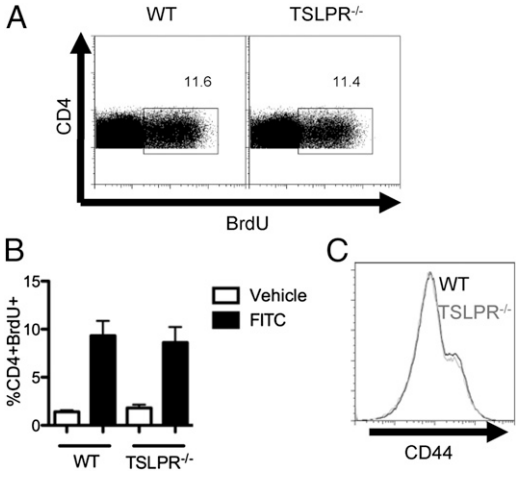

FIGURE 2. CD4 T cells do not require direct TSLP signals to proliferate or become activated in vivo in a TSLP-dependent CHS model. Lethally irradiated TCRβ$^{-/-}$ mice were reconstituted with a 1:1 mix of WT and TSLPR$^{-/-}$ congenically distinct T cell–depleted bone marrow. Chimeric mice were epicutaneously sensitized with 0.5% FITC on the shaven abdomen, and BrdU incorporation by CD4 T cells from skin draining LNs was assessed on day 6 after sensitization. Representative FACS plots (**A**) and graphical depiction (**B**) of BrdU incorporation by WT and TSLPR$^{-/-}$ donor–derived CD4 T cells 6 days after FITC sensitization are shown. Data compiled from three independent experiments. (**C**) Representative histogram depicting CD44 expression by CD4 T cells from within the same FITC/DBP-sensitized chimeric mouse.

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mice to IL-4 reporter mice (KN2) (TSLPR$^{-/-}$ × KN2$^{+/-}$), which have human CD2 knocked into the endogenous IL-4 locus (15). There is a reduced frequency of IL-4–producing Th2 cells in FITC-sensitized TSLPR$^{-/-}$ mice, demonstrating a defect in Th2 differentiation in TSLPR$^{-/-}$ mice after sensitization with FITC/DBP (Fig. 1D, 1E). Taken together, these findings demonstrate a requirement for TSLP in CD4 T cell proliferation, activation, and Th2 differentiation after epicutaneous sensitization with FITC/DBP, a combination known to induce a TSLP-dependent Th2 response (5). This line of experimentation led us to determine whether these defects were due to a CD4 T cell–intrinsic requirement for TSLP.

CD4 T cells do not require direct TSLP signals to proliferate or become activated after allergen sensitization

To address whether CD4 T cells require direct TSLP signals during allergen sensitization, mixed bone marrow chimeras were made in which lethally irradiated TCRβ$^{-/-}$ hosts were reconstituted with a 1:1 mixture of WT and TSLPR$^{-/-}$ bone marrow. In the mixed chimera setting there was equivalent BrdU incorporation (Fig. 2A, 2B) and upregulation of CD44 (Fig. 2C) by WT and TSLPR$^{-/-}$ CD4 T cells within the same FITC/DBP-sensitized host. These findings suggest that the CD4 T cell defect observed in the FITC/DBP-sensitized intact TSLPR$^{-/-}$ mice is cell extrinsic. Furthermore, it suggests that the TSLP expressed in the skin after FITC/DBP sensitization (Supplemental Fig. 1) does not act on naive CD4 T cells in the skin-draining LNs but acts instead on another TSLP-responsive cell type that likely resides in the skin.

