

Effect of Acupuncture on Allergen-Induced Basophil Activation in Patients with Atopic Eczema: A Pilot Trial

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Abstract

Objective and Methods: The crucial symptom of atopic eczema is itch. Acupuncture has been shown to exhibit a significant effect on experimental itch; however, studies focusing on clinical itch in atopic eczema and corresponding mechanisms are lacking. The study design was a unicenter, single-blinded (observer), prospective, randomized clinical pilot trial with an additional experimental part. In 10 patients with atopic eczema, we investigated the effect of acupuncture treatment ($n = 5$) compared to no treatment ($n = 5$) on itch intensity and *in vitro* basophil CD63 expression upon allergen stimulation (house dust mite and timothy grass pollen) in a pilot trial.

Results: Mean itch intensity in a visual analog scale was rated significantly lower in the acupuncture group ($-25\% \pm 26\%$ [day 15–day 0]; $-24\% \pm 31\%$ [day 33–day 0]) than in the control group ($15\% \pm 6\%$ [day 15–day 0]; $29\% \pm 9\%$ [day 33–day 0]). From day 0 (before treatment) to day 15 (after 5 acupuncture treatments) as well as day 33 (after 10 acupuncture treatments), the acupuncture group showed less CD63 positive basophils than the control group regarding stimulation with house dust mite and grass pollen allergen at various concentrations (5 ng/mL, 1 ng/mL, 0.5 ng/mL, or 0.25 ng/mL).

Conclusions: Our results show a reduction of itch intensity and of *in vitro* allergen-induced basophil activation in patients with atopic eczema after acupuncture treatment. Reducing basophil activation can be a further tool in investigating the mechanisms of action of acupuncture in immunoglobulin E-mediated allergy. Due to the limited number of patients included in our pilot trial, further studies are needed to strengthen the hypothesis.

Introduction

ACUPUNCTURE IS AN ANCIENT TECHNIQUE that is widely used in the treatment of pain, but also as complementary therapy in various allergic diseases.¹ The most prevalent subjective symptom of inflammatory skin diseases is itch.^{2,3} Acupuncture has been shown to exhibit a significant effect on experimental itch in healthy volunteers and patients with atopic eczema,^{4–6} as well as clinical itch in patients suffering from allergic rhinitis⁷ or uremic pruritus.⁸ However, studies focus-

ing on the mechanisms of acupuncture in the pathophysiology of itch are lacking. Basophils play a part in the allergic response as they have immunoglobulin E (IgE) on their surface, and release chemical mediators causing allergic symptoms.⁹ Upon challenge with specific allergens that cross-link membrane-bound IgE antibodies, basophils upregulate the expression of different activation markers such as cluster of differentiation (CD)63.^{10,11} The aim of our study was to evaluate possible effects of acupuncture on *in vitro* basophil activation in patients suffering from chronic itch with atopic eczema in a pilot trial.

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Materials and Methods

Trial design

The study design was a unicenter, single-blinded (observer), prospective, randomized clinical pilot trial with an additional experimental part. Acupuncturist and observer were different individuals.

Subjects

Ten (10) patients with atopic eczema (8 male, 2 female; mean age: 25.2 ± 4.5 years) with a history of atopic eczema >10 years and disease severity of SCORAD (Scoring Atopic Dermatitis score) greater than 20 as well as allergic rhinitis with sensitization to *Phleum pratense* and *Dermatophagoides pteronyssinus* were included in the study (Table 1). Patients with intake of drugs or topical treatment with potentially systemically active topical immunosuppressive agents were excluded or asked to stop medication 10 days prior to the study and during the whole study period in order to avoid possible side-effects or treatment-related changes. The study was approved by the local ethics committee of the Technische Universität München and conducted according to Declaration of Helsinki principles; participants had given their written informed consent prior to inclusion in the study.

Interventions

After block randomization, the following procedures were carried out before treatment (day 0), after 15 days (5 acupuncture treatments, respectively), and after 33 days (10 acupuncture treatments, respectively). Acupuncture treatments were carried out twice a week by an experienced acupuncturist. Individual acupuncture points were chosen, however, always including the following acupuncture points: *Quchi* (LI 11), *Hegu* (LI 4), *ZuSanLi* (St 36), and *Xuehai* (Sp 10). Stainless steel needles (0.25×40 mm) were inserted 2–3 cm for a period of 20 minutes. Patients in the control group received no acupuncture intervention, and showed up only for study examination visits.

