

ORIGINAL PAPER

Increased expression of collagen receptors: $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins on blood eosinophils in bronchial asthma

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Clinical and
Experimental
Allergy

Summary

Background Eosinophils are one of the major effector cells in bronchial asthma. Their infiltration of airways correlates with the asthma severity. Recruitment and activation of eosinophils are partially mediated by integrins $\alpha_4\beta_1$ and $\alpha_4\beta_7$. Collagens type I and IV constitute important components of extracellular matrix and vascular basement membrane, respectively. Therefore, collagen-binding integrins ($\alpha_1\beta_1$ and $\alpha_2\beta_1$) may also play a role in eosinophil lung infiltration.

Objective To evaluate the possible presence of $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins on peripheral blood eosinophils from asthmatic subjects.

Methods Collagen receptors were studied on eosinophils separated by immunomagnetic CD16-negative method from healthy donors ($n = 13$) and patients with moderate persistent atopic bronchial asthma ($n = 15$). Surface receptor identification was performed by flow cytometry and cell adhesion assay.

Results Eosinophils isolated from the patients showed increased expression of both $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins as compared with healthy controls. Moreover, adhesive function of eosinophils to collagen type IV was inhibited by snake venom disintegrins: viperistatin and obtustatin. These disintegrins contain KTS active motif and are specific inhibitors of $\alpha_1\beta_1$ integrin.

Conclusion We demonstrated for the first time that collagen receptors: $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins are overexpressed on the surface of peripheral blood eosinophils of asthmatic subjects.

Further studies may reveal potential application of KTS-disintegrins or their structural analogs for therapy of bronchial asthma.

Keywords asthma, disintegrin, eosinophil, extracellular matrix, integrin

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Introduction

Bronchial asthma is one of the most common respiratory diseases, affecting approximately 5% of adults and up to 10% of children. The prevalence of asthma is constantly increasing [1]. Despite the substantial progress in therapy made in the last 20 years up to 40% of adult patients remain symptomatic, and up to 5% have a condition which is 'difficult to control' [2]. It is probably because pathogenesis of asthma is largely unknown and poorly understood. Thus, therapeutic modalities are still focusing

on the control of airway inflammation and asthma symptoms, rather than targeting cause(s) of disease.

Eosinophils are one of the major effector cells in chronic airway inflammation, characteristic for bronchial asthma [3, 4]. They increase in the airways and bronchoalveolar lavage (BAL) fluid after allergen challenge and support asthmatic inflammatory reaction through the release of eosinophil-derived cationic proteins, lipid mediators, reactive oxygen species and cytokines [5]. Eosinophils play also an important role in airway remodelling – another feature of asthma [6, 7]. However, the mechanisms that are involved in eosinophil recruitment to the lung, as well as the pathways of their activation have not been fully elucidated.

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In general, cell migration through blood vessels and into inflamed tissues is mediated by cell-surface receptors and their extracellular matrix ligands. Among these receptors, a major role is played by the integrins – a family (24 members) of heterodimeric glycoproteins composed of non-covalently associated α and β subunits. They are involved in physiological control of both, cell–cell and cell–extracellular matrix interactions, participating this way in various immunological processes (e.g. leukocyte diapedesis), endothelial cell functions, angiogenesis, and platelet aggregation [8]. Their relevance to lung physiology and pathology is also well documented [9]. So far more than 10 adhesion molecules have been identified on the surface of human eosinophils [10].

Collagens form a structural scaffold for all tissues. The basement membrane of small vessels contains collagen IV, while collagen I (and III) is a major component of extracellular matrix in the lungs [11, 12], where it increases in some asthmatic subjects, possibly as a part of remodelling process mediated by eosinophils [13]. Major collagen receptors include $\alpha_1\beta_1$ and $\alpha_1\beta_2$ integrins. They are widely distributed on many cells, mainly leucocytes, where they appear in a delayed manner (hence their former name: ‘very late antigens’ – VLA) after immunological activation [11]. Both integrins perform a wide range of biological functions. They are involved in: tissue remodelling, up-regulation of collagen synthesis, formation of new blood vessels; they also support attachment to the basement membrane and extravasation of leukocytes on their way into inflammatory sites [11]. The $\alpha_1\beta_1$ integrin binds preferentially to collagen IV, while integrin $\alpha_2\beta_1$ to collagen I. Involvement of collagen receptors in the lymphocytic inflammatory response has been recently reported [14]. Moreover, beneficial effects of anti- $\alpha_1\beta_1$ monoclonal antibodies have been described in certain animal models of immunologically mediated diseases, including inflammatory bowel disease [15, 16], arthritis [14], and allergen-induced leukocyte recruitment to the lungs associated with late airway response in sheep [17]. This latter finding points out, that integrin collagen receptors may serve as therapeutic targets in controlling inflammation in bronchial asthma.

