

Thymic stromal lymphopoietin converts human epidermal Langerhans cells into antigen presenting cells that induce pro-allergic T cells

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Background: Thymic stromal lymphopoietin (TSLP) endows human CD11c⁺ dendritic cells (DCs) from peripheral blood with the capacity to induce pro-allergic T cells. TSLP is present at high levels in the epidermis of atopic dermatitis where it appears to trigger emigration of epidermal Langerhans cells (LCs); however, nothing else is known about the influence of TSLP on LCs.

Objective: Effects of TSLP on human epidermal LCs were investigated.

Methods: LCs were isolated by trypsinization from healthy human skin, highly enriched by immunomagnetic techniques (via CD1a) and cultured for 2 days. Additionally, migratory LCs were obtained by emigration from epidermal sheets for 3 days.

Results: The addition of TSLP promoted survival and maturation of LCs obtained by trypsinization, as indicated by their increased expression of CD83, CD86, and high levels of MHC II. TSLP markedly increased numbers of migratory LCs. Allogeneic naïve CD4⁺ T cells, cocultured with migratory TSLP-LCs produced less IFN- γ and IL-10 and more IL-4, IL-5, IL-13, and TNF- α . Finally, TSLP-LCs secreted markedly more of the T_H2 T-cell-attracting chemokine CCL17/thymus and activation-regulated chemokine.

Conclusion: These cytokine patterns correspond to those described for TSLP-treated blood DCs. They highlight a direct effect of TSLP on epidermal LCs.

Clinical implications: Our data emphasize a critical role for LCs in the triggering of atopic dermatitis. Furthermore, they underscore the interest in TSLP as a potential therapeutic target in atopic diseases. (J Allergy Clin Immunol 2007;119:1016-1023.)

Key words: Atopic dermatitis, dendritic cells, Langerhans cells, thymic stromal lymphopoietin

Langerhans cells (LCs) are dendritic cells (DCs) residing primarily in epithelia, foremost in the epidermis.^{1,2} The T-cell stimulatory function of LCs *in vitro* is firmly established.³ Evidence also exists that LCs function as antigen-presenting cells *in vivo*, even though this is currently under discussion.^{4,5} Nevertheless, it is still likely that in both contact dermatitis^{6,7} and atopic dermatitis^{8,9} LCs serve as critical antigen-presenting cells. In the latter condition, LCs express high-affinity IgE receptors that allow them to focus low amounts of allergen and render them more efficient in processing and presenting them to T cells.¹⁰ Thus, it is of interest to further study the role of LC in atopic disease.

Thymic stromal lymphopoietin (TSLP) is an IL-7-like cytokine that seems to be critically involved in allergic disease.^{11,12} It is highly expressed by keratinocytes in the lesions of acute and chronic atopic dermatitis but not in other types of skin inflammation. Human blood DCs treated with TSLP undergo phenotypic and functional maturation. CD4⁺ helper T cells¹³ as well as CD8⁺ T cells¹⁴ that are stimulated by TSLP-treated DCs in an antigen-specific manner secrete a pro-allergic pattern of cytokines, that is, increased amounts of IL-4, IL-5, IL-13, and TNF- α and reduced levels of IFN- γ and IL-10. Notably, TSLP-treated CD11c⁺ blood DCs produce thymus and activation-regulated chemokine (TARC)/CCL17, a T_H2 T-cell-attracting chemokine.¹³ The observations in human DCs are corroborated by mouse studies that also point to a critical role for TSLP in the pathogenesis of atopic disease. When TSLP is transgenically expressed in the epidermis under the control of a keratin promoter, mice develop symptoms strongly resembling atopic dermatitis, that is, eczematous lesions containing infiltrating inflammatory cells, an increase in T_H2 CD4⁺ T cells expressing cutaneous homing receptors, and higher levels of IgE in the serum.¹⁵ A similar phenotype is observed when expression of TSLP is induced in the epidermis by rendering mice deficient for retinoid X receptors selectively in keratinocytes.¹⁶ The

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Abbreviations used

- DC: Dendritic cell
 FITC: Fluorescein isothiocyanate
 IDEC: Inflammatory dendritic epidermal cell(s)
 LC: Langerhans cell
 PE: Phycoerythrin
 TARC: Thymus and activation-regulated chemokine
 TSLP: Thymic stromal lymphopoietin

role of LCs in contributing to the observed pathology has not been investigated in these mouse models.

