

Short communication

Urinary leukotriene levels are increased during exacerbation of atopic eczema/dermatitis syndrome. Relation to clinical status

Background: Leukotrienes are potent mediators of allergic inflammation and their role in the pathogenesis of allergic disorders, particularly asthma, is well established. Their importance in the pathogenesis of atopic eczema/dermatitis syndrome (AEDS) is still unclear. We aimed to compare urinary cysteinyl leukotriene (Cys-LT) levels during exacerbation and remission of AEDS in relation to clinical status, IgE levels, and eosinophil counts.

Methods: Urinary Cys-LTs were measured by direct enzyme immunoassay in 17 adult patients with AEDS and in 17 healthy controls in whom atopy had been excluded. Cys-LTs were compared during exacerbation and remission of AEDS in relation to the clinical status measured by SCORAD. Total IgE levels were measured by enzyme-linked immunoassay (ELISA).

Results:

Mean clinical score during the exacerbation was 64.3 ± 3.1 and during remission 22.4 ± 4 ($P < 0.01$). Cys-LTs levels were significantly higher during the exacerbation of AEDS than in the control group (230.9 ± 20.8 vs 123.2 ± 9.9 pg/mg creatinine; $P < 0.005$). During the remission, the difference between AEDS patients and the control group was not significant (96.3 ± 8.7 vs 123.2 ± 9.9 pg/mg creatinine; $P = 0.8$). During AEDS exacerbation Cys-LTs levels were significantly correlated with the clinical status ($r_s = 0.73$, $P < 0.01$) and with eosinophil counts ($r = 0.86$; $P < 0.01$) but not with the duration of the disease, age of patients, or IgE levels.

Conclusions: Our results point to enhanced biosynthesis of Cys-LTs during the AEDS exacerbations. Inflammatory cells, e.g. eosinophils are the most probable source of Cys-LTs. A strong correlation between Cys-LT levels and clinical status may in part explain preliminary clinical observations of efficacy of leukotriene antagonists in alleviating symptoms of AEDS.

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Leukotrienes, arachidonic acid derivatives, are potent mediators of allergic inflammation and their role in the pathogenesis of allergic disorders, particularly asthma, is well established (1). Recent in-vitro and in-vivo studies have suggested that leukotrienes may also play a role in allergic inflammatory skin reactions, including atopic eczema/dermatitis syndrome (AEDS), a multifactorial inflammatory dermatosis of unclear pathogenesis (2). A key role in the pathogenesis of AEDS is played by IgE-mediated mechanisms (3, 4) but overproduction of IgE cannot fully explain the distinct features of AEDS.

An important role of cysteinyl leukotrienes (Cys-LTs) in AEDS has been suggested by early studies

showing that intradermal challenge with leukotriene (LT)-D₄ causes a wheal and flare response (5). Several recent in-vitro studies showed that peripheral blood leukocytes (mainly eosinophils and basophils) isolated from AEDS subjects release increased amounts of Cys-LT in response to food additives, opsonized zymosan as well as interleukin (IL)-3 or allergens (4, 6, 7). *Staphylococcus aureus* enterotoxins, which play an important role in AEDS exacerbations, have also been demonstrated to induce Cys-LT release from basophils (8). The level of leukotriene release is enhanced in allergen challenged skin compared to nonchallenged skin slices (9).

The studies described above suggest an important role for leukotriene production in the pathogenesis of exacerbations of AEDS. The results of clinical studies measuring urinary Cys-LT levels (believed to reflect global Cys-LT production) in AEDS patients are,

Abbreviations: AEDS: atopic eczema/dermatitis syndrome; CysLTs: cysteinyl leukotrienes; IgE: immunoglobulin E; LT: leukotriene.

however, conflicting. Some studies show increased Cys-LT production in AEDS (10, 11), but others do not confirm this (12–14).

Despite these controversies, several investigators used antileukotriene drugs in the treatment of AEDS exacerbations. Case reports show their clinical efficacy (15).

We studied urinary excretion of Cys-LTs in AEDS subjects during the exacerbation, and during the long-term remission. Cys-LT levels were also compared in the AEDS patients and the healthy, nonatopic controls. We also assessed the relationships between urinary cysteinyl leukotriene levels and the clinical severity of the skin lesions, total serum IgE levels, or eosinophil counts.

