

Multiple-checkpoint inhibition of thymic stromal lymphopoietin-induced T_H2 response by T_H17-related cytokines

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Background: The interplay between allergy and autoimmunity has been a matter of long debate. Epidemiologic studies point to a decreased frequency of allergy in patients with autoimmune diseases. However, recent studies suggest that IL-17 and related cytokines, which play a central role in autoimmunity, might also promote allergy.

Objective: To address this controversy, we systematically studied the interactions between T_H17-related cytokines and the thymic stromal lymphopoietin (TSLP)-mediated proallergic pathway.

Methods: We used human primary dendritic cells (DCs), T cells, and skin explants. A novel geometric representation and multivariate ANOVA were used to analyze the T_H cytokine profile.

Results: We show that IL-17A specifically inhibits TSLP production but increases proinflammatory IL-8 production in human skin explants exposed to TNF- α and IL-4. This inhibitory activity was confirmed in cultured skin explants of atopic dermatitis lesions. At the T-cell level, T_H17-polarizing cytokines (IL-1 β , IL-6, TGF- β , and IL-23) inhibited T_H2 differentiation induced by TSLP-activated DCs. This led to a global dominance of a T_H17-polarizing environment over TSLP-activated DCs, as revealed by clustering and computational analysis.

Conclusions: Our data indicate that T_H17-related cytokines are negative regulators of the TSLP immune pathway. This might explain the decreased frequency of allergy in patients with autoimmunity and suggests new means of manipulating proallergic responses. (J Allergy Clin Immunol 2012;■■■:■■■-■■■.)

Key words: Thymic stromal lymphopoietin, T_H17, dendritic cells, T cells, autoimmunity, allergy

Allergic and autoimmune diseases are both associated with a deregulated immune response. T_H cell subsets are key elements involved in the physiopathology of these diseases. Classically, allergic responses leading to asthma, allergic rhinitis, and atopic dermatitis (AD) are characterized by an inflammatory T_H2 immune response. Thymic stromal lymphopoietin (TSLP), an epithelial cell-derived cytokine, plays a critical role in the initiation and chronicity of the allergic immune responses. TSLP induces an inflammatory T_H2 immune response by conditioning dendritic cell (DC) maturation both in human subjects¹⁻³ and mice.⁴ By contrast, autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, and psoriasis, are characterized by the presence of a T_H17 response. T_H17 cells produce IL-17A and IL-17F but also other proinflammatory factors, such as IL-6, IL-22, IL-21, and IL-26.⁵ The cytokines that polarize human naive T cells into T_H17 cells include IL-1 β , IL-6, TGF- β , and IL-23.⁵

The interplay between allergy and autoimmunity has been a matter of long debate. Several epidemiologic studies reported an inverse relationship between AD, allergic rhinitis, or asthma and psoriasis,⁶ rheumatoid arthritis,⁷ multiple sclerosis,⁸ or type 1 diabetes mellitus.^{9,10} This suggests that autoimmune inflammation might suppress the development or reduce the severity of atopy and *vice versa*. However, other studies did not confirm these conclusions and suggest that allergy and autoimmunity share some common physiopathologic mechanisms.^{9,11-14} The immune mechanisms explaining these epidemiologic findings are poorly understood, especially in the human system. In particular, whether T_H17-related cytokines promote or inhibit chronic allergic inflammation remains unclear.

In our study we considered TSLP-activated DC (TSLP-DC)-induced T_H2 differentiation as a prototypical proallergic pathway and T_H17-related cytokines as associated with autoimmunity. We addressed systematically the role of T_H17-related cytokines in the

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Abbreviations used

AD:	Atopic dermatitis
DC:	Dendritic cell
MANOVA:	Multivariate ANOVA
Med:	Complete medium
TSLP:	Thymic stromal lymphopoietin
TSLP-DC:	TSLP-activated DC

TSLP pathway at 3 critical checkpoints: (1) TSLP production by the epithelia, (2) TSLP-induced DC activation, and (3) TSLP-DC-induced T_H2 cell differentiation.

METHODS**Human skin explants**

Human skin samples were obtained in compliance with French legislation and cultured as previously described.¹⁵ AD biopsy specimens (3–4 mm in diameter) were obtained from the Department of Dermatology, Tenon Hospital, Paris, France, after obtaining informed consent from the patients. This study was approved by the Internal Review Board and Clinical Research Committee of the Institut Curie. After removing subcutaneous fat tissue, 5-mm skin punch specimens were cultured in RPMI containing 10% FCS (Hyclone, Logan, Utah), 1% pyruvate, nonessential amino acids, and penicillin/streptomycin (ie, complete medium [Med]) with combinations of the following soluble factors (all from R&D Systems, Minneapolis, Minn): TNF- α (100 ng/mL), IL-2 (100 ng/mL), IL-4 (25 ng/mL), IL-6 (100 ng/mL), IL-17A (100 ng/mL), IL-17B (100 ng/mL), IL-17C (100 ng/mL), IL-17D (100 ng/mL), IL-17E (100 ng/mL), IL-17F (100 ng/mL), IL-21 (100 ng/mL), IL-22 (100 ng/mL), IL-26 (100 ng/mL), IFN- γ (1000 U/mL), TGF- β (2 ng/mL), and IL-10 (100 ng/mL). After 48 hours, culture supernatants were collected and frozen until assayed. In some experiments, after 24 hours of stimulation in the presence of TNF- α and IL-4, the medium was changed, and IL-17A (100 ng/mL) was added or not for an additional 48 hours. Then supernatants were collected and frozen until analysis. AD punch biopsy specimens were cut into 2 pieces and cultured with or without IL-17A (100 ng/mL). After 48 hours, biopsy specimens were kept in Tissue-Tek (Sakura Finetek USA, Inc, Torrance, Calif) to analyze TSLP expression *in situ* by means of immunohistochemistry.

Blood DC purification and culture

CD11c⁺ DCs were purified to greater than 99% by means of FACS sorting from buffy coats from healthy donors (Saint Antoine-Croizatier Blood Bank, Paris, France), as previously described.¹⁵ Freshly sorted CD11c⁺ DCs were cultured in Yssel medium (gift of Hans Yssel, INSERM) containing 10% FCS and penicillin-streptomycin (Invitrogen, Carlsbad, Calif), ie, complete medium, Med. Cells were seeded at 1.10^6 /mL in flat-bottom 96-well plates with different combinations of human recombinant TSLP (50 ng/mL), IL-1 β (10 ng/mL), IL-6 (20 ng/mL), IL-17A (100 ng/mL), IL-23 (100 ng/mL), and TGF- β (1 ng/mL; all from R&D Systems). CD11c⁺ DCs were harvested after 24, 48, or 72 hours of culture; stained with murine anti-human CD80-phycoerythrin or CD80-fluorescein isothiocyanate, CD40-fluorescein isothiocyanate, CD86-phycoerythrin-Cy5, HLA-DR-Alexa700, and matched isotype controls (Becton Dickinson, Franklin Lakes, NJ); and analyzed with the LSRII flow cytometer. Dead cells were excluded based on side- and forward-scatter characteristics. Data analysis was performed with FlowJo software (version 8.3.3).