The original study on the effects of TSLP on DCs¹³ reported that, in the TSLP-expressing patches of inflamed skin, LCs were almost absent. Instead, these authors found many LCs in the underlying dermis in a phenotypically mature state (as defined by the DC-LAMP/CD208 expression),¹³ which suggests that TSLPs may have induced their emigration from the epidermis and their maturation. Based on these observations, Soumelis et al¹³ hypothesized that these LCs eventually migrate to the lymph nodes and induce pro-allergic T_H2 T cells there, in analogy to what has been shown for blood DCs. Indeed, such T_H2 cells were recently found in the dermis of atopic eczema in close association with DC-LAMP-expressing DCs.¹⁷ However, none of the mentioned studies, including the original report by Soumelis et al,¹³ has functionally investigated LCs isolated from the epidermis. It is not known whether TSLP acts on LC in the same way as on blood DCs, which prompted us to directly study the effects of TSLP on LCs obtained from the human epidermis either by migration from epidermal explants or by trypsinization and subsequent enrichment.

METHODS

Media and reagents

The culture medium used for human skin cultures was RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, 2 mmol/L L-glutamine (Sigma Chemicals, St. Louis, Mo), and 50-μg/mL gentamycin (PAA, Linz, Austria). Recombinant TSLP (15 ng/mL final working concentration in all experiments) and a neutralizing anti-TSLP-antibody were purchased from R&D Systems (Minneapolis, Minn). For phenotypical analyses by flow cytometry fluorescein isothiocyanate (FITC)- and phycoerythrin (PE)-conjugated mouse anti-human mAbs were used: anti-CD1a, anti-CD40, anti-CD80, anti-CD86, anti-HLA-DR (all from BD-Biosciences, San Diego, Calif), anti-CD83 (Beckman-Coulter, Fullerton, Calif), anti-CCR7 (R&D Systems), anti-OX40L (Ancell, Bayport, Minn), and anti-CD205/DEC-205 (clone MG38,¹⁸ gift of Dr. R. M. Steinman, The Rockefeller University, NY). Isotype controls included the appropriate fluorochrome-conjugated mouse IgG1 (from BD-Biosciences and Ancell), mouse IgG2a (R&D Systems), and mouse IgG2b (DAKO, Glostrup, Denmark) reagents.

Isolation and enrichment of LCs

Normal human split-thickness skin (0.3 mm) was obtained as discarded material from plastic surgery (reduction mammoplasty and reduction abdominoplasty) after local ethical committee approval.

Epidermal cell suspensions were prepared as previously described in detail.¹⁹ Trypsin-EDTA (Gibco-Invitrogen, Paisley, UK) was used at a final concentration of 0.05% trypsin and 0.53 mmol/L EDTA at 4°C for 16 hours. LCs were isolated from epidermal cell suspensions by positive selection using a mouse anti-human CD1a antibody directly coupled to super-paramagnetic microbeads (clone HI149, IgG1) (Miltenyi Biotech, Bergisch Gladbach, Germany) according to the manufacturer's protocol. Purity of CD1a⁺ cells was consistently greater than 90%.

Skin explant culture

This culture was based on established methods.^{19,20} Standardized pieces of normal skin were prepared by means of an 8-mm punch from split-thickness skin (0.3 mm). Epidermis and dermis were split before the onset of culture by the enzyme Dispase I (final concentration 1.2 IU/mL, 30 minutes/37°C; Roche Applied Sciences, Basel, Switzerland). Epidermis was floated on 1-mL culture medium in 24-well plates (1 explant per well) for 3 days at 37°C in the absence or presence of TSLP ("TSLP-LC"). Migratory LCs that had migrated into the culture medium during this time were harvested, counted, and further evaluated by flow cytometry and/or LC-T-cell cocultures.

Determination of cell yields, viability, and migration of LCs

Freshly isolated, highly enriched LCs were cultured side by side in the presence or absence of TSLP and in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) for 2 days. A total of 0.5×10^6 LC were cultured in 48-well plates in 1 mL of medium. Cells were counted under the hemacytometer, and viability was determined by trypan blue dye exclusion.

To analyze migration of LCs, Dispase-procured epidermal sheets of standardized size (8 mm in diameter) were cultured side by side in the presence or absence of TSLP for 3 days. LCs that had migrated into the culture medium were counted. LCs were readily and unequivocally identified under the hemacytometer by their pronounced hairy shape. Dead cells were excluded by trypan blue staining.

Purification of naïve T cells and cocultures with LCs

Peripheral blood mononuclear cells (PBMCs) for naïve T cells were obtained from healthy anonymized blood bank donors. Their state of atopy was not assessed. PBMCs were incubated with a mixture of mAbs, including CD14, HLA-DR, CD56, CD8, CD19, CD45RO, and CD40 (all from BD-Biosciences). Petri dishes were coated for 1 hour with AffiniPure goat anti-mouse IgG (10 μg/mL; Jackson ImmunoResearch Laboratories, Avondale, Pa). Naïve CD4⁺CD45RA⁺ T cells were isolated using a panning technique described previously.²¹ This technique was repeated twice to obtain greater than 95% pure CD4⁺ T cells. LCs cultured under different conditions (medium alone, TSLP, neutralizing anti-TSLP antibody) were washed twice and cocultured with naïve CD4⁺CD45RA⁺ T cells in 48-well plates at a 6:1 ratio (6×10^5 T cells; 1×10^5 viable LC) for 6 days before being restimulated for cytokine analyses.

