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Mechanical injury polarizes skin dendritic cells to elicit a Th2 response by inducing cutaneous TSLP expression

Michiko K. Oyoshi[*], **Ryan P. Larson**[†,‡], **Steven F. Ziegler**[†,‡], and **Raif S. Geha**[*]

[*]Division of Immunology, Children's Hospital and Department of Pediatrics, Harvard Medical School, Boston, MA 02115

[†]Department of Immunology, University of Washington School of Medicine, Seattle, WA 98185

[‡]Immunology Program, Benaroya Research Institute at Virginia Mason, Seattle, WA 98101

Abstract

Atopic dermatitis (AD) is characterized by intense scratching and a Th2 dominated systemic and local immune response to cutaneously introduced antigens. Because scratching inflicts mechanical injury to the skin, we examined the effect of mechanical injury inflicted by tape stripping on the capacity of skin dendritic cells (DCs) to polarize T cells towards a Th2 phenotype. DCs isolated from skin 6 hrs after tape stripping elicited significantly higher production of IL-4 and IL-13, and significantly lower production of interferon-γ (IFN-γ) by OVA stimulated $CD4^+$ DO.11.10 cells, than DCs isolated from unmanipulated skin, and expressed significantly more mRNA for the Th2 skewing molecules IL-10 and the Notch ligands Jagged1 and Jagged2, but significantly less mRNA for the Th1 skewing cytokine IL-12. $CD11c^+FITC^+$ cells isolated from draining lymph nodes (DLN) of shaved and tape stripped mouse skin 24 hrs after painting with FITC polarized T cells towards Th2 significantly more than $CD11c^+FITC^+$ cells isolated from DLN of shaved non tape stripped skin, and expressed significantly more IL-10, Jagged1 and Jagged2 mRNA, but significantly less IL-12 mRNA. Tape stripping significantly increased TSLP levels in the skin. Studies in $TSLPR^{-/-}$ mice demonstrated that TSLP played an essential role in the Th2 polarization effect of tape stripping on skin DCs. These results suggest that mechanical injury inflicted by scratching in patients with AD polarizes skin DCs to elicit a Th2 response by upregulating local expression of TSLP.

INTRODUCTION

Atopic dermatitis (AD) is characterized by dry skin, intense scratching and allergic inflammation of the dermis with infiltration by T cells and accumulation of eosinophil derived products (1). Acute AD skin lesions express the Th2 cytokines IL-4, IL-5, and IL-13, while chronic lesions also express the Th1 cytokine IFN-γ, and exhibit dermal thickening and increased deposition of collagen (2). AD patients have evidence of systemic Th2 dominated immune responses with elevated serum IgE present in more than 85% of the patients and evidence of antigen specific IgE in more than half (3).

The hallmark of AD is dry itchy skin due to a disrupted skin barrier with evidence of increased transepidermal water loss (4, 5). About 20% of AD patients have mutation in the skin specific gene filaggrin, which is important for maintaining skin hydration (6–10). Skin dryness in AD results in intense scratching which inflicts mechanical injury to the skin.

[*]Address correspondence and reprint requests to: Raif S. Geha, Karp 10, Division of Immunology, Children's Hospital, 300 Longwood Avenue, Boston, Massachusetts 02115, USA., Phone: (617) 919 2482, Fax: (617) 730 0528, raif.geha@childrens.harvard.edu.

Disruption of the skin barrier in AD allows the entry of antigens which otherwise are excluded by a normal skin barrier. Epicutaneous (EC) sensitization with allergens is thought to play an important role in the pathogenesis of AD. This is supported by the observation that application of allergen to the abraded uninvolved skin of patients with AD provokes an eczematous rash with eosinophilic infiltration (11).

Dendritic cells (DCs) are essential for the generation of an immune response. In the skin there are two major types of DCs: Langerhans cells in the epidermis and interstitial (dermal) DCs in the dermis. Langerhans cells form a DC network in the epidermis where they sample antigens that get through the skin barrier. Antigen that reaches the interstitial spaces is taken by dermal DCs. Following antigen uptake, and in the presence of danger signals generated by microbial antigens from the skin flora, and/or mechanical injury, inflammatory mediators such as IL-1 and TNF-α are released (12, 13) and cause the conversion of the immature skin DCs into mature professional APCs by upregulating the expression of MHC class II molecules and key co-stimulatory molecules, such as CD80 and CD86, and reducing the capacity of DCs to take up antigen. DCs that capture antigen in the skin express CCR7, and migrate to draining lymph nodes (DLN), which express the chemokines CCL19 and CCL21 (14), where they present antigenic peptides to naïve recirculating T cells. Interaction between antigen-laden DCs and antigen specific T cells leads to T cell proliferation and differentiation. In the case of $CD4^+$ T cells, differentiation leads to the generation of Th1 cells that secrete IFN-γ, Th2 cells that secrete IL-4, IL-5, and IL-13 and other Th subsets, which include Th17, Th22 and Th9 (15–17).

Tape stripping mimics scratching in that both inflict mechanical injury to the skin. We have examined the effect of mechanical injury inflicted by tape stripping on the ability of skin DCs to polarize T cells towards Th2. We demonstrate that DCs isolated directly from tape stripped skin and skin-derived DCs isolated from DLN skew the T cell response towards Th2 compared to control DCs derived from uninjured skin. We also show that tape stripping upregulates thymic stromal lymphopoietin (TSLP) expression in skin and that TSLP plays an essential role in the Th2 polarizing effect of tape stripping on skin DCs.

