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Enhanced Releasability of Prostaglandin E_2 and Leukotrienes B_4 and C_4 from Leukocytes of Patients with Atopic Eczema

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The releasability of arachidonic acid-derived inflammatory mediators (eicosanoids) from peripheral blood leukocytes has been tested in patients with atopic eczema and healthy, non-atopic controls. Spontaneous and stimulated release of prostaglandin E_2 (PGE_2), leukotriene B_4 (LTB_4) and leukotriene C_4 (LTC_4) has been measured after challenge of cells with various concentrations of anti-IgE, Ca-ionophore A23187 and C5a. The maximal stimulation of cells with Ca-ionophore resulted in the generation of high amounts of all eicosanoids, which was essentially equal in both atopic and control groups. On the other hand, enhanced spontaneous and stimulated eicosanoid release was noted after immunological challenge using C5a and anti-IgE in the atopic eczema group. Thus, our data support the hypothesis of enhanced releasability of inflammatory mediators in atopic eczema. *Key word: Eicosanoid releasability.* (Received February 8, 1987.)

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The pathogenesis of atopic eczema is characterized by three abnormal immune phenomena which are interrelated and form a vicious cycle: increased IgE-production, enhanced releasability of inflammatory mediators and T cell regulatory dysfunction (12).

The term "releasability" was first used to describe the capacity of cells to release preformed or newly synthesized mediators. The concept of enhanced releasability of vasoactive mediators in atopic diseases has been put forward by the group of Lichtenstein et al. (10), and has been supported by the findings of several investigators showing the release of increased amounts of histamine from basophils in atopic populations compared to non-atopic controls (reviewed in ref. no. 13). Such an enhanced release of histamine could also be demonstrated directly in the skin of patients with atopic eczema upon immunological, but not upon non-immunological stimulation, although in this study, normal cutaneous histamine concentrations were found (16). This and the fact that antihistamines are rather weak therapeutic agents in influencing the cutaneous inflammation in atopic eczema suggests that other mediators might possibly play a greater role in the development of eczematous lesions in this disease. Among potential candidates for a pathogenetic role in atopic eczema, arachidonic acid metabolites—prostaglandins, HETEs and leukotrienes—deserve particular interest for several reasons. They exert extraordinarily potent inflammatory properties in various tissues (9) including skin (reviewed in ref. no. 18), they are believed to play an important role in immunoregulation (5, 6) and their formation is suppressed by glucocorticosteroids (8), which represent the most effective treatment modality in atopic eczema. In addition, we have recently been able to demonstrate a selective elevation of the 5-lipoxygenase metabolite of arachidonic acid, LTB_4, in suction blister fluid obtained from lesional atopic dermatitis skin (17). We therefore decided to test systematically the releasability of the arachidonic acid metabolites prostaglandin E_2 (PGE_2), leukotriene B_4 (LTB_4) and leukotriene C_4 (LTC_4) from peripheral blood

leukocytes upon challenge with various immunological and pharmacological stimuli. In this study we were indeed able to demonstrate enhanced generation of these inflammatory mediators which supports the concept of altered mediator releasability in atopic eczema.

PATIENTS AND METHODS

Patients

Twenty-seven in- and out-patients of the Department of Dermatology of the Ludwig-Maximilians-University of Munich with atopic eczema were examined. The clinical diagnosis conformed to the criteria put forward by Rajka (11) and Hanifin & Rajka (7). Seventeen patients were male, 10 female, mean age was 28.4 years, age range 9 to 69 years. Sixteen patients had mild atopic dermatitis with lesions confined to predilection sites. Eleven patients suffered from widespread or generalized, acute inflammatory lesions. Serum IgE-levels were elevated in 10 of the 16 patients with mild disease with values ranging up to 800 U/l (normal below 100 U/l), and in all patients with severe disease (values ranging from 140 to 16400 U/l). All patients were off systemic drugs for at least 7 days. In patients treated locally with hydrocortisone cream, treatment was switched to emollients three days before study.

Twenty-two healthy volunteers with negative personal and family history of atopy and no clinical evidence of atopic eczema, allergic rhinitis, conjunctivitis or asthma served as controls. Their mean age was 34.2 years, age range 17 to 73 years. In none, serum IgE values were elevated.