Material and methods

Patients and study protocol

We studied 17 adult patients with atopic eczema/dermatitis syndrome and 17 healthy control subjects matched for age and sex. AEDS was diagnosed on the basis of clinical symptoms (three major, three minor criteria (16)) and positive personal and/or family history of atopy. The clinical status was assessed using the SCORAD system (17). Patients with persistent AEDS were included during exacerbation, manifested by flares of eczema at three or more sites, or generalized skin lesions. Skin tests with standardized allergen extracts (house dust, *Dermatophagoides pteronyssinus*, moulds, feather, grass, tree pollens, foods e.g. milk and eggs, and animal fur allergens) were performed in all patients during the 2–4 months before exacerbation.

The study was approved by the Local Ethics Committee of the Jagiellonian University College of Medicine. All subjects gave written informed consent.

Every parameter was studied at two time points: 1) during the exacerbation, and 2) during long-term remission of AEDS (approximately 4 months after exacerbation), and all were compared to the control group. Remission was achieved in all patients using

oral antihistamines or topical steroids for about a month. None of the patients had used oral corticosteroids in the 6 months before enrolment to the study.

Urinary Cys-LT levels

Cys-LTs were measured in unpurified samples by direct enzyme immunoassay (Cayman Chemical, Ann Arbor, MI) and expressed in picograms (pg) per milligram of creatinine using methodology described before (18, 19). Urinary creatinine was determined using a creatinine test kit.

Measurement of total IgE in serum

The total serum IgE levels were determined by ELISA, using the AlaSTAT total IgE test (DPC Biermann, Bad Neuheim, Germany).

Statistical analysis

Data are expressed as mean $\pm$ SEM. In all cases n refers to the number of patients. Means were compared using Student t -tests or the Wilcoxin test, depending on the distribution of data. Correlations were performed using the Pearson correlation coefficient, or by χ^2 test for relationships between LT levels and concomitant asthma/rhinitis. A value of $P < 0.05$ was considered significant.

Results

Clinical characteristics of patients

Mean clinical SCORAD score during the exacerbation was 64.3 ± 3.1 and during remission 22.4 ± 4.1 ($P < 0.01$). The clinical characteristics of the patients are presented in Table 1. Eight patients (47%) had concomitant asthma and 10 patients (59%) had concomitant intermittent rhinitis; 94% of subjects presented with positive skin prick tests (SPT) indicating involvement of IgE-mediated mechanisms (2). None of the subjects investigated had aspirin hypersensitivity.

Table 1. Clinical characteristics of AEDS patients and control subjects. Values are mean $\pm$ SD or numbers of subjects (with percentage in parentheses)

	AEDS patients	Control group
Number of patients	17	17
Mean age (years)	23.5 ± 9.2 (17–47)	24.2 ± 7.2 (19–35)
Gender (female/male)	3 F 14 M	4 F 13 M
IgE levels (geometric mean; range)	243.7 (22–1048) IU/ml	N/F
Mean duration of AEDS (years)	16.7 ± 12 (2–40)	0
Clinical severity of AEDS (SCORAD):		
Exacerbation	64.3 ± 3.1	0
Remission	22.4 ± 4.0	0
Concomitant diseases:		
Asthma	8 (47.0%)	0
Intermittent rhinitis	10 (58.8%)	0
Positive skin prick tests:		
House dust mite	14 (82.3%)	0
Grass pollens	12 (70.5%)	0
Tree pollens	3 (17.6%)	0
Foods (milk, eggs)	5 (29.4%)	0
Moulds	3 (17.6%)	0
Feather	1 (5.9%)	0
Animal fur allergens	5 (29.4%)	0
Chronic infections	6 (35.2%)	1 (6%)