Analysis of T-cell cytokine production

For enzyme-linked immunosorbent assays (ELISAs), 6-day cocultured cells were transferred to fresh wells and restimulated with plate-bound anti-CD3 (10 μg/mL; BD-Biosciences) and anti-CD28 (2 μg/mL; BD-Biosciences) for another 30 hours for ELISA. Culture supernatants were frozen at -80°C until levels of IL-4, IL-5, IL-10, IL-13, TNF-α, and IFN-γ were measured with ELISA kits (IL-4, IL-5, IL-10, IL-13, and TNF-α from Bender MedSystems, Vienna, Austria; IFN-γ from BD-Biosciences). For analyses of intracellular cytokine production, the primed CD4⁺ T cells were restimulated on

day 6 by adding 50 ng/mL PMA plus 2 μ g/mL of ionomycin for an additional 6 hours. GolgiStop (1 μ g/mL; BD-Biosciences) was also added during these 6 hours. Cells were washed and stained with the combination of PE-labeled mAbs to IL-4, IL-10, IL-13, and FITC-labeled anti-IFN- γ and allophycocyanin-labeled anti-TNF- α (all from BD-Biosciences) using a cell permeabilization kit (Fix&Perm, An der Grub, Kaumberg, Austria).

Mixed leukocyte reaction

Graded doses of LC were added to 2×10^5 allogeneic CD4⁺CD45RA⁺ T cells in 96-well, flat-bottom culture plates for 6 days. Proliferation was determined by the addition of 1 μ Ci [³H]thymidine (specific activity 247.9 GBq/mmol = 6.7 Ci/mmol; New England Nuclear, Boston, Mass) during the last 16 hours of the culture period and subsequent measurement of incorporated radioactivity in a scintillation counter (Perkin Elmer Life Sciences, Boston, Mass). Results were calculated as means for triplicate wells.

Determination of LC-derived IL-12p70 and CCL17/TARC

Epidermal explants were cultured in the absence or presence of TSLP or in the presence of a neutralizing anti-TSLP antibody for 3 days. The resulting migratory LCs were then plated at 1×10^6 /mL in the presence or absence of CD40 ligand-expressing cells for another 48 hours, that is, from day 3 to day 5. Murine myeloma cells transfected with the human CD154/CD40 ligand molecule (P3xTBA7 cells) were used to ligate the CD40 molecule on the surface of LC.²² Wild-type cells served as a negative control (P3x63Ag8.653-WT). These cells were a gift from Dr. R. A. Krocze (Berlin, Germany). Supernatants were taken at 48 hours and were stored at -80°C until assayed for IL-12p70 and CCL17/TARC by ELISA (BD-Biosciences for IL-12p70; R&D Systems for CCL17).

Statistical analyses

All experiments were performed at least 3 times with similar results. *P* values are from a nonparametric 2-tailed Wilcoxon matched pair test and from a nonparametric 2-tailed Friedman test followed by the Dunn multiple comparison test.

RESULTS

TSLP promotes maturation of human LCs. Freshly isolated, highly enriched LCs expressed substantial levels of MHC II, low levels of C86, and no CD40 and CD83. When cultured for 48 hours in the presence and absence of TSLP and GM-CSF, both populations upregulated surface CD86 to a larger extent as compared with LCs cultured in medium alone. Percentages of LC (ie, CD1a⁺ cells) expressing CD86 at high levels were 30-44-44% in experiment 1, 11-35-31% in experiment 2, and 45-63-47% in experiment 3 for culture in medium alone, TSLP, and GM-CSF, respectively. A subpopulation of freshly isolated LC upregulated CD83 spontaneously without experimental stimulation. Both TSLP (*P* < .01, *n* = 7) and GM-CSF (*P* < .05, *n* = 7) further enhanced CD83 expression of LC (Fig 1, A). Neither treatment, however, led to CD83 expression on the entire population of CD1a⁺ cells. Soumelis et al¹³ showed that TSLP-activated CD11c⁺ cells express high levels of CD40 and CD80. In contrast, freshly isolated LCs cultured with TSLP showed no neo-expression of CD40 and CD80 (not shown). Thus, this

finding distinguished CD11c⁺ DCs isolated from human blood and LCs isolated and enriched from human skin.

Alternatively, enriched populations of LC (up to 70% of viable cells) could be obtained without enzymatic treatment by migration from epidermal sheets. The phenotypic properties of migratory LCs differed from LCs isolated via CD1a sorting. All emigrants highly expressed the costimulatory molecule CD86, maturation marker CD83, chemokine receptor CCR7, and C-type lectin receptor DEC-205 (CD205). They also moderately expressed CD40 (Fig 1, B). From the “default” maturation that occurs in these explant cultures, an additional maturation-enhancing effect of TSLP could not be well appreciated except for 1 marker. In response to TSLP, expression levels of CD80 increased further in migratory LCs as compared with LC cultured in medium alone (Fig 1, B). This result was previously described for CD11c⁺ blood DCs.¹³ Ito et al²³ implicated OX40L-OX40 interactions in the induction of TNF- α and IL-4 producing T cells activated with TSLP stimulated CD11c⁺ blood DCs. Unlike CD11c⁺ blood DCs, migratory LCs with and without activation by TSLP displayed no differences in their expression levels of OX40L (Fig 1, C).

