

Report

Medium-dose ultraviolet A1 phototherapy and mRNA expression of TSLP, TARC, IL-5, and IL-13 in acute skin lesions in atopic dermatitis

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Abstract

Background The mechanisms responsible for the efficacy of ultraviolet A1 (UVA1) in the treatment of atopic dermatitis (AD) are not fully understood.

Objectives This study was designed to investigate mRNA expression of thymic stromal lymphopoietin (TSLP), thymus- and activation-regulated chemokine (TARC), interleukin-5 (IL-5), and IL-13 in AD before and after UVA1 therapy, to determine correlations among them, and to examine whether UVA1 influences their expression and whether it is associated with UVA1 efficacy.

Methods Twenty-five patients with AD underwent medium-dose UVA1 phototherapy. Before and after UVA1, biopsies from acute skin lesions were studied using reverse transcription and real-time polymerase chain reaction.

Results Levels of mRNA TSLP correlated with those of TARC, IL-5, and IL-13, and levels of TARC correlated with those of IL-5 and IL-13, both before and after UVA1. Expression of IL-5 correlated with that of IL-13 only before UVA1. SCORAD (SCORing of Atopic Dermatitis) indices correlated with levels of TARC and IL-5 before irradiation. After UVA1, no mRNA level correlated with the SCORAD index. Phototherapy with UVA1 improved SCORAD values ($P < 0.001$) and increased expression of TARC ($P < 0.05$) but did not affect mRNA expression of TSLP, IL-5, or IL-13.

Conclusions Expression levels of the mediators TSLP, TARC, IL-5, and IL-13 in AD are interrelated. Phototherapy with UVA1 improves SCORAD indices and increases expression of TARC but has no direct effects on the expression of other molecules. It is likely that UVA1 also interferes with or acts via intermediators on the link between IL-5 and IL-13.

Introduction

Together with progress in the immunology of atopic dermatitis (AD), the development of ultraviolet A (UVA; 320–400 nm) generators by Plewig *et al.*¹ and Mützhas *et al.*² in the early 1980s facilitated not only the photo-testing and diagnosing of photodermatoses but also the treatment of skin disorders. Ultraviolet A2 radiation (320–340 nm) displays biological effects similar to those of UVB (290–320 nm), whereas the effects of UVA1 (340–400 nm) are more similar to those of visible light (400–700 nm).³ According to a recent meta-analysis, phototherapy with medium-dose UVA1, if available, is recommended to control exacerbations of AD, whereas UVB, specifically narrowband UVB (nb-UVB), should be used in the management of the chronic stage of the disease.⁴ However, not all the mechanisms responsible for the efficacy of UVA1 in the treatment of AD are well

understood. Initially, Krutmann *et al.* revealed that high-dose UVA1 (130 J/cm²) reduced elevated serum levels of eosinophil cationic protein in 15 patients with AD.⁵ High-dose UVA1 also reduced the relative number of immunoglobulin E (IgE)-positive intraepidermal Langerhans cells, whereas medium-dose UVA1 irradiation resulted in a decrease of dermal Langerhans cells, activated eosinophils, and CD4 cell count, as well as a relative increase in CD8+ lymphocytes in 15 patients with AD.^{6,7} Phototherapy with UVA1 was also found to be associated with a subsequent reduction in mRNA expression of interleukin-5 (IL-5), IL-13, and IL-31 in a study of 21 patients with AD.⁸ However, few studies have addressed the influence of UVA1 on other pathogenic pathways in lesional skin in AD. Thymic stromal lymphopoietin (TSLP) was found to be expressed by keratinocytes in AD and also to induce production of thymus- and activation-regulated chemokine (TARC), IL-5, and

IL-13.⁹ In addition, the combined action of proinflammatory cytokines (tumor necrosis factor- α [TNF- α] or IL-1 α) and Th2 cytokines (IL-4 and IL-13) on keratinocytes was found to produce TSLP.¹⁰ Acute and chronic skin lesions and unaffected skin from patients with AD featured increased numbers of cells found to be positive for IL-13 mRNA.¹¹ Increased production of IL-5 and IL-13 was also revealed in peripheral blood mononuclear cells of AD patients.¹² Interleukin-13 can cause dermal infiltration of CD4⁺ T cells, mast cells, eosinophils, macrophages, and Langerhans cells, as well as upregulation of TSLP.¹³ Thus, via feedback, a vicious circle seems to be completed.

