

Skin eosinophilia in patients with allergic asthma, patients with nonallergic asthma, and healthy controls

II: 20-hydroxy-leukotriene B₄ is a potent in vivo and in vitro eosinophil chemotactic factor in nonallergic asthma

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Background: In allergic and nonallergic asthma, eosinophils play an important effector role. However, because the pathogenesis of these types of asthma seems different, the mechanisms responsible for the tissue mobilization of those cells may be different. The in vivo and in vitro migratory response of eosinophils from patients with allergic and nonallergic asthma toward 20-hydroxy-leukotriene B₄ (20-OH-LTB₄), which is reported here, illustrates this.

Methods: By means of the Rebuck skin window technique the in vivo skin mobilizing capacity of intracutaneously applied buffer, LTB₄, and 20-hydroxy (OH)-LTB₄ was evaluated in healthy subjects (n = 6), subjects with allergic asthma (n = 14), and subjects with nonallergic asthma (n = 17). Also the in vitro chemotactic responsiveness of eosinophils from the circulation of both patient groups (both n = 8) toward buffer, LTB₄, and 20-OH-LTB₄ were tested by use of a microchemotaxis chamber technique.

Results: Although none of the substances were capable of inducing macroscopic observable skin reactions, intracutaneously applied 20-OH-LTB₄ had an almost similar capacity to mobilize eosinophils in the skin of the subjects with nonallergic asthma as allergens had in subjects with allergic asthma. In 92% of the tested subjects with nonallergic asthma significant skin eosinophilia was observed. By contrast, LTB₄ did not induce significant skin eosinophilia in both patient groups compared with buffer solution. This in vivo eosinophil mobilizing capacity of 20-OH-LTB₄ in subjects with nonallergic asthma was confirmed by in vitro chemotaxis studies. Dose ranges of both LTB₄ and 20-OH-LTB₄ proved to be potent chemoattractants for eosinophils from patients with nonallergic asthma, but not for those of healthy subjects and those with allergic asthma.

Conclusions: Our results indicate that 20-OH-LTB₄ may be involved in the tissue mobilization of eosinophils in nonallergic asthma and that in vitro 20-OH-LTB₄ (and LTB₄) may act as potent chemotactic factors on eosinophils from those patients. (*J ALLERGY CLIN IMMUNOL* 1993;91:634-42.)

Key words: Asthma, skin eosinophilia, skin window, chemotaxis, leukotriene B₄, 20-hydroxy-leukotriene B₄

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Abbreviations used

HSA:	Human serum albumin
PBS:	Phosphate-buffered saline solution
LTB ₄ :	Leukotriene B ₄
20-OH-LTB ₄ :	20-hydroxy-leukotriene B ₄
RP-HPLC:	Reversed-phase, high-performance liquid chromatography

Eosinophils are thought to actively contribute to the pathophysiologic makeup of asthma.¹ They are abundantly present in the circulation, lung tissue, and spu-

TABLE I. Patient characteristics

Group	No.	Age* (yr)	M/F	Allergy†	Eosinophils* (no./ μ l)
Normal subjects	6	32 $\pm$ 5	3/3	–	190 $\pm$ 32
Allergic asthma	14	30 $\pm$ 4	6/8	+	499 $\pm$ 63 [†]
Nonallergic asthma	17	45 $\pm$ 7	7/10	–	523 $\pm$ 71 [†]

*Mean $\pm$ SEM; $p < 0.05$ compared with control values.

†Allergy (–), No positive intracutaneous skin tests to a panel of five different allergens (house dust mite allergen, grass pollen, tree pollen, animal dander, molds), negative RASTs to this allergen panel and normal total IgE levels; (+), positive intracutaneous skin reactions to the forementioned allergen panel, positive RASTs and elevated total serum IgE levels.

tum of these patients.² Activated eosinophils are present in the lung tissue, in particular in the submucosa, indicating that they may contribute to epithelial damage and as a consequence induce increased bronchial hyperresponsiveness to histamine or methacholine.^{3,4} Increased levels of eosinophil-derived mediators observed in the circulation,⁵ bronchoalveolar lavage fluid,⁶ and sputum⁷ of those patients also indicate eosinophil activation. Beside eosinophils, mainly mononuclear cells (T lymphocytes, monocytes) seem to infiltrate the lung tissue of asthmatic individuals.^{8,9} Also the mononuclear cell types seem to be in an activated state.¹⁰ Why predominantly these two cell types (eosinophils and mononuclear cells) infiltrate the diseased tissue is not yet fully understood.