TSLP enhances survival of epidermal LCs. Soumelis et al¹³ showed that TSLP maintains the survival of CD11c⁺ DCs from human blood in 24-hour cultures. We cultured freshly isolated, highly enriched LCs in medium only, in the presence of TSLP, and in the presence of GM-CSF. After 24 hours of culture, no significant difference in LC numbers could be retrieved from the different culture conditions (data not shown). After 48 hours of culture, however, survival of LCs cultured in the presence of TSLP was significantly higher than in cultures with medium only as shown in Fig 2, A (*P* = .0078, *n* = 8). Also, a significantly higher survival of LCs was found in the presence of TSLP as compared with GM-CSF (Fig 2, B; *P* = .0078, *n* = 8). Therefore, we conclude that TSLP is a survival-supporting cytokine for epidermal LCs, similar to GM-CSF, which is a known survival factor for murine and human LCs.^{24,25}

TSLP increases migration of migratory LCs from epidermal explant cultures. Between 3,000 and 12,000 viable LCs emigrated into the culture medium per epidermal sheet (approximately 0.5 cm²). In each experiment, migration of LC was enhanced in the presence of TSLP (*P* = .0156, *n* = 7) as compared with emigration in medium alone (Fig 2, C). Migration of LC from epidermal explant cultures was also augmented by TSLP when assessed on day 4 of the culture (Fig 2, D).

TSLP increases the immunostimulatory capacity of freshly isolated LCs. The immunostimulatory capacity of LC was tested in the allogeneic mixed leukocyte reaction. LCs that had migrated from epidermal sheets in the presence of TSLP stimulated naïve CD4⁺ CD45RA⁺ T cells efficiently. From the “default” maturation of LC in these epidermal explant cultures, TSLP-LCs were only slightly and insignificantly better than LCs that had migrated in the absence of TSLP (Fig 3, A). In contrast, freshly isolated, highly enriched LCs cultured for 48 hours

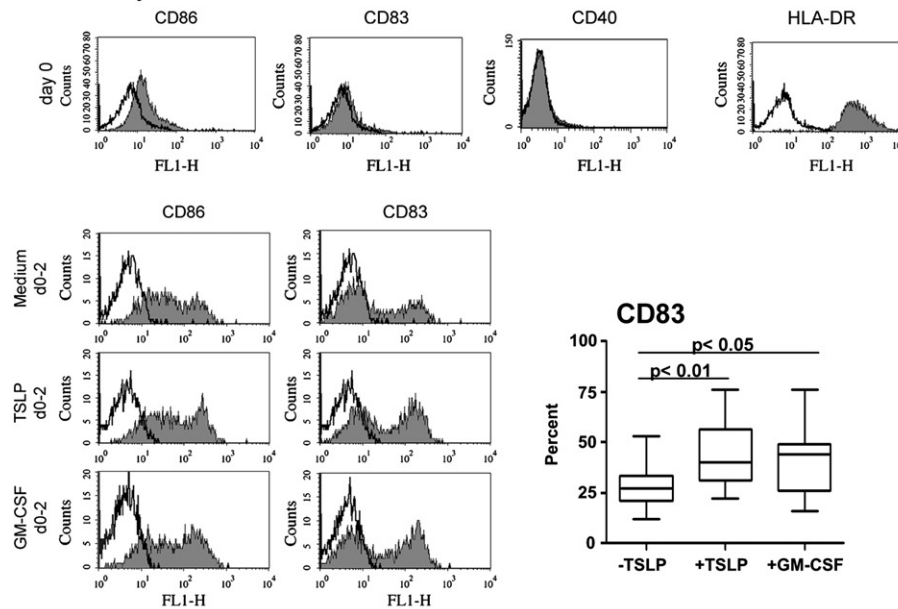
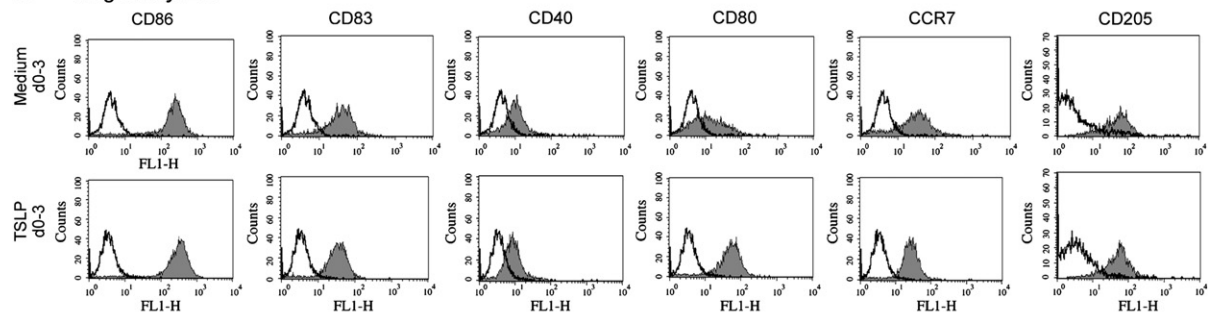
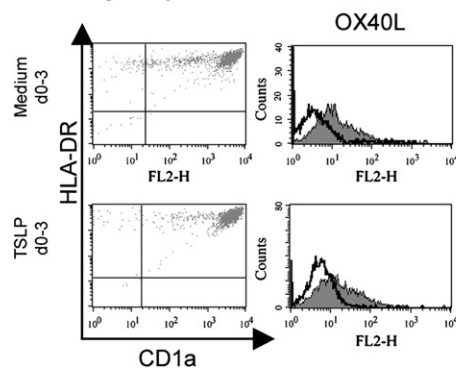
A *freshly isolated LC***B** *migratory LC***C** *migratory LC*

