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Pruritogenic Effects of Mitogen-stimulated Peripheral Blood Mononuclear Cells in Atopic Eczema

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The etiology of atopic pruritus is unclear and seems mostly histamine-independent. In order to investigate non-mast cells as possible sources of pruritogenic agents, peripheral blood mononuclear cells from 12 atopic eczema patients and 12 controls were incubated in vitro for 24 h with phytohemagglutinin or concanavalin A (both at 10 μg/ml) or with medium alone, and each subject was tested with his own cell supernatants and lysates by prick testing and by application on tape-stripped skin. Histamine (0.1%) and substance P (500μM) were tested in comparison, and reactions were observed for up to 24 h. Cell supernatants were also analysed for their contents of several cytokines. Lymphocyte cell extracts or supernatants failed to cause symptoms in controls but induced whealing in 6 and itching in 3 patients on prick testing within 5 min, lasting for 30 min in 2 patients and persisting for 6 h in 1 patient. Histamine caused itching in all controls and in 7 patients within 5 min on prick testing, with decreasing reactivity at later times. Substance P yielded results with lower values. With all three types of test reagents, fewer subjects reacted on tape stripped skin. High levels of interleukins 2 and 6, low levels of interferon and no detectable levels of interleukin 4 and tumour necrosis factor were measured in stimulated cell supernatants and extracts, with even lower levels in subjects exhibiting skin reactivity. These findings thus provide evidence that as yet unidentified mononuclear cell products may be involved in whealing and itching associated with atopic eczema. ***Key words: itching; cytokines; histamine; substance P.***

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Atopic eczema patients are known to suffer from severe itching that is only poorly ameliorated by treatment with antihistamines, mast cell degranulation inhibitors or interferons (1–5). Instead, it responds well to corticosteroid or cyclosporine therapy, agents that affect both mast cell and lymphocyte functions (6–8). Since atopic eczema is characterized by a prominent epidermal and dermal mononuclear infiltrate including lymphocytes and FcεRI- and IgE-bearing antigen presenting cells (9–11) and since these cells are currently discussed as prominent effector cells in the pathology of atopic eczema (12,13), it has been suggested repeatedly in the past that their products might explain the histamine-independent pathology of the disease, including the pruritus (see e.g. 1,7).

In the present study, we therefore tested supernatants of mitogen-stimulated peripheral blood mononuclear cells (PBMC) of atopic eczema patients and controls for their effects on the induction of cutaneous erythema, whealing and itching on each individual's own skin. In addition, the cell supernatants and extracts were analysed for their contents of several cytokines. The data reported here provide the first insights into the possible role of cytokines in atopic pruritus. They also help to validate a simple model for further studying possible lymphocyte-dependent pruritogenic effects.

MATERIAL AND METHODS

Twelve adult patients with atopic eczema fulfilling the diagnostic criteria for the disease (14) and 12 age-matched healthy volunteers with no history of atopy were fully informed about the purpose of the study and agreed to participate. The skin of the forearms of the patients had to be uninvolved for testing.

At least 1 week before skin testing, 10 ml of venous blood were taken from each subject and the PBMC were separated by differential centrifugation, as previously described (15). PBMC were adjusted to 1×10^6/ml and incubated in vitro for 24 h at 37°C and 5% CO_2 with either medium alone (Dulbeccos MEM, Gibco, Eggenstein, Germany) or 10 μg/ml of either phytohemagglutinin (PHA) or concanavalin A (Con A) (both from Sigma, Deisenhofen, Germany). Supernatants and cells were then separated by contrifugation, the unstimulated cells lysed by 3 cycles of freezing and thawing, and after sterile filtration, all samples were stored in aliquots at −20°C until testing.

Skin tests were performed on each subject with his own PBMC supernatants and cell lysates as well as with histamine (0.1 mg/ml) and substance P (500 μM) as positive controls. Prick tests were done on the lower arms, as previously described (16). At least 1 week after prick testing, skin of the forearm was tape-stripped, as previously described (17), and 10 μl of the test substances and controls were applied to the stripped skin. Test sites were evaluated at 5 and 30 min and at 1, 6 and 24 h for erythema and redness. A positive test was defined as ≥ 9 mm^2 for erythema and ≥ 2 mm^2 for swelling. Subjects were also questioned regarding itching which was graded on a scale from 0–3 (0 = none, 1 = barely discernible, 2 = moderate, 3 = intense).

Analysis of cytokines in the cell supernatants and lysates was kindly performed by ELISAs in the laboratory of Dr. H. Gallati, Hoffmann La Roche, Basel, Switzerland.

Because of the pilot nature of the trial and the small number of patients and subjects, statistical analysis was only descriptive.

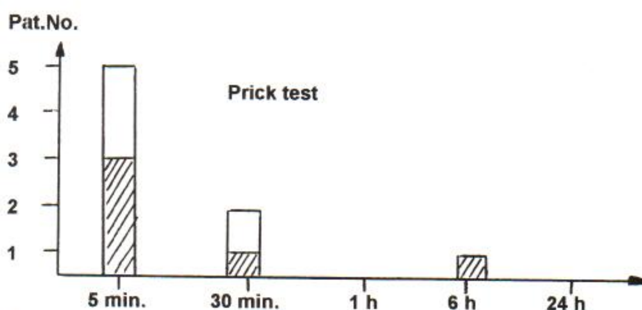

Fig. 1. Number of patients reacting to PBMC preparations at different time points with whealing (open column) or pruritus (hatched column).

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Table I. *Number of subjects responding to skin prick testing with histamine or substance P at different time points*
E = erythema; S = swelling; P = pruritus.