Two mechanisms may be considered with respect to the rather selective eosinophil infiltration in the tissue: (1) the presence of eosinophil selective chemoattractants and (2) an eosinophil-selective adhesion-transendothelial migration principle. So far, most of the chemoattractants described have not shown selectivity for eosinophils, since they also act as chemotactic factors for neutrophils.^{11–13} Recently interleukin-5 (IL-5) has been reported to be an eosinophil-selective chemotactic factor.^{14,15} This cytokine is also capable of inducing selective eosinophil adherence to endothelial monolayers in a manner dependent on CD11 and CD18.¹⁶ Neutrophils are also capable of adhering to endothelial layers via mechanisms mediated by CD11 and CD18. They even are able to migrate across these layers as a consequence of the adherence.¹⁷ Therefore other principles must be operational to explain the selective eosinophil tissue accumulation. One such mechanism could be via the interaction of very late antigen expression on the eosinophil and vascular cell adhesion molecule-1 expression on the endothelial layer.^{18–21} However, the exact principle has to be further clarified.

Originally, *in vitro* experiments showed platelet-activating factor (PAF) to be a rather selective and potent eosinophil chemotaxin.^{22,23} *In vivo* studies with PAF indicated that PAF mobilized eosinophils after intracutaneous application in the skin at the site of

application.^{24,25} The skin reaction observed after PAF application even paralleled the early- and late-phase skin reaction induced by allergen.²⁴ Although these findings indicate that PAF mobilizes eosinophils in the skin of allergic individuals, it was not capable of mobilizing eosinophils in the skin of individuals with nonallergic asthma,²⁶ although these patients generally have higher blood eosinophil counts.

To find a possible eosinophil mobilizing agent in nonallergic asthma we have tested leukotriene B₄ (LTB₄) and 20-hydroxy LTB₄ (20-OH-LTB₄). The choice of these compounds was based on our *in vitro* chemotaxis experiments with PAF and LTB₄, which showed that LTB₄ had the same chemotactic potency for eosinophils of normal individuals as PAF.¹¹ 20-OH-LTB₄ was chosen as a control because it is the less active metabolite of LTB₄ with respect to neutrophil and eosinophil chemotaxis.^{27,28}

It is surprising to note that 20-OH-LTB₄, but not LTB₄, proved to be a potent chemotactic factor for eosinophils in the skin of patients with nonallergic asthma and in *in vitro* chemotaxis studies with blood eosinophils from these patients.

MATERIAL AND METHODS

Subjects

The subjects participating in this investigation were characterized on the basis of their history, clinical symptoms, results of skin prick tests to a panel of five allergens (house dust mite, grass pollen, tree pollen, animal dander, and molds), specific serum IgE determinations for the same allergen panel, and total IgE levels. When results were positive, they were classified as having allergic asthma, when results were negative, they were classified as having nonallergic asthma. Their characteristics are summarized in Table I. Their asthma met the definition of the American Thoracic Society.²⁹ When this investigation was performed the patients did not show clinical symptoms of asthma and were mainly using symptomatic asthma treatment. Five out of the 17 patients with nonallergic asthma who were tested and three out of the 16 patients (two out of these 16 patients have been omitted from the study) with allergic asthma who were tested were using topical steroid treatment. For the *in vitro* chemotaxis studies blood was collected from eight patients in the allergic and nonallergic asthma groups: only

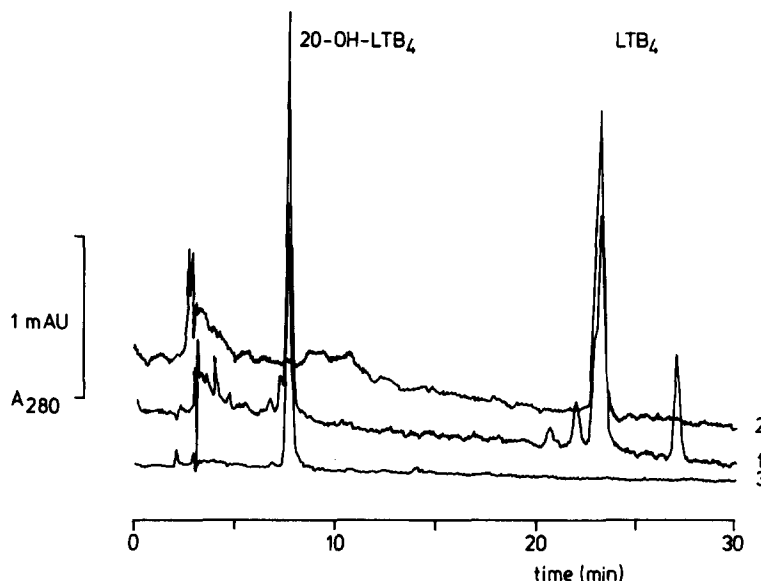


FIG. 1. RP-HPLC chromatogram shows purity of LTB_4 and 20-OH-LTB_4 used for *in vivo* skin reactions.

two patients of both groups used topical steroids. None of the participating patients was using oral steroids or had used this treatment at least 14 days before the skin testing was performed. Other medication (antihistamines, cromoglycate), when necessary, was stopped at least 12 hours before skin testing was performed. This investigation was approved by the ethical committee of the Hochgebirgsklinik Davos Wolfgang. All participating subjects gave their informed consent.