Therefore, the aim of the present study was to investigate whether levels of mRNA expression of TSLP correlate with those of TARC, IL-5, and IL-13 in specimens of acute skin lesions from patients with AD before and after UVA1 therapy, to determine whether UVA1 decreases the expression of these mediators, and to confirm whether this expression corresponds with the clinical efficacy of UVA1.

Materials and methods

Protocol

The study included 25 patients with AD (14 men and 11 women) with a mean $\pm$ standard deviation (SD) age of 32.4 ± 11.5 years (range: 18–61 years). Diagnoses of AD were based on the classification of Hanifin and Rajka.¹⁴ In the clinical assessment of AD, to help minimize bias, all SCORAD (SCORing of Atopic Dermatitis) indices were evaluated by a single person (KM) who was unaware of the results of the molecular studies.¹⁵ Patient characteristics are shown in Table 1.

Exclusion criteria denied the participation of individuals who were pregnant, had glaucoma, cataract, claustrophobia, photosensitivity, or solar urticaria, or had used phototoxic or photoallergic drugs, immunosuppressants, retinoids, glucocorticoids, or sunbeds or other artificial sources of UV irradiation during the 6 months prior to the start of the study or

during it. The study was conducted in accordance with the Declaration of Helsinki of 1975, as revised in 1983, and was approved by the local ethics committee (RNN/16/14/KE).

Each patient's SCORAD index was evaluated on the day before phototherapy and before skin biopsy, and for a second time after the last UVA1 irradiation but before the second skin biopsy. The protocol for medium-dose UVA1 phototherapy, delivered using the Medisun 24000 (Schulze & Böhm GmbH, Brühl, Germany), comprised one exposure daily, on 20 weekdays (Mondays, Tuesdays, Wednesdays, Thursdays, Fridays).^{16,17} The starting dose on day 1 was 10 J/cm², which was followed by doses of 20, 30, and 45 J/cm², respectively, on the subsequent 3 days. The dose of 45 J/cm² was continued for the remainder of the treatment. Topical management was limited to the use of emollients. No systemic drugs for AD were administered. Biopsies were taken from acute skin lesions, which were clinically determined to be acute when subscores for erythema, edema, and oozing added to >1 point on the SCORAD index. The first biopsy was taken before phototherapy and the second within 2 hours after the last UVA1 irradiation. Skin samples were frozen at -70°C until assay.

Total RNA extraction

Total RNA was extracted from biopsied skin samples using RNeasy Mini Kits (Qiagen GmbH, Hilden, Germany), according to the manufacturer's protocol. In the initial step, a buffer containing β -mercaptoethanol was added to Eppendorf tubes containing frozen samples. The samples were then homogenized using a Qiagen homogenizer (TissueRuptor; Qiagen GmbH) and centrifuged for 3 minutes at 14,000 rpm. RNA was quantified using a Picodrop spectrophotometer (Picodrop Ltd, Hinxton, UK). The quality of RNA samples was analyzed by measuring the ratio of absorptions at 260 nm vs. 280 nm. The purified total RNA was either used immediately for cDNA synthesis or stored at -80°C .

Reverse transcription

Generation of cDNA was performed with High Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Inc., Foster City, CA, USA), according to the reverse transcription protocols given by the manufacturer. Briefly, 2 μg DNase-treated total RNA was used as starting material, to which was added 2 $\times$ RT master mix containing 2 μl of 10 $\times$ RT Buffer, 0.8 μl of 25 $\times$ dNTP Mix (100 mM), 2 μl of 10 $\times$ RT random primers, 1 μl MultiScribe™ reverse transcriptase, and 1 μl RNase inhibitor per 20- μl reaction. Reverse transcription was performed in conditions optimized for use with this kit (25 $^{\circ}\text{C}$ for 10 min, 37 $^{\circ}\text{C}$ for 120 min, 85 $^{\circ}\text{C}$ for 5 min). The samples were stored at -20°C .

mRNA expression

Quantification of mRNA expression of the selected genes and β -actin (*ACTB*) as an endogenous control was performed using

Table 1 Characteristics of patients with atopic dermatitis

Characteristic	
Age, years, mean $\pm$ SD	32.4 ± 11.5
Gender, female/male, <i>n</i>	11/14
SCORAD index, mean $\pm$ SD	60.16 ± 14.71
Site of skin biopsy	Acute skin lesions for which subscores for erythema, edema and oozing add to >1 point on the SCORAD index

