

Effect of a specific cysteinyl leukotriene-receptor 1-antagonist (montelukast) on the transmigration of eosinophils across human umbilical vein endothelial cells

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Summary

Background Leukotrienes have been implicated in the selective infiltration of eosinophils into the bronchial mucosa in asthma.

Objective We studied whether eosinophil transmigration through cultured human umbilical vein endothelial cells (HUVECs) can be blocked by a specific cysteinyl LT1-receptor-antagonist.

Methods Unstimulated and stimulated eosinophils from patients with asthma and normal controls were subjected to confluent human umbilical vein endothelial cell (HUVEC) monolayers separating the upper and lower chamber of Transwell™-culture plates. Unstimulated eosinophils or cells pre-incubated in the presence of the eosinophil activating cytokines GM-CSF or IL-13 were placed in the upper chambers while PAF, a potent chemoattractant factor for eosinophils, was added to the lower chamber. Migration of eosinophils was quantified by a β -glucuronidase assay.

Results The assumption that eosinophils express CysLT1 (cysteinyl-leukotriene 1)-receptors was based on our demonstration of mRNA-expression for the CysLT-1-receptor by polymerase chain reaction on purified eosinophils. The chemotactic response to PAF was significantly reduced when eosinophils were pre-incubated with montelukast for 15 min. When eosinophils were pre-incubated with GM-CSF and/or IL-13, the migratory response to PAF was also significantly reduced by montelukast.

Conclusion From these data we conclude that the specific cysteinyl LT1-receptor antagonist montelukast can inhibit PAF-induced eosinophil transmigration through cultured HUVEC monolayers.

Keywords: eosinophils, montelukast, chemotaxis, HUVEC

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Introduction

The pathogenesis of bronchial asthma has been associated with the selective infiltration of eosinophils into the bronchial mucosa and large numbers of eosinophils appear in the BAL fluid of patients with asthma following allergen

provocation [1]. A potential cause of the bronchoconstriction in asthma following allergen challenge has been attributed to the release of leukotrienes by eosinophils [2,3] and it has been shown that eosinophils produce leukotrienes in response to mediators such as PAF [4,5]. The mechanisms governing this accumulation of eosinophils in the bronchial interstitium but also their appearance in BAL-fluid and sputum of patients with asthma are still incompletely understood. A number of mediators, including PAF, C5a, fMLP, RANTES and MCP-3 have been

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described as chemotactic for eosinophils [6]. Incubation of eosinophils in agarose gel in the presence of a chemotactic LTD₄ gradient has also been shown to cause eosinophil chemotaxis and this could be inhibited by the addition of a specific cysteinyl LT1-receptor-antagonist [7]. Furthermore, leukotrienes can cause a predominantly eosinophil containing infiltrate *in vivo* [8].

Recently, it has been shown that cysteinyl-leukotriene-inhalation can increase eosinophil numbers in bronchial biopsies of patients with asthma, suggesting that these leukotrienes are indeed chemotactic for eosinophils *in vivo* [9]. There is also evidence that inhibition of cysteinyl-leukotrienes by a specific cysteinyl LT1-receptor antagonist can reduce the influx of eosinophils into sputum [10], bronchial epithelium [11] and BAL-fluid [12] following segmental allergen challenge. Furthermore, eosinophil numbers in peripheral blood have been reported to decrease following treatment with a specific cysteinyl LT1-receptor antagonist, and infiltration of eosinophils into tracheal strips has been reduced by the addition of a leukotriene-synthesis-inhibitor *in vitro* [13–15]. Whether these actions are due to a reduction in the concentration gradient of leukotrienes or whether leukotrienes can in fact enhance eosinophil chemotaxis to a given stimulus remains as yet unclear. In order to elucidate whether eosinophil chemotaxis can be directly influenced by interfering with the CysLT1-receptor we investigated the effect of the specific cysteinyl LT1-receptor-antagonist montelukast on the transmigration of eosinophils across human umbilical vein endothelial cells *in vitro*.