Naive CD4⁺ T-cell purification and culture

Purification of naive CD4⁺ T cells (CD4⁺CD25[−]CD45RA⁺CD45RO[−]) from adult blood was performed as previously described.¹⁶ Naive T cells were cocultured with DCs in complete Yssel medium at a ratio of 5:1 in medium alone or with IL-1 β (10 ng/mL), IL-6 (20 ng/mL), IL-17A (100

ng/mL), IL-23 (100 ng/mL), and TGF- β (1 ng/mL). T cells were seeded at 250,000/mL in round-bottom 96-well plates. After 6 days, cells were washed twice in PBS 1 $\times$ and restimulated with anti-human CD3/CD28-coupled Dynal beads (T-cell expander, 1 bead per cell, Invitrogen) for 24 hours at a cell density of 10^6 cells/mL. Supernatants were collected and frozen until assayed.

Cytokine secretion assays

Culture supernatants were analyzed within 1 month of collection by using protein ELISA kits for TSLP, IL-8, IL-17, IL-22 (R&D Systems), IL-21 (Peprotech, Rocky Hill, NJ), and cytometric bead assay Flex Sets (BD Biosciences) for IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-13, IL-17A, TNF- α , and IFN- γ , according to the manufacturer's instructions. With a detection limit for TSLP ELISA of 30 pg/mL, all the data at less than that concentration were considered zero.

Immunohistochemistry

Frozen skin sections 5 μ m thick were stained with a goat anti-human TSLP antibody (clone poly 5159; BioLegend, San Diego, Calif) or a goat IgG isotype control (i5000; Vector Laboratories, Burlingame, Calif) followed by a polyclonal rabbit anti-goat antibody linked to horseradish peroxidase (DAKO, Glostrup, Denmark) and revealed by using peroxidase (Vector Laboratories).

Statistical analysis: Multivariate analysis

The concentrations of individual cytokines in different conditions were compared by using a paired 2-tailed Student *t* test or a Wilcoxon signed-rank test when more than 10 donors were included. When the analysis of a given experiment involved more than 20 pairwise comparisons, the resulting *P* values were corrected for multiple testing by using the Benjamini-Hochberg false discovery rate, as implemented in the Matlab function *mafdr* in the Bioinformatics toolbox. Statistical significance was retained for a false discovery rate of less than 0.1. The multivariate statistical analysis was done with the Matlab statistical toolbox. For multivariate ANOVA (MANOVA), a data matrix was created containing 40 rows (4 T_H profiles with 10 donors each) and 13 columns (cytokine concentrations). The matrix was used as input to the function *manova1*. Intergroup distances were computed by using the output *gmdist*. Clustering was done with the function *manovacluster*. The star plots¹⁷ were generated with the function *glyphplot*. The data were first standardized by shifting and scaling each cytokine separately onto the interval (0.1). Each T_H profile was then represented as a star-shaped figure, with 1 spoke for each measured cytokine. The length of a spoke is proportional to the average cytokine concentration in each profile.

RESULTS**IL-17A and IL-17F inhibit TSLP production by human skin explants**

The first checkpoint in which T_H17 -related cytokines can interact with the TSLP- T_H2 pathway is the production of TSLP by epithelial cells. The regulation of the production of human TSLP and, in particular, the influence of the T_H -related cytokines still need to be defined. For that purpose, healthy human skin explants were cultured in the presence of different combinations of T_H -related cytokines (Fig 1). Human skin explants were not able to produce TSLP when cultured in medium alone (Fig 1, A). As we previously showed,¹⁵ TNF- α and IL-4 induced the production of TSLP by human skin explants (Fig 1, A). In this setup known negative regulators of T_H2 inflammation, such as IL-10, TGF- β , and IFN- γ , as well as IL-2, did not modulate TSLP production (Fig 1, A). On the contrary, we found that addition of exogenous IL-17A inhibited up to 70% of the production of TSLP induced by

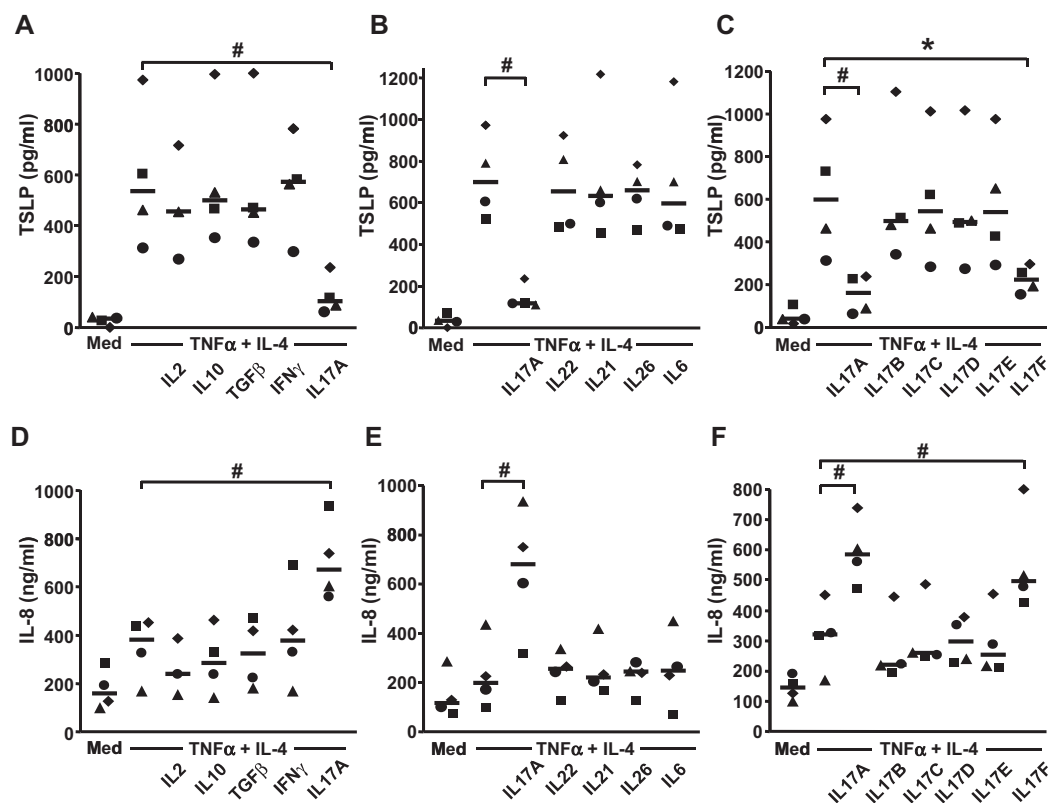


FIG 1. IL-17A and IL-17F specifically inhibit TSLP production induced by TNF- α and IL-4 in human skin explants. **A-F**, TSLP (**A-C**) and IL-8 (**D-F**) quantification by means of protein ELISA. Each symbol represents an independent experiment ($n = 4$). Horizontal bars indicate the median. Statistical significance is retained for pairwise comparison between culture condition groups. * $P < .05$ and false discovery rate > 0.1 . # $P < .05$ and false discovery rate < 0.1 .