SD, standard deviation.

standard TaqMan® Gene Expression Assays (Applied Biosystems, Inc., Carlsbad, CA, USA). Hs00263639_m1, Hs00171074_m1, Hs00174200_m1, and Hs00174379_m1 were used to assess mRNA expression of TSLP, TARC, IL-5, and IL-13, respectively; Hs99999903_m1 was used for the endogenous control. Real-time TaqMan quantitative polymerase chain reaction (qPCR) was performed in 96-well optical plates using the HT 7900 Fast Real-Time PCR System (Applied Biosystems, Inc.) and analyzed using Sequence Detection System Version 2.3 (Applied Biosystems, Inc.). The results for target genes were displayed by ΔC_t to represent the differences in cycle threshold numbers between the target gene and endogenous control.

Statistical analysis

Statistical analysis was performed using Statistica Version 10.0 (Statsoft Polska Sp. zoo, Krakow, Poland). Data estimation with the Shapiro–Wilk test did not confirm a Gaussian distribution for studied parameters. Therefore, non-parametric tests were used. Kendall tau correlation coefficients were estimated to find correlations. The Wilcoxon signed-rank test was used to compare two repeated measurements in a sample of AD patients before and after UVA1 phototherapy. In all calculations, a P -value of <0.05 was considered to indicate statistical significance.

Results

Correlations before UVA1 irradiation

Before UVA1 phototherapy, mRNA levels of TSLP correlated with those of TARC ($\tau = 0.28$, $P < 0.05$) and IL-13 ($\tau = 0.34$, $P < 0.05$) (Fig. 1a,b).

In addition, mRNA levels of TARC correlated with those of IL-5 ($\tau = 0.60$, $P < 0.05$) and IL-13 ($\tau = 0.44$, $P < 0.05$) (Fig. 2a,b).

Prior to UVA1, mRNA expression of IL-5 correlated with that of IL-13 ($\tau = 0.48$, $P < 0.05$) (Fig. 3a).

Correlations after UVA1 irradiation

After UVA1 phototherapy, mRNA levels of TSLP continued to correlate with those of TARC ($\tau = 0.44$, $P < 0.05$), IL-5 ($\tau = 0.34$, $P < 0.05$), and IL-13 ($\tau = 0.34$, $P < 0.05$) (Fig. 4a–c).

In addition, mRNA expression of TARC correlated, as prior to UVA1 therapy, with that of IL-5 ($\tau = 0.36$, $P < 0.05$) and IL-13 ($\tau = 0.54$, $P < 0.05$) (Fig. 5a,b).

By contrast, mRNA expression of IL-13 did not correlate with that of IL-5 after UVA1 irradiation (Fig. 3b).

Impact of UVA1 on mRNA expression

Treatment with UVA1 phototherapy resulted in a significant increase in mean $\pm$ SD mRNA expression of TARC in comparison with baseline values (before and after

phototherapy, respectively: 5.38 ± 2.80 and 6.46 ± 2.73 ; $P < 0.05$) (Fig. 6).

After UVA1 phototherapy, no significant difference was found in mean $\pm$ SD mRNA expression of TSLP (before and after phototherapy, respectively: 7.20 ± 1.64 and 7.25 ± 1.42), IL-5 (before and after phototherapy, respectively: 14.40 ± 2.77 and 14.92 ± 2.36), or IL-13 (before and after phototherapy, respectively: 9.73 ± 2.27 and 10.54 ± 2.76).

SCORAD index and UVA1 irradiation

The administration of UVA1 phototherapy significantly improved the mean $\pm$ SD SCORAD index from 60.16 ± 14.71 before treatment to 30.71 ± 14.41 after treatment ($P < 0.001$) (Fig. 7).

Before UVA1 phototherapy, SCORAD index was correlated with mRNA expression of TARC ($\tau = 0.35$, $P < 0.05$) and IL-5 ($\tau = 0.46$, $P < 0.05$). After UVA1 phototherapy, no correlation was found between the SCORAD index and mRNA expression of any of the cytokines.

