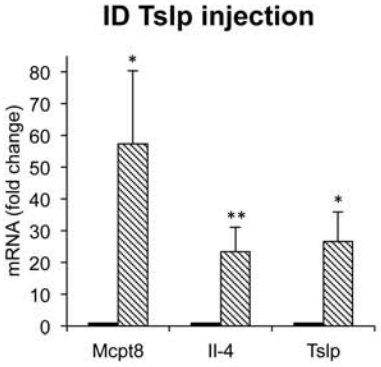
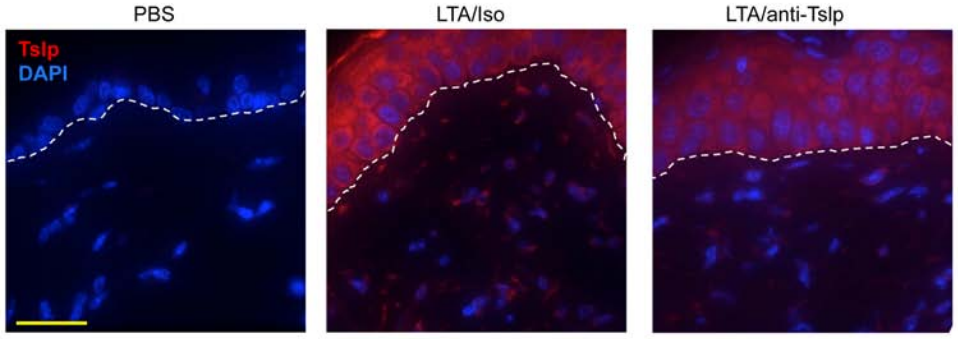
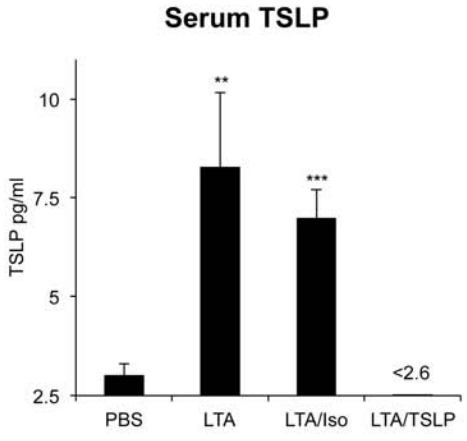


Supplemental Figure 1. Intradermal injection of Tslp recruits basophils, induces *Il-4* and auto-amplifies *Tslp* gene expression in the skin. Mice were ID injected two times with 5 mg of Tslp in PBS or sham control (PBS only) with a 24 hour interval between injections. Skin samples at site of injection were harvested 24 hours after the second Tslp injection and mRNA was analyzed by RT-PCR for expression of *Mcpt8* (basophils), *Il-4*, and *Tslp*. Data are Mean± SEM, n = 6. *P<0.05; **P<0.01 compared to PBS control.



Supplemental Figure 2. LTA mediated dermal Tslp induction is suppressed by a blocking anti-TSLP mAb. Mice were pre-treated with IP injection of isotype control or anti-TSLP blocking mAb on days 0, 3 and 6, as schematically shown in Figure 2. On day 6, mice were also injected ID with LTA or PBS. Skin samples at the injection site were harvested 48 hours post LTA treatment. Red stain corresponds to Tslp (cy3), blue stain corresponds to nuclei (DAPI). Dashed line represents epidermal-dermal junction. Bar = 80 μm. Mean fluorescent intensity (MFI) for Tslp in LTA/Isotype treated epidermis (92) dermis (6.6), and in LTA/anti-Tslp epidermis (27), dermis (2.5).



Supplemental Figure 3. LTA mediated increases in serum Tslp levels are suppressed by a blocking anti-TSLP mAb . Mice were pre-treated with Isotype control (Iso) or Tslp blocking mAb (anti-TSLP) and subsequently treated with PBS or LTA as described in Figure 2. Blood was harvested 48 hours after PBS or LTA treatment and analyzed by ELISA for the expression of Tslp. All mice in the LTA treated group and the LTA/Isotype group had detectable levels of Tslp. None of the mice pretreated with anti-Tslp blocking antibody had detectable levels of Tslp. Data are Mean± SEM, n = 6. **P<0.01; ***P<0.001 as compared to PBS control.