Reagents

Ficoll-paque and Percoll were obtained from Pharmacia AB (Uppsala, Sweden). All *in vitro* chemotaxis experiments were carried out in Geys' balanced salt solution supplemented with 1.5 mmol/L CaCl_2 , heparin (10 IU/ml), 5 mmol/L glucose, and 1.0% human serum albumin (HSA) (wt/vol)/, pH 7.4. All other chemicals were reagent grade.

Skin tests

On the volar surface of both forearms cutaneous wheal and flare reactions were induced by 50 μl of a solution containing the following: (1) buffer (0.3% HSA) in sterile phosphate-buffered saline solution (PBS), (2) LTB_4 (60 ng in buffer), and (3) 20-OH-LTB_4 (60 ng in buffer). The leukotrienes were stored in absolute ethanol at -20°C . The ethanol was evaporated immediately before use, and the leukotrienes were reconstituted in buffer solution for application to the skin or chemotaxis buffer. The purity of the leukotrienes used was regularly controlled by means of reversed-phase, high-performance liquid chromatography (RP-HPLC) (Fig. 1).^{30, 31} RP-HPLC analysis was performed with a CP spher 8 $\mu\text{-C18}$ column (250 $\times$ 4.6 mm; Chrompack, Middelburg, The Netherlands). The solvent system was tetrahydrofuran-methanol-water-acetic acid (25:30:45:0.1, by vol.), which had been brought to pH 5.5 with ammonia. The aqueous phase contained 0.1%

EDTA to prevent binding of cations to the column. A flow rate of 0.8 ml/min was maintained, and the effluent was monitored at 280 nm. The identity of LTB_4 and 20-OH-LTB_4 was confirmed by coelution with reference compounds and by ultraviolet spectrum. A reference mixture containing both LTB_4 and 20-OH-LTB_4 was used in parallel: 1: reference mixture; 2: LTB_4 ; 3: 20-OH-LTB_4 . LTB_4 was obtained from Sigma Chemical Co. (St. Louis, Mo.), and 20-OH-LTB_4 was obtained from Cascade Biochem Ltd. (Berkshire, U.K.). When allergen was applied to the skin instead of the leukotrienes, the concentration used was exactly the same as used in a previous study.²⁶ Allergen was obtained from HAL (Haarlems Allergenen Laboratorium, Haarlem, The Netherlands). In all skin tests the individual's writing arm was used to evaluate the macroscopic skin reactions, whereas the other arm was used to evaluate the skin eosinophilia after applying skin windows for 24 hours.

Macroscopic evaluation of the skin reactions

On one forearm the skin reactions were read after 20 minutes, 6 hours, and 24 hours. The skin reactions were quantified by measuring the maximum diameter of the wheal and flare reaction.

Skin window evaluation of the skin reaction

Twenty minutes after intracutaneous application of the test solutions, the skin was cleaned with ethanol and the epidermis was scraped off over an area of ≈ 5 mm in diameter until a sheen of tissue fluid was noted without bleeding. After skin debris was removed, the site was covered with a glass coverslip with a piece of hard paper of the same size on top, and then the coverslip and paper were taped to the skin.²⁶ Twenty-four hours after application the glass coverslip was removed, air-dried, stained with May-Gruenwald-Giemsa, and mounted onto a microscope slide. Then, in five randomly chosen different areas, which at first sight

TABLE II. Skin window tests

Group	No.	Buffer	Grass pollen
Healthy subjects	6	1 ± 1	1 ± 1
Allergic asthma	14	2 ± 1	15 ± 3*
Nonallergic asthma	17	1 ± 1	0.4 ± 0.2

Eosinophils accumulated in the skin window (relative proportion/500 counted cells) after intracutaneous application of buffer and grass pollen.