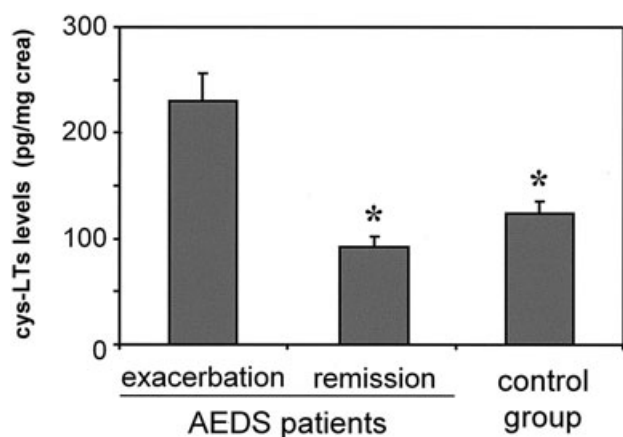


Figure 1. Mean urinary cysteinyl leukotriene (Cys-LT) levels in patients with AEDS ($n=17$) during exacerbation and remission of the disease and in comparison with control subjects ($n=17$). Cys-LTs were measured in patients during exacerbation and 3 months after stopping successful therapy with antihistamines and topical steroids; Bar graphs show means $\pm$ SEM; * $P<0.01$ by Students t -test.

Urinary Cys-LT levels in AEDS

Urinary Cys-LTs were detectable in all urine samples analyzed and were significantly higher during exacerbation than during remission of the disease (230.9 ± 20.8 vs 96.3 ± 8.7 pg/mg creatinine; $P=0.005$) (Fig. 1). Moreover, there was a significant difference in leukotriene excretion between AEDS patients during exacerbation and the control group (123.2 ± 9.9 pg/mg creatinine). This difference was no longer observed during the long-term remission (Fig. 1).

We did not find significant differences in Cys-LT levels between males and females ($P=0.7$). There was no correlation between Cys-LT levels and age of the patients or the duration of the disease ($P=0.6$).

Analysis of the relationship between Cys-LT levels and clinical severity of the disease using the SCORAD scale revealed a significant correlation between these parameters ($r=0.7$; $P=0.03$; Fig. 2A). Similarly there was a significant correlation between urinary Cys-LT levels and eosinophil counts ($r=0.86$; $P=0.02$; Fig. 2B) suggesting that eosinophils could be an important source of Cys-LTs in AEDS.

The relationship between Cys-LT levels with IgE levels did not reach statistical significance ($r=0.4$; $P=0.09$) during disease exacerbation. Interestingly, no additional increment of Cys-LT levels during AEDS exacerbations was associated with concomitant asthma (227 ± 43.4 vs 231.5 ± 36.6 ; $\chi^2=NS$) or rhinitis (226.7 ± 26.5 vs 233.6 ± 46.7 ; $\chi^2=NS$) in subjects with AEDS. However none of the patients studied presented exacerbation of either asthma or rhinitis, respectively.

Discussion

Our study shows that in adult patients with AEDS, Cys-

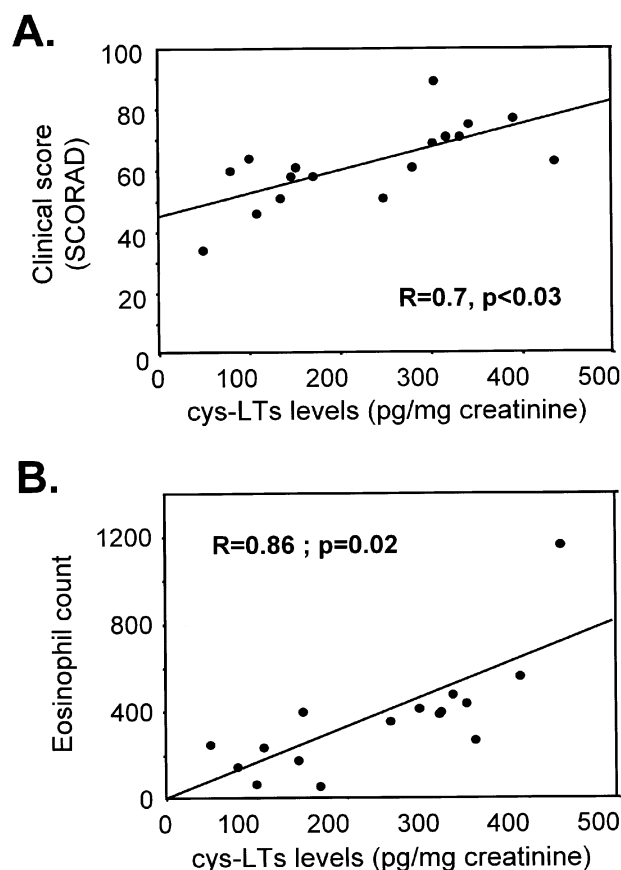


Figure 2. Relationship between urinary cysteinyl leukotriene (Cys-LTs) levels and the severity of the skin lesions measured using SCORAD (panel A) and eosinophil counts (panel B) during exacerbation of atopic eczema/dermatitis syndrome (AEDS).