FIG 1. Phenotype of LCs activated with TSLP. LCs were identified by their CD1a expression. Fluorescence of CD1a-gated cells is depicted in the histograms. Filled histograms represent staining of experimental antibodies; open histograms are isotype controls. **A**, Freshly isolated LCs were cultured with medium alone, with TSLP, and with GM-CSF for 48 hours. TSLP markedly increased the percentage of CD83-expressing LCs. One representative experiment of 3 complete experiments (ie, with all antibodies) is shown in the histograms. CD83 analyses were performed in 4 additional experiments. **B**, Maturation-associated molecules CD40, 86, 83, 205, and CCR7 were expressed at high levels already in the absence of TSLP. Only CD80 expression was further enhanced by TSLP. One representative experiment of 5 is shown. **C**, Migratory LCs are highly positive for HLA-DR and CD1a, and there is no differential expression of OX-40L.

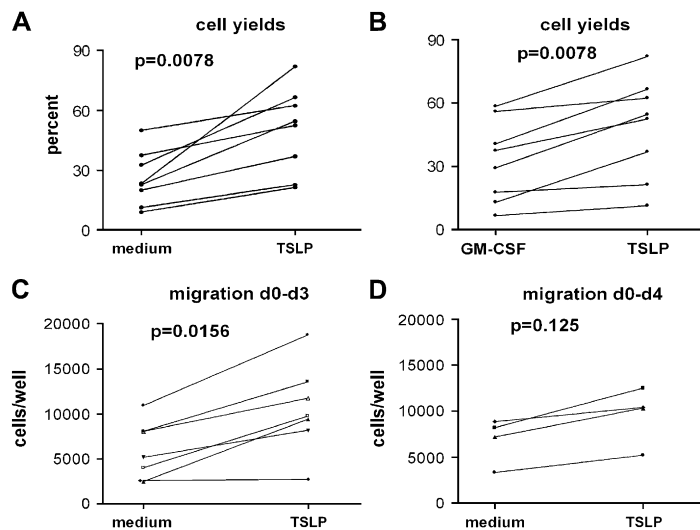


FIG 2. TSLP improves viability of cultured LCs and enhances migration of human skin emigrants. Highly enriched LCs were cultured side by side in the presence or absence of TSLP (**A**) or in the presence of TSLP or GM-CSF (**B**) for 2 days. Cell yields, that is, percent surviving LCs of LC plated at the onset of culture are indicated ($n = 8$; $P = .0078$ for control vs TSLP; $P = .0078$ for GM-CSF vs TSLP). **C**, Dispase-procured epidermal sheets were cultured side by side in the presence or in the absence of TSLP for 3 days. LCs that had migrated into the culture medium were counted ($n = 7$, $P = .0156$). **D**, Cultures were conducted as in **C**, but for a total of 4 days ($n = 4$, NS).

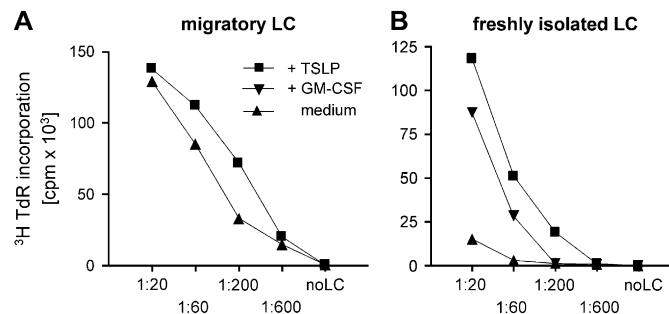


FIG 3. Naïve CD4 T-cell proliferation induced by TSLP-treated migratory LCs (**A**) and by freshly isolated LCs (**B**). Different numbers of LCs were cocultured with naïve allogeneic T cells. Freshly isolated LCs cultured in the presence of TSLP or GM-CSF stimulate T cells more efficiently (**B**). One representative experiment of 3 each is shown.

in the presence of TSLP and GM-CSF stimulated naïve CD4⁺ CD45RA⁺ T cells more efficiently than their counterparts cultured in the absence of TSLP (**Fig 3, B**).