Materials and methods

Patients

Twelve allergic asthmatics, five males and seven females, mean age 25.6 ± 1.3 years and eight non-allergic donors, six males and two females, mean age 25.8 ± 2.4 years with no history of asthma, were studied. All patients suffered from allergic asthma as defined elsewhere [16]. All had a history of intermittent wheeze, chest tightness, cough and sputum production either spontaneously or exercise-induced, and either a clinical history for bronchial hyperreactivity or a positive bronchoprovocation test with carbachol. All patients had previously been diagnosed as asthmatics by an independent physician. Each patient had either a positive skin prick test to common aeroallergens (Allergopharma, Reinbek, Germany), an elevated total IgE level (173.6 ± 54.8 kU/L) or elevated specific IgE levels of >0.35 kU/L (Kabi Pharmacia CAP System, Uppsala, Sweden) to their respective allergen as well as a history of reversible bronchoconstriction after inhalation of these particular allergens. Only four patients had a low total

Table 1. Patient characteristics

	Sex	Age	FEV ₁ (baseline) (L)	FEV ₁ % pred	IgE (kU/L)	Current therapy
1	F	33	2.81	90	71.8	None
2	M	29	5.27	112	575	β ₂
3	M	25	4.07	93	375	None
4	F	32	2.00	91	–	β ₂ , ICS
5	M	29	4.27	79	34.3	β ₂ , ICS
6	M	21	4.51	93	375	β ₂ , T
7	F	24	2.59	90	77.9	None
8	F	28	2.92	84	199	None
9	F	21	3.28	100	105	None
10	F	23	3.57	109	54.6	β ₂ , ICS
11	F	19	2.40	73	174.0	β ₂
12	M	23	6.24	130	–	ICS

FEV₁ = forced expiratory volume in one second; % pred. = percentage of predicted value; IgE = immunoglobulin E; β₂ = β₂ agonists; ICS = inhaled corticosteroids; T = theophylline.

IgE level but a clear history of allergen induced bronchoconstriction.

Baseline FEV₁ (forced expiratory volume in 1 s) in percentage of predicted and current therapy are listed in Table 1. None of the patients had been taking non-steroidal anti-inflammatory drugs (NSAIDs) prior to the study. Normal, non-allergic donors with no history of asthma, eczema, rhinoconjunctivitis, chronic sinusitis or any other disease associated with eosinophilia served as controls. None of them had been taking any NSAIDs, antihistamines or any other drug prior to the study. All patients and normal subjects gave their written informed consent. The study protocol was approved by the Ethics Committee of the University of Freiburg.

Isolation of human eosinophils

Eosinophils were isolated from the peripheral blood of normal controls and patients with asthma. Briefly, whole blood was diluted 1 : 1 with PBS (Gibco, Paisley, UK) and aliquots of 25 mL were layered onto 20 mL of isotonic Percoll (density 1.080 g/mL) (Pharmacia, Uppsala, Sweden) in 50 mL plastic tubes (Falcon Plastics, Oxnard, CA) and centrifuged for 30 min with 1000 g at 4 °C. The supernatant and mononuclear cells at the interphase were discarded and the pellet containing neutrophils and eosinophils retained. Contaminating erythrocytes were removed by hypotonic lysis. The granulocytes were washed twice in PBS containing 2% fetal calf serum (FCS) (Gibco, Paisley, UK). Purification of eosinophils was then

performed by negative selection with anti-CD16-coated magnetic microspheres (MACS, Miltenyi, Bergisch-Gladbach, Germany). CD16 microbeads (5 μ L per 1×10^7 neutrophils) were added to the cell suspension and incubated for 30 min at 4 °C under constant gyration. The cell suspension was then added onto the separation column. Eosinophils were eluted from the magnetic field with 40 mL ice-cold PBS/2% FCS. Purity of eosinophil preparations thus reached $\geq 99\%$ with some contaminating mononuclear cells and neutrophils. Eosinophil viability as assessed by propidium-iodide staining was consistently $> 97\%$.

Isolation of human umbilical vein endothelial cells (HUVECs)

Endothelial cells were obtained from human umbilical cord veins. Both ends of the umbilical vein were cannulated and perfused with PBS/EDTA 0.03% several times in order to remove remaining blood. Then the umbilical cord veins were filled with 5 mL of trypsin/PBS solution (PromoCell, Heidelberg, Germany). After incubation at 37 °C for 30 min, the endothelial cells were removed by flushing the cord veins with 5 mL PBS/EDTA 0.03% and collected in 10 mL PBS/10% FCS. The cells were sedimented and resuspended in endothelial cell growth medium (PromoCell).

Cell culture

HUVECs from the first or second passage were cultured on collagen-coated (2% collagen in PBS for 20 min at 37 °C) Transwell™ culture inserts (6.5 mm² diameter polycarbonate membrane with 5 μ m pores from Costar, Cambridge, MA, USA) in endothelial growth medium. Confluence of the monolayer was confirmed by light microscopy. Four hours before transmigration assays were performed, the medium was replaced by RPMI-1640 without glutamine (Seromed, Germany) containing 0.1% BSA.