* $p < 0.05$ compared with control values.

were rich in eosinophils, 100 cells were differentiated, and the relative proportion of neutrophils, mononuclear cells, and eosinophils per 500 counted cells was quantified by light microscopy (magnification $\times 200$). A significant eosinophilia was considered to occur when the difference in the counted number of eosinophils as compared with the buffer solution was more than 2. In the differential count only intact cells were counted, and no distinction was made between partially degranulated and nondegranulated cells. In our hands this method of quantification of skin eosinophilia proved to be reliable and reproducible. This is extensively documented in a previous report.²⁶

Cell isolation

Blood was collected from healthy individuals (100 to 200 ml) and asthma patients (30 to 50 ml) in 0.4% (wt/vol) trisodium citrate (pH 7.4). Mononuclear cells were removed via separation over isotonic Ficoll-paque (1.077 gm/ml, pH 7.4). After isotonic lysis of the erythrocytes in an ice-cold solution containing 155 mmol/L NH_4Cl , 10 mmol/L KHCO_3 , and 0.1 mmol/L ethylenediamine tetraacetic acid (EDTA) (pH 7.2), the mixed granulocytes were washed and resuspended in RPMI supplemented with HSA (1% wt/vol) and incubated 30 minutes at 37° C to restore initial densities of the cells. After this incubation period the cells were washed and resuspended in PBS supplemented with HSA (1% wt/vol) and trisodium citrate (0.4% wt/vol). After preincubation of the cell suspension for 5 minutes at 37° C, *N*-formyl-methionyl-leucyl-phenylalanine (FMLP, Sigma Chemical Co.) (10 nmol/L) was added to the cell suspension, and the incubation was continued for 10 minutes at 37° C before 1 ml of cell suspension (containing 50 to 80 10^6 cells) was layered on 4 ml of an isotonic Percoll solution (density, 1.082 gm/ml). To prevent cell contamination of the eosinophils with cell debris and remaining erythrocytes, 1 ml of Percoll (density, 1.100 gm/ml) was brought under the Percoll layer of 1.082 gm/ml. After centrifugation (20 minutes, 1000 g_{max} , room temperature) the eosinophils were collected from the interface. The eosinophils were washed and resuspended in Geys' buffer and kept at room temperature until use. The FMLP treatment does not influence eosinophil function as is extensively documented elsewhere.³²⁻³⁴ The purity of the cells was always more than 90%, and the recovery ranged from 40% to 70%.

Chemotaxis assay

Chemotaxis was carried out with use of a modified Boyden chamber assay with a 48-well microchemotaxis chamber

(Neuroprobe, Cabin John, Md.). Chemotaxins or Geys' buffer (30 μl) were placed in the lower compartments. Two filters (cellulose nitrate) were placed between the lower and the upper compartments. The lower filter had a pore width of 0.45 μm (Millipore type HA; Millipore Corp., Bedford, Mass.), and the upper filter had a pore width of 8 μm (Sartorius SM 113; Sartorius AG, Göttingen, Germany). Before use, the filters were soaked in Geys' buffer. Purified eosinophils were placed in the upper compartment (25 μl of 5×10^6 cells/ml). The chemotaxis chambers were subsequently incubated for 2.5 hours at 37° C, unless otherwise stated. After incubation the upper filters were removed, fixed upside down in butanol-ethanol (20%/80%, vol/vol) for 10 minutes, and stained with Weigert solution (1% hematoxylin [vol/vol] in 95% ethanol [vol/vol] and an acidic FeCl_3 -solution [70 mmol/L] mixed in a volume ratio of 1:1). The filters were dehydrated with ethanol, made transparent with xylene, and fixed upside down. The number of cells per 10 high-power fields was determined by light microscopy (magnification $\times 400$). In this way the number of cells that had passed the upper filter was determined.

Statistical analysis

The results of the relative proportion of the various cell types present on the glass coverslip of the skin window chambers are expressed as mean $\pm$ SEM. Statistical analysis was performed by analysis of variance (ANOVA) of single-factor experiments with repeated measures to study the treatment effect. Individual comparisons of the mean effects were performed with the Newman-Keuls and the unpaired Student *t* test; *p* values less than 0.05 were considered significant.

RESULTS

Macroscopic evaluation of the skin reactions

Skin reactions to LTB_4 and 20-OH- LTB_4 were read after 20 minutes, 6 hours, and 24 hours. Almost none of the tested individuals (2 of 39) showed a positive skin reaction to LTB_4 or 20-OH- LTB_4 . These two patients both had allergic asthma and were omitted from the study. One of those patients showed an immediate skin reaction to the buffer solution (positive dermographism), and the other patient underwent (beyond our knowledge) a bronchial challenge with histamine shortly before the skin testing. In contrast, skin reactions to allergen were positive in all patients with allergic asthma.