Patients were advised to continue their topical therapy as before the study (potentially systemically active agents were not allowed; topical class I and II corticosteroids were allowed).

Outcome parameters

The severity of atopic eczema was evaluated by SCORAD (range 0–103). It is a clinical tool developed by the European Task Force on Atopic Dermatitis for assessing the extent, severity, and subjective symptoms of atopic dermatitis.¹² Itch was rated on a visual analog scale (VAS) between 0 and 100, and blood samples (36 mL) were drawn to measure basophil activation (at least 1 hour after a meal).

Basophil activation test (BAT)

The FLOW-CAST Basophil Activation Test® (Bühlmann Laboratories AG, Schönenbuch, Switzerland) was used for the quantitative determination of *in vitro* basophil activation.¹³ Fifty microliters (50 µL) heparinized whole blood was first incubated with 20 µL stimulation buffer for 10 minutes at 37°C and then with 50 µL of allergen extract solution (*D. pteronyssinus* or *P. pratense*, Bühlmann Laboratories AG,®

TABLE 1. CLINICAL AND IMMUNOLOGIC CHARACTERISTICS OF PATIENTS WITH ATOPIC ECZEMA BEFORE ENROLLMENT IN THE STUDY

	Patient										p-Value
	1	2	3	4	5	6	7	8	9	10	
	Acupuncture group					Control group					Acup vs. con
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Age (years)	29	29	31	43	24	31	23	24	27	28	0.20
SCORAD	80	28.3	68.6	46.5	40	53	20.5	46	21	26	0.07
Prick test wheal size (mm ²)											
<i>D. pteronyssinus</i>	16	108	8	49	108	58	35	9	78	25	0.36
Timothy grass pollen	25	35	14	25	35	27	45	110	60	32	0.09
Spec IgE (IU/mL, Immulite 2000)											
<i>D. pteronyssinus</i>	>100	>100	0.927	>100	86.4	44	18.2	1.5	>100	25	0.34
Timothy grass pollen	26.3	26.4	66.8	53.8	>100	43	>100	>100	>100	76.8	0.04
Recombinant Bet v 1	7.56	24	11	11.7	40.6	19	86	57.9	77	16.7	0.12

SD, standard deviation; Acup, acupuncture; con, control; SCORAD, Scoring Atopic Dermatitis score; D., *Dermatophagoides*; Spec, Specific; IgE, immunoglobulin E; Bet v 1, major birch pollen allergen, Betula v 1.

Schoenenbuch, Switzerland) diluted in buffer at a final concentration of 5 ng/mL, 1 ng/mL, 0.5 ng/mL, or 0.25 ng/mL, 50 μ L stimulation buffer (negative control), or a highly specific monoclonal anti-Fc ϵ RI antibody (positive control) for 40 minutes at 37°C. The degranulation process was stopped by 1-mL blocking buffer on ice. Twenty microliters (20 μ L) of phycoerythrin-conjugated anti-IgE and fluorescein isothiocyanate conjugated anti-gp53 were added and incubated for 30 minutes in an ice bath. Erythrocytes were destroyed by adding 2 mL lysing solution (B-BAT-LYR; Becton-Dickinson) for 10 minutes at room temperature. Cells were washed twice with washing solution and resuspended in 200 μ L washing solution. Flow cytometric analysis was performed within 2 hours using a FACScan (Becton-Dickinson Immunocytometry System, Heidelberg, Germany) and Cell-Quest TM software. According to the instructions of the manufacturer, the basophil population was gated by the presence of phycoerythrin-conjugated anti-IgE, and the expression of gp53 (CD63) was analyzed on this gated cell population. Acquisition was performed on 1000 cells for each sample, and results are given as the percentage of basophils expressing gp53.

Statistical analysis

If not mentioned otherwise, mean values $\pm$ 95% confidence intervals are given. After having checked all parameters as normally distributed by the Kolmogorov-Smirnov one-sample test, differences between treatment groups were evaluated by the unpaired samples *t*-test. *P*-values less than 0.05 were considered as statistically significant. All tests were performed two-tailed. The analyses were performed using SPSS version 13 (SPSS Inc., Chicago, IL).