Transmigration assay

Eosinophil chemotactic activity was measured in Transwell™ culture plates (Costar). Freshly isolated eosinophils from asthmatic subjects and healthy donors were purified as described and then placed (100 000 cells/well) in the upper chambers (on top of the HUVEC layer) while PAF (10^{-5} M) was added to the lower chamber. After incubation for 2 h at 37 °C in a humidified atmosphere with 5% CO₂, the filter unit with the HUVEC layer was removed and migration of eosinophils into the lower chamber was quantified by assaying for β -glucuronidase activity. Briefly, cells were lysed with 1% Triton (Sigma,

Deisenhofen, Germany) and incubated for 12 h with 100 μ L p-nitrophenyl- β -D-glucuronide (3 mg/mL) (Sigma). The reaction was stopped by adding 100 μ L glycine buffer (3% glycine, pH 10.3). The optical density was measured by an ELISA reader at 405 nm and compared with previously established standard curves using defined cell counts. The results are expressed as a chemotactic index which is defined as the number of cells measured in the bottom well in response to PAF with or without pre-incubation with montelukast and/or GM-CSF/IL-13 divided by the number of cells which transmigrated spontaneously in response to medium alone. According to this definition the spontaneous, unstimulated migration of eosinophils resulted in a chemotactic index of 1. Measurements were performed in duplicates and are expressed as means. Montelukast was used in our experiments in concentrations of 10^{-5} M which were also chosen in previous *in vitro* studies [17].

Survival assay

Eosinophil viability was assessed by propidium iodide. 10^5 cells were washed once in PBS/2% FCS, resuspended in 200 μ L of a propidium iodide solution (0.5 μ g/mL in PBS) and left for 5 min. Relative proportions of viable to non-viable cells were determined by flow-cytometry (FACScan, Becton Dickinson, Heidelberg, Germany) of at least 3000 cells.

RNA isolation, reverse transcription and PCR amplification

Total cellular RNA was extracted from cultured eosinophils (1×10^6 cells) following lysis with PqGold (PqLab, Erlangen, Germany). The upper phase was transferred and the RNA was precipitated with iso-propanol. The total RNA sediment was reverse transcribed using ProStar™ first strand synthesis Kit from Stratagene (Heidelberg, Germany). One-tenth of the cDNA was amplified by PCR for 35 cycles (denaturation at 94 °C, annealing at 67 °C, extension at 72 °C, each for 1 min) in a Biometra T-gradient thermo-cycler under standard conditions using Taq-Polymerase from Finnzymes (Espoo, Finland). PCR primers were designed according to the gene sequence AC: AF119711; PCR-product length: 410 bp. One-fifth of the PCR reaction product was separated by flat-bed electrophoresis in 2% agarose gels (USB, Cleveland, USA) and detected by ethidium-bromide staining.

Forward PCR-primer: CTTCTGCCACATGCCATGAC
Reverse PCR-primer: CCTACACACAAACCTGGCT

Statistics

The results are expressed as mean $\pm$ standard error of the

mean (SEM). Statistical comparisons of migrated eosinophils and viability were performed using the Wilcoxon signed rank test. A P -value of <0.05 was considered as statistically significant.

Results

Spontaneous transmigration of human eosinophils through the endothelial cell barrier

Transendothelial migration of freshly isolated eosinophils from asthmatic subjects and healthy donors was assessed as described. There was no statistically significant difference between the spontaneous migration of unstimulated eosinophils from normal controls ($26.8 \pm 4.8 \times 10^3$ cells, $n = 8$) and patients with asthma ($24.4 \pm 5.0 \times 10^3$ cells, $P = n.s.$, $n = 12$) across HUVECs in the absence of PAF (Fig. 1).