Leukocyte preparation

Cell preparation was performed as previously described (14). 40 ml of venous blood was drawn through a wide bore needle into a heparinized syringe containing 12 ml dextran 60 (Macrodex, Pharmacia, Uppsala) and sedimented for 90 min at room temperature. The supernatant containing leukocytes was removed, the leukocytes pelleted by centrifugation for 10 min at 4°C and 400×g and resuspended in 25 mM Tris buffer with 120 mM Na^+, 4 mM K^+, 1 mM Mg^{++} μ, 0.6 mM Ca^{++}, pH 7.4. The cells were washed three times in this buffer and suspended at a final concentration of 5×10^5 cells/ml. The preparation contains a mixture of blood leukocytes and approximately 20% of the peripheral blood platelet count. There was no significant difference in differential blood count between atopic patients and controls.

Stimulation of leukocytes

Stimulation of cells was done as previously described (15). Cells were challenged with anti-IgE, Ca-ionophore A23187, C5a or buffer alone. Cell suspensions were added to Eppendorf vials containing the appropriate dilutions of the stimulants or solvent alone. Incubations were carried out at 37°C for 30 min in open vials and terminated by cooling the vials on ice. Cells were centrifuged for 10 min at 4°C and 400×g and the supernatants stored at −70°C until assay, which was performed within one week.

Anti-IgE-challenge was performed by adding ε-specific goat-anti-human-IgE preparation (Behring-Werke, Marburg, FRG) at dilutions of 10^{-2}, 10^{-3} and 10^{-4}, corresponding to final concentrations of 5000, 500 and 50 IU/ml. Ca-ionophore A23187 (Calbiochem, La Jolla, USA) was dissolved as a stock solution in ethanol and further diluted with buffer. The final concentrations of ionophore were 200, 20 and 2 μM. Controls were performed with solvent alone. Ethanol concentration was always below 1% and had no effect upon eicosanoid generation.

C5a activity-containing serum was prepared according to Vallota and Müller-Eberhard (23). This preparation will be referred to as "C5a" for the sake of brevity. The serum was added to leukocyte suspensions concentrated and at 10^{-1} and 10^{-2} dilutions.

Radioimmunoassays of eicosanoids

Prostaglandin E_2, leukotrienes B_4 and C_4 were measured by specific radioimmunoassays as described in detail previously (17), using a PGE_2-RIA-kit (New England Nuclear, Dreieich, FRG), LTB_4 antibody raised by Salmon et al. (19) and LTC_4 antibody described by Aehringhaus et al. (1). The detection limits for LTB_4, LTC_4 and PGE_2 were 145 pg/ml, showing a cross-reaction of 2% with 12-HETE and below 0.33% with other eicosanoids. 55 pg/ml and 0.25 pg/ml, respectively. The identity of the immunoreactive eicosanoids has been confirmed by HPLC as described (17).

Statistical analysis

Experiments were performed in duplicates. Results were expressed as mean ± SEM. Statistical significance was assessed by Student's *t*-test and chi-square-test, respectively.

RESULTS

Releasability of prostaglandin E_2

Incubation of leukocytes at 37°C led to a measurable release of PGE_2 already in the absence of stimuli. This spontaneous PGE_2 release was similar in atopics and controls (40.9±8.2 vs. 37.1±5.5 pg/ml) (Table I).

Stimulation of cells with anti-IgE proved to be a weak stimulus for PGE_2 synthesis. At the highest and lowest anti-IgE concentrations used, PGE_2 synthesis exceeded the spontaneous release only in a few samples, the difference being not significant. However, at an anti-IgE concentration of 500 IU/ml, cells from patients with atopic eczema generated tenfold amounts of PGE_2 (corrected for spontaneous release) as compared to controls (26.3±9.8 vs. 2.6±2.0 pg/ml, $p<0.05$).

Challenge of cells with Ca-ionophore A23187 and C5a yielded almost equivalent amounts of PGE_2 in a clearly dose dependent manner. The release of PGE_2 was somewhat higher in controls at the maximal ionophore concentration (180.9±31.3 vs. 73.1±13.6 pg/ml, $p<0.005$), but at all other concentrations of both stimuli, the values were not different.