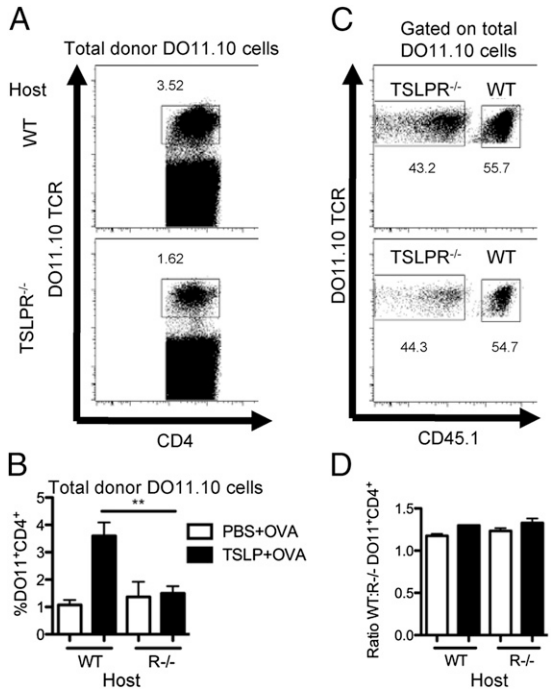

FIGURE 3. Allergen-specific CD4 T cells do not require direct TSLP signals to expand after intradermal administration of TSLP and protein allergen. WT (CD45.1$^+$) and TSLPR$^{-/-}$ (CD45.2$^+$) congenically distinct DO11.10$^+$CD4$^+$ T cells were mixed 1:1 and 10^6 total DO11.10$^+$CD4$^+$ T cells were adoptively transferred into WT and TSLPR$^{-/-}$ hosts, which were subsequently immunized intradermally with 5 μg TSLP plus 5 μg OVA (T+O). Skin-draining LNs were harvested on day 6 after allergen sensitization and analyzed. Representative plots depicting total (WT and TSLPR$^{-/-}$) DO11.10$^+$CD4$^+$ T cells in WT (*top*) and TSLPR$^{-/-}$ (*bottom*) hosts (**A**) as well as graphical depiction (**B**) of frequency of total donor DO11.10$^+$CD4$^+$ T cells (WT and TSLPR$^{-/-}$ combined) are shown. Representative FACS plots (**C**) and graphical depiction (**D**) of ratios of relative frequencies of WT to TSLPR$^{-/-}$ DO11.10$^+$CD4$^+$ T cells in the total donor CD4 T cell pool are shown. Data are representative of two independent experiments with three or more mice per group. Statistical significance was calculated using a one-way ANOVA. $^{**}p < 0.01$.

TSLP-dependent intradermal sensitization and challenge to protein allergen occurs in the absence of TSLPR on allergen-specific CD4 T cells

To further understand the effect of TSLP on naive allergen-specific CD4 T cells during allergen sensitization, we sensitized WT and TSLPR$^{-/-}$ hosts intradermally with TSLP and OVA in the presence of coadoptively transferred congenically distinct CFSE-labeled WT and TSLPR$^{-/-}$ OVA-specific DO11.10 CD4 T cells. This model allows for the tracking of WT and TSLPR$^{-/-}$ allergen-specific CD4 T cells in the same host inflammatory environment as well as limiting any observed effects to TSLP alone, as there are a variety of other inflammatory mediators induced after treatment with FITC/DBP (16). As expected, we observed a significant reduction in the expansion of total donor (WT and TSLPR$^{-/-}$ combined) DO11.10 CD4 T cells that reside in the OVA plus TSLP–sensitized TSLPR$^{-/-}$ host as compared with those cells in WT hosts (Fig. 3A, 3B). Analysis of donor WT and TSLPR$^{-/-}$