Migration of eosinophils through a HUVEC layer in response to a chemotactic gradient of platelet activating factor

Since platelet-activating factor (PAF) has been described as a potent chemotactic stimulus for eosinophils we examined the migratory response of isolated peripheral blood eosinophils from patients with asthma and normal controls in response to a chemotactic gradient of PAF. Addition of PAF to the lower chamber of the Transwell™ culture plates strongly augmented the movement of eosinophils from

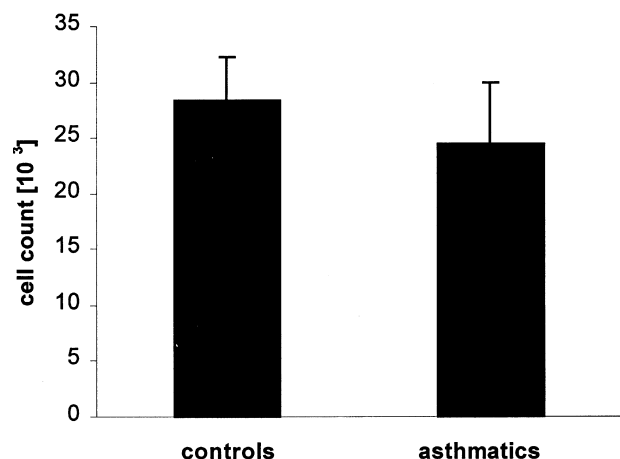


Fig. 1. Comparison of the spontaneous migration of eosinophils from healthy donors and from asthmatics. Isolated eosinophils from healthy donors and from asthmatics were transferred in the upper chamber of the Transwells™ with endothelial monolayers and incubated for 2 h. Data are shown as mean $\pm$ SEM of migrated cells. Controls, $n = 8$, and asthmatics, $n = 12$; $P = n.s.$ (cell-count $\times 10^3$).

normal controls (mean chemotactic index $\pm$ SEM: 2.8 ± 0.4 ; $n = 8$) as well as asthmatic subjects (chemotactic index 2.4 ± 0.2 ; $n = 12$) compared to the spontaneous transendothelial cell movement (index = 1) (both: $P < 0.01$) (Fig. 2). The migratory response of eosinophils to PAF was slightly lower in cells from patients with asthma (mean cell-count of migrated cells $\times 10^3 \pm$ SEM: 54.4 ± 8.6 ; $n = 12$) when compared with normal controls ($71.2 \pm 11.9 \times 10^3$ cells; $n = 8$) although this difference failed to reach statistical significance.

Influence of montelukast on transendothelial chemotactic migration of eosinophils

To study whether the migratory activity of eosinophils can be affected by a leukotriene-receptor antagonist, eosinophils were pre-incubated with the selective and highly specific CysLT1-receptor antagonist montelukast before cells were subjected to the transmigration assay. The transmigration of eosinophils in response to PAF was significantly attenuated in cells from patients with asthma as well as normal controls when cells were incubated with montelukast in a concentration of 10^{-5} M for 15 min before the cells were added to the transmigration wells (chemotactic index/controls: 2.8 ± 0.4 without montelukast vs. 1.6 ± 0.1 with montelukast, $P < 0.01$, $n = 8$, Fig. 2a; chemotactic index/asthmatics: 2.4 ± 0.2 without montelukast vs. 1.3 ± 0.2 with montelukast, $P < 0.01$, $n = 12$, Fig. 2b).

Interestingly, in five of 12 cases the migratory response of eosinophils to PAF decreased below the baseline level of spontaneous migration following incubation with montelukast which, however, was not seen in controls (Fig. 2b).

Effect of GM-CSF on pre-stimulated eosinophils from healthy donors

In order to partially mimic the *in vivo* environment of eosinophils from patients with asthma, eosinophils from healthy donors were pre-incubated in the presence of GM-CSF which has been measured in elevated concentrations in bronchoalveolar lavage fluid in asthma and which has been shown to upregulate several functions of normal eosinophils. However, pre-incubation with GM-CSF did not lead to a significant increase in the migratory response towards the chemotactic gradient of PAF (migration index of eosinophils from normal controls without GM-CSF: 2.8 ± 0.4 ; with GM-CSF: 2.1 ± 0.2 , $P = n.s.$, $n = 8$) (Fig. 3a).

