

Thymic stromal lymphopoietin links keratinocytes and dendritic cell–derived IL-23 in patients with psoriasis

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Background: Thymic stromal lymphopoietin (TSLP) is a major proallergic cytokine that promotes T_H2 responses through dendritic cell (DC) activation. Whether it also plays a role in human autoimmune inflammation and associated pathways is not known.

Objective: In this study we investigated the potential role of several epithelium-derived factors, including TSLP, in inducing IL-23 production by human DCs. We further dissected the role of TSLP in patients with psoriasis, an IL-23–associated skin autoimmune disease.

Methods: The study was performed in human subjects using primary cells and tissue samples from patients with psoriasis and healthy donors. We analyzed the production of IL-23 *in vitro* by blood and skin DCs. We studied the function for TSLP and its interaction with other components of the inflammatory microenvironment *in situ* and *ex vivo*.

Results: We found that TSLP synergized with CD40 ligand to promote DC activation and pathogenic IL-23 production by primary blood and skin DCs. *In situ* TSLP was strongly expressed by keratinocytes of untreated psoriatic lesions but not in normal skin. Moreover, we could demonstrate that IL-4, an

important component of the T_H2 inflammation seen in patients with atopic dermatitis, inhibited IL-23 production induced by TSLP and CD40 ligand in a signal transducer and activator of transcription 6–independent manner.

Conclusion: Our results identify TSLP as a novel player within the complex psoriasis cytokine network. Blocking TSLP in patients with psoriasis might contribute to decreasing DC activation and shutting down the production of pathogenic IL-23. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: Thymic stromal lymphopoietin, dendritic cells, IL-23, psoriasis, CD40 ligand, skin inflammation

Psoriasis is a chronic skin inflammatory disease characterized by hyperplasia of the epidermis (acanthosis), thickening of the cornified layer (hyperkeratosis), and incomplete keratinocyte differentiation (parakeratosis).¹ Although the initial events triggering a psoriatic lesion are still unknown, skin dendritic cells (DCs) and antigen-specific T cells are centrally involved in the physiopathologic process.²

Among the cytokines that could mediate immune inflammation in patients with psoriasis, IL-23 is gaining much attention. IL-23, a heterodimeric cytokine comprising IL-12p40 and IL-23p19,³ is produced by monocytes, macrophages, neutrophils, and DCs. Indeed, intradermal injection of IL-23 induced the development of epidermal acanthosis in mice.⁴ Moreover, IL-23 induced IL-17– and IL-22–producing T cells,^{5,6} numbers of which have been shown to be increased in psoriatic lesions.⁷ Additionally, the clinical efficacy of the anti-IL-12p40 mAb against psoriasis⁸ and the association of a single nucleotide polymorphism in the IL-23 receptor gene in patients with psoriasis⁹ support the important pathogenic role of IL-23 in these patients.

Although IL-23 downstream functions have been well characterized, the factors inducing IL-23 production in patients with psoriasis have not been elucidated. In this study we report the unexpected finding that the proallergic cytokine thymic stromal lymphopoietin (TSLP) synergizes with T cell–derived CD40 ligand (CD40L) to promote IL-23 production in patients with psoriasis.

METHODS

Patients

After obtaining informed consent from patients, 4- to 6-mm punch skin biopsy specimens were taken from either patients with psoriasis (n = 10, Table I) or patients with atopic dermatitis (AD; n = 8) for immunohistology

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Abbreviations used

AD:	Atopic dermatitis
CD40L:	CD40 ligand
DC:	Dendritic cell
GAPDH:	Glyceraldehyde-3-phosphate dehydrogenase
STAT:	Signal transducer and activator of transcription
TBS:	Tris-buffered saline
TSLP:	Thymic stromal lymphopoietin

and from patients with psoriasis ($n = 5$) for skin cell purification. Patients did not receive any treatment: no topical treatment 2 weeks before the biopsy, no systemic antibiotics 4 weeks before the biopsy, no systemic immunosuppressive drugs 12 weeks before the biopsy, and no systemic biological agents 12 weeks before the biopsy. Healthy age- and sex-matched control subjects were also included in the study. Skin samples were immediately frozen in liquid nitrogen and stored at -80°C for immunohistology. Local ethic committees of the Heinrich-Heine University, Dusseldorf, Germany, the University of Rome Tor Vergata, Rome, Italy, and IDI-IRCCS, Rome, Italy, approved the study.

Immunohistology

Frozen skin sections were stained with 5 $\mu\text{g/mL}$ monoclonal rat anti-human TSLP (clone 12F3; kind gift from Professor Yong-Jun Liu), 0.6 $\mu\text{g/mL}$ rabbit anti-human CD3 (Dako, Glostrup, Denmark), and 10 $\mu\text{g/mL}$ mouse anti-human CD40L (Dendritics, Mount Pleasant, Mich) or corresponding IgG isotypes, followed by goat anti-rat (BD PharMingen, San Jose, Calif) and goat anti-rabbit and horse anti-mouse (Vector Laboratories, Burlingame, Calif) biotinylated polyclonal antibodies, respectively. Staining was revealed with the Vectastain ABC peroxidase system (Vector Laboratories). Polyclonal goat anti-human TSLP (5 $\mu\text{g/mL}$; poly 5159; BioLegend, San Diego, Calif) followed by rabbit anti-goat horseradish peroxidase (Dako) was detected by using 3-3'-diamino-benzidine-tetrahydrochloride revelation (magnification 20x and 40x and bar scale indicates 50 and 25 μm , respectively in all pictures of immunohistology).

Cytokine analysis

IL-23 production was quantified by using protein ELISA kits (eBioscience, San Diego, Calif) in culture supernatants (sensitivity, ≥ 7 pg/mL). IL-6, IL-12p40, IL-12p19, and CD86 expression was analyzed by using real-time quantitative RT-PCR, as previously described.⁵ The following probes were used: *IL6* HS 00174131_m1, *IL12p40* HS 00233688_m1, *IL12p19* HS00372324_m1, and *CD86* HS00199349_m1. For each sample, cycle threshold values were normalized to mRNA of the ribosomal protein *L34* gene, and data are reported as the ratio of the target gene to *L34* (Hs00241560_m1).