TNF- α and IL-4 (**Fig 1, A**). Similar results were obtained with serum-free media (data not shown).

Next we checked whether the inhibition of TSLP production was specific to IL-17A or shared by other T_H17 -derived cytokines. IL-6, IL-22, IL-21, and IL-26 are all reported to be produced by T_H17 cells.^{16,18,19} These T_H17 -derived cytokines did not modulate TSLP production (**Fig 1, B**). Among the IL-17 family members, only IL-17F inhibited TSLP production similar to IL-17A (**Fig 1, C**).

T_H17 cells are considered proinflammatory actors. IL-17A was previously found to enhance the production of IL-8 by keratinocytes.²⁰ As a control for the IL-17A proinflammatory effect, we quantified the production of IL-8 by human skin explants in the same set of experiments as above. Opposite to the inhibition of TSLP production, IL-17A or IL-17F increased IL-8 production by 3.5-fold (**Fig 1, D and F**). None of the other T_H -related cytokines tested affected the production of IL-8 (**Fig 1, D**). The differential regulation of TSLP and IL-8 by IL-17A and IL-17F was dose dependent, with a significant effect starting at the dose of 10 ng/mL (See **Fig E1** in this article's Online Repository at www.jacionline.org).

To further substantiate the ability of IL-17A to inhibit TSLP, we assessed whether IL-17A could inhibit established TSLP production. For that purpose, healthy skin explants were cultured in the presence of TNF- α and IL-4 for 24 hours to induce TSLP production. After 24 hours, the tissues were washed and fresh medium with or without IL-17A was added for an additional 48

hours. The TNF- α plus IL-4-primed skin in Med kept producing TSLP, whereas in the presence of IL-17A, TSLP production was inhibited up to 60% (see **Fig E2** in this article's Online Repository at www.jacionline.org). We concluded that IL-17A not only prevents but also inhibits established TSLP production.

To validate our findings in terms of physiopathologic relevance, we addressed the inhibitory effect of IL-17A on TSLP expression in skin explants from AD lesions. After 48 hours of culture with and without IL-17A, TSLP was measured by using ELISA of culture supernatants and *in situ* immunohistochemistry. TSLP was not detectable by using ELISA, even in untreated AD skin explants, because of the small amount of tissue (data not shown). However, as previously published, TSLP was detected in AD untreated skin lesions at high levels *in situ* by using immunohistochemistry (**Fig 2, A**). IL-17A significantly inhibited TSLP expression in AD skin lesions after 48 hours (**Fig 2, A and B**). The observation of a bimodal distribution of response to IL-17A treatment suggested that subgroups of patients might behave differently. After reviewing the clinical characteristics of the patients (see **Table E1** in this article's Online Repository at www.jacionline.org), we could show that IL-17A significantly inhibited TSLP expression in lesions from patients with moderate (**Fig 2, C**) but not severe (**Fig 2, D**) AD. This suggested that patients with severe AD might be resistant to IL-17A inhibition.

Our results show that IL-17 suppresses both *de novo* and established TSLP production while promoting IL-8-mediated inflammation.

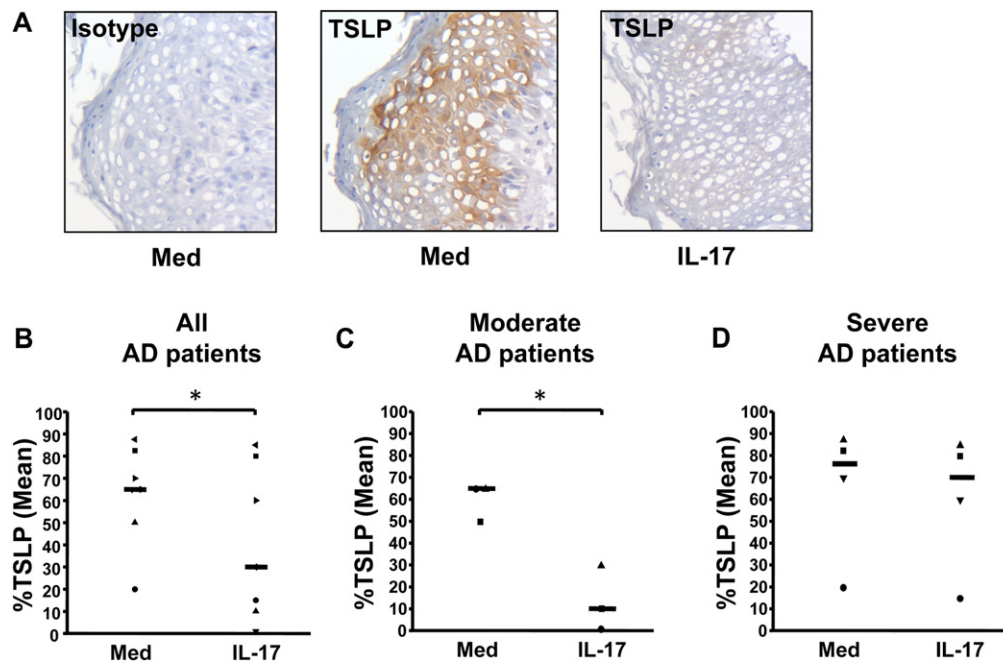


FIG 2. IL-17A inhibits established TSLP production by human atopic dermatitis (AD) skin explants. **A**, Frozen sections of cultured AD skins stained by means of immunohistology for TSLP. **B–D**, Percentages of staining are shown for all patients (Fig 2, **B**) or divided into patients with moderate (Fig 2, **C**) and severe (Fig 2, **D**) AD. Each symbol represents a different donor ($n = 7$). Horizontal bars indicate the median. * $P < .05$.

T_H17-polarizing cytokines enhance costimulatory molecule and CCL22 expression by TSLP-DCs

DCs are the main target of human TSLP.² DC activation by TSLP represents the second checkpoint at which interactions with T_H17-related cytokines can occur. Blood CD11c⁺ DCs were cultured for 48 hours in the presence of TSLP with or without T_H17-related cytokines and analyzed for the expression of the costimulatory molecules CD40, CD80, and CD86. As previously found,² TSLP induced a strong DC activation characterized by increased expression of CD40, CD80, and CD86 (Fig 3, **A**). IL-17A had no effect on TSLP-induced DC activation (see Fig E3, **A** and **B**, in this article's Online Repository at www.jacionline.org).