MATERIALS and METHODS

Mice

Balb/c wild type (WT) mice were purchased from Charles River Laboratory (Wilmington, MA). DO11.10 TCR transgenic mice and $TSLPR^{-/-}$ mice on Balb/c background were bred in our facility. All mice were kept in a specific pathogen-free environment and fed an OVA-free diet. All procedures performed on the mice were in accordance with the Animal Care and Use Committee of the Children's Hospital Boston.

Analysis of skin DCs

Ears of Balb/c WT mice were left unmanipulated or tape stripped 12 times with transparent bio-occlusive dressing (Tegaderm, Westnet Inc.). Preparation of ear cells was performed as described previously (18). Briefly, six hrs after tape-stripping, ears were floated on 0.25 % trypsin/2.2 mM EDTA (Invitrogen) at 37°C for 45 min. Ears were then cut into small pieces which were incubated in RPMI 1640 containing 10% FCS at 37°C for 2 hrs. After filtration through a 70 μm cell strainer, single cell suspensions were washed and incubated with Fc block (2.4G2). Surface markers were stained with mAbs for B220, CD11b, CD11c, CD19, CD40, CD80, CD86, Langerin, and MHC class II (all from eBioscience), then ran on a FACSCanto (Becton Dickinson, Mountain View, California, USA) and the results were analyzed using FlowJo software (Tree Star Inc.). For functional assay, $CD11c^+$ cells were isolated using AutoMacs (Miltenyi Biotec).

In vivo assay for migration of skin DCs

For migration of skin DCs to DLN, the dorsal skin of anesthesized mice was shaved or shaved and tape-stripped 6 times followed by application of 100 μL of 10 mg/ml FITC dissolved in 1:1 acetone/dibutylphthalate (DBP). At 2, 6, 24 and 48 hr after FITC application, inguinal and axillary lymph nodes were removed and incubated with 300 U/ml type I collagenase (Worthington Biochemical) and 100 U/ml DNase I (Sigma) in PBS at 37 °C for 60 min. After filtration through 70 μm cell strainer, single cell suspensions were stained with mAbs then analyzed by FACS as described above.

Proliferation and polarization assays

$CD11c^+$ cells were isolated using AutoMacs (Miltenyi Biotec) and were stained with PE-conjugated CD11c antibody (eBioscience). $CD11c^+FITC^+$ or $CD11c^+FITC^-$ cells were sorted on a FACS Vantage SE (BD). Splenic $CD4^+$ T cells from DO11.10 mice were purified by $CD4^+$ Microbeads separation using AutoMacs (Miltenyi Biotec). 1×10^5/well of $CD4^+$DO11.10 T cells and 1×10^4/well of DCs were cultured in the presence of 2 μM of OVA $peptide_{323–339}$ (OVAp) for 96 hrs in RPMI 1640 (Invitrogen) supplemented by 10% FCS, 1 mM sodium pyruvate, 2 mM L-glutamine, 0.05 mM 2-ME, 100 U/ml penicillin, and 100 μg/ml streptomycin. Proliferation was assessed by the incorporation of $[^3H]$-thymidine, 1 μCi/well added during the last 8 hrs of 96 hr cultures. The supernatants were harvested and stored at −70 °C for measurements of cytokines by ELISA. Mouse ELISA kits were purchased from eBioscience (IL-4, IL-5, IFN-γ) and R&D Systems (IL-13).

Quantitative PCR (Q-PCR)

Purified DCs from skin or DLN were suspended in lysis buffer solution provided in the RNAqueous extraction kit (Ambion Inc.). $CD4^+$ T cells were purified with Microbeads (Miltenyi Biotec) after cocultured with $CD11c^+$ cells, then suspended in lysis buffer. Reverse transcription was performed using iScript cDNA synthesis kit (BioRAD). PCR reactions were run on an ABI Prism 7300 (Applied Biosystems) sequence detection system platform. Taqman primers and probes were obtained from Applied Biosystems. The housekeeping gene β_2-microglobulin or GAPDH was used as a control. Relative gene expression was determined using the method described by Pfaffl (19).

Measurement of TSLP levels in the skin

The skin was homogenized using a Polytoron RT-3000 (Kinematica: AG) in T-PER tissue protein extraction reagent (Thermo Scientific) supplemented with a protease inhibitor cocktail (Roche). Protein concentration was quantified with Nanodrop (Thermo Scientific). ELISA for TSLP protein was performed according to manufacturer's protocol (R&D systems).

Statistical analysis

Statistical significance was determined by using GraphPad Prism, version 4.0a (La Jolla, CA). Statistical differences were determined by student's *t* test (between 2 groups) and a one-way analysis of variance (ANOVA) (between multiple groups). A P value of < 0.05 was considered to indicate statistical significance.

RESULTS

Mechanical injury polarizes skin DCs to elicit a Th2 response

DCs were isolated from ear skin 6 hours after tape stripping and compared to DCs isolated from unmanipulated ear skin. We used the 6 hr time point in order to capture DCs from injured skin prior to their migration to LN, and at a time when expression of cytokines (IL-1,

IL-6 and TNFα) in tape stripped skin has peaked ((12, 13, 20, 21) and data not shown). The percentages of $CD11c^+$ DCs in tape stripped skin and unmanipulated skin were comparable (Fig. 1A). The two DC populations were comparable in subset composition, as evidenced by FACS analysis of myeloid DCs ($CD11c^{hi}CD11b^+B220^-CD19^-Langerin^-$), plasmacytoid DCs ($CD11c^{lo}CD11b^-B220^+CD19^-Langerin^-$) and Langerhans cells ($CD11c^+CD11b^+B220^-CD19^-Langerin^+$) (Fig. 1B). DCs from unmanipulated and tape stripped skin had comparable surface expression of CD40, CD80, CD86, MHC class II and OX40L (Fig. 1C). We previously showed that CCR7 expression is not detectable on DCs from unmanipulated skin but is induced on skin DCs 6 hrs after tape stripping CCR7 (21).