Results

Ten (10) patients with atopic eczema (8 male, 2 female; mean age: 25.2 $\pm$ 4.5 years) were enrolled. None of the patients had been on systemic immunosuppressive drugs within 3 months prior to the study. None of the patients dropped out or experienced a serious adverse event. The corresponding clinical characteristics are shown in Table 1. There were no significant differences between groups except for specific IgE to timothy grass pollen, which was 43 $\pm$ 31 IU/mL in the acupuncture group compared to 77 $\pm$ 10 IU/mL in the control group (*p* = 0.04).

SCORAD

Compared to day 0 (before treatment), on day 15 as well as day 33 mean SCORAD was rated slightly lower in the acupuncture group (-6.1 ± 10.1 [day 15–day 0]; -5.6 ± 17.6 [day 33–day 0]) than in the control group (4.4 ± 1.6 [day 15–day 0]; 3.5 ± 3.4 [day 33–day 0]) with no significant differences between groups.

Itch intensity (VAS)

Compared to day 0 (before treatment) on day 15 (*p* = 0.024) as well as day 33 (*p* = 0.022), mean itch intensity was rated significantly less in the acupuncture group ($-25\% \pm 26\%$ [day 15–day 0]; $-24\% \pm 31\%$ [day 33–day 0]) than in the control group ($15\% \pm 6\%$ [day 15–day 0]; $29\% \pm 9\%$ [day 33–day 0]).

TABLE 2. MEAN VALUES OF THE PERCENTAGE OF CD63-POSITIVE BASOPHILS, ITCH INTENSITY (% OF VAS), AND SCORAD (SCORE) IN THE ACUPUNCTURE AND CONTROL GROUPS AT DAYS 0, 15, AND 30

Group	Day	Negative (%CD63 basophils)	Positive (%CD63 basophils)	D1 5 ng/mL (%CD63 basophils)	D1 1 ng/mL (%CD63 basophils)	D1 0.5 ng/mL (%CD63 basophils)	D1 0.25 ng/mL (%CD63 basophils)	T1 5 ng/mL (%CD63 basophils)	T1 1 ng/mL (%CD63 basophils)	T1 0.5 ng/mL (%CD63 basophils)	T1 0.25 ng/mL (%CD63 basophils)	Itch intensity (%VAS)	SCORAD (score)
Acupuncture	0	26.0 $\pm$ 25.4	63.7 $\pm$ 19.2	46.4 $\pm$ 23.0	46.5 $\pm$ 26.4	39.7 $\pm$ 28.6	37.8 $\pm$ 26.5	61.6 $\pm$ 26.3	45.3 $\pm$ 30.5	41.9 $\pm$ 32.0	42.2 $\pm$ 32.1	55 $\pm$ 22	52.7 $\pm$ 21.2
	15	26.3 $\pm$ 15.4	37.1 $\pm$ 6.5	41.7 $\pm$ 15.6	33.2 $\pm$ 24.3	29.4 $\pm$ 20.6	28.5 $\pm$ 20.3	43.3 $\pm$ 22.6	36.6 $\pm$ 19.0	30.5 $\pm$ 17.5	27.6 $\pm$ 17.2	29 $\pm$ 25	46.6 $\pm$ 16.6
	30	32.8 $\pm$ 20.3	65.6 $\pm$ 7.0	53.4 $\pm$ 18.8	48.0 $\pm$ 18.5	42.2 $\pm$ 15.7	37.2 $\pm$ 18.8	63.8 $\pm$ 16.6	47.1 $\pm$ 19.0	41.9 $\pm$ 22.8	38.8 $\pm$ 24.1	31 $\pm$ 25	47.0 $\pm$ 23.6
Control	0	14.9 $\pm$ 11.9	47.8 $\pm$ 11.0	35.6 $\pm$ 11.0	22.5 $\pm$ 17.6	18.7 $\pm$ 14.6	18.4 $\pm$ 16.4	50.6 $\pm$ 17.8	32.9 $\pm$ 19.4	28.4 $\pm$ 18.5	20.9 $\pm$ 13.9	11 $\pm$ 7	25.1 $\pm$ 13.9
	15	23.8 $\pm$ 13.2	50.3 $\pm$ 9.3	53.3 $\pm$ 20.6	34.9 $\pm$ 19.0	26.3 $\pm$ 15.1	25.9 $\pm$ 15.7	67.9 $\pm$ 6.8	51.1 $\pm$ 10.1	36.2 $\pm$ 12.6	25.5 $\pm$ 14.8	26 $\pm$ 12	32.1 $\pm$ 14.1
	30	26.9 $\pm$ 20.0	69.3 $\pm$ 12.0	65.2 $\pm$ 15.6	38.7 $\pm$ 16.0	34.8 $\pm$ 19.5	26.8 $\pm$ 22.5	57.8 $\pm$ 29.0	49.5 $\pm$ 28.9	48.2 $\pm$ 30.7	38.1 $\pm$ 27.4	40 $\pm$ 27	31.2 $\pm$ 14.8