TABLE III. Skin window tests

Test substance	Dilutions	Patient group								
		Allergic asthma*				Nonallergic asthma*				
LTB ₄	Undiluted (60 ng)	0	3			0	2	6	12	
	× 10	2	2			4	1	0	10	
	× 100	3	0			5	0	0	0	
20-OH-LTB ₄	Undiluted (60 ng)	10	1	3		16	10	14	11	6
	× 10	6	0	3		5	2	4	3	2
	× 100	7	1	2		6	0	0	0	0

Eosinophils (relative proportion/500 counted cells) accumulated in the skin window when dilutions of LTB₄ (60 ng) and 20-OH-LTB₄ (60 ng) were applied intracutaneously.

*Every dilution range represented was tested in a separate patient.

Skin window evaluation

Cellular deposits on skin windows of healthy subjects and subjects with allergic asthma and patients with nonallergic asthma after intracutaneous application of buffer or allergen. When buffer or allergen was applied to the skin of healthy subjects, patients with allergic asthma, and those with nonallergic asthma, the cellular deposits on the glass coverslips demonstrated the presence of neutrophils, eosinophils, and mononuclear cells. Only in patients with allergic asthma did allergen induce significant skin eosinophilia. In healthy subjects or patients with nonallergic asthma, few eosinophils were mobilized (Table II).

Skin eosinophilia induced by concentration ranges of LTB₄ and 20-OH-LTB₄ after intracutaneous application to patients with allergic asthma and those with nonallergic asthma. Concentration ranges of LTB₄ and 20-OH-LTB₄ were applied intracutaneously to test the capacity to mobilize eosinophils in the skin window. Results are presented in Table III. Optimal eosinophil mobilizing capacity in patients with nonallergic asthma was reached at the highest concentration (60 ng) tested of both LTB₄ and 20-OH-LTB₄. In patients with allergic asthma LTB₄ or 20-OH-LTB₄ was hardly capable of mobilizing eosinophils at the highest concentrations. On the basis of these data we carried out all tests with 60 ng of LTB₄ and 20-OH-LTB₄.

Cellular deposits on skin windows of healthy subjects, subjects with allergic asthma, and those with nonallergic asthma after intracutaneous application of buffer, LTB₄, and 20-OH-LTB₄. After intracutaneous application of buffer, LTB₄, or 20-OH-LTB₄ to healthy subjects and subjects with allergic asthma and those with nonallergic asthma, the relative proportions of various cell types, differentiated per 500 counted cells, that had accumulated on the glass coverslip 24 hours after the skin window was applied, were quantified. The results are presented in Figs. 2, A, B, and C.

In healthy subjects, the relative proportion of neu-

trophils (per 500 counted cells) mobilized by LTB₄ and 20-OH-LTB₄ was 357 ± 37 and 317 ± 23 (mean $\pm$ SEM, $n = 6$). This was significantly more than was mobilized by buffer (i.e., 200 ± 52 , mean $\pm$ SEM, $n = 6$; Fig. 2, A). Relatively few eosinophils were mobilized. In two of the six tested individuals 20-OH-LTB₄ seemed to have the capacity to mobilize some eosinophils (NS), whereas in the other four no eosinophils could be detected at all (Fig. 2, B). As a consequence of the preferential neutrophil mobilizing capacity of LTB₄ and 20-OH-LTB₄, the relative proportions of mononuclear cells mobilized onto the glass coverslip were significantly less compared with those mobilized by buffer (145 ± 38 and 179 ± 25 versus 299 ± 52 ; Fig. 2, C).

Although more patients with allergic asthma ($n = 14$) than healthy individuals ($n = 6$) were tested, the cellular distributions observed on the glass coverslips were almost similar to those observed in healthy subjects. The relative proportions of neutrophils mobilized on the glass coverslip by LTB₄ and 20-OH-LTB₄ were slightly less than the ones observed in healthy individuals (buffer, 226 ± 35 ; LTB₄, 325 ± 19 ; and 20-OH-LTB₄, 271 ± 32). Only LTB₄ mobilized significantly more neutrophils than buffer (Fig. 2, A). As in healthy individuals few eosinophils were mobilized by LTB₄ and 20-OH-LTB₄ (Fig. 2, B). Also in patients with allergic asthma the relative proportion of mononuclear cells mobilized by LTB₄ and 20-OH-LTB₄ was less than the proportion mobilized by buffer solution (buffer, 273 ± 35 ; LTB₄, 174 ± 18 ; 20-OH-LTB₄, 228 ± 32). Again, only LTB₄ was significantly less.