DC polarization was assessed by examining the capacity of skin $CD11c^+$ cells to elicit cytokine secretion in purified $CD4^+$ DO11.10 T cells stimulated with OVAp. DCs from tape stripped skin induced significantly higher secretion of the Th2 cytokines IL-4 and IL-13 compared to DCs from unmanipulated skin (Fig. 1D). Induction of IFN-γ secretion by the two DC populations was not significantly different. DCs from tape stripped skin and unmanipulated skin elicited comparable proliferation in OVAp stimulated T cells (Fig. 1E).

Mechanical injury polarizes skin DC emigrants in DLN to elicit a Th2 response

Following cutaneous introduction of antigen, interaction between antigen-laden skin-derived DCs and recirculating T cells takes place in skin DLN. We examined whether mechanical injury also polarizes antigen-laden skin DCs that emigrate to DLN to elicit a Th2 response. Back skin of mice was shaved and tape stripped (STS) or shaved alone, then painted with FITC in acetone:DPB (1:1) solvent. As previously reported (22, 23), FITC painting of shaved skin caused the appearance of $CD11c^+FITC^+$ DCs in DLN starting at 6 hrs and peaking at 24 hrs (Fig. 2A). The percentage of $CD11c^+FITC^+$ DCs in DLN of STS skin peaked earlier (6 hrs) and at a higher level, but by 24 hrs the percentages of $CD11c^+FITC^+$ DCs in DLN of shaved and STS were comparable. There were few $CD11c^+FITC^+$ DCs in DLN at 48 hrs. We compared DCs in DLN of shaved and STS skin 24 hrs after FITC painting. Fig. 2B shows a representative FACS analysis of $CD11c^+FITC^+$ DCs in DLN 24 hrs after FITC painting. The subset composition of $CD11c^+FITC^+$ DCs was comparable in DLNs of shaved and STS skin (Fig. 2C). The two populations of $CD11c^+FITC^+$ DCs also expressed comparable levels of CD40, CD80 CD86 and OX40L, but $CD11c^+FITC^+$ DCs from DLN of STS skin expressed higher levels of MHC class II and also CCR7, consistent with more vigorous mobilization of $FITC^+$ DCs from STS skin (Fig. 2D). $CD11c^+FITC^+$ DCs from DLN of both STS skin and shaved skin expressed higher levels of CD40, CD80, CD86 and MHC class II than their $CD11c^+FITC^-$ counterparts (Supplemental Fig. 1),

$CD11c^+$ DCs were isolated from DLNs by AutoMACS 24 hrs after FITC painting. $CD11c^+FITC^+$ cells, which likely represent hapten-laden skin DCs emigrants, and $CD11c^+FITC^-$ cells, were sorted by FACS and examined for their ability to polarize $CD4^+$ DO11.10 T cells stimulated with OVAp. Fig. 2E shows that $CD11c^+FITC^+$ cells from DLNs of STS skin induced significantly higher secretion of the Th2 cytokines IL-4 and IL-13, and significantly lower secretion of the Th1 cytokine IFN-γ compared to $CD11c^+FITC^+$ cells from DLNs of shaved skin. $CD11c^+FITC^+$ cells from DLNs of both STS and shaved skin induced significantly higher secretion of Th2 cytokines, and significantly lower secretion of IFN-γ, compared to their $CD11c^+FITC^-$ counterparts. $CD11c^+FITC^-$ cells from DLN of STS and shaved skin were comparable in their ability to induce the secretion of Th2 and Th1 cytokines. There was no significant difference between any of the $CD11c^+$ subpopulations in their ability to support T cell proliferation (Fig. 2F). These results suggest that mechanical injury by tape stripping causes DCs that carry antigen from skin to DLNs to skew naïve T cells towards a Th2 response.

The transcription factors GATA-3 and T-bet play critical roles in driving the differentiation of naïve $CD4^+$ T cells into Th2 and Th1 cells respectively (24) (25). $CD4^+$DO11.10 T cells cultured with DCs isolated from skin DLNs 24 hrs after FITC painting and OVAp for 4 days were purified and their content of GATA-3 and T-bet mRNA was assessed by Q-PCR. $CD11c^+FITC^+$ DCs from DLNs of STS skin induced significantly higher expression of GATA-3 mRNA, and conversely, lower expression of T-bet mRNA in T cells, compared to $CD11c^+FITC^+$ DCs from DLNs of shaved skin (Fig. 2G). $CD11c^+FITC^+$ cells from DLNs of both STS and shaved skin induced significantly higher GATA-3 mRNA, and significantly lower T-bet mRNA than their $CD11c^+FITC^-$ counterparts (Fig. 2F). There was no significant difference in mRNA expression of GATA-3 and T-bet mRNA in T cells cultured with $CD11c^+FITC^-$ DCs from STS and shaved skin. These results suggest that increased GATA-3 expression and decreased T-bet may underlie the skewing of naïve T cells towards Th2 when they are educated by DCs derived from mechanically injured skin.

