

Letter to the Editor

Epicutaneous application of house dust mite induces thymic stromal lymphopoietin in non-lesional skin of patients with atopic dermatitis

To the Editor:

Aeroallergens, including house dust mite (HDM) allergens, have been implicated in the pathogenesis of atopic dermatitis (AD). Epicutaneous application of HDM induces eczema in the nonlesional skin of 40% to 50% of patients with AD in the Atopy Patch Test (APT).¹ Thymic stromal lymphopoietin (TSLP) has also been suggested to play an important role in the pathogenesis of AD. TSLP acts on dendritic cells (DCs) and induces the expression of cell-surface activation markers and the production of chemokines such as CCL17/thymus and activation-regulated chemokine (TARC).² In turn, TSLP-primed DCs skew naive T cells toward a T_H2-type cytokine-producing profile (IL-4, IL-5, IL-13, and TNF).³ TSLP is produced by epithelial cells, such as keratinocytes, on induction by proinflammatory cytokines, microorganisms, and mechanical injury.⁴ Interestingly, the cysteine protease Der p1, which is a major allergen of HDM, has been shown to induce TSLP expression *in vitro* in human bronchial epithelial cells.^{5,6} However, the effect of HDM on TSLP expression in nonlesional AD skin *in vivo* has not been investigated thus far. Here, we demonstrate *in vivo* that the epicutaneous application (APT) of HDM resulted in the induction of TSLP expression in patients with AD.

Ten patients, with mild to moderate AD (mean SCORing Atopic Dermatitis score 15.7 ± 6.6 ; age, 30-67 years; male/female ratio, 7/3), and treated only with topical corticosteroids, were recruited (Table I) and subjected to the APT. All patients were previously sensitized to HDM (determined by ImmunoCAP or ImmunoCAP-ISAC; Phadia AB, Uppsala, Sweden). Six of the 10 patients with AD showed a positive APT result to HDM. One of the patients with a positive APT result was excluded, because the petrolatum-only (solvent control) was also found to be positive. From all patients, four 4-mm skin punch biopsies were

obtained: (1) nonlesional skin biopsy, (2) lesional skin biopsy, (3) skin biopsy at 24 hours, and (4) skin biopsy 48 hours after the initiation of the APT. From patients with a positive APT result, an additional skin biopsy was taken 48 hours after petrolatum-only. The APTs were performed with HDM extract, containing *Dermatophagoides pteronyssinus* allergens Der p1 and 2 (index of reactivity = 200/g) in petrolatum (Stallergènes, Antony, France). APTs were performed on the patients' back. The biopsied skin area had not been exposed to high dosages of ultraviolet light or topical corticosteroids for at least 2 weeks before inclusion in the study. Nonlesional skin biopsies were taken at least 10 cm from lesional or HDM-exposed skin. The study was approved by the institutional review board of University Medical Center Utrecht, and experimental procedures were performed according to the Declaration of Helsinki principles. All patients provided written informed consent.

Total RNA was isolated from biopsy cryosections of patients with a positive APT result ($n = 5$) by using a protocol adapted from the RNeasy micro kit (Qiagen, Valencia, Calif). TSLP and TARC gene expression levels were determined by quantitative real-time PCR by using the following primers: TSLP forward, AGTGGGACCAAAAGTACCGAGTT; TSLP reverse, GGATTGAAGGTTAGGCTCTGG, CCL17/TARC (Hs00171074-m1; Life Technologies, Carlsbad, Calif). Increases in mRNA expression were calculated by using the ΔCt method. The equation $1.8^{(\text{Ct of a set of housekeeping genes} - \text{Ct of the gene of interest})} \times 10,000$ was used to obtain normalized values. Immunohistochemistry for TSLP was performed on skin sections of all patients ($n = 10$), as described previously.³

Four of the 5 patients with a positive APT result showed a positive patch test result after 24 hours, whereas the clinical response of 1 patient was delayed. After 48 hours, all APT results were positive ($n = 5$). Positive APT reactions were characterized by erythema, induration, and papules (and/or vesicles). Patients' control skin test results with petrolatum-only application were negative, except for the excluded patient described above.