In this report, we present evidence that $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins exist on the surface of peripheral blood eosinophils of healthy subjects and increase their expression in asthma patients. Moreover, we show that $\alpha_1\beta_1$ integrin-dependent adhesive properties of eosinophils could be strongly inhibited by KTS-disintegrins – selective, low molecular weight $\alpha_1\beta_1$ inhibitors, isolated from viper venoms [11, 18].

Methods

Patients' characteristics

Fifteen patients with atopic bronchial asthma in a moderate persistent stage of their disease, according to GINA [1],

were recruited for the study. All subjects were in stable clinical condition. Their atopic status was confirmed by a positive skin testing for at least two standard inhaled allergens (Allergopharma, Reinbeck, Germany). Clinical symptoms were well controlled by inhaled glucocorticoids (500–1000 μ g/day of fluticasone or equivalent), long-acting β_2 -mimetics and oral theophylline. Only two patients were on oral methylprednisolone (4 mg; for more than 3 months in both). All subjects demonstrated greater than 15% improvement in their forced expiratory volume in 1 s after 200 μ g of inhaled salbutamol. Current smokers and ex-smokers were excluded from the study, as were the patients suffering from diabetes mellitus, heart failure, cancer, and renal or hepatic failure. Healthy non-atopic, non-smoking volunteers (workers of the Jagiellonian University Hospital; $n = 13$) with no history of chronic respiratory (or any other) diseases served as controls. All subjects gave informed consent to participate in the study, which had been approved by The Jagiellonian University Ethical Committee.

In patients and controls eosinophil number in whole blood as well as serum levels of IgE and eosinophil cationic protein (ECP) (UniCAP System, Pharmacia, Uppsala, Sweden) were measured. Clinical and laboratory characteristics of the subjects studied are shown on Table 1.

Isolation of peripheral blood eosinophils

For eosinophil isolation, blood was collected on ethylenediaminetetraacetic acid from the antecubital vein. Purification of eosinophils by density gradient centrifugation and CD16 immunomagnetic negative separation was performed as described previously [19]. Briefly, blood was diluted and layered onto 1.119 and 1.077 g/mL Histopaque gradient (Sigma Chemicals Co., St Louis, MO, USA), then centrifuged at 300 g by 30 min at 20 °C. The upper layer containing the mononuclear cells was discarded, while the lower layer, above the red blood cells, which contained mainly granulocytes, was collected. Remaining red blood cells were removed by lysis with 155 mM NH_4Cl and 10 mM KHCO_3 buffer. After washing twice in phosphate-buffered saline (PBS) and 0.5% albumin, granulocytes were incubated with anti-CD16 antibodies conjugated to magnetic beads (MACS CD 16 Micro Beads, Miltenyi Biotec, Auburn, CA, USA) in a concentration of 50 μ L per 5×10^7 cells (total volume about 200 μ L) and incubated for 45 min on ice. Finally, negative magnetic separation of eosinophils was performed using magnetic column CS and magnet-activated Vario MACS cell sorter (Miltenyi Biotec).

Purity of eosinophils was > 95% (May–Grunwald–Giemsa staining) and viability > 97%. Contaminating cells included neutrophils (1–4%) and lymphocytes (3–4%). Only freshly separated eosinophils were used in further experiments.

Table 1. Characteristics of subjects studied

	Asthmatics; n = 15	Controls; n = 13	
Age (years)	35 ± 14	31 ± 7	NS
Sex (M : F)	7 : 8	5 : 8	NS
Blood eosinophils (/mm ³)	433 ± 505	86 ± 61	<i>P</i> < 0.05
Total IgE (IU/L)	232 (63; 381)	88 (34; 102)	<i>P</i> < 0.05
Serum ECP (UG/L)	5.12 ± 2.82	2.96 ± 3.85	NS
Asthma duration (years)	6 ± 2		
FEV ₁ (L)	3.4 ± 0.7	4.5 ± 0.3	<i>P</i> < 0.05
FEV ₁ (% predicted)	72 ± 12	103 ± 11	<i>P</i> < 0.05

M : F = male/female number,

Results are presented as mean ± standard deviation, except for IgE (skewed distribution), which is given as median with lower and upper quartile.