Mechanical injury causes increased expression of IL-10 and Notch ligands by skin DCs

We previously showed that IL-10 is important for the Th2 response to EC sensitization of tape stripped skin, and that DC-derived IL-10 is important for Th2 polarization *in vitro*, and in *vivo* (26). Notch signaling in T cells by Jagged1 and Jagged2 ligands on APCs has been reported to enhance Th2 cell differentiation (27, 28). DCs from tape stripped ear skin expressed significantly higher amounts of IL-10, Jagged1 and Jagged2 mRNA, than DCs from unmanipulated ear skin (Fig. 3A). In contrast, they expressed significantly lower amounts of mRNA for IL-12, which plays an important role in Th1 cell differentiation (29, 30).

Similarly, $CD11c^+FITC^+$ DCs from DLN of STS skin expressed significantly higher levels of IL-10, Jagged1 and Jagged2 mRNA, and significantly lower levels of IL-12 mRNA, compared to $CD11c^+FITC^+$ DCs from DLN of shaved skin (Fig 3B). The same differences were seen when $CD11c^+FITC^+$ DCs were compared to their $CD11c^+FITC^-$ counterparts in DLN of STS skin. They was no significant difference in expression of IL-10, Jagged1, Jagged2 and IL-12 mRNA between $CD11c^+FITC^+$ DCs and $CD11c^+FITC^-$ DCs from DLN of shaved skin. These results indicate that mechanical injury induces increased expression of the putative Th2 skewing molecules IL-10, Jagged1 and Jagged2 and decreased expression of the Th1 skewing cytokine IFN-γ in skin DCs and in DCs that carry hapten and emigrate from skin to DLN.

Tape stripping causes elevation of TSLP levels in the skin

TSLP is expressed by keratinocytes (31). We examined the effect of mechanical injury on TSLP protein levels in back skin. Shaving and tape stripping (STS) caused a significant increase in skin TSLP levels after 6 hrs, with a return to baseline by 24 hrs (Fig. 4). In contrast, shaving alone caused no detectable increase in skin TSLP levels either at 6 hrs or 24 hrs.

TSLP is important for the Th2 polarizing activity of DCs from mechanically injured skin

To examine whether TSLP plays a role in the Th2 polarization of skin DCs by mechanical injury, we examined the effect of tape stripping on the polarization of skin DCs from $TSLPR^{-/-}$ mice. DCs isolated from unmanipulated and tape stripped skin of $TSLPR^{-/-}$ mice expressed comparable levels of CD40, CD80, CD86, MHC class II and OX40L as their counterparts isolated from the skin of WT controls (Supplemental Fig. S2). In contrast to DCs isolated from tape stripped ear skin of WT mice, DCs isolated from tape stripped ear skin of $TSLPR^{-/-}$ mice did not promote IL-4 and IL-13 production by OVAp stimulated $CD4^+$DO11.10 cells, compared to DCs isolated from unmanipulated skin (Fig. 5A). There was no difference between DCs isolated from unmanipulated skin of $TSLPR^{-/-}$ mice and

WT controls in their ability to support cytokine production by T cells. Lack of TSLPR had no detectable effect on the capacity of DCs from unmanipulated or injured skin to drive IFN-γ production.

We examined whether TSLP is necessary for the upregulation of IL-10, Jagged1 and Jagged2 mRNA expression in skin DCs following mechanical injury. In contrast to DCs from tape stripped skin of WT mice, DCs from tape stripped ear skin of $TSLPR^{-/-}$ mice failed to upregulate expression of IL-10, Jagged1 and Jagged2 mRNA compared to DCs from unmanipulated ear skin (Fig. 5B). Addition of recombinant TSLP failed to upregulate IL-10, Jagged1 and Jagged2 mRNA expression in DCs isolated from unmanipulated skin of WT mice (data not shown), suggesting that TSLP must synergize with factors released following mechanical skin injury to cause upregulation of these molecules.

We also examined the role of TSLP in the Th2 polarization of $CD11c^{+}FITC^{+}$ DCs isolated from DLNs of FITC painted STS skin. In contrast to $CD11c^{+}FITC^{+}$ DCs isolated from DLN of FITC painted STS skin of WT mice, $CD11c^{+}FITC^{+}$ DCs isolated from DLN of FITC painted STS skin of $TSLPR^{-/-}$ mice did not promote IL-4, IL-13 production by OVAp stimulated $CD4^{+}$DO11.10 cells (Fig. 5C). Conversely, unlike $CD11c^{+}FITC^{+}$ DCs from DLN of STS skin of WT mice, $CD11c^{+}FITC^{+}$ DCs from DLN of STS skin of $TSLPR^{-/-}$ mice did not exhibit a diminished capacity to drive IFN-γ production. There was no detectable difference between $CD11c^{+}FITC^{-}$ DCs isolated from DLN of FITC painted shaved skin of $TSLPR^{-/-}$ mice and WT controls in their ability to support cytokine production by T cells. Taken together, these results indicate that TSLP plays an important role in the polarization of skin DCs by mechanical injury to drive a Th2 response.

DISCUSSION

We have demonstrated that mechanical injury inflicted by tape stripping polarizes skin DCs to elicit a Th2 response and that TSLP is essential for this polarizing effect.