TSLP transcript expression was analyzed on RNA extracted from homogenized skin (24 donors of each group: healthy skin; nonlesional AD; lesional AD; nonlesional psoriasis; and lesional psoriasis) and reverse transcribed by using standard protocols. Real-time PCR was conducted with 10 μL of cDNA (1.25 ng/ μL) with Power SYBR Green Master Mix (Applied Biosystems, Foster City, Calif). cDNA was analyzed for the expression of human glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*; forward, ATCAGCAATGCCTCTGCAC; reverse, TGGCATGGACTGTGGTCATG) and *TSLP* (forward, CACCGTCTCTTGTGCAATCG; reverse, TAGCCTGGGACCAGATAGC; kind gift from M. Rescigno) by using the ABI Prism 7000 Sequence Detection System (Applied Biosystems). Samples underwent the following stages: stage 1, 50°C for 2 minutes; stage 2, 95°C for 10 minutes; and stage 3, 95°C for 15 seconds, followed by 60°C for 1 minute. Stage 3 was repeated 40 times. For each skin sample, cycle threshold values were normalized to *GAPDH* mRNA, and data were reported as the *TSLP/GAPDH* ratio.

Cell purification from blood and culture

Blood CD11c⁺ DCs or naive CD4 T cells were purified to 99% by using fluorescence-activated cell sorting from buffy coats of healthy adult volunteer blood donors (Crozier Blood Bank), as previously described.^{5,10} Freshly sorted cells were cultured in RPMI (Invitrogen, Carlsbad, Calif) containing 10% FCS, 1% sodium pyruvate, 1% HEPES, and 1% penicillin-streptomycin. DCs were seeded at $0.5 \times 10^6/\text{mL}$ in flat-bottom 96-well plates and stimulated with several stimuli: 50 ng/mL TSLP; 10 ng/mL IL-7; 25 ng/mL TNF- α ; 100 ng/mL IL-6; 100 ng/mL IL-15; 10 ng/mL IL-1 β (R&D Systems, Minneapolis, Minn); 10 ng/mL IL-4 (PeproTech, Rocky Hill, NJ); 10 $\mu\text{g/mL}$ human β -defensin 1, 2, and 3 (Phoenix Pharmaceuticals, Burlingame, Calif); 1 $\mu\text{g/mL}$ IL-18 (MBL, Woburn, Md); 1 $\mu\text{g/mL}$ LL-37 (Innovagen, Lund, Sweden) coupled with 1 $\mu\text{g/mL}$ self-RNA; 1 $\mu\text{g/mL}$ CD40L (Alexis-Enzo Life Sciences, Farmingdale, NY); and 1 $\mu\text{g/mL}$ resiquimod (R848; InvivoGen, San Diego, Calif). In some experiments cells were pretreated for 1 hour with the signal transducer and activator of transcription (STAT) 6 inhibitor AS 1517499 (Axon Medchem, Groningen, The Netherlands) at indicated concentrations. In some experiments DCs were cocultured with naive CD4 T cells preactivated for 6 hours with anti-CD3/CD28 (1 bead per cell; Life Technologies, Carlsbad, Calif). PBMCs from healthy donors unstimulated and stimulated for 1 hour with phorbol 12-myristate 13-acetate (1 $\mu\text{g/mL}$) and ionomycin (500 ng/mL) were used as controls in some experiments.

Cell purification from human skin and culture

Normal breast skin samples were obtained from patients undergoing mastectomy at Curie Hospital (Paris, France) in accordance with institutional ethical guidelines. Psoriatic skin samples were obtained from patients at the University of Rome Tor Vergata (Rome, Italy) and IDI-IRCCS (Rome, Italy). The samples were kept on ice in PBS and processed within the first 10 hours after collection. The skin was cut into manageable strips of 4×1 cm, pinned, and lengthened over an aluminum-covered polystyrene sphere. By using a skin knife, very thin layers of whole skin were cut. Then the pieces were spread, epidermis upward, in a Petri dish containing 10 mL of X-Vivo medium (Lonza, Basel, Switzerland) supplemented with 10% heat-inactivated FCS, 1% penicillin/streptomycin, and 1.6 mg/mL Collagenase D (Roche, Mannheim, Germany). This was incubated overnight at 37°C . The suspension of digested skin was then filtered through a 70- μm cell strainer (BD), washed twice in ice-cold PBS, and stained for analysis (total skin cells) or for cell sorting (DCs) on a FACSaria III cytometer (BD Bioscience). Total DCs from whole skin were defined as 4'-6-diamidino-2-phenylindole dihydrochloride-negative CD45⁺HLA-DR⁺ cells (antibodies from BD Biosciences) after exclusion of the monocyte-macrophage population by using autofluorescence. Cells were seeded at $1 \times 10^6/\text{mL}$ in V-bottom 96-well plates in the presence or absence of 50 ng/mL TSLP (R&D Systems), 1 $\mu\text{g/mL}$ CD40L (Alexis), and 1 $\mu\text{g/mL}$ resiquimod (R848; InvivoGen). After 24 hours, culture supernatants were collected and stored at -80°C .

Biopsy specimens were extensively washed in PBS without calcium and magnesium, minced with a scalpel, and placed in culture at 37°C with 5% CO_2 in RPMI 1640 complemented with 2 mmol/L glutamine, 1 mmol/L sodium pyruvate, 1% nonessential amino acids, 0.05 mmol/L 2-mercaptoethanol, 100 U/mL penicillin, and 100 $\mu\text{g/mL}$ streptomycin (Lonza) with 5% autologous plasma and 60 U/mL recombinant human IL-2 (Novartis, Origgio, Italy) to obtain enriched skin T cells. Medium was replaced every third day, and after 8 to 10 days, T cells that emigrated from tissue samples were collected and placed in starvation with minimal IL-2 before phenotypic characterization.

Flow cytometric analysis

Blood DCs were harvested after 24 hours of culture and resuspended in an EDTA-containing medium. Blood DCs were stained with mouse anti-human CD86-fluorescein isothiocyanate (BD Biosciences), and skin cells were stained with anti-human CD3-allophycocyanin (Miltenyi Biotec, Bergisch Gladbach, Germany) and CD40L-phycoerythrin (eBioscience). Data were analyzed by using flow cytometry (Cyan; Beckman Coulter, Brea, Calif). Dead cells were excluded based on positivity for LIVE-DEAD Fixable Aqua (Life Technologies).

TABLE I. Quantification of TSLP expression in biopsy specimens from patients with psoriasis

Patient no.	Sex	Age (y)	PASI score	% TSLP (average)	% TSLP (max)
1	M	51	31.4	85	95
2	M	50	13.2	20	70
3	M	59	19.5	90	95
4	M	40	19.5	85	90
5	M	40	18.6	80	90
6	M	45	8	80	90
7	M	55	9	70	90
8	M	35	15	40	60
9	M	52	6.2	50	70
10	M	40	10.2	75	90

In situ TSLP expression was quantified by means of immunohistology in 10 independent samples from patients with psoriasis.