SUPPLEMENTAL

MATERIALS AND METHODS

Quantitative real-time PCR (RT-PCR)

Total RNA was isolated by RNeasy Mini Kits (Qiagen, Valencia CA) according to the manufacturer's protocol. One microgram of RNA was reverse transcribed using the Qiagen Quantiscript kit according to manufacturers protocol. RT-PCR was performed and analyzed by the dual-labeled fluorogenic probe method by using an ABI Prism 7300 sequence detector (Applied Biosystems, Foster City CA). Probes for mouse Tslp, Il-4, Il-5, Il-13, Il-17a, Il-22, Il-23, Il-25, Il-33, Ifn-γ, Tnf-a, and beta actin were purchased from Applied Biosystems. Amplification reactions were performed in MicroAmp optical plates (Applied Biosystems) in a 25-µL volume. All reactions were normalized to beta actin.

Cell staining and Microscopy

Paraffin-embedded skin biosies were cut at 5 µm on frosted microscope slides. Slides were deparaffinized and then rehydrated. Skin sections were then blocked with 5% BSA in Super Block (ScyTek Laboratories, Logan, UT). Slides were then stained with antibodies directed against Mcpt8 (TUG8) (Biolegend, Dedham, MA; cat #647404, 1:500); or Tslp (ThermoFisher, Rockford, IL; cat #PA5-20321, 1:1000) or equal amounts of isotype control antibodies. Slides were incubated at 4℃ overnight and then washed with PBS/Tween 0.05%. Secondary Cy-3 conjugated antibody (Jackson Labs; West Grove, PA) was added for 1 hour and cell nuclei were visualized with DAPI (Sigma). Images were taken with a Leica

Microscope (Wetzlar, Germany) at 40x magnification using SlideBook software (Intelligent Imaging Innovations, Denver, CO).

Mice

All procedures performed on mice were in accordance with the NIH guidelines for humane treatment of animals and were approved by the IACUC of National Jewish Health. Mice were kept under pathogen free conditions. Intradermal injections were performed on C57BL/6 x FVB mice aged 2-6 months. Mice were briefly anesthetized with Isoflurane, shaved, and injected with 100 micrograms of *S. aureus* derived LTA (InvivoGen; San Diego, CA cat #tlrl-pslta), in a 100 µl volume of PBS into the back. 48 hours later, mice were euthanized by carbon dioxide inhalation, and the treated skin region was excised using a 6 mm biopsy punch (Miltex Inc.). Epicutaneous application was performed by anesthetizing with Isoflurane, shaving, followed by 5 tape strips and application of 100 micrograms LTA in a 100 µl volume of PBS in a Tagaderm dressing. Mice were harvested 48 hours later. Biopsies were then preserved in TRI reagent (MilliporeSigma) for RNA extraction, or in formalin buffered saline for paraffin-embedding for Histology. Administration of the Tslp blocking mAb clone 28F12 (Biolegend cat# 515202 or ThermoFisher cat# 16-5491-85) or isotype control (BioLegend) was performed by 3 separate IP injections of 500 µg mAb in a 150 µl volume. The first 2 injections were performed 6 and 3 days prior to LTA treatment. The third injection was 1 hour prior to LTA treatment. Mice were harvested 48 hours after the final LTA injection. For direct injection of

recombinant Tslp (R&D Systems cat # 555-TS-10) into mice, 5 micrograms recombinant Tslp was ID injected two times with a 24 hour interval between injections. Skin samples at site of injection were harvested 24 hours after the second Tslp injection. Serum Tslp levels were measured by Quantikine ELISA kit for mouse (R&D Systems cat #MTLP00).

Statistical analyses.

All statistical analysis was conducted using Graph Pad Prism. Comparisons of expression levels were performed using analysis of variance (ANOVA) techniques and Student's *t* tests as appropriate.

Supplemental Figure 1. Intradermal injection of Tslp recruits basophils, induces Il-4 and auto-amplifies Tslp gene expression in the skin. Mice were ID injected two times with 5 μg of Tslp in PBS or sham control (PBS only) with a 24 hour interval between injections. Skin samples at site of injection were harvested 24 hours after the second Tslp injection and mRNA was analyzed by RT-PCR for expression of Mcpt8 (basophils), Il-4, and Tslp. Data are Mean± SEM, n = 6. *P<0.05; **P<0.01 compared to PBS control.

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