GM-CSF pre-incubated eosinophils from asthmatics

Several lines of evidence suggest that eosinophils from

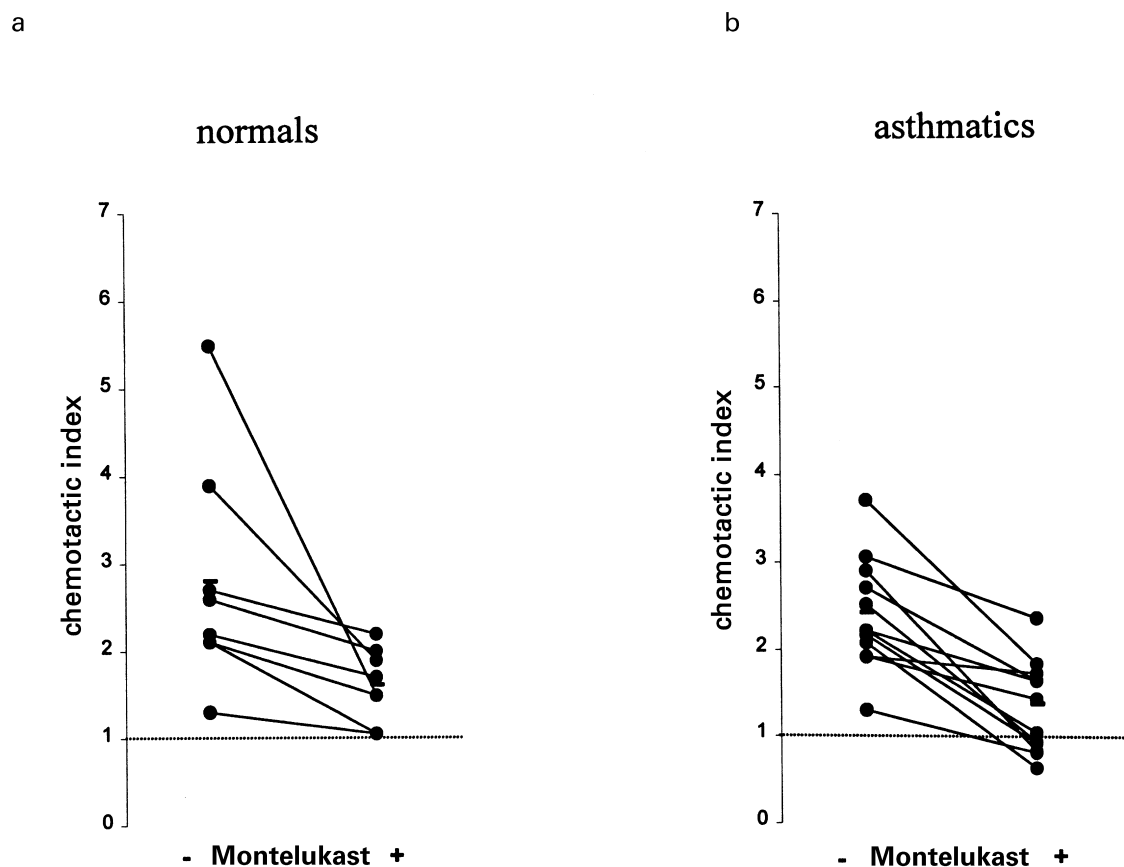


Fig. 2. Influence of montelukast on PAF-induced chemotactic migration of eosinophils through the endothelial monolayer. Eosinophils were incubated for 15 min in the absence or presence of 10^{-5} M montelukast. Thereafter, eosinophils were transferred to TranswellsTM and exposed for a further 2 h to 10^{-5} M PAF in the lower chamber. Data are presented as a chemotactic index of either subject from 8 and 12 independent experiments with eosinophils from healthy donors (a) and asthmatics (b), respectively. The addition of montelukast to eosinophils from asthmatics reduced the chemotactic index in 5 of 10 experiments below that of the spontaneous migration (index 1) graphically expressed as dotted line (b) (black bars indicate mean values).

patients with asthma have been exposed to elevated concentrations of GM-CSF. We therefore investigated whether the addition of GM-CSF can further augment the chemotactic response of eosinophils to PAF. Interestingly, similar to eosinophils from normal controls there was no significant change in the chemotactic response of eosinophils from patients with asthma to PAF when cells were pre-incubated in the presence of GM-CSF (migration index of eosinophils from asthmatics without GM-CSF: 2.4 ± 0.2 ; with GM-CSF: 3.5 ± 0.6 , $P = n.s.$, $n = 12$) (Fig. 3b).

IL-13 pre-incubated eosinophils from asthmatics

Since IL-13, which can activate eosinophils has been shown to appear in elevated concentrations in the BAL fluid following allergen provocation and has been correlated with the influx of eosinophils into the bronchial lumen

we investigated whether the addition of IL-13 to eosinophils from patients with asthma can augment their migratory response *in vitro*. Again, there was no significant increase in the chemotactic response to PAF when cells from patients with asthma were pre-incubated in the presence of IL-13 (migration index of eosinophils from asthmatics without IL-13: 2.4 ± 0.2 ; with IL-13: 2.4 ± 0.2 , $P = n.s.$, $n = 12$). In addition, no further increase was observed when cells were incubated in the presence of GM-CSF and IL-13 (migration index of eosinophils from asthmatics without additional GM-CSF/IL-13: 2.4 ± 0.2 ; with GM-CSF and IL-13: 2.4 ± 0.3 , $n = 7$).