M, Male; PASI, Psoriasis Area Severity Index; % TSLP (average), percentage of the epidermal surface positively stained for TSLP across the entire section; % TSLP (max), percentage of the epidermal surface positively stained for TSLP in the most positive field.

Western blot analysis

For protein extraction, CD4 T cells or DCs were resuspended in RIPA buffer (20 mmol/L Tris-HCl [pH 7.5], 150 mmol/L NaCl, 1 mmol/L EDTA, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, 1 mmol/L Na_3VO_4 , 1 mmol/L dithiothreitol, 10 mmol/L β -glycerophosphate, 10 mmol/L NaF, 1 mmol/L phenylmethylsulfonyl fluoride, and protease inhibitor cocktail [Sigma-Aldrich, St Louis, Mo]). After 10 minutes on ice, cell lysates were centrifuged for 10 minutes at 12,000g and 4°C, and the supernatants were collected and used for Western blot analyses. Cell extracts were diluted in Laemmli buffer and boiled for 5 minutes. Proteins were separated on 7% SDS-PAGE gels and transferred to polyvinylidene difluoride Hybond-P membranes (Amersham, GE Healthcare, Little Chalfont, United Kingdom) by using a wet-blotting apparatus (Bio-Rad Laboratories, Hercules, Calif). Membranes were saturated for 1 hour at room temperature with either 5% nonfat dry milk in PBS or 5% BSA in Tris-buffered saline (TBS), both containing 0.1% Tween-20. Membranes were incubated with the following antibodies overnight at 4°C: mouse anti-STAT6 (1:500 dilution in PBST/BSA 5%; BD Biosciences) and rabbit anti-phospho-STAT6 (Tyr 641; 1:1000 dilution in TBST/BSA 5%; Cell Signaling, Danvers, Mass). Secondary anti-mouse or anti-rabbit IgGs conjugated to horseradish peroxidase (GE Healthcare) were incubated with the membranes for 1 hour at room temperature at a 1:10,000 dilution in either PBS or TBS containing 0.1% Tween 20. Immunostained bands were detected by using a chemiluminescent method (Santa Cruz Biotechnology, Dallas, Tex).

Statistical analysis

All statistics were performed with a 2-tailed Student *t* test (or Mann-Whitney *U* test) with GraphPad Prism software (version 5.03; GraphPad Software, La Jolla, Calif). Statistical significance was retained for *P* values of less than .05.

RESULTS

TSLP and TNF- α are epithelium-derived factors that synergize with CD40L to induce IL-23 production by DCs

Through our search for factors that trigger DCs to produce IL-23 in patients with psoriasis, we systematically stimulated primary human blood DCs with epithelium-derived factors known to play a role in skin inflammation. These included cytokines (TSLP, IL-7, TNF- α , IL-1 β , IL-6, IL-15, and IL-18) and antimicrobial peptides (LL-37 coupled with RNA and

human β -defensin 1, 2, and 3). The pharmacologic Toll-like receptor 8 agonist resiquimod (R848) was used as a positive control for IL-23 induction in DCs. We analyzed IL-23 production in 24-hour DC supernatants and the expression of surface CD86 as a maturation marker. Although TSLP, TNF- α , and LL-37 coupled to RNA, as well as R848, were able to induce DC maturation by upregulating CD86 (Fig 1, A), only R848 induced significant levels of IL-23 production as a single agent (Fig 1, B).

Given the importance of infiltrating T cells in the psoriatic lesions and the interaction between DCs and T cells, we hypothesized that T cell-derived factors might also contribute to IL-23 production by DCs. We primed DCs with epithelium-derived factors and subsequently stimulated them with CD40L. We found that TSLP and TNF- α priming in the presence of CD40L synergistically induced increased DC maturation and IL-23 production compared with CD40L alone (Fig 1, C and D). In contrast, IL-23 production and CD86 upregulation mediated by R848 were not enhanced by CD40L. To experimentally validate that TSLP and CD40L synergy induces IL-23 in skin DCs, we purified DCs from the skin of healthy donors and analyzed IL-23 production. Costimulation with TSLP and CD40L synergistically induced IL-23 production by primary skin DCs compared with stimulation with TSLP or CD40L alone (Fig 1, E). Thus skin DCs behave similarly to blood DCs for TSLP- and CD40L-induced IL-23 production.

To add physiologic relevance to this result, we stimulated TSLP-primed DCs with CD40L-expressing T cells. We found that preactivated naive CD4 T cells expressed high levels of CD40L (see Fig E1 in this article's Online Repository at www.jacionline.org) and in combination with TSLP-activated DCs induced IL-23 production in a synergistic manner (Fig 1, F). This suggests that psoriatic skin-infiltrating T cells might provide the CD40L signal to DC *in situ*.

TSLP is expressed in human psoriatic skin

To determine whether TSLP could be involved in inducing IL-23 in patients with psoriasis, we analyzed the expression of TSLP in psoriatic skin. Immunohistochemistry revealed that TSLP-specific staining was completely absent in the skin of healthy subjects. Importantly, TSLP was highly expressed in the epidermis of patients with psoriasis at similar levels as seen in AD skin and human tonsils (Fig 2, A). A similar TSLP staining pattern was found in both diseases, with an absence of TSLP expression in the basal layer and variable staining in the differentiated keratinocyte layers.

Independent samples from 10 different donors were assessed and were all positive for TSLP (Table I). Patients had not received any treatment for at least 2 weeks before the biopsy, excluding a confounding effect of therapy. The specificity of the TSLP staining was confirmed by using 3 methods: (1) a similar staining pattern was obtained by using 2 different anti-TSLP antibodies from different clones, species, and isotypes (see the Methods section and Fig 2, A); (2) preincubation of the anti-TSLP antibody with recombinant TSLP suppressed the staining (Fig 2, B); and (3) rat isotype (Fig 2, A) and goat isotype (see Fig E2 in this article's Online Repository at www.jacionline.org) control-stained psoriatic lesions did not show any signal.