LT levels are significantly higher during exacerbation of the disease than during clinical remission, or in the control nonallergic subjects. Moreover, the urinary Cys-LT levels correlate with clinical severity of the skin lesions during the exacerbation of AEDS, suggesting an association with the intensity of skin inflammation. Simultaneously, no additional increase in Cys-LT levels was related to the concomitant asthma or rhinitis although these factors have been previously associated with increased severity of AEDS.

Previous studies, which investigated leukotriene synthesis in AEDS showed conflicting results. Fauler et al. (11) was first to study urinary leukotriene(LT)-E₄ excretion in AEDS and found a three-fold increase during exacerbation compared to remission of the disease. Sansom et al. did not confirm these findings (12). Both of these studies were conducted using comparable methods and similar populations of patients with severe, generalized lesions. However urinary LT-E₄ levels in the control subjects, found by Sansom et al., were significantly higher than in other studies (210 pg/mg creatinine) which could partly explain the discrepancy.

Recently Hishinuma et al. (10) compared AEDS

patients with healthy controls, showing increased urinary Cys-LTs in atopic eczema/dermatitis syndrome. This study was conducted on a larger population ($n=20$) than those of previous authors, but no clinical assessment was performed and the clinical status of the disease was unclear. In the present study we analyzed urinary Cys-LT production within the same group of subjects during both exacerbation and remission, and these patients were very closely monitored clinically. We compared this study group with 17 healthy individuals.

Potential sources of leukotrienes in AEDS include mainly eosinophils, mast cells, and macrophages (4). These cells are found in excessive amounts within skin lesions of AEDS. Langerhans cells may also be involved in the inflammatory reaction and may be a source of arachidonic acid derivatives in the skin. Changes in the cell membrane composition of the skin have been shown in AEDS, which might influence arachidonic acid metabolism (20). In the present study, Cys-LT levels were correlated with eosinophil counts, indicating eosinophils as the most likely source of Cys-LTs in AEDS. Shimizu et al. (21) suggested that the enhanced Cys-LT generation in AEDS may result from increased numbers of eosinophils rather than from enhanced release from individual cells.

One of the most important observations of our study was the correlation between Cys-LT level and skin lesion severity. This may suggest an important link between the degree of skin inflammation and Cys-LT production, as well as justify the use of leukotriene antagonists to alleviate AEDS symptoms. Indeed, intradermal injection of LT-D₄ causes a

wheal-and-flare response that can be blocked by LT-D₄ antagonists (5). The relationship between skin lesion severity and Cys-LT production might also indicate sources of Cys-LTs in AEDS other than from eosinophils. Indeed, LT-B₄ and LT-C₄ have been detected previously in the skin of AEDS patients (20, 22), and LT release from basophils has been shown to be increased in AEDS patients when compared to control subjects (4, 21).

The importance of the IgE-dependent mechanisms in increased Cys-LT production in AEDS is still unclear. We did not find a significant correlation between urinary Cys-LT secretion and total IgE level. However, previous studies have shown that although IgE is important in the development of AEDS the tests investigating IgE-dependent mechanisms (including histamine release) do not always correlate closely with the severity of AEDS.

Findings of the present study suggest that Cys-LTs play a crucial part in the pathogenesis of AEDS. Based on these data and on the case reports of clinical benefit gained from antileukotriene therapy in AEDS (15, 23), randomized and placebo-controlled clinical trials of LT antagonists and LT synthesis inhibitors seem to be warranted (15, 24). Antileukotriene drugs could be used in combination with antihistamines, corticosteroids or other anti-inflammatory drugs in the treatment of persistent AEDS exacerbations.

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