We demonstrate that DCs isolated from skin subjected to mechanical skin injury by tape stripping are polarized to drive a significantly stronger Th2 response compared to DCs isolated from non-injured skin. Similarly, $FITC^{+}$ DCs isolated from DLN of tape stripped skin are also polarized to drive a significantly stronger Th2 response than $FITC^{+}$ DCs isolated from non-injured skin. Since $FITC^{+}$ DCs in DLN of FITC painted skin likely represent skin DCs emigrants, taken together the data indicates that mechanical injury exerts a Th2 skewing effect on skin DCs, including those that migrate to DLN, where they present antigen to re-circulating T cells. In addition to skewing the T cell response towards a Th2 phenotype, $FITC^{+}$ DCs from DLN of injured skin inhibit Th1 skewing. We were unable to detect inhibition of the Th1 response by DCs isolated directly from injured skin. Inhibition of Th1 polarization by skin DCs following mechanical could require longer time compared to potentiation of Th2 polarization, and thus might not have been evident in DCs that were isolated from skin only 6 hrs after injury.

It has been reported that increased CD86 expression by DCs may favor Th2 polarization, while increased expression of CD80 and CD40 may enhance Th1 polarization (32, 33). DCs isolated directly from tape stripped skin versus unmanipulated skin, and $FITC^{+}$ DCs isolated from DLN of FITC-painted STS skin versus shaved skin had comparable expression of CD40, CD80 and CD86 costimulatory molecules, DC subset composition and ability to support T cell proliferation to antigen. Thus, differences in the expression of costimulatory molecules, DC subsets distribution, or ability to present antigen could not have accounted for the Th2 polarizing effect of mechanical injury on skin DCs. The failure to detect upregulation of costimulatory molecules on DCs isolated from skin 6 hrs after tape

stripping, suggests that this time period may not have been sufficient to detect upregulation of these molecules following mechanical injury. Comparable upregulation of costimulatory molecules on $FITC^+$ DCs from DLN of FITC-painted STS and shaved skin is consistent with previous observations that application of hapten in solvent to shaved mouse skin causes upregulation of these molecules (34–37).

In vivo administration of OX40L blocking mAb to mice inhibits TSLP driven allergic inflammation, but also results in depletion of $OX40L^+$ cells (38). We did not detect upregulation of OX40L expression by skin DCs from tape stripped skin. Furthermore, OX40L expression was comparable on $FITC^+$ DCs isolated from draining LN of FITC painted STS skin and FITC painted shaved skin. This makes it unlikely that OX40L was important in Th2 skewing by DCs from mechanically injured skin.

Higher MHC class II expression by $FITC^+$ DCs from DLN of STS skin versus shaved skin, but not by DCs isolated from skin 6hrs after injury versus unmanipulated skin, suggests that mechanical injury promotes delayed increased expression of MHC class II. This could be related to induction of TNF-α expression in the skin (12, 13), which is known to upregulate MHC class II expression on DCs both by itself, and in synergy with ATP, potentially released by injured skin cells (39, 40). There is no data in the literature that links increase MHC class II expression to Th2 polarization. An additional effect of mechanical injury was a vigorous mobilization of skin DCs to DLN, evidenced by the observation that shaving followed by tape stripping caused a more vigorous efflux of $CD11c^+FITC^+$ DCs in DLN than shaving alone. Tape stripping induces vigorous expression of IL-1β and TNF-α in skin, two cytokines that are known to promote DC migration from skin to DLN (12, 13, 41).

DCs isolated directly from injured skin and skin-derived $FITC^+$ DC emigrants isolated from DLN of FITC-painted injured skin upregulated the expression of mRNA for the Th2 polarizing molecules IL-10, Jagged1 and Jagged2. Conversely, they downregulated the expression of mRNA for the Th1 polarizing cytokine IL-12. We previously showed that DC derived IL-10 is important for Th2 polarization *in vitro*, and that antigen pulsed DCs deficient in IL-10 are impaired in their ability to elicit a Th2 response in recipient mice to EC challenge with antigen (26). This suggests that IL-10 expression by DCs from mechanically injured skin may be important for their Th2 polarizing activity. We have found that DCs isolated from injured skin of $IL\text{-}10^{-/-}$ mice fail to polarize a Th2 response (data not shown). However, since IL-10 is expressed abundantly by keratinocytes, bone marrow chimeras are necessary to formally demonstrate the role of DC derived IL-10 in the polarization by DCs from mechanically injured skin. A number of studies, but not all, have implicated Jagged1 and Jagged2 expression by DCs in Th2 polarization (27, 28, 42–45). Further experiments are needed to elucidate the role of these Notch ligands in the Th2 polarization of skin DCs by mechanical injury. OX40L upregulation on human DCs has been shown to promote Th2 skewing (46, 47). Given the pivotal role of IL-12 in driving Th1 polarization (29, 30), decreased expression of IL-12 mRNA by $CD11c^+FITC^+$ DCs in DLN of STS skin likely underlies their impaired ability to drive Th1 cells. Decreased feedback inhibition of Th2 polarization by IFN-γ may have contributed to Th2 skewing by DC s from injured skin.