TSLP primes migratory LCs for the production of CCL17/TARC, but not of IL-12, in response to CD40 ligation. To investigate the capacity of migratory LCs to release CCL17/TARC, a typical T_H2-attracting chemokine and IL-12p70, LC populations were cocultured with CD40L-transfected cells, and released cytokines were measured by ELISA. Low levels of TARC were detected in the cultures of migratory LCs in the absence of TSLP and irrespective of the presence of CD40L-expressing cells or of neutralizing anti-TSLP antibodies. This finding corresponds to the constitutive production of CCL17 that was described for LC-like cells derived from human monocytes.²⁶ In contrast, LCs that had migrated from the sheets in the presence of TSLP secreted

substantial amounts of CCL17 (up to 15 ng per mL and 10⁶ cells) in response to CD40 ligation ($P < .05$, $n = 5$) (**Fig 4**). Migratory LCs released no IL-12p70 under any conditions tested (data not shown).

TSLP-treated migratory LCs induce an inflammatory T_H2 cytokine profile in naïve CD4⁺ helper T cells. The capacity of LCs migrated from epidermal sheets in the presence of TSLP to polarize naïve CD4⁺ T cells was compared with migratory LCs generated in the absence of TSLP or in the presence of a neutralizing anti-TSLP antibody. Naïve allogeneic CD4⁺/CD45RA⁺ T cells purified from adult peripheral blood were cultured with LCs at a 6:1 ratio for 6 days. They were restimulated for 30 hours with anti-CD3 and anti-CD28, and cytokines released into the culture supernatants were measured by ELISA. Compared with migratory LCs generated in medium alone, TSLP-LC induced naïve CD4⁺ T cells to

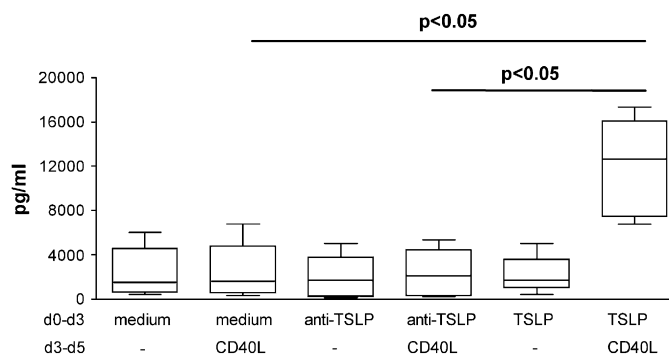


FIG 4. CCL17/TARC production by TSLP-treated migratory LCs in response to CD40 ligation. Epidermal explants were cultured for 3 days in the absence or presence of TSLP and in the presence of a neutralizing anti-TSLP antibody. The resulting mature LCs were cocultured with CD40 ligand-expressing cells, and supernatants were assayed for CCL17. In response to CD40 ligation, TSLP-treated migratory LCs produced significantly more CCL17/TARC than LCs without TSLP treatment ($n = 5$, $P < .05$). CD40 ligation of LCs that had migrated in the absence of TSLP or in the presence of anti-TSLP antibody did not induce increased CCL17 secretion.

produce higher amounts of IL-4 ($P < .05$, $n = 5$), IL-13 ($P < .05$, $n = 4$), IL-5 (not significant, $n = 4$), and TNF- α ($P < .05$, $n = 4$), but less of the Th1 cytokine IFN- γ ($P < .05$, $n = 4$) and the anti-inflammatory cytokine IL-10 ($P < .05$, $n = 4$) (Fig 5, A). These cytokine secretion patterns became also evident in flow cytometry analyses of intracellular T-cell cytokines (Fig 5, B). Flow cytometry experiments ($n = 6$) were performed with LCs from different donors as those used for ELISA analyses.

DISCUSSION

In this study, we describe distinct effects of TSLP on human epidermal LCs. Similar to what has been reported for DCs isolated from peripheral blood,¹³ TSLP treatment of LCs leads to (1) increased viability; (2) heightened maturation; (3) enhanced migratory capacity; (4) pronounced production of CCL17/TARC; and (5) induction of an inflammatory Th2 cytokine profile in CD4⁺ helper T cells.