Next we considered that a T_H17-polarizing environment might also participate in the modulation of allergic responses. Hence we studied the effect of T_H17-polarizing cytokines on the TSLP-induced DC activation. On the basis of our previous report,¹⁶ we used the combination of IL-1 β , IL-6, TGF- β , and IL-23 (referred to as the T_H17 cocktail) for T_H17 polarization. The T_H17 cocktail further enhanced the expression of CD40, CD80, and CD86 by DCs in the presence of TSLP (Fig 3, **A**). Among the T_H17-polarizing cytokines, IL-1 β was primarily responsible for this enhanced activation (Fig 3, **A**). As described, TSLP induced the production of the chemokines CCL17 (thymus and activation-regulated chemokine) and CCL22 (macrophage-derived chemokine; Fig 3, **B**).²¹ We checked whether the T_H17 cocktail was able to inhibit chemokine production by TSLP-DCs. The T_H17 cocktail did not modulate TSLP-induced production of CCL1 but increased by 2.8-fold the production of CCL22 compared with TSLP alone (Fig 3, **B**). Moreover, although TSLP-DCs did not produce the proinflammatory cytokine IL-8 (Fig 3, **B**), the combination of TSLP and the T_H17 cocktail upregulated

IL-8 production by 15-fold (Fig 3, **B**). Our results suggest that a T_H17-promoting microenvironment might enhance the proinflammatory activity of TSLP-DCs, as well as their capacity to stimulate T cells.

T_H17-polarizing cytokines inhibit the T_H2 differentiation induced by TSLP-DCs

The induction of an inflammatory T_H2 response by TSLP-DCs represents the final step of a possible effect of the T_H17-related cytokines in allergy. Naive CD4⁺ T cells were cocultured for 6 days with allogeneic medium- or TSLP-DCs with or without the T_H17 cocktail to address this question. T_H profiles were determined by means of cytokine analysis in culture supernatants after a 24-hour polyclonal restimulation. As expected, TSLP-DCs were able to induce the differentiation of inflammatory T_H2 cells characterized by the production of T_H2 cytokines (IL-4, IL-5, and IL-13) and TNF- α and decreased quantities of IL-10 (Fig 4 and see Fig E3, **C**). Although IL-17A did not affect the TSLP-DC-induced T_H2 profile (see Fig E3, **C**), the T_H17 cocktail drastically inhibited the production of T_H2 cytokines by more than 90% (Fig 4, **A**). IL-10 production was also decreased when T cells were differentiated with TSLP-DCs in the presence of the T_H17 cocktail compared with that seen in TSLP-DCs alone (Fig 4, **A**). On the contrary, T_H17-promoting cytokines increased IL-17A and IL-9 production by T cells cocultured with TSLP-DCs. All other cytokines measured, such as IFN- γ , IL-6, and TNF- α , were not modulated by the T_H17 cocktail (data not shown). The suppressive effect of the T_H17 cocktail on the TSLP-DC-induced T_H2 profile was not due to cell death because no difference was observed in T-cell expansion and viability between all conditions

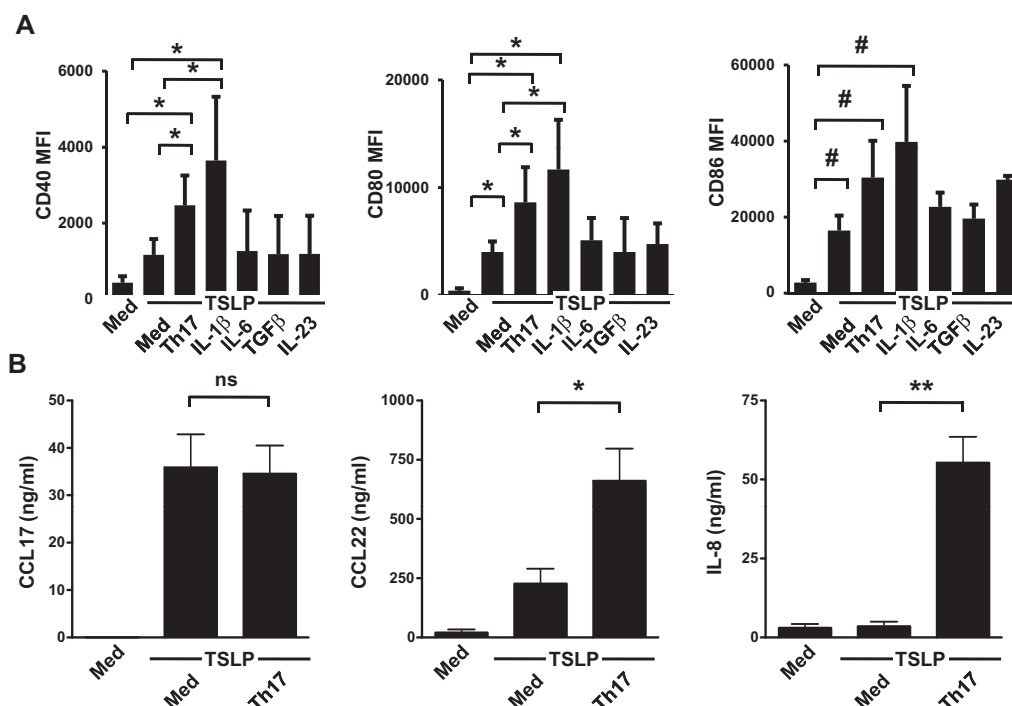


FIG 3. T_H17-polarizing cytokines enhance the maturation and production of CCL22 and IL-8 by TSLP-DCs. **A**, CD40, CD80, and CD86 expression assessed by means of flow cytometry (mean fluorescence intensity [MFI] level, mean $\pm$ SEM, $n = 3$). **B**, CCL17, CCL22, and IL-8 quantification by means of ELISA or cytometric bead assay in 48-hour DC supernatants (mean $\pm$ SEM, $n = 5$). Statistical significance is indicated for pairwise comparison of culture condition groups. * $P < .05$; ** $P < 0.01$; # $P < 0.05$ and false discovery rate < 0.1 .

(data not shown). Among the cytokines of the T_H17 cocktail, none was sufficient to inhibit the TSLP-DC-induced T_H2 profile (see Fig E4 in this article's Online Repository at www.jacionline.org). This suggests that a complex T_H17 inflammatory environment is required to overcome the TSLP-induced T_H2 responses.

Given this profound modulation of the TSLP-DC-induced T_H differentiation by T_H17 cytokines and because we analyzed at least 13 cytokines in total, we generated a geometric representation to allow a global visualization of the data. We represented 1 diagram per profile in which the length of a spoke was proportional to the measurement of cytokine concentration (Fig 4, B). Such representation allowed us to recapitulate and highlight the differences between T_H subsets. T_H2-related cytokines and TNF- α characterized the T cells differentiated by TSLP-DCs, whereas the cytokines IL-17A, IL-22, and TNF- α characterized the T cells differentiated by addition of the T_H17 cocktail (Fig 4, B). Such a multiparametric representation revealed that IL-9 and TNF- α were the 2 main cytokines that characterized best the T cells differentiated with the combination of TSLP-DCs and the T_H17 cocktail (Fig 4, B).

Overall, we showed that T_H17-polarizing cytokines inhibited the TSLP-DC-induced T_H2 profile while switching it to another inflammatory cytokine profile with increased IL-9, TNF- α , and IL-17A levels.