Shaving followed by tape stripping, but not shaving alone, caused a significant increased in skin TSLP levels within 6 hrs. Upregulation of TNF-α and release of TLR3 ligands by commensal bacteria in response skin injury are known to upregulate TSLP expression by keratinocytes (48, 49). TSLP played an important role in the polarization of skin DCs induced by tape stripping. DCs isolated directly from injured skin of $TSLPR^{-/-}$ mice and as $FITC^+$ skin DC emigrants from DLN of FITC-painted injured skin of these mice failed to cause Th2 polarization compared to control DCs derived from uninjured skin of $TSLPR^{-/-}$

mice. These results indicate that TSLP released in the skin following mechanical injury is essential for the Th2 polarizing effect of mechanical injury on skin DCs. DCs from tape stripped skin of TSLPR$^{-/-}$ mice failed to upregulate mRNA expression of the putative Th2 skewing molecules IL-10, Jagged1 and Jagged2, suggesting that failure to upregulate these molecules contributed to the failure of these DCs to cause Th2 skewing TSLP increases OX40L expression by human DCs (46, 47). Upregulation of OX40L expression by mouse DCs has not been reported. Cuture of DCs from unmanipulated mouse skin with recombinant TSLP for 18 hrs upregulated CD40, CD80, CD86, MHC class II, but not OX40L, expression (data not shown). Furthermore, we did not detect a difference in OX40L expression between DCs isolated from tape stripped skin of TSLPR$^{-/-}$ mice and WT controls, and in a previous study found no differences in OX40L expression by FITC^{+} DCs isolated from DLN of FITC painted shaved skin of TSLPR$^{-/-}$ mice and WT controls (50). A recent study suggested that Th2 polarization of mouse naïve CD4^{+} T cells driven by Langerhans cells does not require OX40L signaling (51). Thus, it is unlikely that OX40L expression was important for TSLP mediated Th2 skewing by DCs from mechanically injured skin.

Although FITC^{+}DCs from draining LN of FITC painted shaved skin caused significantly less Th2 skewing than FITC^{+}DCs from DLN of FITC painted STS skin, they nevertheless were more potent in causing Th2 skewing than FITC^{-} DCs isolated from the same lymph nodes. This is consistent with the observation that painting with FITC in acetone:DBP upregulates TSLP expression in the skin (34–37). At the DC:T ratio of 1:10 we used in this study and in a previous study (50), we detected no difference between CD11c^{+}FITC^{-} DCs isolated from DLN of FITC painted shaved skin of TSLPR$^{-/-}$ mice and WT controls in their ability to support Th2 cytokine production by T cells. However, in a recent study, in which a lower DC:T ratio of 1:25 was used, we observed a modest (<20%), but significant decrease in the ability of CD11c^{+}FITC^{-} DCs isolated from DLN of FITC painted shaved skin of TSLPR$^{-/-}$ mice to support Th2 cytokine production by T cells (34), suggesting a role for TSLP in the Th2 response to FITC painting of the skin in the absence of mechanical injury inflicted by tape stripping.

TSLP has been shown to be important for cutaneous Th2 cytokine expression in mice EC sensitized with OVA, as well as in the cutaneous hypersensitivity response to the haptens oxazolone and FITC (35, 50). However, TSLP was not essential for the systemic Th2 response of mice to chronic EC sensitization with OVA, as evidenced by comparable serum levels of IgE and IgG1 OVA specific antibodies, and comparable production of Th2 cytokine by splenocytes in TSLPR$^{-/-}$ mice and WT controls EC sensitized with OVA (50). This finding suggests that factors other than TSLP may play an important role in Th2 polarization in response to repeated tape stripping and application of antigen over seven weeks in this chronic EC sensitization model. Nevertheless, TSLP was still important for cutaneous Th2 cytokine expression in this model (50), demonstrating a role for TSLP in the effector phase of allergic skin inflammation.

Recently, it was reported that application of dust mite antigens to the skin causes accumulation of basophils in DLN, which have been proposed to play an important role in Th2 skewing (52–55). We did not detect accumulation of basophils in skin DLN 24 hrs after shaving or shaving and tape stripping, as assessed by enumerating cells that express FcεRI and CD49b (data not shown). Thus, it is unlikely that basophils contribute to the Th2 polarizing effect of mechanical injury.

Scratching of the skin elicits skin flares in patients with AD and TSLP levels are increased in AD skin lesions (56, 57). To the extent that mechanical injury inflicted by tape stripping mimics that inflicted by scratching, our results suggest that scratching of dry skin in patients

with atopic dermatitis polarizes skin DCs to elicit a Th2 response by upregulating local expression of TSLP. Together, with the previously demonstrated role of TSLP in promoting Th2 cytokine secretion by skin infiltrating T cells (50), our results suggest that neutralization of TSLP may provide a therapeutic strategy that targets both the induction and effector arms of the immune response to cutaneously introduced antigens in patients with AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviation

AD	atopic dermatitis
DBP	dibutylphthalate
DC(s)	dendritic cells
DLN	draining lymph node
EC	epicutaneous
TSLP	thymic stromal lymphopoietin
Q-PCR	quantitative PCR
STS	shaved and tape stripped

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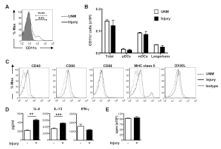

Figure 1. Th2 polarizing effect of tape stripping on skin DCs

A. FACS analysis of CD11c expression by cell isolated from unmanipulated (UNM) and tape stripped (Injury) ear skin. Isotype control was shown as gray shade. **B.** Total number of $CD11c^+$ cells and their subset composition. pDCs:plasmacytoid DCs, mDCs:myeloid DCs. Columns and error bars represent mean and SEM (n = 4). **C.** Representative FACS analysis of CD40, CD80, CD86, MHC class II and OX40L expression by $CD11c^+$ cells. Similar results were obtained in three independent experiments. **D**. Polarization of $CD4^+$ DO11.10 cells by DCs isolated from unmanipulated (−) and tape stripped (+) ear skin. **E.** Proliferation of $CD4^+$ DO11.10 cells by DCs isolated from unmanipulated (−) and tape stripped (+) ear skin. Columns and error bars represent mean and SEM (n=4 per group). **p<0.01, *** p<0.001.