Discussion

The mediator TSLP is a glycoprotein produced by divergent cell types, especially damaged epithelial cells, including keratinocytes from patients with AD.^{9,18} This molecule activates both primary afferent neurons and immune cells to promote inflammatory responses in the skin and to induce itching.^{9,18} TSLP produced in the skin stimulates Langerhans cells, namely immature dendritic cells, which then migrate to regional lymph nodes and express TARC.⁹ This attracts naive CD4⁺ lymphocytes, and their interaction with Langerhans cells switches the Th2 phenotype.⁹ In addition, activated human CD4⁺ T cells have been found to display TSLP receptors, and TSLP-induced lymphocytes are known to produce IL-5 and IL-13.^{9,19}

Another molecule engaged in AD is TARC. This protein is a chemokine that binds to T cells and induces chemotaxis, thus playing a role in the trafficking and activation of mature T cells.²⁰ Serum levels of TARC are known to be significantly higher in patients with AD than in healthy people.^{21,22} Variations in pruritus have been found to correlate positively with before and after ratios of serum TARC levels in patients with AD.²³ However, data on its mRNA expression in skin specimens are sparse. In the present study, UVA1 phototherapy resulted in both a significant increase in mRNA expression of TARC and an improvement in SCORAD index in comparison with the baseline assessment. Whether increased expression of TARC may have some beneficial effect in the treatment of AD remains speculative because the lack

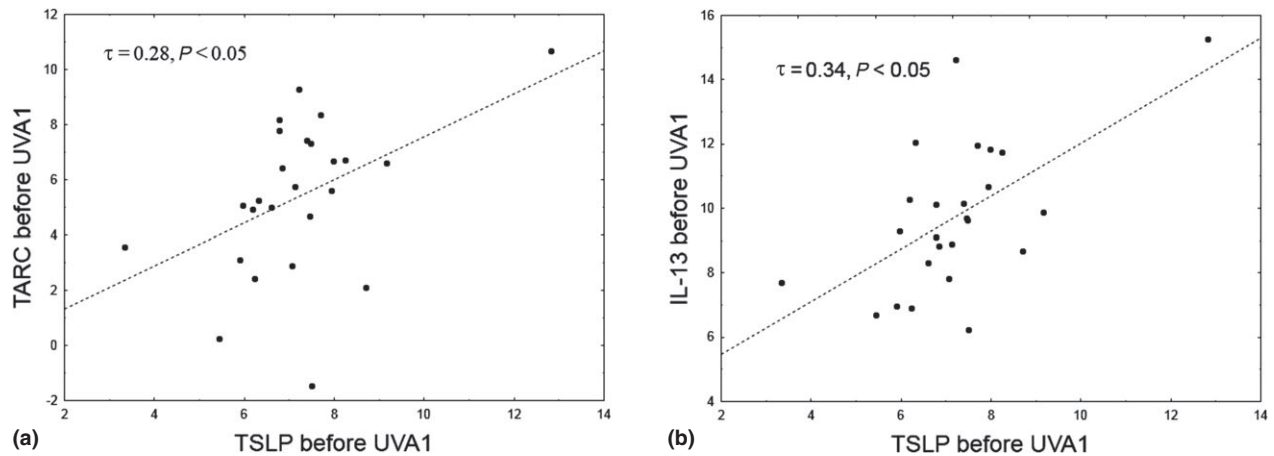


Figure 1 Correlations between mRNA levels of (a) TSLP and TARC, and (b) TSLP and interleukin-13 (IL-13) in acute skin lesions of patients with atopic dermatitis before ultraviolet A1 (UVA1) phototherapy