Migratory LCs were used for most experiments of this study. This model, where LCs are initially confronted with experimentally added TSLP, still within the epidermal environment, seems to be more physiological. It may also correspond better to the recently described mouse models.^{15,16} Two points reassured that the observed effects on T cells could be attributed to LCs rather than to contaminating non-LC leukocytes or to residual keratinocytes. (1) As described previously,²⁰ we found that virtually all HLA-DR⁺ cells in the populations that had migrated from the epidermal sheets coexpressed CD1a (Fig. 1C), a reliable marker for LCs within the epidermal context. (2) Epithelial cells do not express TSLP receptors,²⁷ and therefore, it is unlikely that TSLP would act indirectly via keratinocytes.

Relevance for the pathogenesis of atopic dermatitis

TSLP is present in the epidermis of human atopic dermatitis (but not in normal keratinocytes or in other

inflammatory dermatoses) where it seems to induce migration of LCs. This result was shown by an immunohistochemistry approach.¹³ Furthermore, it was shown that TSLP-treated human blood DCs induce an inflammatory Th2 cytokine pattern in T cells.¹³ These findings led to the hypothesis that TSLP may be a “master switch” for allergic inflammation.¹² LCs were not tested in those studies. Our data link these 2 sets of observations in that we show here that TSLP elicits essentially the same effects in LC obtained from the human epidermis as previously described for blood DCs. This result may indeed render them capable of inducing or maintaining atopic disease.

Overexpression of TSLP in the epidermis of mice leads to the development of a phenotype that is similar to atopic eczema (more eosinophils and mast cells in the skin; IgE in the serum; increased numbers of Th2 cells with homing receptors for the skin; increased expression of Th2-associated cytokines in the skin).^{15,16} Based on our data, it is tempting to speculate that epidermal LCs may indeed be responsible for the atopy-related phenomena. This notion is supported by recent observations that LC-like cells, derived from peripheral blood monocytes of atopic dermatitis patients, induce larger numbers of IL-4-producing autologous, allergen-specific T cells than inflammatory dendritic epidermal cells (IDECs) analyzed in parallel.²⁶ Also, it has been described that repeated stimulation of T cells with cultured murine LCs leads to the outgrowth of IL-4-producing Th2 lines.²⁸ More studies using newly devised mouse models, where LCs can be selectively ablated,²⁹⁻³¹ will help to further clarify the role of LCs in atopic dermatitis.

A hypothetical scenario for the development of atopic dermatitis

In synopsis with previous reports, our data allow us to devise a hypothetical sequence of events that may lead to atopic dermatitis. TSLP produced by keratinocytes in atopic dermatitis lesions induces maturation and migration of LCs to draining lymph nodes. This result may hold true