ECP, eosinophil cationic protein; FEV₁, forced expiratory volume in 1 s, NS, not significant.

Binding of purified peripheral blood eosinophils to monoclonal immunoglobulin G antibodies

Binding of purified peripheral blood eosinophils to monoclonal antibodies was studied *in vitro* by a measurement of acid phosphatase activity from the adhered and subsequently lysed cells (according to the BD Cell Adhesion Protocol Manual). This enzyme is stable, independent of cell activation and not releasable. Briefly, mouse anti-human monoclonal IgG antibodies (mAbs) (1 µg/100 µL per well) were immobilized in PBS overnight at 4 °C on 96-well plate. AEF3.3 (anti- α_1) mAb was kindly provided by Dr Lobb (Biogen Inc., Cambridge, MA, USA). All other mAbs were purchased: P1E6 (anti- α_2) from Chemicon Inc. (Temecula, CA, USA), FIB504 (anti- β_7) from BD Pharmingen (San Diego, CA, USA), mouse IgG anti keyhole limpet haemocyanin antigen (isotypic control) from Becton Dickinson Biosciences (San Jose, CA, USA) while HP2/1 (anti- α_4) and Lia1/2 (anti- β_1) from Beckman Coulter Inc. (Fullerton, CA, USA). After immobilization of mAbs, wells were blocked [1% bovine serum albumin (BSA)] for 1 h at room temperature. Purified eosinophils were suspended in HBSS containing 1 mM Mg²⁺, 1 mM Ca²⁺, 1% BSA, and incubated (1 × 10⁵ cells/100 µL per well) for 90 min at 37 °C. After washing, attached cells were lysed using 50 mM sodium acetate, 12 mM *p*-nitro-phenyl phosphate and 0.2% Triton X-100 solution (all purchased from Sigma-Aldrich, St Louis, MO, USA). After 90 min the reaction was stopped (1 N NaOH) and plate read at 405 nm using microplate reader Elx 800 (Bio-Tek Instruments, Winooski, VT, USA). Absorbance of the wells coated with isotypic control (mouse IgG anti keyhole limpet haemocyanin antigen) was subtracted to obtain a final result. The number of adhered cells was calculated from a standard curve, calculated individually for every subject studied. All experiments were performed in duplicates.

Purification of snake venom disintegrins

Obtustatin and viperistatin were purified from venom of *Vipera lebetina obtusa* and *Vipera palestinae*, respectively,

using reverse-phase HPLC as described earlier [20]. Briefly, 10 mg of venom in 300 µL of 0.1% TFA (trifluoroacetic acid) was applied onto C18 column, and eluted with an acetonitrile gradient (0–80%) in 0.1%TFA over 45 min at a 2 mL/min flow rate. Fractions containing disintegrin activity (1 mg in 500 µL of 0.1% TFA) was reapplied to the same column and eluted with 'flatter' acetonitrile gradient (20–80%) over 70 min. The yield of purified obtustatin was approximately 12 mg/1 g of crude venom. Purity was assessed by SDS-PAGE and MALDI-TOF mass spectrometry using an Applied Biosystems DE-Pro spectrometer (Wistar Institute, University of Pennsylvania, mass spectrometry facility).

Desintegrins showing no binding to $\alpha_1\beta_1$ and $\alpha_2\beta_1$ (echistatin and VL05) were purified in a similar manner [21, 20] and served as a negative control.

Flow cytometry

One hundred microlitre samples of eosinophil suspension (1 × 10⁶ cell/mL) were incubated for 30 min at 4 °C in HBSS buffer (1% BSA) with mAbs in a concentration of 1 µg/100 µL. After washing, cells were incubated with fluorescein isothiocyanate-labelled goat anti-mouse IgG (1 µg/100 µL; Jackson Immuno Research Laboratories, West Grove, PA, USA) and analysed on Epics XL flow cytometer (Beckman Coulter Intl, Nyon, Switzerland). To discriminate eosinophils from other contaminating cells, double staining using PE-conjugated mouse anti-IL-5 receptor (CDw125) IgG mAb was used (BD Pharmingen). Results were expressed as a mean of fluorescence intensity (MFI). Isotypic control was examined for all subjects studied and final MFI was expressed as a difference between MFI of a given integrin tested and that of individual isotypic control.