In patients with nonallergic asthma ($n = 17$) the relative proportion of neutrophils mobilized onto the glass coverslip by LTB₄ exceeded that of buffer (291 ± 22 versus 201 ± 27 [$p < 0.05$]), whereas for 20-OH-LTB₄ it was the reverse (159 ± 25 versus 201 ± 27 [NS]; Fig. 2, A). In contrast, in this patient group an increased proportion of eosinophils appeared

on the glass coverslip after intracutaneous application of 20-OH-LTB₄ compared with LTB₄ and buffer solution (12 ± 2 vs 3.3 ± 1.0 and 2.5 ± 0.9 , respectively [$p < 0.05$]). This increase is much stronger than the increase observed in any of the other groups studied (healthy individuals and those with allergic asthma). Only two out of the 17 tested patients showed fewer than two eosinophils difference with the buffer solution on the glass coverslip, which was negative according to our definition. All other tested individuals were strongly positive (Fig. 2, B). In this patient group the relative proportion of mononuclear cells mobilized by LTB₄ was significantly less than after buffer, whereas with 20-OH-LTB₄, an almost statistically significant increased proportion of mononuclear cells appeared on the glass coverslip compared with the buffer (buffer, 291 ± 28 ; LTB₄, 205 ± 23 ; 20-OH-LTB₄, 330 ± 26).

When the skin window data obtained from the subjects with allergic asthma and those with nonallergic asthma are compared, it will be clear that buffer solution and LTB₄ behave almost similarly. In contrast, striking differences appeared with 20-OH-LTB₄. In patients with allergic asthma a relatively increased proportion of neutrophils and a decreased proportion of mononuclear cells and relatively few eosinophils were mobilized, whereas in patients with nonallergic asthma a decreased proportion of neutrophils and an increased proportion of mononuclear cells was mobilized. In addition, a strongly increased proportion of eosinophils was mobilized. In this patient group no direct correlation appeared to exist between the relative proportion of eosinophils and mononuclear cells present on the glass coverslip of the individual data. Neither was a correlation between blood eosinophil counts and the number of infiltrated eosinophils present.

In vitro chemotactic potency of LTB₄ and 20-OH-LTB₄ for eosinophils from healthy subjects and subjects with allergic and nonallergic asthma

In a separate series of experiments the in vitro chemotactic potency of dose ranges of LTB₄ and 20-OH-LTB₄ for isolated eosinophils from the circulation of healthy subjects and subjects with allergic asthma and those with nonallergic asthma were evaluated. The results are presented in Figs. 3, A and B. These data indicate that eosinophils from individuals with nonallergic asthma show an increased chemotactic responsiveness in vitro toward LTB₄ and 20-OH-LTB₄ (not statistically different from each other) compared with these cells from individuals with allergic asthma or healthy subjects (data for healthy subjects were almost identical to those of individuals with allergic asthma and are therefore not presented in the figure).