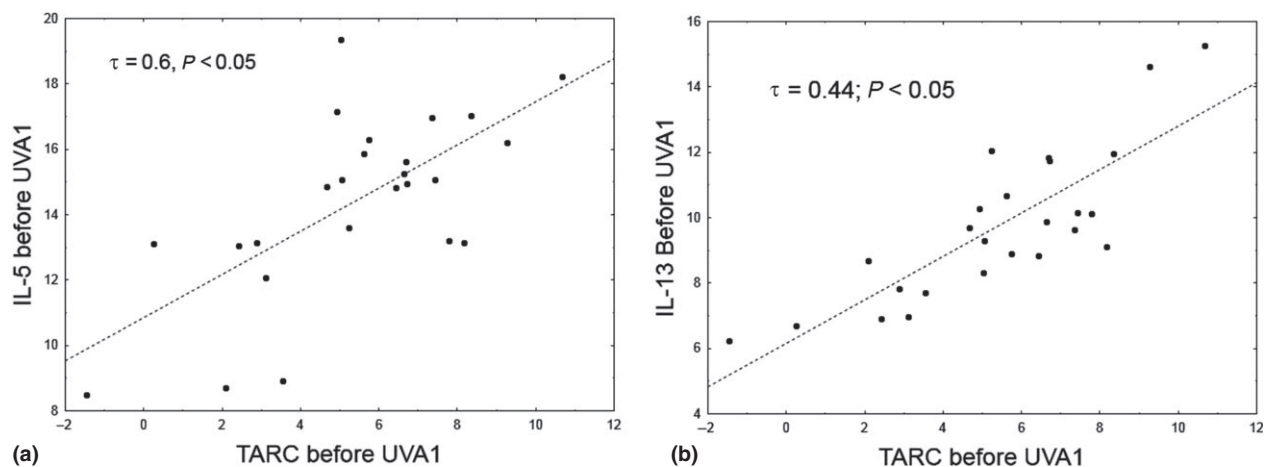


Figure 2 Correlations between mRNA levels of (a) TARC and interleukin-5 (IL-5), and (b) TARC and IL-13 in acute skin lesions of patients with atopic dermatitis before ultraviolet A1 (UVA1) phototherapy

of staining in our study limits our ability to evaluate where and how the inflammatory markers are expressed in the tissue. It is likely that the efficacy of UVA1 is based on its activity with respect to the decreased expression of molecules other than TARC and different effects on the expression of cytokines and chemokines in different cell types in the epidermis and dermis in AD. As expected, mRNA levels of TARC before UVA1 also correlated with those of IL-5 and IL-13. In this pathogenic pathway, IL-5 is a glycoprotein produced primarily by Th2 cells, activated eosinophils, and mast cells, that promotes maturation of eosinophil precursors from both inside and outside the bone marrow.^{24,25} Interleukin-5 has also been found to release mediators by basophils stimulated by major basic protein.²⁶

Our study confirms the expression of TSLP, TARC, IL-5, and IL-13 in the acute skin lesions of patients with AD. The second important finding was that levels of mRNA expression of TSLP correlated with those of TARC, IL-5, and IL-13. In addition, before UVA1 phototherapy, mRNA expression of TARC correlated with expression of IL-5 and IL-13. Almost 20 years ago, Hamid *et al.* noted significantly greater numbers of cells with mRNA expression of IL-13 in acute and chronic skin lesions and unaffected skin from patients with AD compared with normal control skin.¹¹ More recently, Gambichler *et al.*⁸ reported reductions in IL-5, IL-13, and IL-31 mRNA expression after UVA1 phototherapy. However, the main finding of our study is that UVA1 phototherapy does not decrease expression of TSLP, IL-13, or

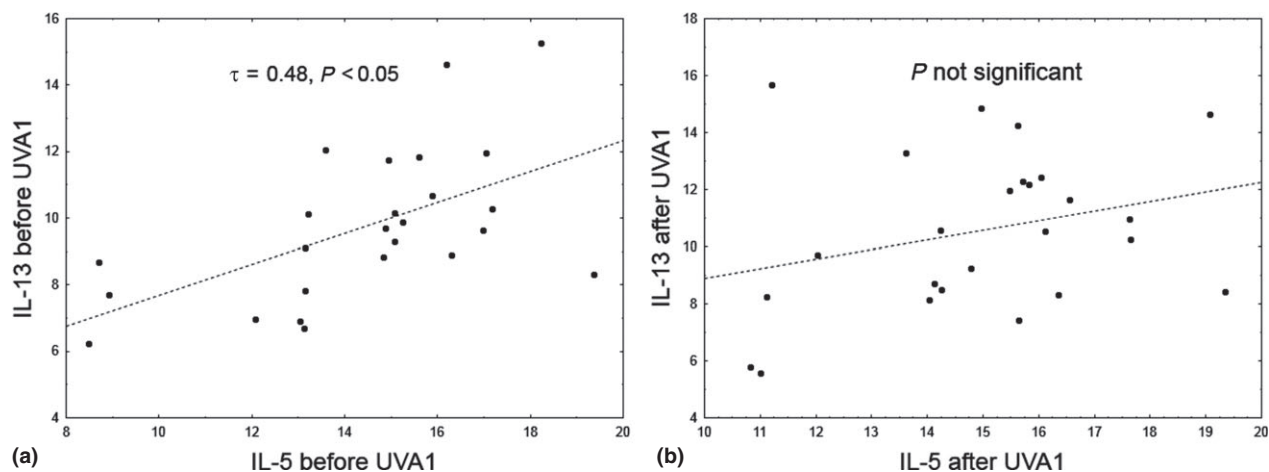


Figure 3 Correlations between mRNA levels of interleukin-5 (IL-5) and IL-13 in acute skin lesions of patients with atopic dermatitis (a) before and (b) after ultraviolet A₁ (UVA₁) phototherapy