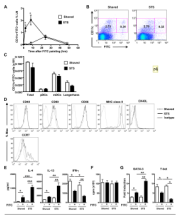

Figure 2. Polarization of T cells by DCs from DLN of shaved and STS skin

A. Kinetic analysis of $CD11c^+FITC^+$ DCs in DLN of shaved and STS skin painted with FITC (n=3 per group). **B.** Representative FACS analysis of $CD11c^+FITC^+$ DCs in skin DLN 24 hrs after FITC painting. **C.** Number and subset composition of $CD11c^+FITC^+$ cells in DLN of shaved and STS skin (n=3). pDCs:plasmacytoid DCs, mDCs:myeloid DCs. (n=3 per group). **D.** Representative FACS analysis of xCD40, CD80, CD86 MHC class II OX40L, and CCR7 expression on $FITC^+$ DCs from DLN 24 hrs after painting shaved and STS skin with FITC. Similar results were obtained in three independent experiments. **E.** Polarization of $CD4^+$ DO11.10 cells by $CD11c^+FITC^+$ DCs and $CD11c^+FITC^-$ DCs isolated from DLN of shaved and STS skin painted with FITC. **F.** Proliferation of $CD4^+$ DO11.10 cells. **G.** Q-PCR analysis of mRNA expression of GATA-3 and T-bet in $CD4^+$ DO11.10 cells purified after 4 days of co-culture with DCs and OVAp. Results are expressed as fold induction compared to $CD4^+$ T cells cultured in the absence of DCs. Columns and error bars represent mean and SEM (n=5 per group). *p<0.05, **p<0.01, *** p<0.001.

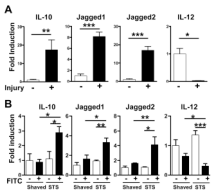

Figure 3. Expression of mRNA for cytokines and Notch ligands Jagged1 and Jagged2 by skin DCs

A–B Q-PCR analysis of mRNA for cytokines and Notch ligands by skin DCs. **A.** DCs isolated from unmanipulated (−) and tape stripped (+) ear skin. Results are expressed as fold induction compared to DCs from unmanipulated skin. **B.** $CD11c^{+}FITC^{+}$ and $CD11c^{+}FITC^{-}$ from DLN of shaved and STS skin for cytokines and Notch ligands. Results are expressed as fold induction compared to $CD11c^{+}FITC^{-}$ cells from DLNs of shaved skin. Columns and error bars represent mean and SEM (n=5 per group). *p<0.05, **p<0.01, *** p<0.001.