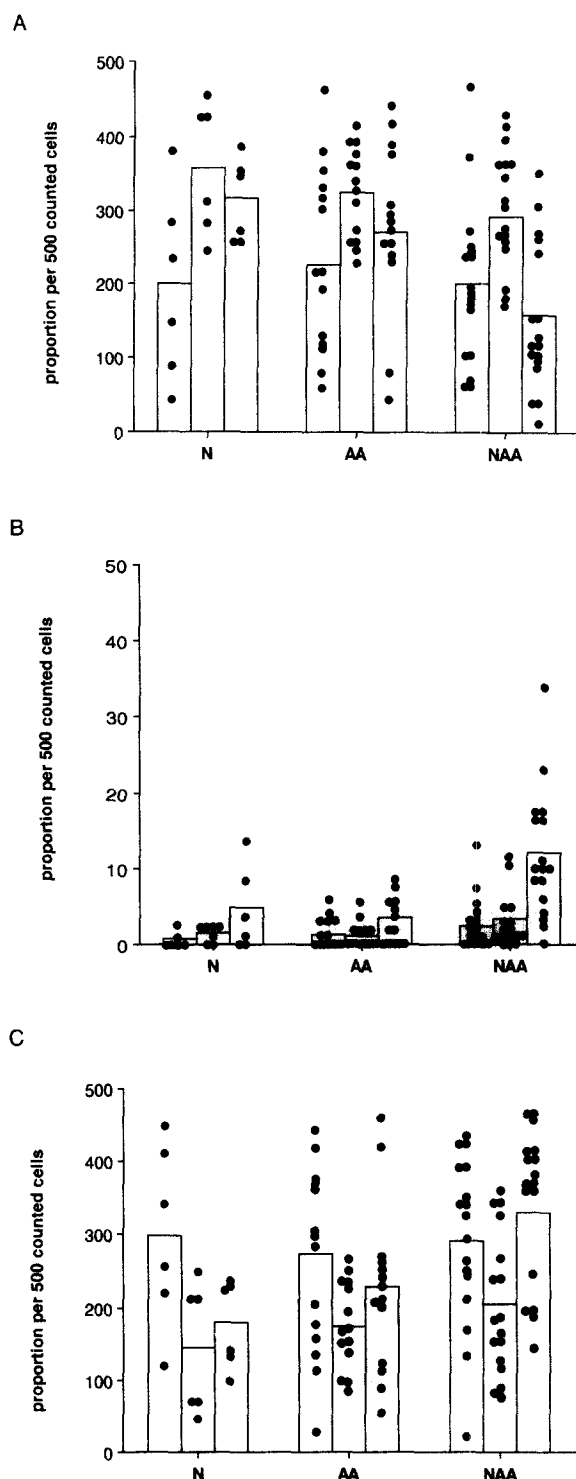


FIG. 2. Graphic representation of individual skin window results. On the Y-axis the proportion of neutrophils (A), eosinophils (B), or mononuclear (C) cells per 500 counted cells are presented. On the X-axis the various groups are presented: N, normal (healthy) subjects ($n = 6$); AA, allergic asthmatic subjects ($n = 14$); NAA, nonallergic asthmatic subjects ($n = 17$). For each group three bars are presented: left bar, data after buffer application; middle bar, data after LTB₄ application; right bar, data after 20-OH-LTB₄ application. Bars represent the arithmetic mean value of the individual data. For statistical evaluation see text.

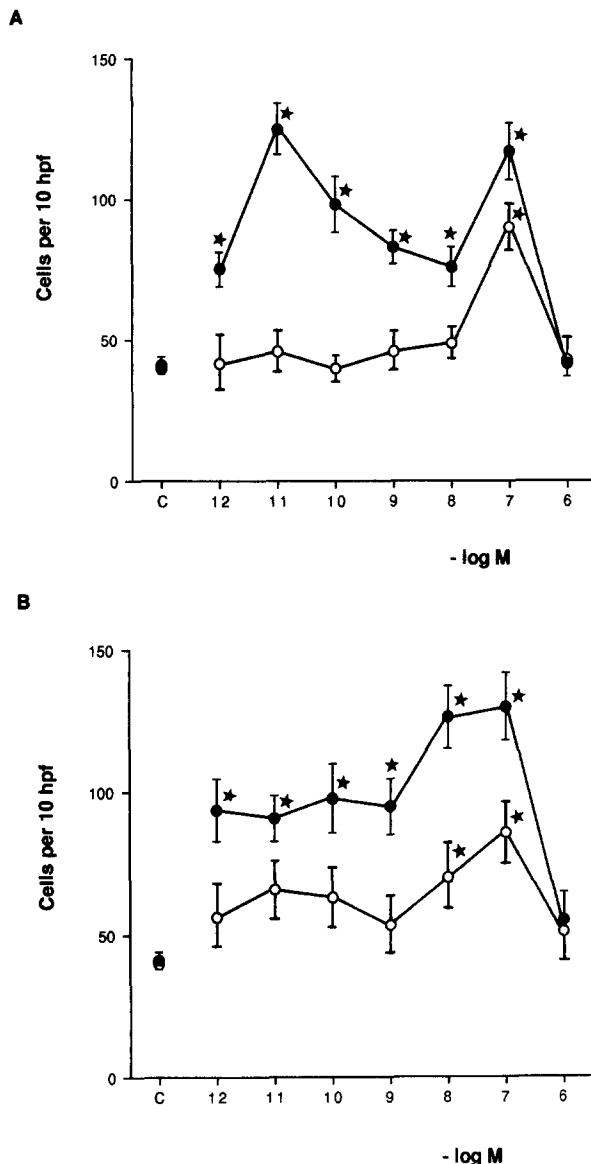


FIG. 3. In vitro eosinophil chemotactic potency of LTB₄ (B) and 20-OH-LTB₄ (A) toward eosinophils from allergic (○) and nonallergic (●) asthmatic subjects. On the Y-axis the number eosinophils per 10 high-power fields are plotted (mean values $\pm$ SEM, $n = 8$). On the X-axis the buffer value (C) and the dose range of the chemoattractant are presented. * $p < 0.05$ compared with the buffer value (unpaired Student t test).

At most concentrations tested the chemotactic responses of eosinophils from individuals with nonallergic asthma were not only significantly increased compared with the buffer value but were also compared with the corresponding value obtained with eosinophils from subjects with allergic asthma. To both chemoattractants a significant chemotactic response already existed at concentrations as low as 10^{-12} mol/L.