Dominance of the T_H17-polarizing cytokines over the TSLP-induced T_H2 differentiation

Our data showed that the integration of TSLP and T_H17-derived signals by T_H cells led to a profile that is different

from that of prototypical T_H2 and T_H17. We sought to establish the relative dominance of TSLP versus T_H17 cytokines in driving T_H cell polarization. In other words, is the T_H cytokine profile obtained with the combination of TSLP-DCs and T_H17 cytokines closer to the one generated by TSLP-DCs or to that of T_H17 cells? We used the MANOVA method²² to detect statistical differences between multiple groups (T_H subsets), each of which was characterized by a multivariate mean (cytokine profile). Using a Matlab implementation of MANOVA, we identified 4 statistically different groups ($P < .05$). This confirmed that each culture condition generated well-defined T_H subsets (Fig 5, A). A dendrogram was made by using the Mahalanobis distances between the multivariate group means to quantify the degree of similarity between these different T_H subsets (Fig 5, B). We found the closest similarity between Med-DCs plus T_H17 and TSLP-DCs plus T_H17 (Fig 5, B). The cytokine profile of TSLP-DC-activated T cells was well apart from those cocultured in the presence of the T_H17 cocktail in the presence or absence of TSLP-DCs. These findings suggest a dominance of the T_H17-polarizing environment over TSLP conditioning, suggesting that T_H17-induced autoimmune inflammation might override TSLP-induced allergic responses.

DISCUSSION

In this report we demonstrate that T_H17-related cytokines are negative regulators of the TSLP immune pathway at 2 levels: (1) the production of TSLP by epithelial cells and (2) the TSLP-mediated T_H2 differentiation.

IL-17 has 6 family members: IL-17A to IL-17F.²³ Among these cytokines, IL-17A and IL-17F share the highest amino

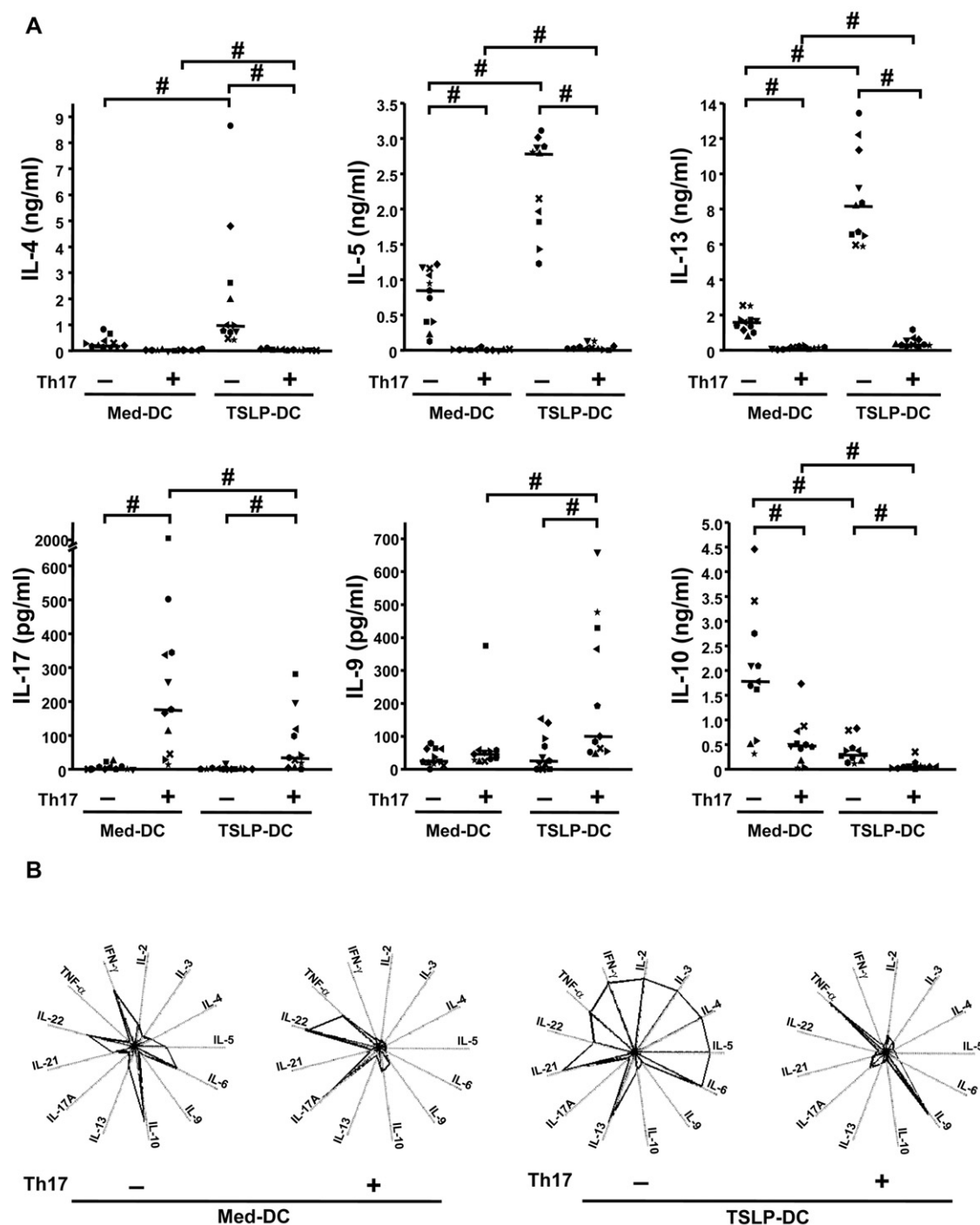


FIG 4. T_H17 -polarizing cytokines inhibit the TSLP-DC-induced inflammatory T_H2 differentiation. **A**, The T_H profile was assessed based on cytokine production (median, $n = 11$, pairwise comparison). $\#P < .05$ and false discovery rate < 0.1 . **B**, Star-shaped figure of T_H profile. The length of a spoke is proportional to the average of a cytokine concentration of that profile.

acid sequence homology and signal through the same receptor.²⁴ In most systems IL-17A and IL-17F have similar biological effects, such as inducing the expression of proinflammatory factors.²³ IL-25 (IL-17E) is the most divergent between the IL-17 family members and promotes a T_H2 immune response.²⁵ Our data obtained on primary human skin explants showed that IL-17A and IL-17F both inhibited, to a similar extent,

the production of TSLP. By contrast to IL-17A and IL-17F, we found that IL-25 did not affect TSLP production. Our results differ from those of a recent study reporting that IL-17F did not affect TSLP production by nasal epithelial cells, whereas IL-25 enhanced this production.²⁶ These discrepancies might be due to the different epithelial systems (ie, skin vs nasal epithelium) or the different stimuli used for