Moreover, we found that TSLP expression was also increased at the transcriptional level in psoriatic lesions

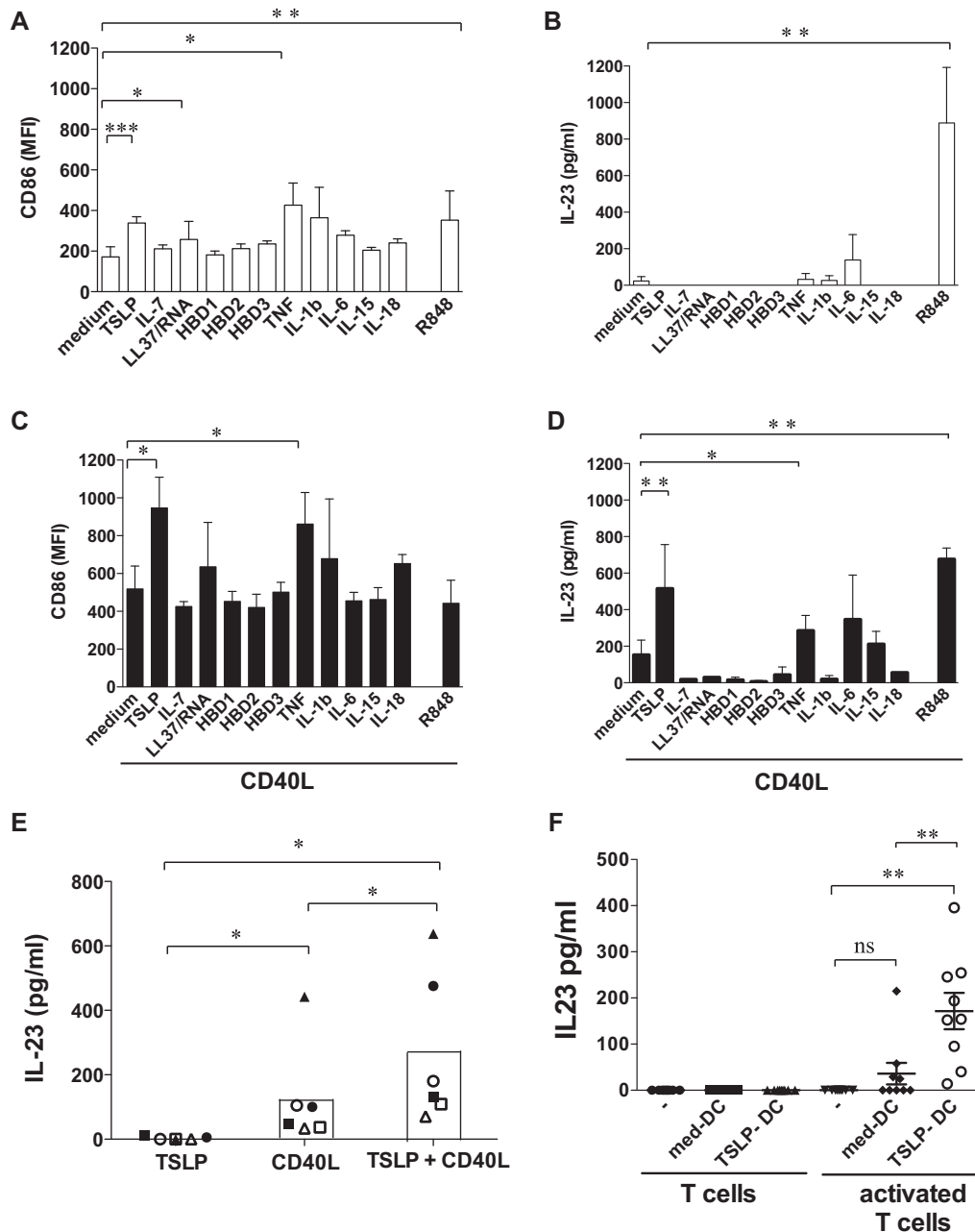


FIG 1. TSLP and TNF- α are epithelium-derived factors able to activate DCs and prime for IL-23 production by DCs. **A-D**, In blood DCs activated for 24 hours without (Fig 1, A and B) or with (Fig 1, C and D) CD40L, IL-23 was measured by means of ELISA (Fig 1, B and D), CD86 was analyzed by means of flow cytometry (Fig 1, A and C). **E** and **F**, IL-23 produced by skin DCs activated for 24 hours (Fig 1, E) or by blood DCs cocultured for 14 hours with anti-CD3/CD28-activated naive CD4 T cells was measured by means of ELISA (Fig 1, F). Results of 6 independent experiments are shown. MFI, Mean fluorescent intensity; ns, $P > .05$. * $P < .05$, ** $P < .005$, and *** $P < .001$.

compared with that seen in normal and nonlesional skin (see Fig E3 in this article's Online Repository at www.jacionline.org).

TSLP has already been associated with AD for its role in inducing a proallergic T_H2 response.¹⁰ Here we report that TSLP is also produced in patients with psoriasis, a T_H17 - and IL-23-related immune disease. We quantified the *in situ* TSLP expression in healthy, AD, and psoriatic skin, and we found comparable levels in patients with the 2 diseases (Fig 2, C).

CD40L is similarly expressed on lymphocytes from psoriatic and AD skin

Because TSLP acted in synergy with CD40L to induce IL-23 production by DCs *in vitro* and TSLP was expressed in both patients with psoriasis and those with AD, we hypothesized that a differential expression of CD40L could explain the exclusive production of IL-23 in psoriatic skin.¹¹

Immunohistochemical analysis revealed that CD40L⁺ cells were present in the dermis of patients with psoriasis and those