D, *Dermatophagoides pteronyssinus*; T, timothy grass pollen; VAS, visual analog scale; SCORAD, Scoring Atopic Dermatitis score.

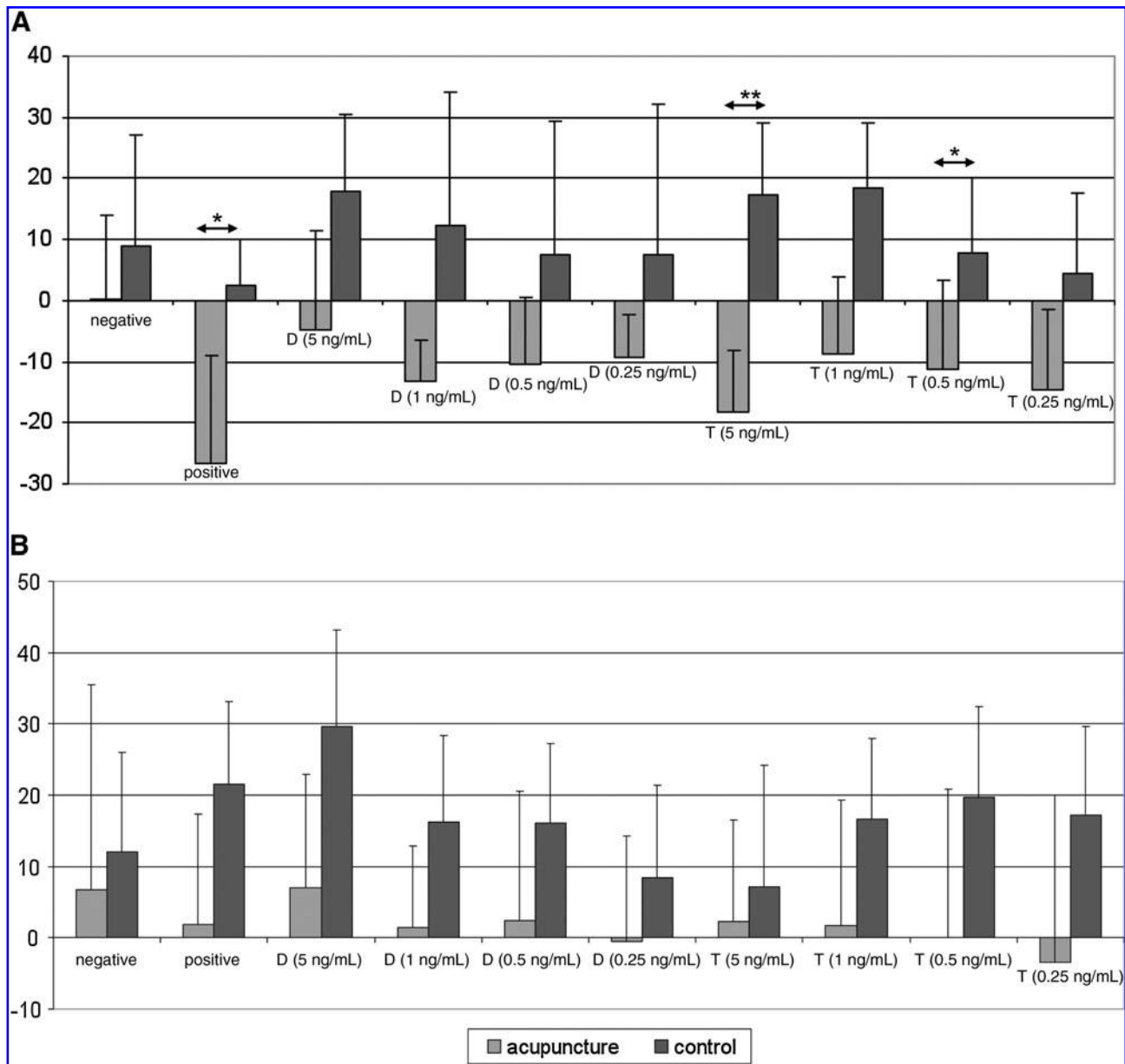


FIG. 1. Mean differences in the percentage of CD63-positive basophils between days 0 and 15 (A) as well as between days 0 and 30 (B) in the acupuncture treatment group and the control group. Negative values represent a decrease, positive values an increase in CD63-positive basophils over time. D, *Dermatophagoides pteronyssinus*; T, timothy grass pollen extract. (* $p < 0.05$; ** $p < 0.01$).

Basophil activation test

Compared to day 0 (before treatment) on day 15 (after 5 acupuncture treatments) as well as day 33 (after 10 acupuncture treatments), the acupuncture group showed a reduction in the percentage CD63 positive basophils compared to the control group for all performed tests and concentrations (positive control, *D. pteronyssinus* (D) (5 ng/mL, 1 ng/mL, 0.5 ng/mL or 0.25 ng/mL) and timothy grass pollen (T) (5 ng/mL, 1 ng/mL, 0.5 ng/mL, or 0.25 ng/mL).

Mean differences in the percentage of CD63 positive basophils between day 0 and day 15 were significantly higher in the acupuncture treatment group compared to the control group for positive control stimulation with anti-FcεRI anti-

body ($p = 0.028$) as well as timothy grass pollen 5 ng/mL ($p = 0.002$) and 0.5 ng/mL ($p = 0.012$). Results are shown in Table 2 and Figure 1.

Discussion

In this pilot study, we observed significant reductions of basophil activation as well as itch intensity by acupuncture. While basophil activation was generally reduced in the acupuncture group, it reached statistically significant levels for timothy grass stimulation as well as the positive control stimulation. The general reduction of basophil activation by acupuncture—even in the positive control stimulation with anti-FcεRI antibody—points to a reduction of spontaneous

activation of basophils and an allergen-independent mechanism of acupuncture action. We have no explanation for the relatively stronger effect of acupuncture at day 15 compared to day 33 of treatment; further studies are needed for clarification.

Although there are several studies on the effect of acupuncture on experimental itch,^{4,5,14–16} so far no controlled study investigated the effect of acupuncture on basophil activation or on itch in atopic eczema. Only a few studies have so far investigated the effect of acupuncture or related techniques on atopic eczema.^{17–22}