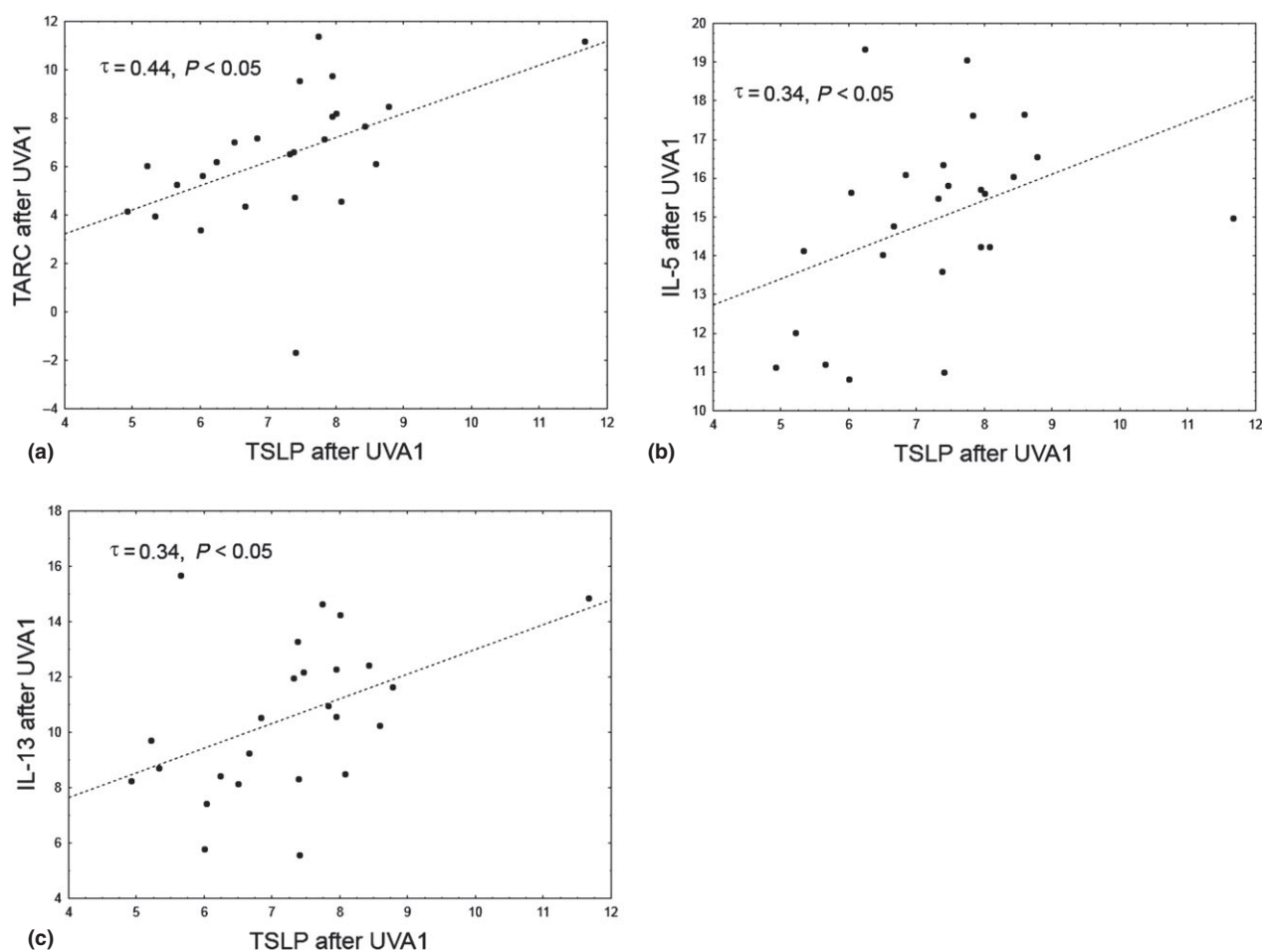


Figure 4 Correlations between mRNA levels of (a) TSLP and TARC, (b) TSLP and interleukin-5 (IL-5), and (c) TSLP and IL-13 in acute skin lesions of patients with atopic dermatitis after ultraviolet A₁ (UVA₁) phototherapy