Inhibition of cell adhesion

Bovine collagen IV (Chemicon Inc.) was immobilized overnight at 4 °C on 96-well ELISA plate (0.5 µg collagen

IV/100 μ L of 0.02 M acetic acid per well). Eosinophils prepared for adhesion were mixed with different concentrations of disintegrins and incubated by 15 min at 37 °C. Further adhesion procedure was performed as described above. For these experiments eosinophils obtained from four asthmatic subjects were used. All experiments were performed in duplicates.

To exclude non-specific inhibition of adhesion, two other disintegrins, not binding to collagen receptors were used: (a) echistatin (in a concentration up to 5 μ M), with high affinity binding to $\alpha_{IIb}\beta_{III}$ and $\alpha_v\beta_3$ [21] and (b) VLO5 (also in a concentration up to 5 μ M), a potent inhibitor of $\alpha_4\beta_1$, $\alpha_4\beta_7$ and $\alpha_9\beta_1$ [20]. Adhesion inhibition was also studied using specific mAbs: anti- α_1 (as a positive control), anti- α_2 , anti- α_4 , anti- β_7 and the mouse isotypic control – all in a concentration of 1 μ g/100 μ L.

We also studied the effect of obtustatin and viperistatin on eosinophil adhesion to rhVCAM-1 (purchased from RLD Systems, Minneapolis, MN, USA). The rhVCAM-1 was immobilized overnight at 4 °C on 96-well ELISA plate (0.5 μ g/100 μ L of PBS). Next steps were performed as described above.

Statistics

Normally distributed results were reported in tables and figures as a mean $\pm$ standard deviation (SD). Values which were not normally distributed were presented as median with lower and upper quartile. Comparisons between experimental groups were tested using Mann–Whitney *U*-test, while relationships were tested using Spearman Rank Correlations. Two-sided 5% level of significance was used. All statistical testing was performed by a Statistica StatSoft (Tulsa, OK, USA) software.

Results

Significant positive correlation was found between the number of peripheral blood eosinophils and serum ECP levels ($R = 0.62$, $P = 0.008$). Asthma patients showed higher whole blood eosinophil counts and total serum IgE concentration than healthy controls, while serum ECP levels were similar in both groups (Table 1).

Expression of eosinophil integrins using adhesion assay

The adhesion of eosinophils mediated by various integrins studied on immobilized monoclonal antibodies is presented in Fig. 1. In patients with asthma a significant increase in a number of adhered cells was observed for α_1 ($P = 0.007$), α_2 ($P = 0.025$) and β_7 ($P = 0.014$) integrin subunits as compared with controls. On the other hand, adhesion to mAbs against α_4 and β_1 integrin subunits did not differ between healthy subjects and asthmatics. These results suggest that the expression of both collagen receptors ($\alpha_1\beta_1$ and $\alpha_2\beta_1$) is elevated on the surface of eosinophils in moderate persistent

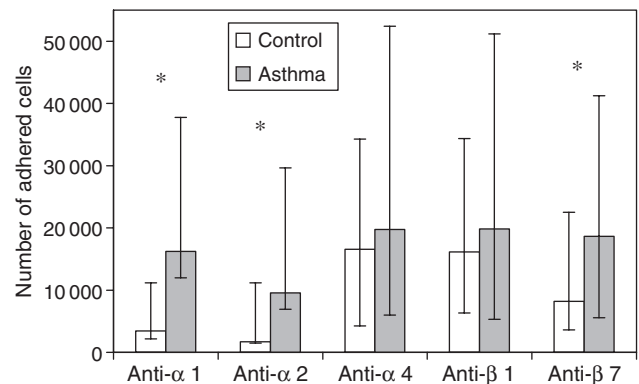


Fig. 1. Adhesion of human eosinophils to immobilized monoclonal antibodies. Results are expressed as a median with lower and upper quartile; * $P < 0.05$.

asthma. A positive correlation was also observed between the number of cells adhered to anti- α_1 mAb and the number of peripheral blood eosinophils ($R = 0.53$, $P = 0.007$). Such relationship was not detected for any other integrin studied or for IL-5 receptor (CDw125).