Time	Patients			Controls		
	E	S	P	E	S	P
Histamine						
5 min	12	12	7	12	12	12
30 min	6	7	2	10	11	3
1 h	1	5	0	4	4	1
6 h	1	1	1	0	0	0
24 h	0	0	0	0	0	0
Substance P						
5 min	6	9	4	5	10	8
30 min	0	5	1	3	7	1
1 h	0	4	0	0	2	0
6 h	1	1	2	0	0	0
24 h	0	0	0	0	0	0

Table II. *Number of subjects responding to histamine or substance P on tape-stripped skin at different time points*
E = erythema; S = swelling; P = pruritus.

Time	Patients			Controls		
	E	S	P	E	S	P
Histamine						
5 min	8	6	6	4	5	6
30 min	6	4	2	3	3	2
1 h	3	2	0	1	1	1
6 h	1	1	0	0	0	0
24 h	0	0	0	0	0	0
Substance P						
5 min	2	3	2	1	2	2
30 min	1	3	1	1	1	1
1 h	0	1	1	0	0	0
6 h	0	2	1	0	0	0
24 h	0	0	0	0	0	0

RESULTS

On skin testing with the lymphocyte supernatants and cell lysates, none of the normal controls showed any reaction at all. Of the atopic eczema patients, 6 had skin reactions, 5 on prick testing (Fig.1) and 1 additional patient on tape-stripped skin (not shown). Reactions occurred mostly at 5 min and consisted of whealing in 5 and additional itching (grade 2) in 3 patients. In 2 patients, whealing and additional itching, respectively, were still noted at 30 min, and in 1 patient, whealing and itching persisted for up to 6 h (Fig.1). Erythema always occurred in association with whealing and itching and is not shown separately.

Of the patients who had reacted on prick testing, only 1 exhibited itching and whealing also on tape-stripped skin. One additional patient reacted with whealing only.

The reactions occurred in all cases with supernatants of cell lysates, in 4 patients with control cell supernatants, and in 2 patients with cell supernatants stimulated with either of the two mitogens.

The skin test reactions to histamine and substance P in patients and controls are summarized in Table I for prick tests and Table II for tape-stripped skin. As can be seen, all control subjects reacted within the first 5 min on prick testing with histamine, and slightly fewer with substance P. Although whealing was also observed in all cases, fewer patients experienced associated pruritus with histamine, an effect that was also evident with substance P (Tables I and II). In all tests, the number of reactions was lower at 30 min, with a further decrease at 1 h. Late reactions at 6 h were noted only in 1 patient, with no further rèactions at all at the 24-h reading.

In Table III, data on the cytokine analyses of the test samples are summarized. Measurable cytokine levels were only detected for IL-2, IL-6 and IFNτ. Mean values tended to be slightly higher in patients vs controls, although the two groups did not differ regarding spontaneous release or levels in unstimulated cell extracts (not shown). On separate analysis of the samples that had induced a positive skin reaction in patients, cytokine levels in these samples were found to be even lower than in those of all patients or control subjects (Table III).

DISCUSSION

The present data show that unstimulated and mitogen-stimulated mononuclear leukocyte products can indeed induce skin test reactions in atopic eczema patients, but not in the nonatopic controls. Cell extracts rather than secreted products were primarily active in these tests.

The lack of reactivity of half of the atopics to their PBMC is nevertheless unsatisfying. It cannot be attributed to faulty skin testing methodology, since prick tests to histamine were positive in all patients and controls (Table I). Lower numbers of patients experienced pruritus in all tests (Table I), an observation that has been observed before with histamine and substance P in atopic patients with other test systems (18,19).

Compared to the results with prick tests, the data obtained with tape-stripped skin were generally less satisfying, possibly due to technical reasons such as the variably efficient removal of the epidermal barrier.

Reactions on prick testing occurred always within the first

Table III. *Measurement of cytokines by ELISA in 24 h PBMC supernatants and cell lysates which were used for skin testing in the test subjects*
Means ± 1 SD are shown.

Cytokines	Atopic eczema patients		Controls
	All	Skin test positive	
IL-2, pg/ml	531.5±490.1	50.0±130.0	507±396.6
IL-4, pg/ml	0.0±0.0	0.0±0.0	0.0±0.0

few minutes and rarely persisted beyond 1 h with only few patients and no controls exhibiting late reactions at 6 h and none at 24 h. Since this time pattern was observed with all three types of test substances, it is unlikely that reactions to the cell products were missed due to faulty reading times. The early reactivity on positive skin testing with the cell products also speak against this possibility. The early nature of the reactions does, however, not rule out the possibility that the reactions to the cell products were due to so-called histamine releasing agents. This aspect will have to be more stringently proven by pharmacological modulation of the reactions with potent H1-antagonists.

The failure of half of the patients to react to their PBMC products might also be due to inadequate in vitro stimulation. Incubation with clinically relevant antigens rather than with mitogens might have increased the yield of skin test reactivity. This holds also for the incubation period, since optimal secretion of some cytokines need not be at 24 h (20).

The ELISA data nevertheless show that several cytokines reached considerable levels with the present methodology (Table III), in agreement with recent findings where particularly high levels of IL-6 were measured in PBMC supernatants of atopics and controls and only low IFNτ levels, depending on the type of in vitro stimulation (21). In the present study, cytokine levels were comparable or lower in controls and skin-reactive patients, respectively (Table III). From these findings, one can conclude that IL-2, IL-6 and IFNτ are unlikely mediators of whealing and itching. The possible itch inducing ability of cytokines not measured in the supernatants must remain open at present.

In summary, the present data provide some evidence that PBMC products may indeed be involved in the induction of itching in atopic eczema patients. They also show that skin prick testing is a simple and adequate method to further pursue this question.

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