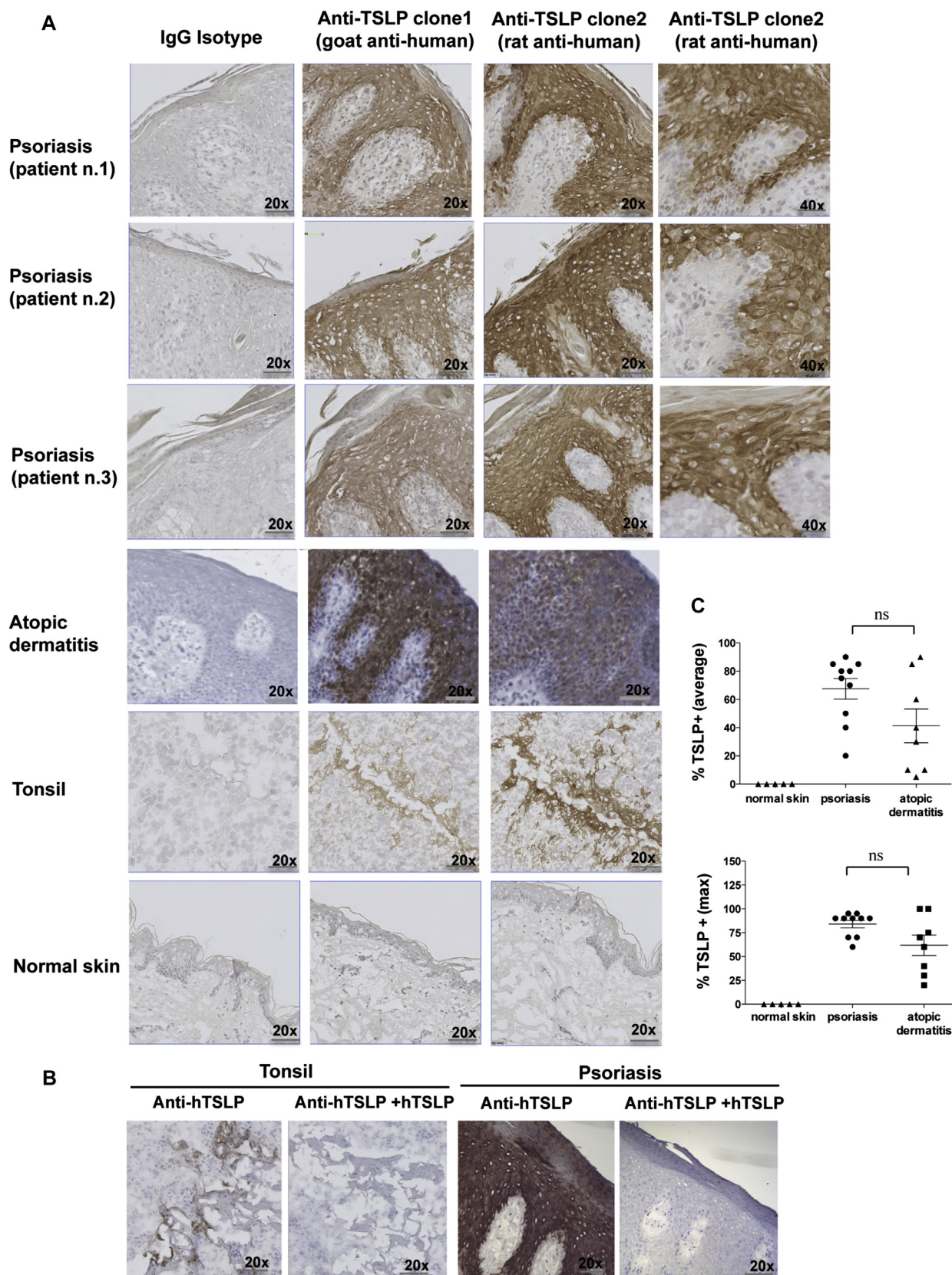


FIG 2. TSLP is expressed in human psoriatic skin. **A** and **B**, Frozen sections were stained with rat IgG isotype or 2 different anti-TSLP antibodies (3 representatives of 10 different donors; Fig 2, **A**) or with anti-TSLP in the absence or presence of 5 μ g/mL recombinant human TSLP (1 representative of 3 different donors; Fig 2, **B**). **C**, Percentages of epidermal surface positively stained for TSLP across the entire section (*average*) and percentages of epidermal surface positively stained for TSLP (*max*) in the most positive field in healthy, AD, and psoriatic skin were compared. *ns*, $P > .05$.