Releasability of leukotriene B_4

In approximately one third of atopics and controls, spontaneous secretion of low amounts of LTB_4 was measured during the 30 min incubation time (Table II).

Anti-IgE-challenge of cells showed little further enhancement of LTB_4 release. In analogy to the results with PGE_2, at the concentration of anti-IgE of 500 IU/ml, the highest amounts of LTB_4 were measured. At this concentration, 12 of 27 atopic dermatitis patients, but only 2 of 16 healthy probands synthesized LTB_4 in excess of spontaneous release during incubations ($p<0.05$). At the other concentrations tested, only low amounts of LTB_4 could be measured in a few samples, the difference being not significant.

Again, much higher and dose-dependent release of LTB_4 was observed with Ca-

Table I. *Releasability of prostaglandin E_2 from peripheral blood leukocytes of patients with atopic eczema and healthy controls*

Values represent mean of duplicate determinations or number of samples above the detection limit of 0.25 pg/ml

Stimulus	Prostaglandin E_2 (pg/ml)	
	Controls (n=11)	Atopic dermatitis (n=18)
None	37.1±5.5	40.9±8.2
Anti-IgE		
10^{-2}	3/11	7/18
10^{-3}	2.6±2.0	26.3±9.9 ($p<0.05$)
10^{-4}	7/11	6/18
Ca-ionophore A23187		
200 µM	180.9±31.3	73.1±13.6 ($p<0.005$)
20 µM	17.1±5.9	11.6±4.8
2 µM	4.9±2.9	9.8±5.0
C5a		
Pure	168.5±17.8	153.0±17.5
10^{-1}	28.5±7.7	20.8±4.2
10^{-2}	5/11	7/18

ionophore and C5a. The LTB_4 concentrations measured after ionophore stimulation were virtually indistinguishable between atopics and controls. In contrast, with C5a, atopic dermatitis patients released approximately 60 and 20% more LTB_4 at the two highest stimulus concentrations compared to control patients, however, the difference failed to reach statistical significance.

Table II. *Releasability of leukotriene B_4 from peripheral blood leukocytes of patients with atopic eczema and healthy controls*

Values represent mean of duplicate determinations or number of samples above the detection limit of 145 pg/ml

	Leukotriene B_4 (pg/ml)	
Stimulus	Controls (n=16)	Atopic dermatitis (n=27)
None	6/16	10/27
Anti-IgE		
10^{-2}	1/16	4/27
10^{-3}	2/16	12/27 ($p<0.05$)
10^{-4}	2/16	9/27
Ca-ionophore A23187		
200 μM	3 719.8±1 681.1	3 524.7±877.7
20 μM	254.3±184.2	207.5±59.3
2 μM	3/16	8/27
C5a		
Pure	2 009.1±738.7	3 215.6±313.3
10^{-1}	347.5±144.9	426.1±110.9
10^{-2}	7/16	10/27

Table III. *Releasability of leukotriene C_4 from peripheral blood leukocytes of patients with atopic eczema and healthy controls*

Values represent mean of duplicate determinations or number of samples above the detection limit of 55 pg/ml

	Leukotriene C_4 (pg/ml)	
Stimulus	Controls (n=13)	Atopic dermatitis (n=23)
None	0/13	6/23 ($p<0.05$)
Anti-IgE		
10^{-2}	3/13	11/23 ($p<0.01$)
10^{-3}	2/13	12/23 ($p<0.01$)
10^{-4}	2/13	4/23
Ca-ionophore A23187		
200 μM	3 820.3±665.4	3 503.0±729.4
20 μM	770.8±341.8	722.5±280.2
2 μM	4/13	7/23
C5a		
Pure	5/13	8/23
10^{-1}	2/13	3/23
10^{-2}	5/13	3/23

Releasability of leukotriene C_4

None of the control subjects released LTC_4 upon incubation with buffer alone as compared to 6 of 23 atopic dermatitis patients ($p<0.05$) (Table III). Only few released LTC_4 after anti-IgE in contrast to approximately half of the atopic patients, whose LTC_4 production exceeded spontaneous release at the two highest concentrations (difference between controls and atopics statistically significant, $p<0.05$). Ca-ionophore proved to be a strong stimulus for LTC_4 generation with equal values for atopics and controls. In contrast to LTB_4 and PGE_2 production, where ionophore and C5a were almost equally potent stimuli, C5a only induced minimal LTC_4 generation in a minority of subjects with no difference between controls and atopics.