Influence of montelukast on pre-incubated eosinophils from healthy controls

Similar to eosinophils from patients with asthma, cells from normal controls were pre-treated with GM-CSF (10 ng/mL)

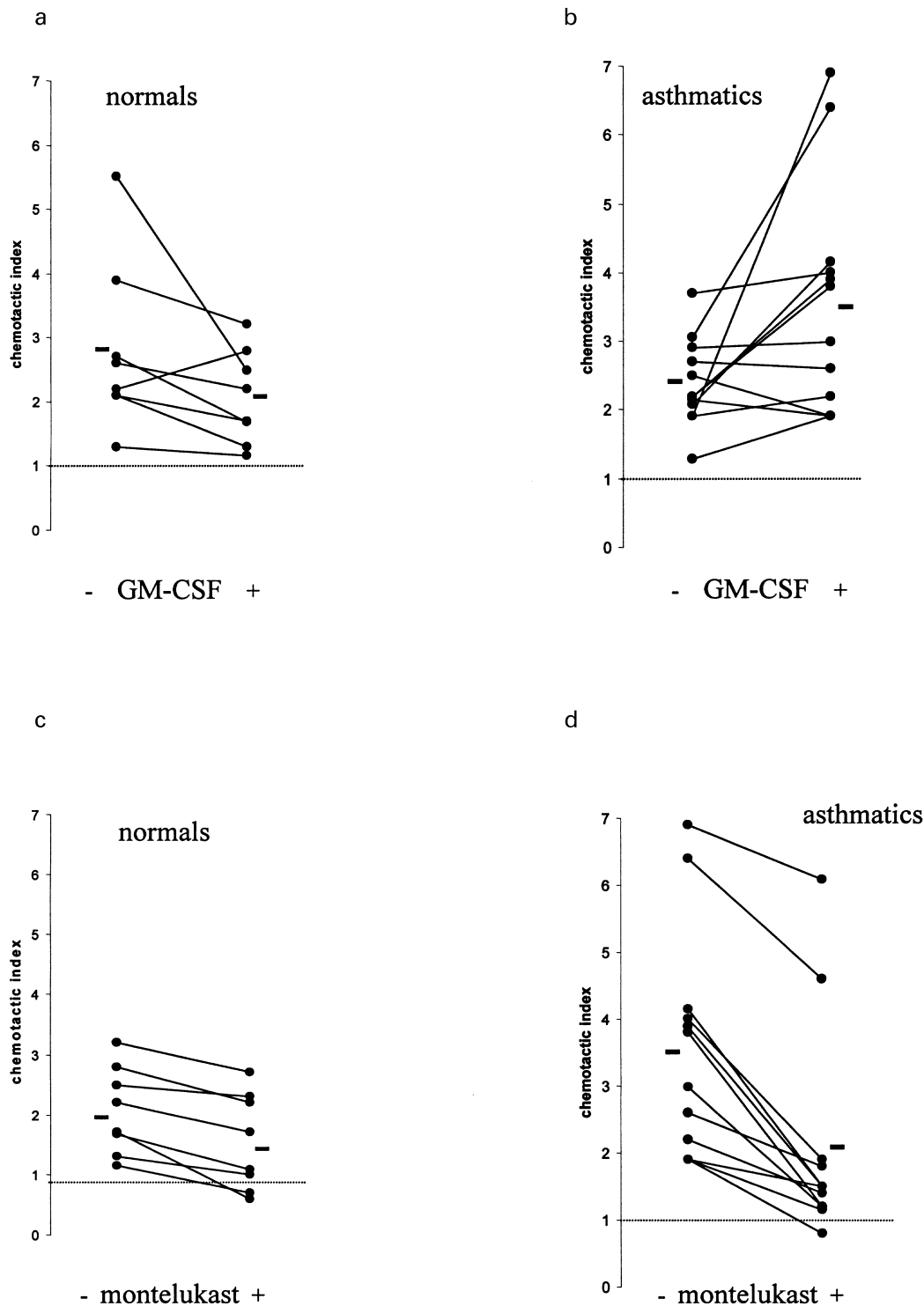


Fig. 3. Influence of GM-CSF on PAF-induced chemotactic migration of eosinophils through the endothelial monolayer. PAF-induced transendothelial migration of eosinophils from controls (Fig. 3a) and asthmatics (Fig. 3b) pre-incubated for 24 h with GM-CSF (10 ng/mL) were compared to the chemotactic response of unstimulated eosinophils. Figure 3 (c) and (d) show the migratory response of GM-CSF-pre-treated eosinophils from controls (3c) and asthmatics (3d), respectively, which were thereafter washed with medium and incubated for a further 15 min in the absence and presence of 10^{-5} M montelukast and transferred to TranswellTM with PAF (10^{-5} M) as chemoattractant in the lower chamber. Data are presented as a chemotactic index of each individual subject (black bars indicate mean values).