DISCUSSION

Pure 20-OH-LTB₄ when applied intracutaneously to the skin of healthy subjects and subjects with allergic asthma and those with nonallergic asthma has the capacity to mobilize a relatively increased proportion of eosinophils in the skin of subjects with nonallergic asthma. This capacity is almost identical to the capacity of allergen to mobilize eosinophils under similar conditions in the skin of individuals with allergic asthma (Table II and Fig. 2, B). In contrast to this reaction, the former reaction is not preceded by an immediate wheal and flare reaction. In vitro chemotaxis experiments demonstrated that this eosinophil mobilizing activity could be the consequence of a direct chemoattractant activity of 20-OH-LTB₄. 20-OH-LTB₄ showed a direct chemotactic activity toward eosinophils from patients with nonallergic asthma and only a very weak chemotactic activity toward eosinophils from patients with allergic asthma. Therefore eosinophils from patients with nonallergic asthma seem to possess receptors sensitive for this compound.

The lack of a macroscopic observable skin reaction after intracutaneous application of 20-OH-LTB₄ suggests that infiltrating and sessile cells in the skin are not activated (directly or indirectly) to release vasoactive mediators. Within 20 minutes after allergen application in subjects with allergic asthma an immediate wheal and flare reaction always occurs. Because in subjects with allergic asthma the same proportion of eosinophils is mobilized as after application of 20-OH-LTB₄ in subjects with nonallergic asthma, these data seem to indicate that the tissue influx of eosinophils is independent of the release of vasoactive mediators by mast cells.

LTB₄ and 20-OH-LTB₄ have been known for their chemoattractant capacity toward neutrophils; 20-OH-LTB₄ is less potent than LTB₄.^{27, 28} Our data suggest that in vivo LTB₄ and 20-OH-LTB₄ possess an almost equal potency to mobilize neutrophils (at least in healthy subjects and subjects with allergic asthma). This provides in vivo evidence for the original report of Dahinden et al.,³⁵ which reported 20-OH-LTB₄ and LTB₄ to be almost equally potent neutrophil chemotactic factors in vitro.

Rosenbach et al.²⁸ reported that in vitro LTB₄ possessed relatively weak chemoattractant activity toward eosinophils, and that 20-OH-LTB₄ possessed an even weaker chemotactic activity than LTB₄, which is in agreement with many findings in neutrophil chemotaxis. Here it is shown that in vitro LTB₄ and 20-OH-LTB₄ are only weakly chemotactic toward eosinophils from healthy subjects and subjects with allergic asthma but strongly chemotactic toward eosinophils

from subjects with nonallergic asthma. In healthy subjects and subjects with allergic asthma LTB_4 seemed slightly more chemotactic than 20-OH- LTB_4 , supporting the data of Rosenbach et al. In subjects with nonallergic asthma 20-OH- LTB_4 seemed slightly more chemotactic for eosinophils than LTB_4 (difference not significant).

Because both LTB_4 and 20-OH- LTB_4 have an almost similar chemoattractant activity toward eosinophils from subjects with nonallergic asthma, the eosinophils seem to possess binding sites that recognize the common part of both compounds. However, as in neutrophils,³⁵ the introduction of the hydroxyl group in LTB_4 seems to increase the binding capacity and thereby the chemoattractant capacity.

Both LTB_4 and 20-OH- LTB_4 are potent chemoattractants for eosinophils from subjects with nonallergic asthma in vitro, whereas only 20-OH- LTB_4 is active in vivo. This seems somewhat contradictory. However, the exact fate of in vivo applied LTB_4 to the skin of subjects with nonallergic asthma is not known. Its conversion may be different from the one into 20-OH- LTB_4 ,³⁶ which could mobilize eosinophils. This possibility seems absent in vitro and needs further investigation.

Our in vitro and in vivo findings raise the question of why eosinophils from subjects with nonallergic asthma and not those of subjects with allergic asthma seem to have relatively high-affinity binding sites for 20-OH- LTB_4 (and LTB_4). A possible explanation could be that circulating eosinophils in these patient groups are exposed to different plasma factors, resulting in differential up and down regulation of particular receptor sensitivities. Therefore these in vivo priming phenomena of eosinophils need more attention in the near future to better understand the pathophysiologic make-up of asthma.^{37, 38}

Taken together, the eosinophil mobilization capacity described herein of the lipid factor 20-OH- LTB_4 in subjects with nonallergic asthma does suggest that this mediator may generate tissue eosinophilia via a direct action on the eosinophil.

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