Comparison of in vitro findings with clinical data

With both PGE_2 and the leukotrienes, no correlation was seen between eicosanoid release, severity of atopic dermatitis and serum-IgE-levels. Therefore, data from all patients were pooled for statistical analysis.

DISCUSSION

The hypothesis of enhanced releasability of vasoactive inflammatory mediators has been put forward (12) in an attempt to explain the inflammatory and immunoregulatory abnormalities characterizing the pathogenesis of atopic disease. Of these mediators, histamine has received most attention up to date. Available data suggest that there is indeed some trend towards higher releasability of histamine from leukocytes of patients with atopic eczema, although this difference has not been found consistently by all investigators (reviewed in ref. no. 13).

A major role for histamine in atopic dermatitis is made questionable by the fact that antihistamines, although they are affective as antipruritic agents, do not significantly influence the course of the disease. This is in contrast to glucocorticosteroids, which are the most potent drugs available for treatment of atopic eczema. Since, among other actions, glucocorticosteroid effects are currently being explained, at least partly, by their inhibition of arachidonic acid release (8), arachidonic acid-derived metabolites might assume a greater role in atopic dermatitis than other previously recognized inflammatory mediators.

Prostaglandin E_2 leads to vasodilation and erythema when injected intracutaneously (20), and exerts potent immunoregulatory, mainly immunosuppressive, actions (reviewed in ref. nos. 5 and 6).

LTB_4 leads to a dermal infiltrate after intracutaneous injection (21), enhances vascular permeability (21) and induces T-suppressor lymphocytes (2).

LTC_4 is assumed to play a key role in bronchoconstriction in atopic asthma (4), and possesses potent vasoactive actions (3). Therefore, both the inflammatory and immunopathological aspects of atopic eczema could, at least partly, be explained by the action of eicosanoids. For these reasons, we decided to test whether in atopic dermatitis there is an enhanced releasability of arachidonic acid-derived inflammatory mediators from peripheral blood leukocytes. For this purpose, we measured the spontaneous release and stimulated generation of PGE_2, LTB_4 and LTC_4 after challenge of cells with anti-IgE, C5a and Ca-ionophore A23187.

The most potent agent inducing eicosanoid release proved to be Ca-ionophore. Using this pharmacological, non-physiological stimulus, eicosanoid release was equal in atopic dermatitis patients and controls apart from minor differences in PGE_2 release at highest stimulus concentrations, which are probably without biological relevance.

However, after submaximal stimulation of cells using anti-IgE and C5a, stimuli simulating more "physiological" conditions, marked differences between atopics and control were noted. There was a higher releasability of all eicosanoids after anti-IgE stimulation. The fact that in many subjects values were near or below the detection limit of our assay suggests that basophils contribute only little to the total amount of eicosanoid release achieved by stimulation with Ca-ionophore or C5a. Obviously, conventional anti-IgE antibodies show only a weak activity at the proposed low affinity receptor on leukocytes other than the basophils (22).

The higher releasability of eicosanoids from leukocytes with anti-IgE, but not Ca-ionophore, can be seen in analogy to our earlier findings of increased histamine releasability from atopic eczema skin slices, which was also enhanced with anti-IgE, but normal with the pharmacological agents acetyl choline and substance 48/80 (16).

C5a was a weak stimulus for LTC_4 generation, but released high amounts of LTB_4, which were again markedly higher in the atopic group. Enhanced spontaneous LTC_4 releasability was also observed in some atopic eczema patients in contrast to controls, although the measurement of LTC_4 in supernatants of unstimulated cells was again limited by the very low concentrations of this mediator.

Our data support the concept of enhanced mediator releasability in atopic eczema. Enhanced production of the biologically extremely potent metabolites of arachidonic acid might explain several aspects of the disease such as cutaneous inflammation, disordered immune regulation, clinical response to glucocorticosteroid treatment and enhanced concentrations of LTB_4 in atopic dermatitis skin.

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