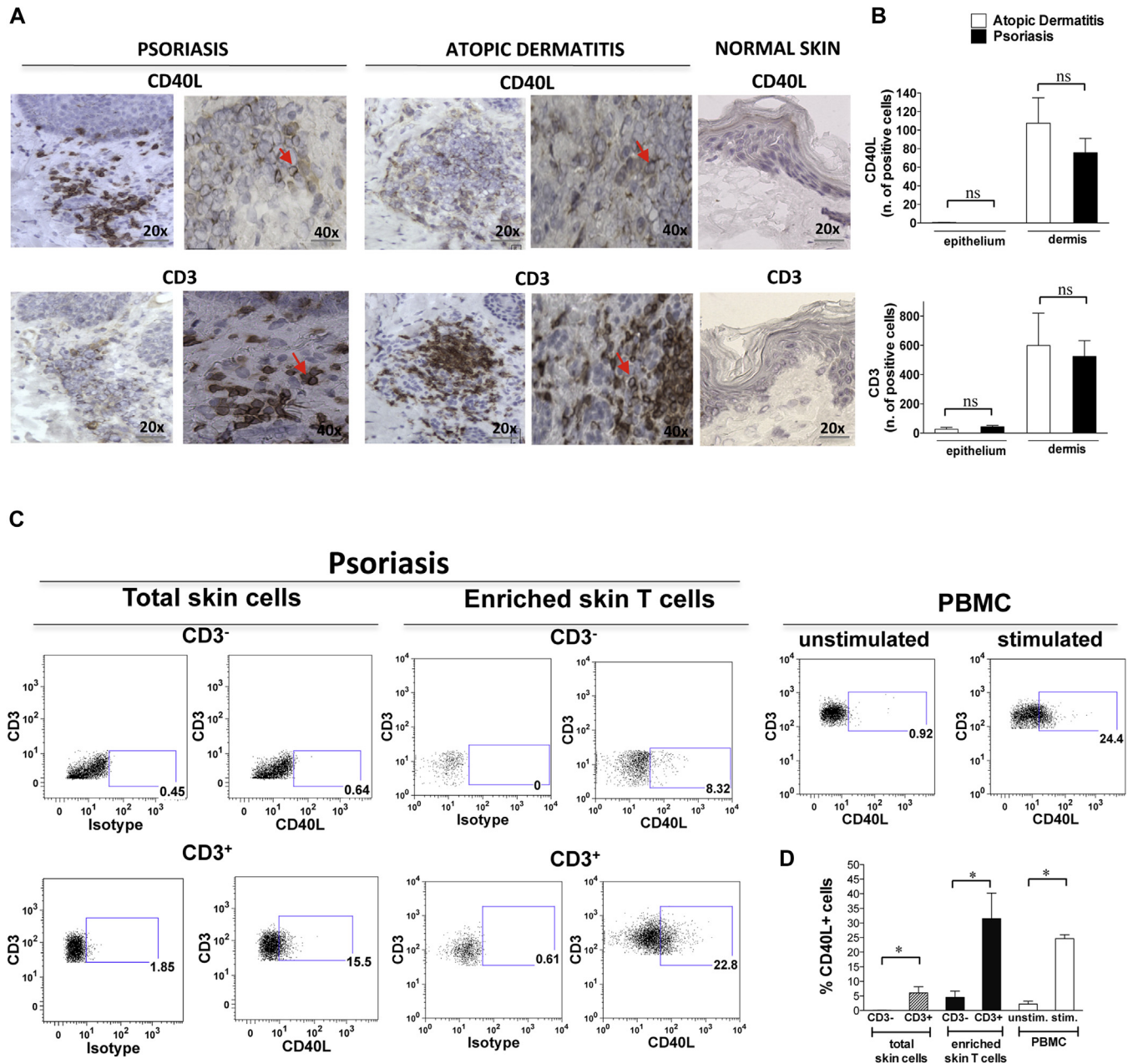


FIG 3. CD40L is similarly expressed on lymphocytes from psoriatic and AD skin. **A**, Frozen sections were stained with anti-CD40L and anti-CD3 on psoriatic, AD, and normal skin (1 representative of 10 patients with psoriasis and 8 patients with AD). **B**, Counts of total positive cells for CD40L and CD3 in skin biopsy specimens from patients with psoriasis and patients with AD were compared. **C** and **D**, CD3 and CD40L expression were evaluated on psoriatic skin cells or PBMCs by using flow cytometry (Fig 3, **C**), and means $\pm$ SDs of 5 independent donors are reported (Fig 3, **D**). ns, $P > .05$. * $P < .05$.

with AD, whereas these cells were absent in healthy skin (Fig 3, **A**). The comparative count of CD3⁺ and CD40L⁺ cells in the epidermis and dermis of both inflamed skin samples revealed that the majority of T cells were infiltrating the dermis and that the number of CD40L⁺ T cells in psoriatic and AD skin was similar (Fig 3, **B**). The red arrows in Fig 3, **A**, showing coexpression of CD3 and CD40L staining suggest that CD40L signal was derived from infiltrating T cells. Coexpression of CD3 and CD40L was confirmed by means of flow cytometric analysis of skin-infiltrating cells from primary psoriatic lesions (Fig 3,

C and **D**). Thus our data suggest that CD40L expression cannot account for the differential production of IL-23 in psoriatic versus AD skin.

IL-4 inhibits TSLP plus CD40L-mediated IL-23 production by DCs independently of STAT6

To explain the specific IL-23 production in patients with psoriasis, we hypothesized that the AD microenvironment could inhibit IL-23 production by DCs. It is known that AD is a

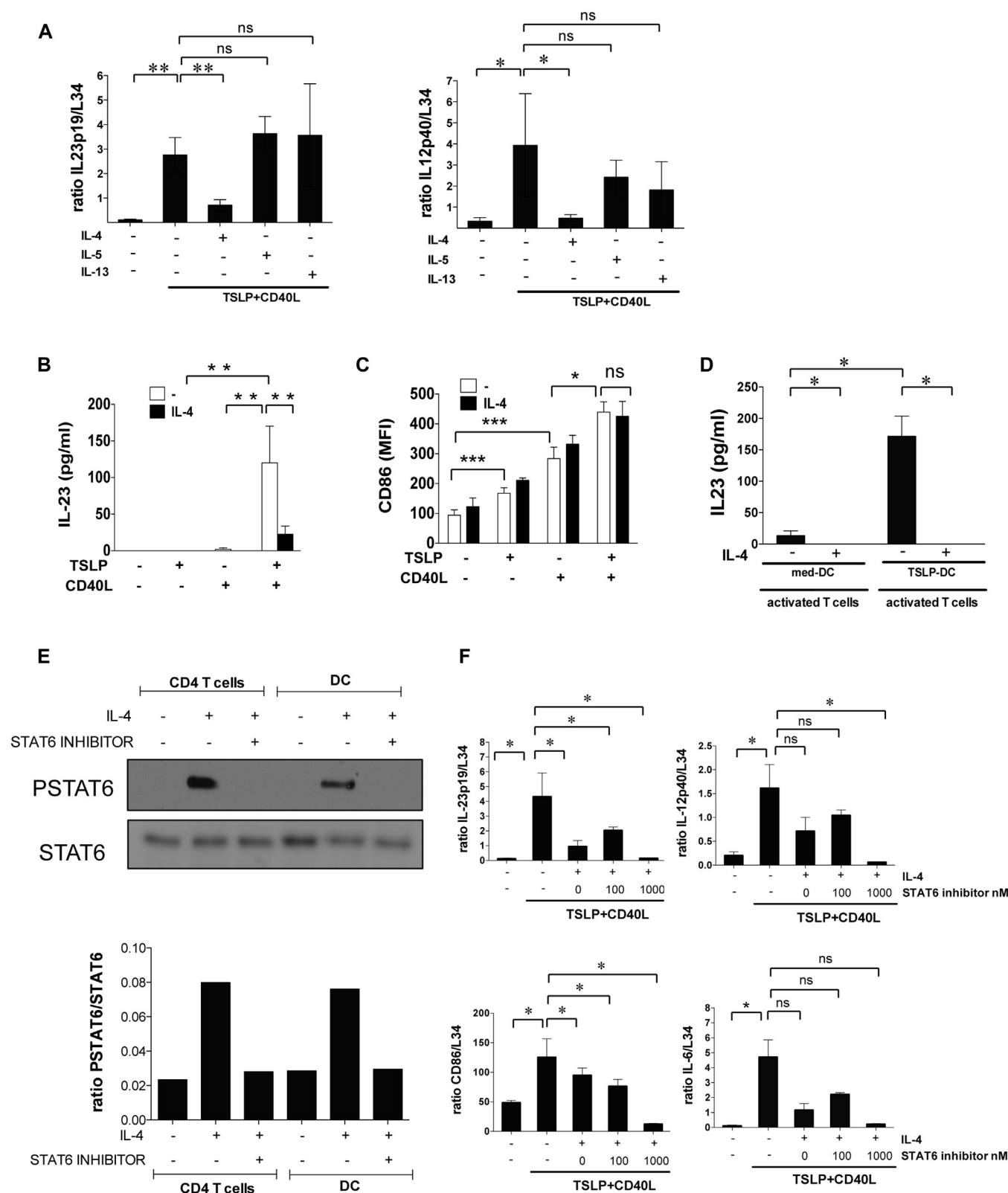


FIG 4. IL-4 inhibits TSLP plus CD40L-mediated IL-23 production by DCs independently of STAT6. **A-C**, IL-23p19 and IL-12p40 expression (Fig 4, A), IL-23 production (Fig 4, B), and CD86 expression (Fig 4, C) by blood DCs cultured for 6 (Fig 4, A) or 24 (Fig 4, B and C) hours in different conditions were analyzed. **D**, IL-23 secretion by DCs cocultured for 14 hours with anti-CD3/CD28-activated naive CD4 T cells was measured by using ELISA. **E**, Inhibition of STAT6 expression in CD4 T cells and DCs by STAT6 inhibitor (1 μ mol/L) was analyzed by using Western blotting. **F**, IL23p19, IL12p40, IL6, and CD86 transcripts were evaluated in DCs incubated for 6 hours and normalized to L34 gene expression (Fig 4, A and F). Means $\pm$ SDs of 4 independent experiments are reported (Fig 4, A-D and F). PSTAT, Phospho-STAT. ns, $P > .05$; * $P < .05$, ** $P < .005$, and *** $P < .001$.