Eosinophil integrin expression by flow cytometry

Flow cytometric analysis was performed as a confirmatory procedure in detecting the presence of collagen receptors on the surface of normal eosinophils and their increased expression in bronchial asthma. As presented in Figs 2 and 3, expression of α_1 and α_2 integrin subunits in patients were also significantly increased as compared with these in healthy controls ($P = 0.049$ and $P = 0.03$, respectively). In general, the flow cytometry data confirmed the results obtained by adhesion studies, except for α_4 integrin unit, which showed significantly increased fluorescent intensity on asthmatic eosinophils as compared with controls ($P = 0.04$).

Effect of KTS-disintegrins on $\alpha_1\beta_1$ integrin-dependent adhesion of eosinophils to collagen IV

Both disintegrins: obtustatin and viperistatin in a dose-dependent manner inhibited adhesion of eosinophils from asthmatic patients to collagen IV (Fig. 4). Obtustatin was effective in concentrations exceeding 0.15 μ M, whereas viperistatin was much more potent on a molecular basis, being effective already above 2.9 nM. Calculated IC50s for this inhibition were: 1.15 μ M and 11 nM, respectively.

For practical reasons dose–response curves were prepared using eosinophils from asthma patients, as cells isolated from healthy subjects adhered poorly (about threefold less than in patients) to collagen IV. It is, however, important to note that both disintegrins studied (concentration range: 100 nM–10 μ M), inhibited completely adhesion of the eosinophils obtained from the healthy subjects to collagen IV (results not shown).

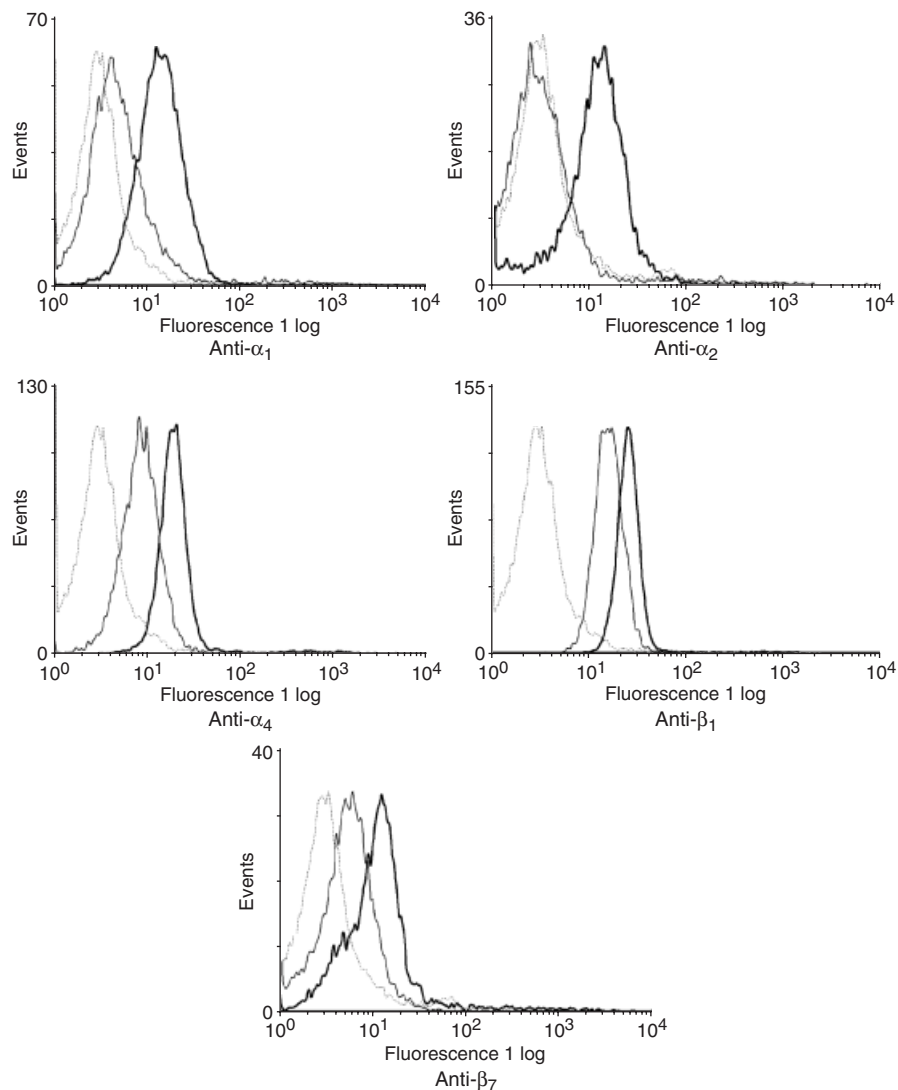


Fig. 2. Flow cytometry histograms of monoclonal antibodies (mAbs) binding to eosinophils isolated from peripheral blood of a healthy subject and an atopic asthmatic. Fluorescence of eosinophils labelled with isotypic control (striped line) vs. specific mAb (solid line). The left peak (thin solid line) represents healthy control; the right peak (thick line) shows an asthma patient.