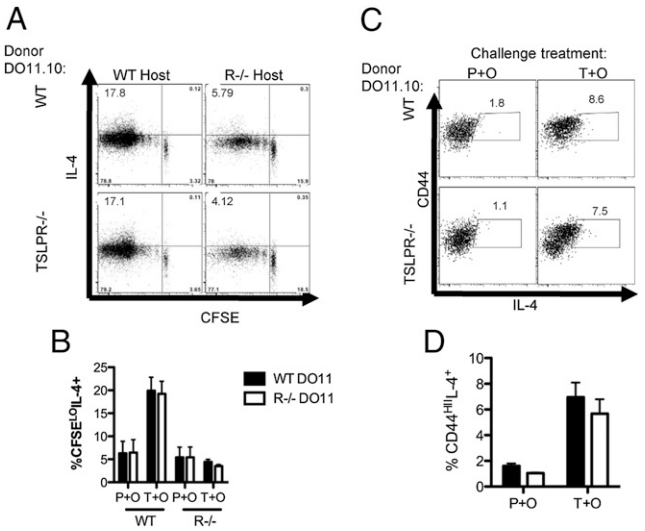

FIGURE 4. Allergen-specific CD4 T cells do not require direct TSLP signals to produce IL-4 after intradermal sensitization or challenge with TSLP and protein allergen. CFSE-labeled WT (CD45.2$^+$) and TSLPR$^{-/-}$ (CD45.1$^+$) congenically distinct DO11.10$^+$CD4$^+$ T cells (10^6 total) were mixed 1:1 and adoptively transferred into WT and TSLPR$^{-/-}$ hosts, which were subsequently sensitized with 5 μg TSLP plus 5 μg OVA (T+O) or PBS plus OVA (P+O) and skin-draining LNs were harvested on day 6 after sensitization. The same mixed adoptive transfer strategy was used for challenge experiments. WT hosts were subjected to T+O sensitization and were intradermally challenged on day 7 after sensitization with T+O or P+O. Skin-draining LNs were harvested 24 h after allergen challenge for analysis. Cells were restimulated with PMA and ionomycin and analyzed for IL-4 expression. Representative plots (**A**) and graphical representation (**B**) of IL-4–expressing CFSE^{lo} WT and TSLPR$^{-/-}$ DO11.10$^+$CD4$^+$ T cells within the same WT or TSLPR$^{-/-}$ host after intradermal sensitization with T+O or P+O are shown. Representative plots (**C**) and graphical depiction (**D**) of CD44$^+$ IL-4–expressing WT and TSLPR$^{-/-}$ DO11.10 CD4 T cells in intradermally T+O-sensitized WT mice that were subsequently challenged intradermally with T+O or P+O. Data are representative of two independent experiments with three or more mice per group.

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DO11.10 CD4 T cells within the same host independently based on their expression of congenic markers demonstrates equivalent expansion of DO11.10 CD4 T cells from both WT and TSLPR$^{-/-}$ donors, resulting in a 1:1 ratio (Fig. 3C, 3D). Therefore the significantly increased expansion observed in the WT host is due to the equivalent expansion of both donor WT and TSLPR$^{-/-}$ DO11.10 CD4 T cells (Figs. 3C, 3D), thus demonstrating an indirect effect of TSLP on allergen-specific CD4 T cell expansion after TSLP-dependent allergen sensitization. Additionally, we observed a significant reduction in IL-4–expressing CD4 T cells in TSLPR$^{-/-}$ hosts, as compared with WT hosts, independent of TSLPR expression by donor DO11.10 CD4 T cells (Fig. 4A, 4B). Consistent with the mixed bone marrow chimera data shown above, these data demonstrate that CD4 T cells respond to TSLP indirectly during allergen sensitization, resulting in proliferation, activation, and Th2 differentiation.

Using a similar coadoptive T cell transfer study, we assessed whether allergen-specific CD4 T cells required direct TSLP signals to respond to allergen challenge. In this scenario only WT hosts were sensitized and challenged because we established that CD4 T cells respond normally to TSLP plus OVA sensitization regardless of TSLPR expression status. WT and TSLPR$^{-/-}$ CD4 T cells responded equivalently to OVA plus TSLP challenge, as shown by equivalent frequencies of IL-4–producing T cells in the skin-draining LNs, demonstrating that CD4 T cells do not require direct TSLP signals during an acute challenge with allergen in the presence of TSLP (Fig. 4C, 4D).

In conclusion, these data support a model where CD4 T cells respond indirectly to TSLP during epicutaneous sensitization and acute allergen challenge in the presence of type 2 inflammatory stimuli. TSLP plays a nonredundant role in promoting Th2 differentiation of CD4 T cells in the LN draining the sites of sensitization and challenge. This is most likely accomplished through the maturation of skin-resident DCs following Ag uptake and migration to the regional LN (5). This model is supported by recent reports from our laboratory and others demonstrating a DC-intrinsic requirement for STAT5 or TSLPR expression, respectively, in mediating TSLP-dependent allergic responses in the skin, providing additional evidence that DCs are the direct target of TSLP in vivo (17, 18). In contrast to our findings, previous work in a skin allergy model suggested a direct role for TSLP on CD4 T cells during allergen challenge resulting in amplification of Th2 cytokine production. This study did not find any deficit in CD4 T cell function after chronic epicutaneous allergen sensitization (9). However, we have clearly demonstrated that CD4 T cells have a cell-extrinsic requirement for TSLP to expand and undergo Th2 differentiation after acute allergen sensitization in the presence of TSLP. It is possible that allergen-specific CD4 T cells sensitized in a chronic manner may have different cytokine requirements during the challenge phase of the response, as we have shown that TSLP treatment of in vitro–differentiated Th2 cells can amplify Th2 cytokine production (19). However, the model presented in this study clearly demonstrates an indirect role for TSLP on allergen-specific CD4 T cell expansion, activation, and Th2 differentiation during allergen sensitization and challenge.

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Disclosures

M.R.C. is an employee of Amgen, Inc. The other authors have no financial conflicts of interest.