T_H2 -mediated disease¹² and that T_H2 factors are absent from psoriatic lesions.¹³ We analyzed IL-23 production and CD86 expression by DCs exposed to combinations of TSLP, CD40L, and the prototypical T_H2 cytokines IL-4, IL-5, and IL-13. We found that IL-5 and IL-13 did not affect IL-23 induction (Fig 4, A), whereas IL-4 totally inhibited IL-23 mRNA (Fig 4, A) and protein expression in DCs (Fig 4, B). However, IL-4 did not modulate TSLP- and CD40L-induced DC maturation (Fig 4, C). A similar inhibitory effect of IL-4 on IL-23 production was found in TSLP-primed DCs stimulated with CD40L-expressing T cells (Fig 4, D). To demonstrate the specificity of the IL-4-mediated inhibitory effects on IL-23 production, we performed a dose titration of IL-4 and found that its inhibitory effect was dose dependent (see Fig E4 in this article's Online Repository at www.jacionline.org).

Because IL-4 is absent from psoriatic skin,¹³ our data provide a potential explanation for the specific induction of IL-23 by TSLP and CD40L synergy in patients with psoriasis but not in those with AD. This result also reveals an important role of IL-4 in the IL-12/IL-23 balance by enhancing IL-12¹⁴ while inhibiting IL-23 production.

To investigate the mechanisms of IL-23 inhibition by IL-4, we pretreated DCs with an inhibitor of STAT6, which is phosphorylated after IL-4 signaling in CD4 T cells and DCs (Fig 4, E). IL-4 inhibited IL-23 expression by DCs at the transcriptional level (Fig 4, F). However, STAT6 inhibition did not rescue IL-23 expression (Fig 4, F). Similar results were found for IL-6, whereas CD86 induction was not modulated by IL-4 (Fig 4, F).

Thus interaction with components of the microenvironment determines a different TSLP function in patients with psoriasis and those with AD.

DISCUSSION

TSLP has been associated with AD,¹⁰ asthma,^{15,16} and allergic rhinitis¹⁷ for its role in inducing a proallergic T_H2 response.¹⁰ Here we report that TSLP is also produced in patients with psoriasis, a T_H17 - and IL-23-related autoimmune disease. Our findings add to the existing complexity of the cytokine and inflammatory networks in patients with psoriasis² and suggest that TSLP might have different immunomodulatory roles, depending on the type of inflammatory environment.

Our results suggest a model in which epithelium-derived TSLP induces DC maturation and primes for subsequent CD40L-induced IL-23 production by DCs. Although the role of TSLP in inducing DC maturation was already described,¹⁰ its contribution in priming for inflammatory cytokine production is not clear. Indeed, TSLP alone does not induce proinflammatory cytokine (TNF- α and IL-12) production by DCs,¹⁰ which was attributed to a lack of activation of the STAT4 and interferon regulatory factor 8 transcription factors.¹⁸ Our data suggest that CD40L might provide missing molecular cofactors promoting TSLP-induced IL-23 production. In line with these data, the combination of TSLP and CD40L synergistically mediated not only IL-23 but also IL-6 (Fig 4, F) and IL-12p70 production.¹⁹

TNF- α and TSLP might both contribute to IL-23 production by DCs. TNF- α , either alone²⁰ or in combination with IL-4 or IL-13,²¹ was shown to promote TSLP production by

keratinocytes in models mimicking the AD microenvironment.¹² Whether this pathway is involved in psoriasis remains to be addressed. Vitamin D analogues were also shown to promote TSLP production by relieving a retinoid X receptor-mediated inhibition in mouse and human keratinocytes²²⁻²⁴ with a potential relevance to psoriasis therapy.²⁴ However, in this last study psoriatic samples were obtained from patients previously treated with steroids, which was shown to suppress TSLP production.²⁵ In our study TSLP was observed in untreated lesions, indicating that production and function are linked to disease physiopathology and not to therapeutic manipulation.

The T cells (5% to 20%) expressing CD40L in our study might represent recently activated T cells because CD40L is an early and transient T-cell activation marker²⁶ that is mostly observed in secondary lymphoid tissues and not at peripheral sites of inflammation.²⁷ It is already known that CD40L is present in psoriatic skin.^{28,29} The expression of CD40L *in situ* in peripheral tissues has been observed in other pathologies, such as the lungs of patients with idiopathic pulmonary fibrosis, in which CD40L⁺ T cells contribute to the persistence of a chronic inflammation.³⁰ In psoriatic skin the expression of CD40 is detected on dermal DCs and Langerhans cells.²⁹ Langerhans cells also express the TSLP receptor and respond to TSLP,³¹ suggesting that both TSLP and CD40L can provide a strong maturation signal for DCs in inflamed skin.

The induction of IL-23 by DCs after TSLP plus CD40L activation is strongly inhibited by IL-4. This result reveals a potential mechanism for the absence of IL-23 production in allergy and other T_H2 -related diseases, contributing to our global understanding of the antagonism between T_H2 and T_H17 pathways. Moreover, this result reveals how a single cytokine (TSLP) might have different physiopathologic functions in different types of inflammation and provides strong evidence that components of the microenvironment might be critical in controlling the functions of inflammatory cytokines. We demonstrated that inhibition of IL-23 induction by IL-4 is STAT6 independent.

In our study we identified that 2 types of interaction with components of the skin inflammatory microenvironment determine the function of TSLP: (1) synergistic interaction with CD40L leading to IL-23 production and (2) antagonistic interaction with IL-4 suppressing IL-23 in a T_H2 environment. This illustrates the importance of considering the complexity of molecular networks to unravel the function of a given cytokine in distinct types of inflammation.

The present study shows that TSLP is produced by keratinocytes in patients with psoriasis and that TSLP, in this context, primes for IL-23 production by DCs. Our findings extend the role of TSLP from allergy to IL-23-driven autoimmunity, with a possible implication of TSLP in other forms of autoimmune inflammation. From a physiopathologic perspective, it will be important to establish links between TSLP and the other cytokines involved in pathogenic inflammatory networks in patients with diverse autoimmune diseases. In patients with psoriasis, TSLP could serve as a therapeutic target to reduce DC activation and production of pathogenic IL-23.