Neither echistatin, nor VL05 inhibited adhesion of eosinophils to collagen IV (data not shown).

Anti- α_1 antibody (in a concentration of 0.5 and 1 $\mu\text{g}/100 \mu\text{L}$) was able to completely inhibit adhesion of cells to collagen IV. Other antibodies used (anti- α_2 , anti- α_4 , anti- β_7 and mouse isotypic control) were devoid of such effect.

While eosinophils strongly adhered to rhVCAM-1 (due most probably to the presence of $\alpha_4\beta_1$ and $\alpha_4\beta_7$), obtustatin and viperistatin were unable to inhibit this adhesion (data not shown).

Discussion

In this study we showed for the first time, that $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins could be detected on the surface of human peripheral blood eosinophils, and that they undergo up-

regulation in patients with moderate persistent atopic bronchial asthma. To ascertain the validity of these findings two independent methods were used: cell adhesion assay (Fig. 1) and flow cytometry (Figs 2 and 3).

Based on these findings, it could be hypothesized that eosinophil $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins might mediate their adhesion to and transmigration through airway blood vessels, as it has been described for other types of leukocytes [9, 11]. Abraham et al. [17] has shown previously that a specific antibody against $\alpha_1\beta_1$ could block the recruitment of eosinophils into airway mucosa in a sheep model of bronchial asthma [17]. Our results may explain, at least in part, the mechanism of such inhibition. In addition, during chronic, asthmatic airway inflammation, both overexpressed integrins may also participate in airway remodelling through their complex involvement

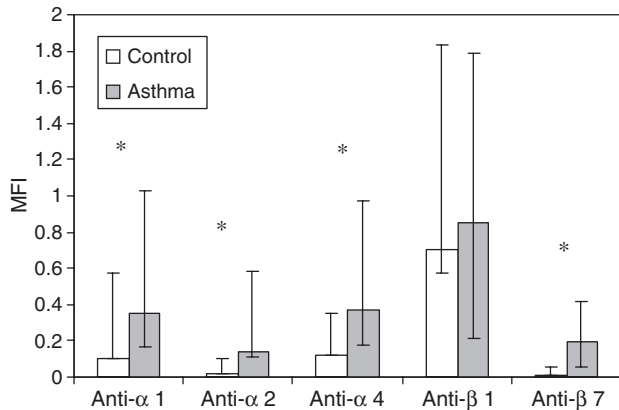


Fig. 3. Binding of monoclonal antibodies to eosinophils, measured by flow cytometry and expressed as a median of fluorescence intensity (MFI). Error bars represent lower and upper quartile of MFI; * $P < 0.05$.

in angiogenesis and fibrosis linked to inflammation [22–24].

It should be noted, however, that preferential recruitment and accumulation of eosinophils within the bronchial mucosa involves also other adhesive molecules, as documented by a successful application of antagonists of $\alpha_4\beta_1$ [25–27] and $\alpha_L\beta_2$ [28] integrins in asthmatic subjects and in experimental models of bronchial asthma.

Increased expression of $\alpha_1\beta_1$ integrin as a result of immune stimulation, has already been observed on monocytes [29], while other adhesion molecules, including α_4 integrin, increase their expression on eosinophils involved in asthmatic airway inflammation [30–32]. Such augmented expression of both collagen receptors in patients with moderate persistent asthma suggests their activation in systemic circulation. Whether indeed eosinophil activation takes place primarily in the asthmatic lung or in the peripheral blood, is still a matter of debate. Some authors were unable to detect activated cells in the peripheral circulation of patients with bronchial asthma [33]. Previously, we have demonstrated overexpression of mRNA for leukotriene C₄ synthase, a crucial enzyme in the production of cysteinyl leukotrienes, in peripheral blood eosinophils of asthmatic subjects [34]. This may indicate at least partial activation of these cells in the systemic circulation. Currently the view dominates that in bronchial asthma eosinophils are primed in the peripheral blood and further activated in the lung tissue [12, 35].