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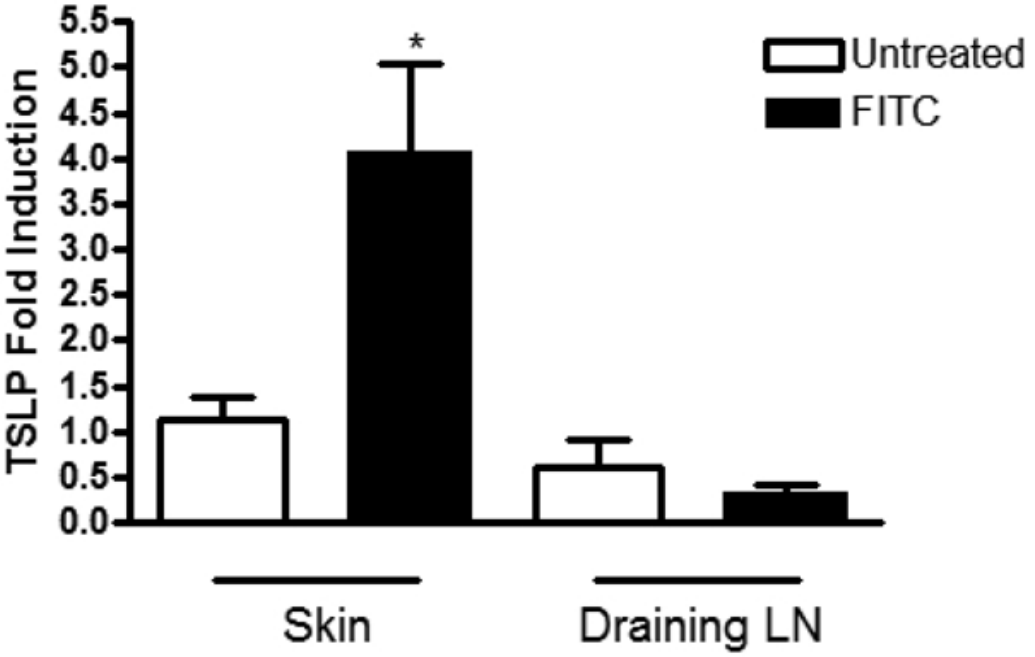
Supplementary Figure 1. Epicutaneous sensitization with FITC/DBP selectively induces TSLP in the skin and not the draining LNs. TSLP mRNA (A) and protein (B) measured in the skin and draining LNs 24 Hours post epicutaneous sensitization with FITC/DBP.

A



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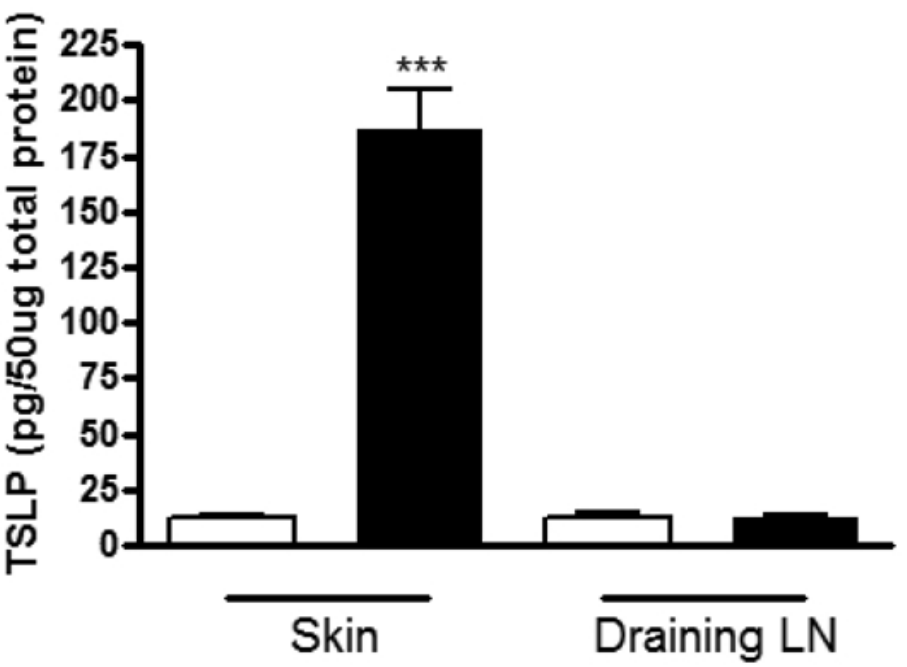

Supplementary Figure 1