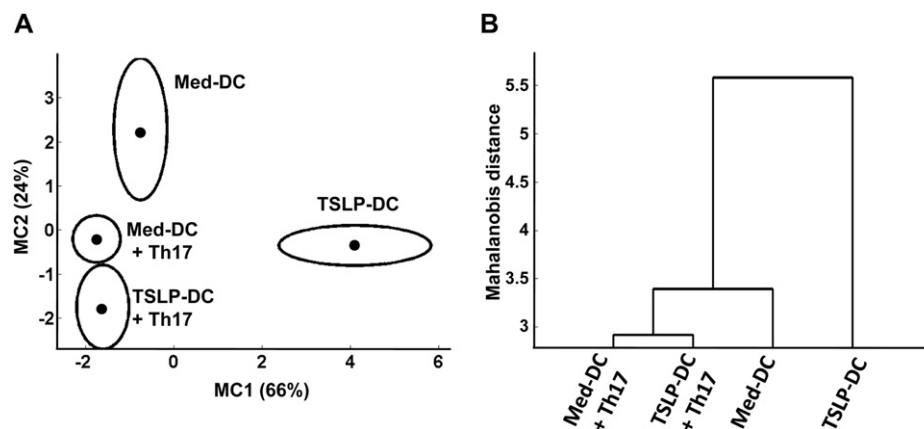


FIG 5. Dominance of the T_H17 -polarizing cytokines over TSLP-DC-induced inflammatory T_H2 differentiation. **A**, MANOVA of T_H profiles ($n = 11$). Points in the plane represent the average of the first ($MC1$) and second ($MC2$) coordinates of each T_H subset. The ellipses around the points represent the SD. **B**, Dendrogram using the Mahalanobis distance between multivariate group means.

TSLP induction: TNF- α plus IL-4 (Fig 1, C) versus double-stranded RNA.²⁶

In a murine model of asthma, IL-17A directly inhibited CCL17 production by bone marrow-derived DCs, which subsequently led to a decrease in T_H2 -secreted cytokines in the regional lymph nodes.²⁷ However, IL-17 promoted the maturation of DC progenitors in mice (ie, increased cell surface expression of CD11c, costimulatory molecules [CD40, CD80, and CD86], and MHC class II).²⁸ By contrast, we found that human IL-17A did not directly affect either the DC activation induced by TSLP or the T_H2 differentiation promoted by TSLP-DCs. IL-17A exclusively acted on the TSLP pathway at the epithelial level by inhibiting TSLP production while enhancing IL-8 production. This is in accordance with the fact that the epithelium is the main target of IL-17, where it induces proinflammatory cytokines (TNF, IL-1, IL-6, granulocyte colony-stimulating factor, and GM-CSF) and chemokines (CXCL1, CXCL5, IL-8, CCL2, and CCL7).²⁴ Our data reveal a novel effect of IL-17 as a negative regulator of TSLP production by keratinocytes, suggesting that allergic T_H2 inflammation can be inhibited by IL-17.

Because reciprocal cross-inhibition regulates T_H cell differentiation,²⁹ we decided to systematically test not only the role of IL-17 but also the role of T_H17 -polarizing cytokines (IL-1 β , IL-6, TGF- β , and IL-23) on TSLP-induced DC activation and on the subsequent TSLP-DC-induced T_H2 differentiation. We show that T_H17 -polarizing cytokines enhanced costimulatory molecule expression and CCL22 production and induced IL-8 production by TSLP-DCs. Our results are in line with a report showing that IL-1 β induced CCL22 production by human macrophages.³⁰ IL-1 β also induces IL-8, TNF- α , and IL-6 on several cell types, such as monocytes, endothelial cells, keratinocytes, and fibroblasts.³¹ In our experiment IL-1 β was the cytokine responsible for the enhanced production of IL-8 by DCs (data not shown). Although T_H17 -polarizing cytokines did not inhibit TSLP-induced activation in DCs, we show for the first time that these cytokines drastically inhibited the TSLP-DC-induced T_H2 differentiation. Reciprocally, TSLP-DCs partially inhibited the production of IL-17A by naive T cells cultured in a T_H17 -polarizing environment. Our results suggest a cross-inhibition in which the final product of the T_H17 pathway (IL-17A) could inhibit the initial step of the TSLP pathway at its source, whereas the T_H17 -inducing

cytokines blocked the final step, leading to TSLP-driven T_H2 cell differentiation. Furthermore, our findings suggest a dominant role of the T_H17 -polarizing environment over the TSLP-DC conditioning that was confirmed by using the MANOVA method.²²

T_H17 -polarizing cytokines inhibited TSLP-DC-induced T_H2 differentiation, leading to a new T_H profile. The use of a geometric representation that allows visualization of the whole cytokine profile permitted us to determine how T cells integrate such a complex environment (TSLP-DCs plus T_H17 -polarizing cytokines). The presence of T_H17 -polarizing cytokines increased IL-17A and also IL-9 production by T cells cocultured with TSLP-DCs. When TGF- β was removed from the T_H17 -polarizing cocktail, IL-9 values decreased to undetectable levels (data not shown), demonstrating that TGF- β played a critical role in IL-9 production but only when combined with TSLP-DCs. Moreover, TNF- α was still highly produced, whereas IL-10 was expressed at low levels by these T_H cells, suggesting that they kept their proinflammatory potential. It was previously shown that IL-9 secretion is induced both in TGF- β plus IL-4-stimulated cells (T_H9 polarization) and in T_H17 -stimulated cells (TGF- β , IL-1 β , IL-6, IL-21, and IL-23).³² By contrast, we show that IL-9 secretion was not induced by the T_H17 -polarizing cytokine cocktail (IL-1 β , IL-6, TGF- β , and IL-23) alone. Only the combination of TSLP-DCs and the T_H17 -polarizing cytokines significantly promoted IL-9 production.

Our work provides a potential mechanism to explain the inverse relation between autoimmunity and allergy, which has been reported in several epidemiologic studies.⁹ Our data are further supported by a recent study in patients with co-occurring AD and psoriasis that shows antagonistic T-cell infiltrates in skin lesions (T_H2 and T_H1/T_H17 , respectively) but never coexistence of T_H2 and T_H17 cells within the same lesion.³³ Our results showed that IL-17A was able to significantly inhibit TSLP in skin lesions from patients with moderate AD, whereas no effect was found in patients with severe AD. This has potential therapeutic implications and suggests that severe AD might be resistant to IL-17 inhibition *in vivo*. Because contrasting roles of IL-17 have been shown in patients with asthma and AD, it will be important to further study whether our model could be applied to the context of all allergic diseases or only to AD.

Finally, our results suggest that a T_H17 response might override TSLP-induced allergic responses. This opens the perspective of developing novel approaches for the treatment of allergy by using T_H17 -related cytokines as therapeutic agents to control TSLP-mediated allergic inflammation.

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Key message

- We demonstrate that T_H17 -related cytokines are negative regulators of the TSLP immune pathway at 2 levels: (1) the production of TSLP by epithelial cells and (2) the TSLP-mediated T_H2 differentiation.