TABLE I. Increased expression of TSLP protein in atopy patch tested skin

Symbol	Pt ID	Patient characteristics						TSLP protein expression				
		Age (y)	M/F	SCORAD score	Atopy	Total IgE (kU/L)	HDM specific IgE (CAP) (kU/L)	NL	APT 24 h	APT 48 h	L	C
△	1	67	M	17	AA	>5000	>100	—	+	+/++	+++	—
▽	2	31	M	14.5	AA, AR, FA	>5000	>100	—	+	++/+++	+++	—
◇	3	63	M	31	AR	3460	58 ISU (ISAC)	—	+	+	+++	—
□	4	37	F	10	—	>5000	>100	—	+	++	+++	—
	5	54	F	10	AA, AR, FA	4061	>100	—	—	—	+++	NA
	6	59	F	16	AA, AR, FA	NA	82 ISU (ISAC)	—	—	—	NA	NA
	7	32	F	7.8	AA, AR	119	7.5	—	—	—	+++	NA
	8	41	M	13.5	AR	NA	31.0	—	—	—	+++	NA
	9	59	F	18.5	AA, AR, FA	NA	98 ISU (ISAC)	—	*	*	+++	NA
○	10	30	F	19	AA, AR, FA	NA	>100	—	++	++	+++†	—

TSLP staining intensity was scored as follows: no staining (—), weak (+), intermediate (++), and strong (+++).

AA, Allergic asthma; AR, allergic rhinitis; C, petrolatum-only (solvent control); F, female; FA, food allergy; ISU, ISAC standardized units; L, lesional; M, male; NA, not available; NL, nonlesional; Pt ID, patient number; SCORAD, SCORing Atopic Dermatitis.

*In patient 9, petrolatum-only (solvent control) was macroscopically positive; therefore, the APT was excluded for analysis.

†Biopsy was taken from recently developed eczema lesion.

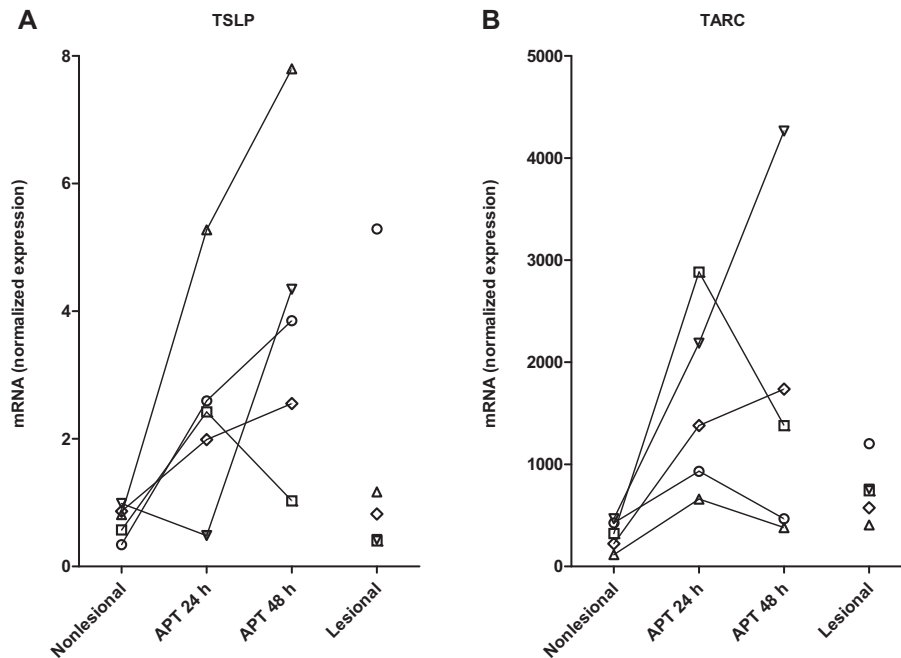


FIG 1. Increased TSLP and TARC expression after epicutaneous application of HDM on nonlesional AD skin. TSLP (**A**) and TARC (**B**) gene expression in nonlesional, lesional, and APT skin ($n = 5$). Symbols represent patients summarized in [Table I](#). **C**, TSLP immunohistochemistry in nonlesional (–) skin, lesional (+++) skin, and 24-hour APT skin (+), and 48-hour APT skin (++) with HDM. Scale bars represent 100 μm .

TSLP gene expression was increased at 24 hours in 4 of the 5 patients (median, 2.4-fold; range, 2.0–5.3-fold change) following HDM application ([Fig 1, A](#)). All patients showed elevated TSLP gene expression at 48 hours following the APT compared with nonlesional skin.