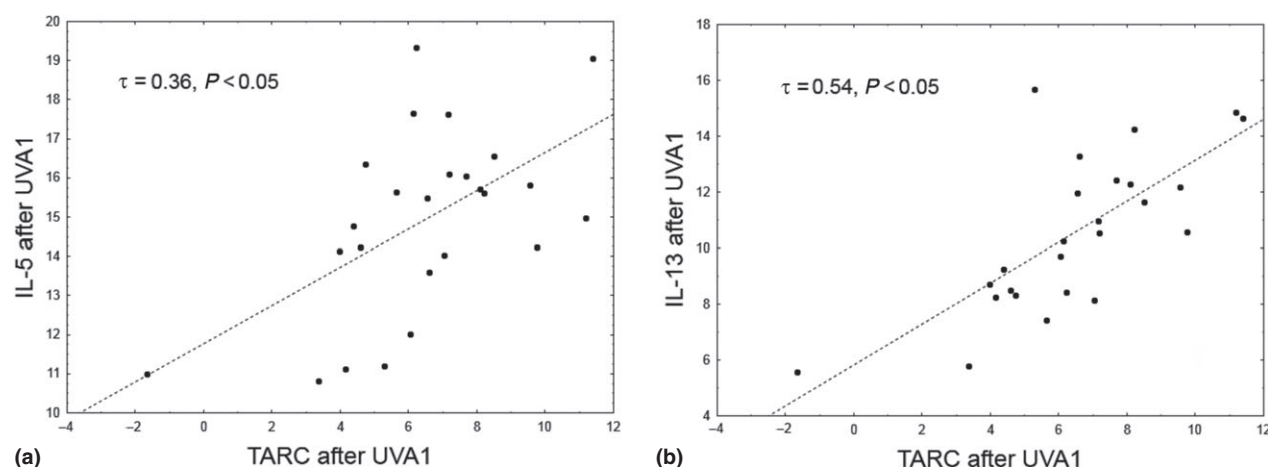


Figure 5 Correlations between mRNA levels of (a) TARC and interleukin-5 (IL-5), and (b) TARC and IL-13 in acute skin lesions of patients with atopic dermatitis after ultraviolet A₁ (UVA₁) phototherapy

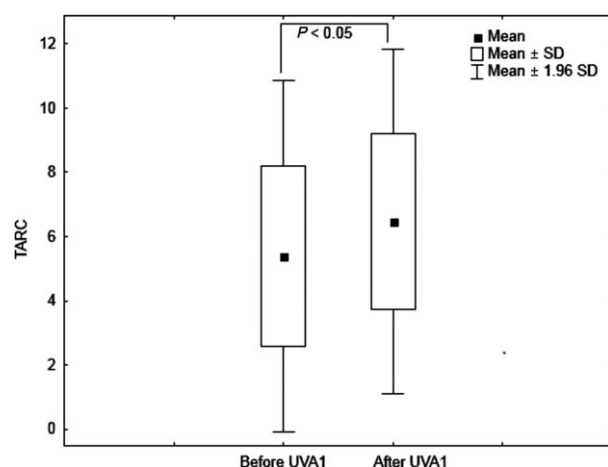


Figure 6 Ultraviolet A₁ (UVA₁) phototherapy induces a significant increase in mRNA expression of TARC in acute skin lesions of atopic dermatitis patients. Expression of TARC is displayed in relative units that represent the differences in cycle threshold numbers between the TARC gene and β -actin (*ACTB*) as an endogenous control. SD, standard deviation

IL-5. Surprisingly, the results of the present study identify a significant increase in mRNA expression of TARC after UVA₁ irradiation. In addition, after UVA₁ exposure, mRNA expression of TSLP continued to correlate with expression of TARC and IL-13 and correlated for the first time with expression of IL-5. Levels of mRNA expression of TARC correlated with those of IL-5 and IL-13 in the same way as they did before UVA₁ exposure.