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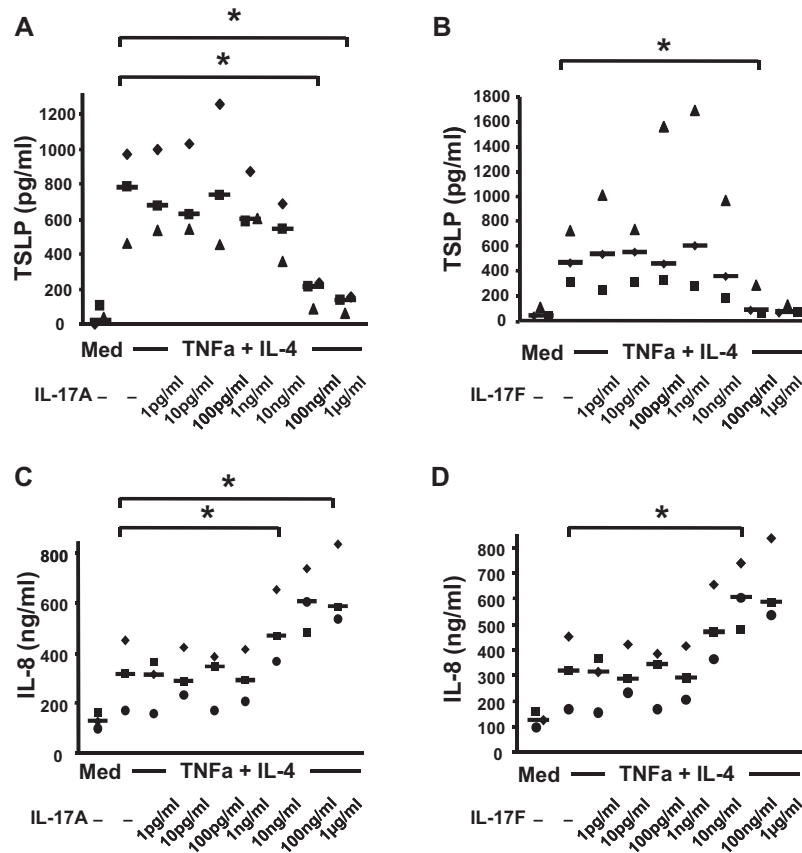


FIG E1. IL-17A and IL-17F dose-dependent inhibition of TSLP in human skin explants. **A-D**, TSLP (Fig E1, A and B) and IL-8 (Fig E1, C and D) quantification by means of protein ELISA. Each symbol represents a different donor assayed in an independent experiment ($n = 3$). Horizontal bars indicate the median. * $P < .05$.

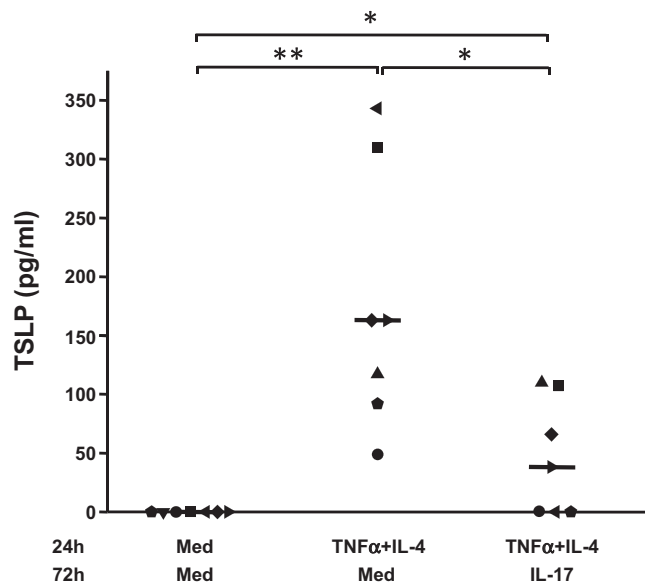


FIG E2. IL-17A inhibits ongoing TSLP production by human skin explants. TSLP quantification by means of protein ELISA is shown. Each symbol represents a different donor assayed in an independent experiment ($n = 7$). Horizontal bars indicate the median. $*P < .05$; $**P < .01$.