Strong TSLP gene expression was found in the lesional skin biopsy of only 1 patient. Interestingly, the biopsy from this patient was taken from a recently developed (<1-week-old) eczema lesion. The other 4 patients' lesional skin biopsies were taken from more chronic lesions and did not show increased gene

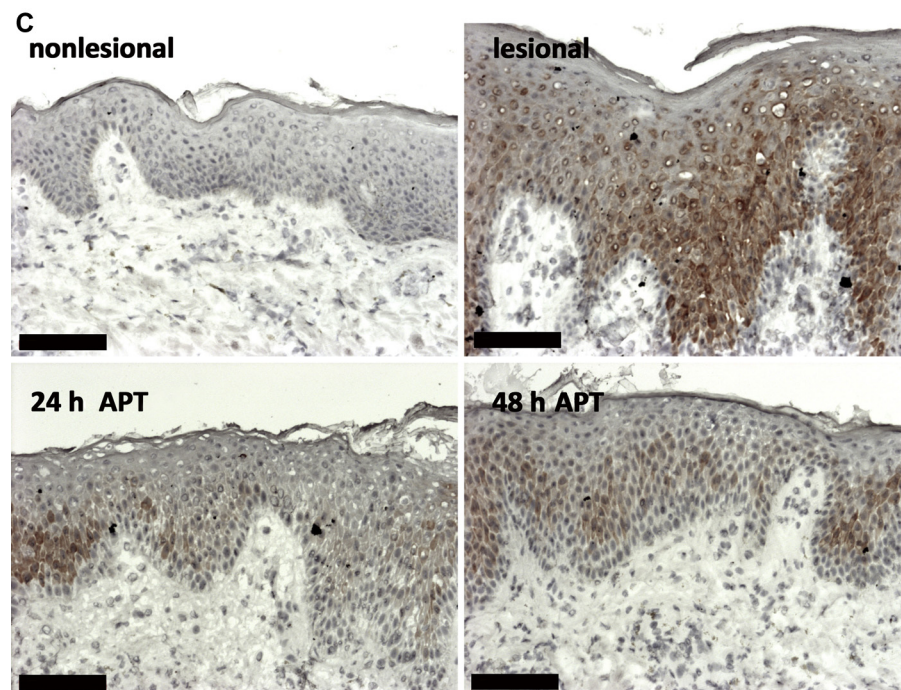


FIG 1. (Continued)

expression for TSLP. This may suggest that TSLP gene transcription occurs in the initial phases of eczema development. However, by using immunohistochemistry, we found increased TSLP protein expression in both the acute and chronic lesional skin of all the patients studied. It is possible that HDM induced TSLP expression through protease-activated receptor-2, which is known to play a role in TSLP induction in epithelial cells.⁶ Protease-activated receptor-2 mRNA was expressed in nonlesional skin but did not change following the APT (data not shown).

Immunohistochemical staining for TSLP was positive and patchy in the suprabasal layers of lesional skin epidermis (Fig 1, C), as shown previously.³ Staining for TSLP was negative in all patients' nonlesional skin biopsies. Interestingly, the APT biopsies of all patients with positive APT results showed staining for TSLP in a focal and patchy pattern in the suprabasal layers. The TSLP staining was more intense and tended to include a larger part of the suprabasal epidermis after 48 hours than after 24 hours (Fig 1, C). The temporal expression pattern following the APT suggests that because of the compromised epithelial barrier function in the skin of patients with AD, HDM could reach the suprabasal layer, which seems more conducive to TSLP induction than the apical layers of the skin. Thus, TSLP staining in APT biopsies was comparable to that in lesional skin with regard to location and pattern. No HDM-specific induction of TSLP was detected in APT biopsies of patients with a negative or nonspecific APT result.

We have previously demonstrated that the expression of CCL17/TARC mRNA is increased in AD lesional skin.⁷ In addition, CCL17/TARC serum levels are elevated in patients with AD and were shown to correlate with disease activity.^{7,8} Because TSLP induces CCL17/TARC in DCs,^{2,3,9} we examined CCL17/TARC expression in HDM patch tested skin as a potential indication of TSLP bioactivity. CCL17/TARC mRNA expression was increased in all patients with a positive APT reaction ($n = 5$) after 24 and 48 hours compared to nonlesional skin (Fig 1, B). Taken together, these results show that epicutaneous application of HDM using the APT results in the induction of TSLP and CCL17/TARC.

In conclusion, we showed that epicutaneous application of HDM resulted in the induction of TSLP and CCL17/TARC gene expression in nonlesional skin of patients with AD with a positive APT result. The expression of TSLP protein by keratinocytes in APT biopsies was comparable to that in lesional AD skin with respect to location and pattern. Our results suggest that TSLP plays a role in the induction of eczema by HDM in patients with AD.

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