for 24 h. Eosinophils were then incubated in the presence or absence of montelukast to investigate the influence of a specific cysteinyl LT₁-receptor-antagonist on pre-activated eosinophils from healthy donors. As shown in Fig. 3(c) the addition of montelukast caused a significant inhibition of eosinophil transendothelial migration compared to cells which were not pre-treated with the leukotriene-receptor-antagonist (chemotactic index/controls: 2.1 ± 0.2 (GM-CSF) vs. 1.5 ± 0.3 (montelukast), $P < 0.05$, $n = 8$).

The effect of montelukast on transmigration of pre-activated eosinophils from asthmatic subjects

Despite the fact that the migratory activity of eosinophils was not significantly changed by GM-CSF and/or IL-13, the addition of montelukast still caused a significant reduction in eosinophil transmigration in this setting (chemotactic index/asthmatics: 3.5 ± 0.6 (GM-CSF) vs. 2.1 ± 0.6 (montelukast), $P < 0.01$ (Fig. 3d) (data not shown); chemotactic index/asthmatics: 2.4 ± 0.4 (IL-13) vs. 1.4 ± 0.1 (montelukast), $P < 0.05$; chemotactic index/asthmatics: 2.4 ± 0.3 (IL-13/GM-CSF) vs. 1.3 ± 0.1 (montelukast), $P < 0.05$, $n = 7$).

Montelukast and eosinophil viability

In order to exclude a cytotoxic effect of montelukast eosinophil viability was measured following the addition of increasing concentrations of montelukast. There was no significant change in the percentage of viable eosinophils

as measured by propidium iodide exclusion when cells were incubated over the dose range of 10^{-9} M to 10^{-5} M montelukast (data not shown). To assess that the cytokines used in our study did indeed activate other aspects of eosinophil physiology, cell viability following incubation with GM-CSF and IL-13 was measured. There was a significant increase in eosinophil viability following incubation with GM-CSF and IL-13 (data not shown).

Detection of CysLT₁ receptor specific mRNA from purified eosinophils

Since the expression of the CysLT₁-receptor has not been described on eosinophils, we amplified the mRNA specific for CysLT₁ using primers designed from the recently described gene sequence of the CysLT₁ receptor [18]. As shown in Fig. 4, expression of the CysLT₁-receptor mRNA is clearly detectable on eosinophils.

Discussion

Several lines of evidence suggest that cysteinyl leukotrienes C₄, D₄, and E₄ play a role in the selective accumulation of eosinophils in asthma. Previous studies have suggested that leukotrienes are directly chemotactic for eosinophils [7]. In these investigations the migratory response of eosinophils to a chemotactic gradient of cysteinyl-leukotrienes was analysed. There is increasing evidence that leukotriene receptor antagonists can reduce the chemotactic response to leukotrienes *in vitro* as well as the influx of eosinophils into the bronchial tree in patients with asthma [7,10–12].

To investigate the effect of the specific cysteinyl LT₁-receptor-antagonist montelukast on eosinophil chemotaxis a modified Transwell™-transendothelial-model according to Kitayama *et al.* was used in this study [6]. In this system human umbilical vein endothelial cells were chosen as the migration barrier instead of naked filter systems in order to also incorporate diapedesis through intact confluent HUVEC layers assuming that this might be a more physiological barrier than the Boyden chamber system.