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Key messages

- TSLP is highly expressed by keratinocytes in patients with psoriasis.
- TSLP synergized with CD40L to induce DC-derived IL-23 production.
- Targeting TSLP is a promising therapy in patients with psoriasis.

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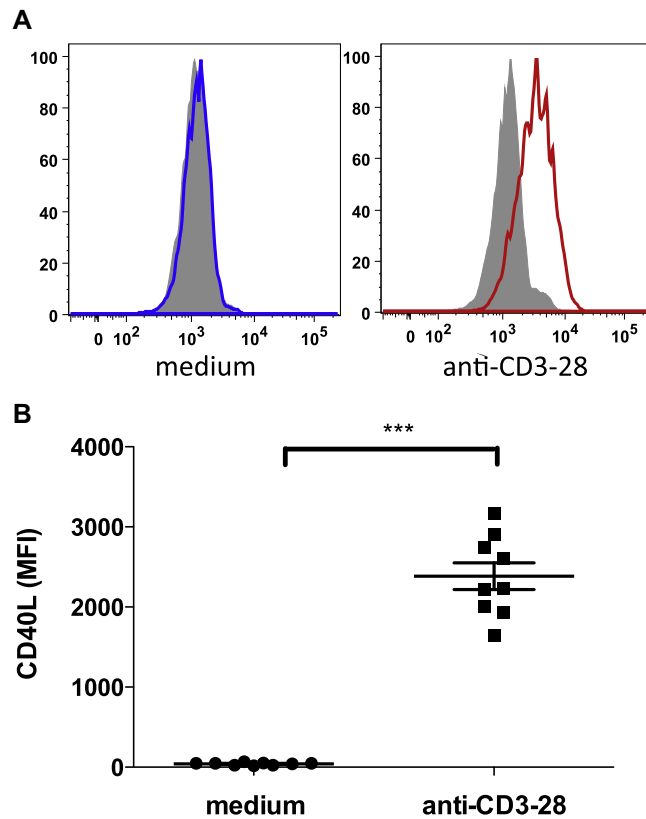


FIG E1. Naive CD4 T cells activated by anti-CD3/CD28 express high levels of CD40L. Expression of CD40L on resting naive CD4 T cells and after 6 hours of stimulation with anti-CD3/CD28 was analyzed by flow cytometry. One representative donor (**A**) and quantification of mean fluorescent intensity (MFI) for 7 donors (**B**) are shown. *** $P < .001$.

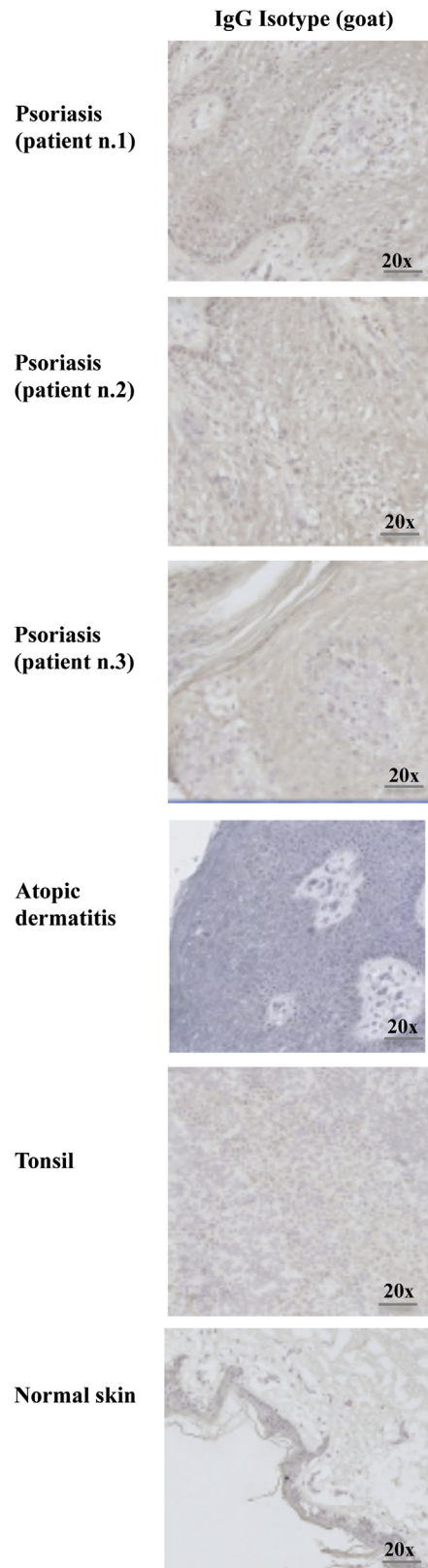
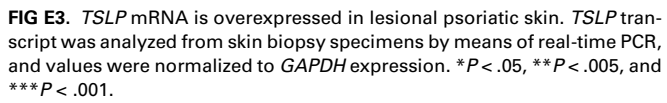


FIG E2. Goat isotype control staining. Frozen sections were stained with goat IgG isotype. Results are from 3 representatives of 10 different donors.



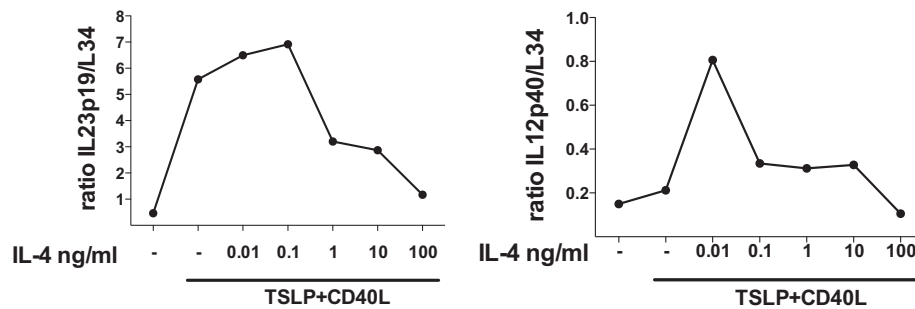


FIG E4. IL-23 inhibition by IL-4 in TSLP plus CD40L-primed DCs is dose dependent. Blood CD11c⁺ cells primed with TSLP or TSLP plus CD40L were stimulated with different doses of IL-4. *IL23p19* and *IL12p40* transcripts were measured by using real-time PCR, and values were normalized to *L34* gene expression. Results are from 1 representative of 3 independent experiments.