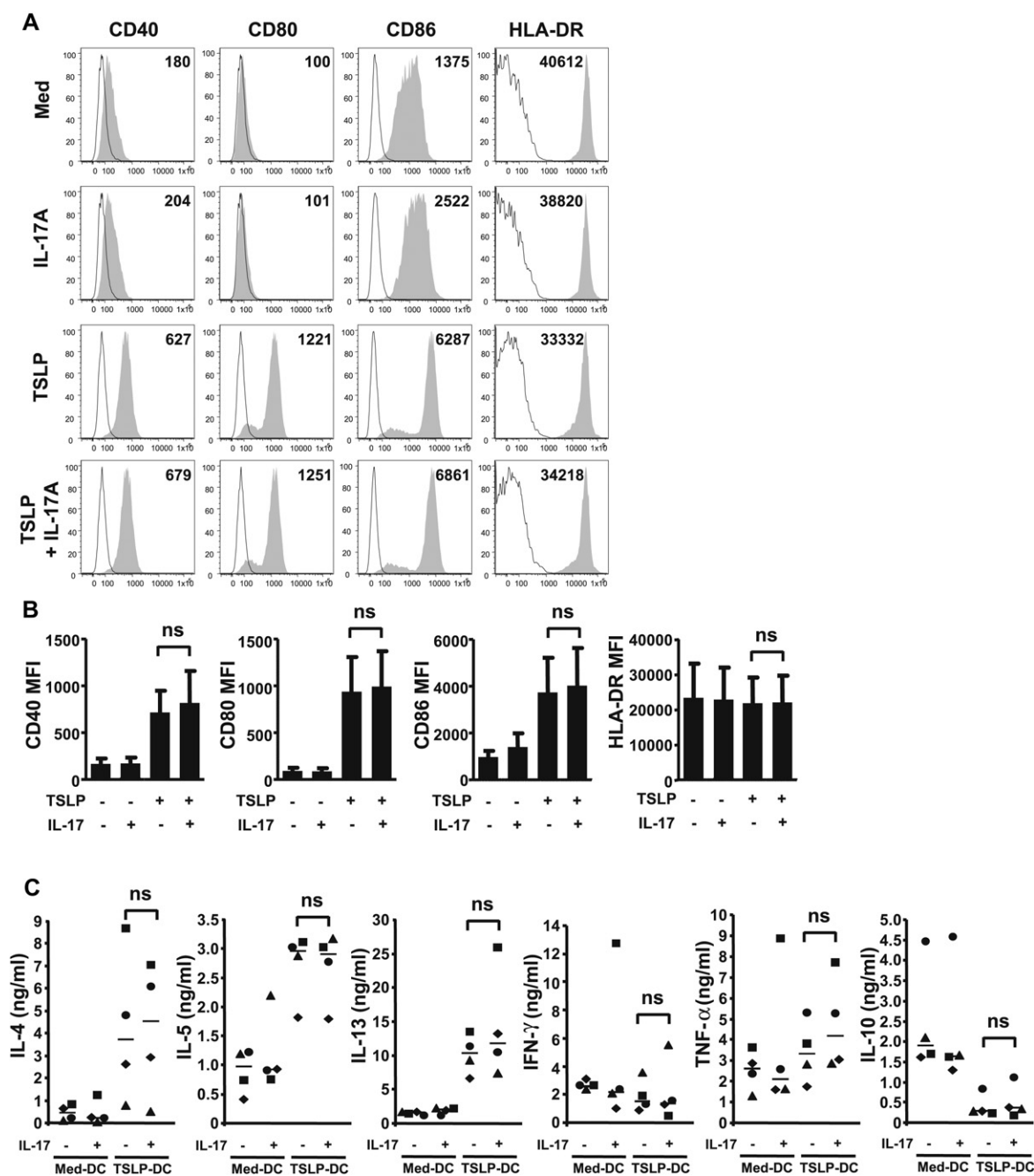


FIG E3. IL-17A does not affect the TSLP-induced DC maturation or the TSLP-DC-induced inflammatory T_H2 response. **A** and **B**, DC activation marker expression from 1 representative of 3 independent experiments. The mean fluorescence intensity (MFI) expression is noted in each corresponding plot (Fig E3, A) and in a histogram (Fig E3, B; MFI $\pm$ SEM). **C**, Cytokine production measured by means of cytometric bead assay ($n = 4$). Horizontal bars indicate the median. ns, $P > .05$.

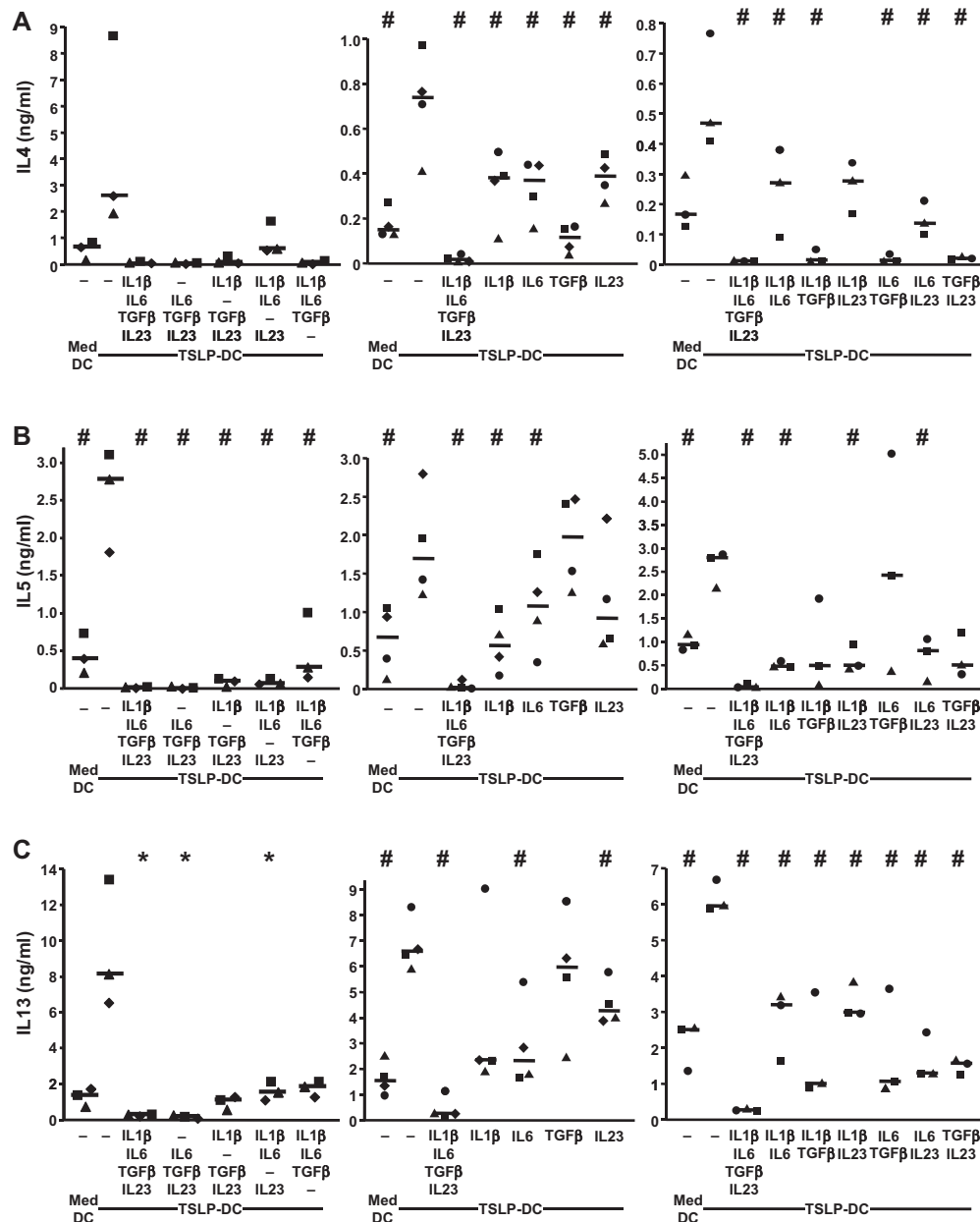


FIG E4. Effect of T_H17 -polarizing cytokines on TSLP-DC-induced inflammatory T_H2 differentiation. Twenty-four-hour TSLP-activated DCs were cocultured for 6 days with naive T cells with or without different combinations of IL-1 β , IL-6, TGF- β , and IL-23. IL-4 (**A**), IL-5 (**B**), and IL-13 (**C**) were quantified by means of cytometric bead assay. Each symbol represents an independent experiment ($n = 4$). Horizontal bars indicate the median. Each condition is compared with the TSLP-DC-Med condition. * $P < .05$; # $P < .05$ and false discovery rate < 0.1 .

TABLE E1. Characteristics of patients with AD

Patient no.	Age (y)	Sex	Body site	Treatment	Severity level	Type of lesions	Total IgE (kUI/L)	Other allergy	TSLP inhibition by IL-17A (%)
1	28	F	Trunk	Phototherapy	Severe	Acute (near patch test)	1684	No	3.0
2	26	M	Trunk	Topical corticoid	Severe	Papulosquamous	6250	Asthma, rhinitis	25.0
3	16	M	Trunk	Topical corticoid	Moderate	Papulosquamous	441	Asthma, rhinitis	80.0
4	23	M	Trunk	Topical corticoid	Moderate	Papulosquamous	2116	Rhinitis	98.5
5	36	F	Arm	Topical corticoid	Moderate	Chronic: lichenification	48	Asthma, rhinitis	53.8
6	17	M	Trunk	Topical corticoid	Severe	Acute (near patch test)	2332	No	2.9
7	32	M	Neck	Topical corticoid	Severe	Chronic: lichenification	315	Asthma	14.3

In patients with moderate AD (boldfaced text), IL-17A inhibits TSLP production.

F, Female; M, male.