Extending previous studies in which eosinophils from animal models of asthma were used we have now studied the migratory response of eosinophils from normal controls and patients with asthma [15,19,20]. Thus, possible pre-stimulation of eosinophil chemotaxis *in vivo* would have been incorporated into our results. Interestingly, however, in this setting there were no major differences in the migratory response of eosinophils from normal controls and patients with asthma to PAF. PAF was chosen in our experiments since it has been established as a chemotactic stimulus for eosinophils in similar experimental settings [21–26] and has been shown to induce leukotriene

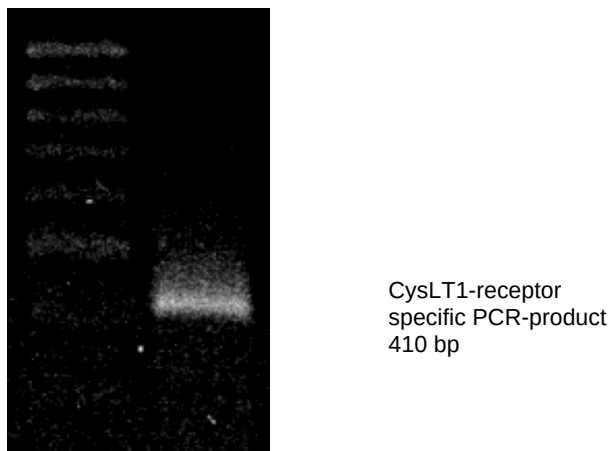


Fig. 4. CysLT₁-receptor specific mRNA expression in human eosinophils. Total RNA was isolated from eosinophils purified from healthy controls, reverse transcribed and amplified with CysLT₁-receptor specific PCR-primer. A representative photograph of an ethidium bromide stained gel with CysLT₁-receptor specific RT-PCR product from three independent experiments is depicted.

synthesis from eosinophils [4,5]. Since eosinophils have been shown to release leukotrienes in response to PAF [22,24–27] and since there is no evidence that montelukast has any direct effect on eosinophil chemotaxis we would assume that in our experiments PAF augmented leukotriene release from eosinophils. This is in line with previous *in vivo* evidence where the effects of PAF administered by inhalation to patients with asthma were inhibited by the leukotriene synthesis inhibitor zileuton [28].

Because endothelial cells do not express 5-lipoxygenase and are thus not able to synthesize leukotrienes *de novo* [29] they are an unlikely candidate for leukotriene production in our experiments. We cannot exclude that transcellular metabolism of leukotrienes in which LTA₄ from eosinophils can be metabolized by endothelial cells to LTC₄, D₄, and E₄, thus causing a concentration gradient towards the HUVEC layer [29]. However, it is most likely that leukotrienes in this study were indeed produced by eosinophils. Since there is no evidence suggesting that these leukotrienes produced by eosinophils caused a chemotactic concentration gradient we speculate that autocrine production of leukotrienes augments the migratory response of eosinophils to another chemotactic stimulus such as PAF.

It can be speculated that in our system leukotrienes produced by eosinophils might have led to PAF generation from endothelial cells, as previously described by McIntyre *et al.* which could further augment the chemotactic concentration of this mediator [30].

In order to mimic inflammatory changes in BAL fluid in our setting, cytokines such as GM-CSF and IL-13 which have been reported in elevated concentrations in BAL-fluid of patients with asthma and have been shown to upregulate eosinophil function in several studies we used GM-CSF and/or IL-13 to pre-incubate eosinophils from normal controls as well as patients with asthma [31–34]. Although it has been reported that leukotriene release from HL-60 cells can be enhanced by GM-CSF suggesting that GM-CSF might also upregulate leukotriene production by eosinophils we were unable to find any increase in the chemotactic response of eosinophils following incubation with GM-CSF [35]. This observation is in line with previous reports which have yielded conflicting results about the chemotactic response of eosinophils following the addition of GM-CSF and/or IL-13. While Ebisawa *et al.* demonstrated augmented movement of eosinophils following pre-incubation with GM-CSF our results are in agreement with Moser *et al.* who could not find an increase in transendothelial migration of eosinophils after pre-incubation with GM-CSF for 24 h [36,37].

Interestingly, the migratory response of both un-stimulated as well as IL-13 and/or GM-CSF stimulated eosinophils was inhibited by the addition of non-toxic concentrations of

montelukast. This reduction in eosinophil migration suggests that eosinophils express functional CysLT₁-receptor which might play a role in the regulation of eosinophil function. In order to substantiate the hypothesis that eosinophils do indeed express such a receptor we performed RT-PCR using specific primers for the newly discovered CysLT₁-receptor [18]. By doing so, our study is the first in demonstrating CysLT₁-receptor mRNA expression in eosinophils. Based on the response of eosinophils to the addition of a specific cysteinyl LT₁-receptor-antagonist *in vitro* and *in vivo* it can be assumed that this mRNA expression indeed translates into the expression of a functional receptor.

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