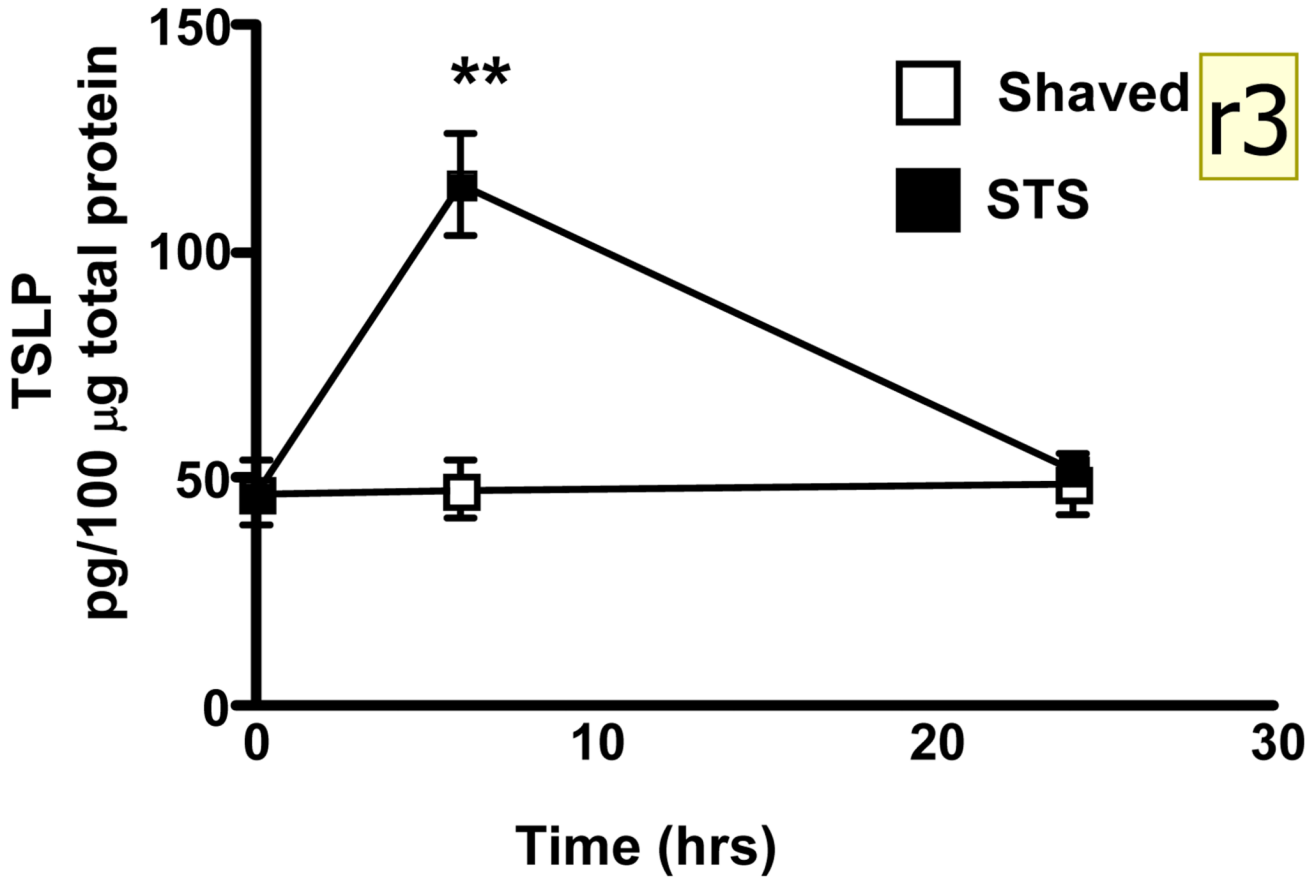

Figure 4. Tape stripping causes elevation of TSLP levels in the skin

Effect of shaving versus shaving and tape stripping on TSLP levels in the skin (n=4 per group). **p<0.01.

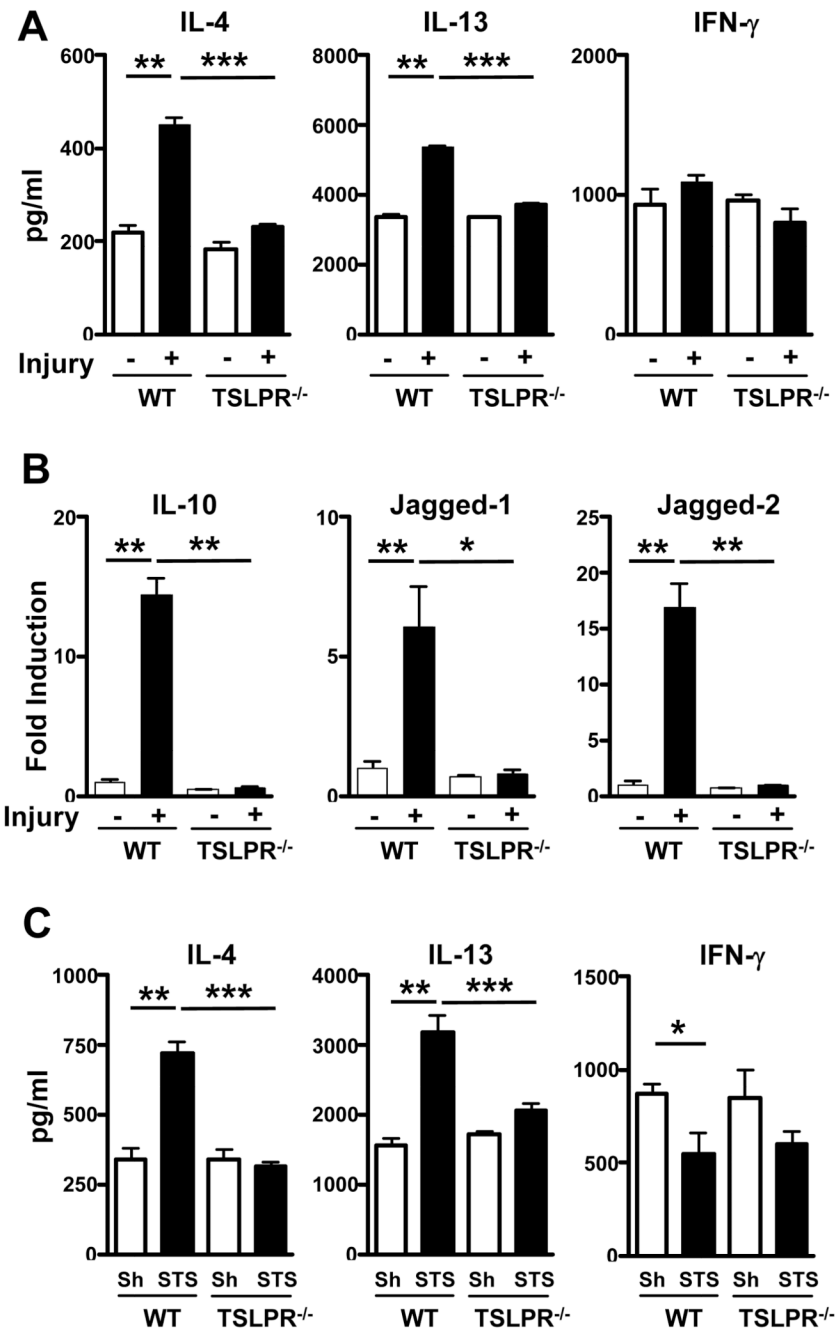

Figure 5. TSLP is important for the Th2 polarizing effect of tape stripping on skin DCs

A. Th cell polarizing activity of DCs from unmanipulated (−) and tape stripped (+) skin of TSLPR$^{-/-}$ mice and WT controls. **B**. Q-PCR analysis of mRNA for IL-10 and Notch ligands by skin DCs isolated from unmanipulated (−) and tape stripped (+) ear skin of TSLPR$^{-/-}$ mice and WT controls. Results are expressed as fold induction compared to DCs from unmanipulated skin. **C.** Th cell polarizing activity of CD11c^{+}FITC^{+} DCs from DLN of shaved (Sh) and STS skin of TSLPR$^{-/-}$ mice and WT controls. Columns and error bars represent mean and SEM (n=4 per group). *p<0.05, **p<0.01, *** p<0.001.