Experimental studies in human and animal models have shown a potential influence of acupuncture on various immunoregulatory substances such as various neurotransmitters and cytokines^{23,24}; as some of them are known to activate basophils or reduce itch,^{25,26} this might be an explanation of the observed effect of acupuncture on basophil activation and itch in the current study. Further experiments have also shown local inhibition of activation and proliferation of mucous mast cells as well as secretion of substance P and vasoactive intestinal peptide in the colon of rats,²⁷ and suppression of IgE production and modulation of Th1/Th2 cell response by electroacupuncture in 2,4-dinitrophenylated keyhole limpet protein immunized mice.²⁸ In the field of pain, acupuncture has been shown to specifically affect brain regions^{29–31} that have also been found to play an important role in the central modulation of itch.^{26,32–34} Future studies will have to focus on the underlying mechanisms.

Limitations of this pilot study are the low number of included patients as well as the lack of a placebo control group controlling for point-specific effects. As patients with potentially systemically active topical immunosuppressive agents were excluded, modulation of itch and especially *in vitro* basophil activation by active treatment with drugs can be regarded as highly improbable.

In this study, patients were treated with an individualized selection of acupuncture points chosen by the acupuncturist evaluating the procedure of acupuncture. Another approach regarding future studies can be a (semi)standardized point scheme characterizing the effect of a special selection of acupuncture points. Future larger-scale studies are also needed to evaluate the effect of acupuncture on clinical and *in vitro* outcome parameters of atopic eczema and corresponding implications.

Reducing basophil activation can be an additional tool in investigating the mechanisms of action of acupuncture in IgE-mediated allergy. Due to the limited number of patients included in our pilot trial, further studies are needed to strengthen the hypothesis.

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Disclosure Statement

No competing financial interests exist.

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