As expected, UVA₁ significantly improved the clinical outcomes of AD and was found to be associated with a decrease in SCORAD index. Therefore, it is likely that the efficacy of UVA₁ is attributable to its action on the expres-

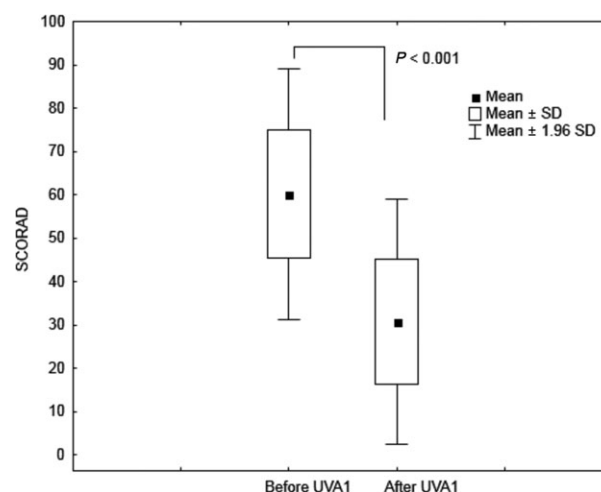


Figure 7 SCORAD indices in patients with atopic dermatitis show a significant decrease after ultraviolet A₁ (UVA₁) phototherapy. SD, standard deviation

sion of molecules other than TSLP, IL-5, and IL-13. Further, the indirect effects of UVA₁ on the studied molecules, via intermediators, should be taken into consideration when interpreting these results. Undoubtedly, TSLP, TARC, IL-5, and IL-13 are expressed in lesional skin, but not all aspects of their roles in the pathogenesis of AD are fully understood.

Interestingly, the results of the present study also reveal a positive correlation in expression levels of IL-5 and IL-13 before UVA₁ phototherapy. Interleukin-13 is an immunoregulatory cytokine for which cDNA was isolated 20 years ago.²⁷ It promotes immunoglobulin class switching to IgE by B cells, thus playing a role in eosinophilic inflammation.²⁸ However, it should be

emphasized that after UVA1 phototherapy, mRNA expression of IL-5 did not correlate with that of IL-13. It is also noteworthy that although SCORAD index values were found to correlate with mRNA expression of TARC and IL-5 before UVA1 phototherapy, no such correlations between SCORAD index and mRNA expression were seen after UVA1. Therefore, it may be inferred that UVA1 may interfere with or act via intermediators on the link between IL-5 and IL-13. In the discussion of possible similarities between the respective effects of UVA1 and UVB treatments, it should be stressed that nb-UVB was found to suppress production of TARC by human keratinocytes, whereas UVB irradiation induced expression of TSLP.^{29,30} With regard to IL-5 and IL-13, production of IL-13 was unaffected in cord blood-derived human mast cells treated with varying doses of UVB, whereas increased levels of IL-5 were found in spleen lymphocytes of mice treated with UVB.^{31,32}

Our study has several limitations. Firstly, only a small group of patients with AD were examined. In addition, only skin biopsies obtained from acute skin lesions were investigated. It would have been of great interest to compare mRNA expression levels in affected skin with those in unaffected skin, chronic skin lesions, and in skin samples from healthy control subjects. Evaluation of the selected molecules at the protein level would also give valuable insight into their properties. However, our results did not indicate any one cell type in the skin as a cellular source of the molecules studied. The skin biopsies contained a heterogeneous population of multiple different cell types. Unfortunately, immunochemistry of proteins was not performed because it was not included in the study protocol; this represents another limitation of the study. Further, an analysis of any gene polymorphisms present in these molecules may well provide additional data on susceptibility for the development of AD. However, such an analysis will have to await future studies. Our intention was to confirm whether TSLP, TARC, IL-5, and IL-13 are expressed and whether their levels of expression correlate with one another in the lesional skin of patients with AD. Another key conclusion is that UVA1 significantly improves SCORAD index and increases expression of TARC without having direct effects on the other molecules studied. However, it is likely that UVA1 can influence the link between IL-5 and IL-13. Undoubtedly, as the results of RT-PCR are comparable and reproducible, further studies conducted in larger populations of AD patients from different sites are required. This reproducibility may allow us to draw conclusions from a more comprehensive evidence base in the future.

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