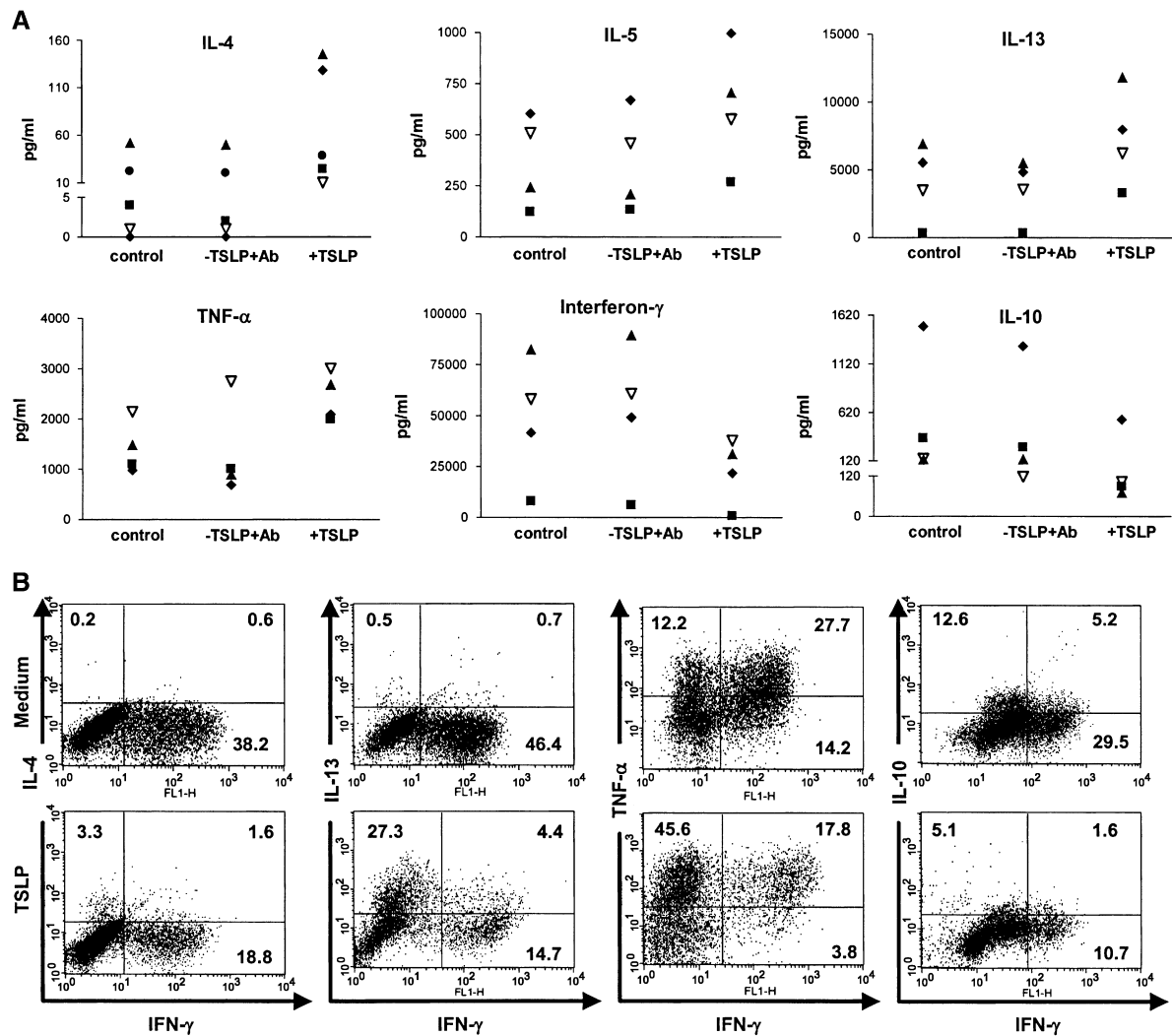


FIG 5. TSLP-treated migratory LCs induce T cells to make less IFN- γ and IL-10 but more IL-4, 5, and 13. **A**, Naive T cells were cocultured with allogeneic mature LCs that had migrated from epidermal sheets in the absence (control) or presence of TSLP or a neutralizing anti-TSLP antibody (-TSLP+Ab) for 3 days. Differences between control- and TSLP-LC-stimulated T-cell cytokine levels were significant for IL-4, IL-13, IL-10, IFN- γ , and TNF- α (see text). The increase in IL-5 was not statistically significant. Individual donors are marked by identical symbols across stimulation conditions. **B**, Intracellular cytokine staining of T cells confirmed the cytokine secretion profiles measured by ELISA. Data are representative for 1 of 6 independent experiments.

in an analogous manner for bronchial epithelium and the development of asthma.³² TSLP was found there as well,³³ and the existence of lung DCs,^{34,35} including LCs,³⁶ is well documented.

In the lymph node, TSLP-“primed” LCs would polarize allergen-specific T_H2 cells. As we found similar levels of OX40 ligand on TSLP-treated and -untreated migratory LCs, it remains to be investigated whether LCs use the same OX40 ligand-dependent mechanism to induce pro-allergic T cells as described for blood-derived DCs.²³ When back in the skin, these T cells may promote the inflammatory process by their high production of TNF- α and their relatively low secretion of IL-10, both hallmarks of the TSLP-induced “inflammatory T_H2 pattern.”¹²

TNF- α has been implicated in T_H2-cell-mediated allergic diseases such as atopic dermatitis and asthma.³⁷⁻³⁹

Influx of T_H2 cells into the skin may additionally be facilitated by the chemokine CCL17/TARC that is provided by TSLP-treated LCs. CCL17 is a ligand for CCR4⁴⁰ and CCR8⁴¹ and serves for the recruitment and migration of cells expressing these receptors such as T_H2 cells.⁴²⁻⁴⁴ CCL17 is indeed selectively expressed in lesional atopic dermatitis skin, and serum levels of CCL17 correlate with the disease activity of atopic dermatitis patients.⁴⁵⁻⁴⁷

These observations cannot explain, however, the shift from a T_H2 pattern in acute atopic dermatitis to a T_H1/T_H0 pattern in chronic atopy.^{9,48} Clearly, TSLP cannot account for it, because it was found to be expressed in lesional

epidermis of both acute and chronic atopic dermatitis.¹³ IDECs may be the critical cells that counterbalance and eventually overrule the T_H2 pattern. Both IDECs grown *in vitro* from monocytes and directly isolated from atopic skin lesion were shown to produce T_H1 -promoting cytokines IL-12 and IL-18.²⁶

Conclusions

Taken together, our data may provide a “missing link” in the hypothesis that TSLP is critically involved in the pathogenesis of atopic dermatitis and asthma.^{11,12} In analogy to blood DCs, where this has been shown previously, epidermal Langerhans respond to TSLP in essentially the same way—release of T_H2 -attracting chemokines (CCL17) and induction of “inflammatory T_H2 cells.”¹² Thus, this study provides a direct functional link among epithelial cells, LCs, and T-cell-mediated immune responses. TSLP, which acts upstream of $CD4^+$ T cell responses, should therefore be considered as a potential target for therapy of atopic dermatitis.

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