The possibility that the magnitude of collagen receptor expression may depend on the severity of the disease has not been specifically addressed in our study. However, experiments performed in a small number of patients suffering from less severe forms of the disease (intermittent and mild persistent asthma) showed similar expression of $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins as in controls (data not shown). It is thus tempting to speculate about some degree of association between the expression of both the integ-

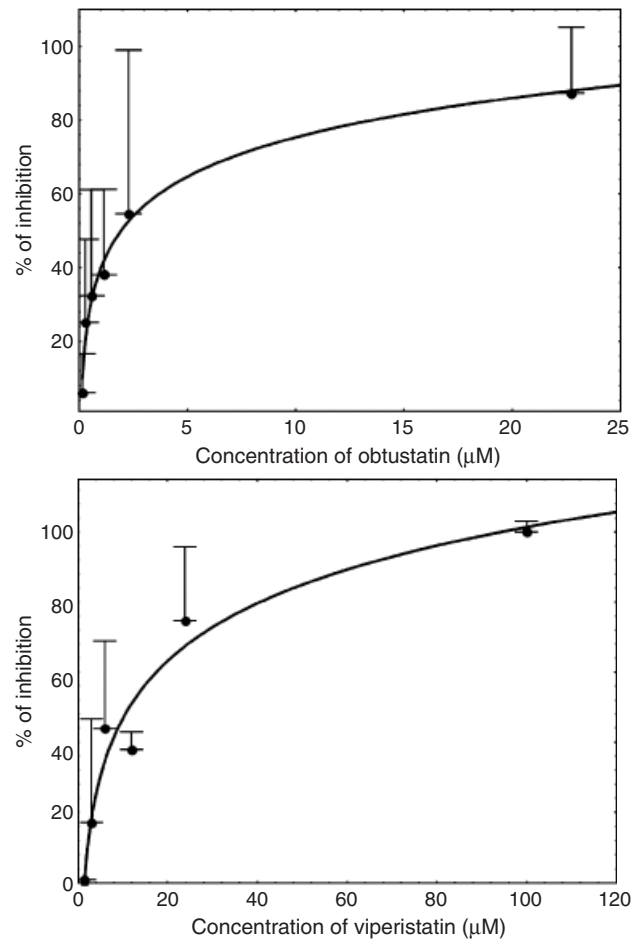


Fig. 4. Effect of obtustatin and viperistatin on the adhesion of eosinophils to immobilized collagen IV. Results are presented as a mean $\pm$ SEM from four representative experiments.

rins studied and the clinical severity of disease; an observation, which has been previously made for other eosinophil integrins in bronchial asthma [31, 32].

It has been documented earlier that in bronchial asthma sputum eosinophils expressed elevated levels of α_4 integrin subunit [30]. Our data concerning the expression of this integrin subunit on blood eosinophils showed a discrepancy between the results of adhesion assay and flow cytometry analysis. It could be explained by a different accessibility of the cell surface receptors to mAbs when an immobilized ligand is used (adhesion), as opposed to the same ligand located on the cells suspended in a solution (flow cytometry).

Up-regulation of α_1 and α_2 subunits was not associated with any significant change in β_1 chain expression. At first it seems difficult to explain how can $\alpha_1\beta_1$ and $\alpha_2\beta_1$ be increased with no change in the expression of β_1 ? However, while α_1 and α_2 subunits associate only with β_1 , this latter chain may form functional complexes with many different α subunits (from α_1 to α_{11}). Thus, the $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins may co-exist on the cell membrane with

other β_1 -containing molecules [8], which may mask changes in β_1 , related solely to changes in collagen receptors.

The potential shortcoming of our study was a small contamination (less than 5%) of our eosinophil preparation used for adhesion assay. So part of adhered cells might belong to contaminating cell fraction. However, flow cytometry analysis examines eosinophil population only, thus confirming the results obtained by adhesion experiments. Therefore, we have all reasons to believe that both collagen receptors are indeed present on peripheral blood eosinophils.

All asthma patients studied were treated with inhaled glucocorticoids (500–1000 μ g/day of fluticasone or equivalent) except for two who were also taking 4 mg of oral methylprednisolone. It is well known, that corticosteroids may reduce integrin expression [36]. One can then speculate that the expression of collagen receptors on the surface of patients' eosinophils would be even higher, were they not taking any steroids at all. While we were unable to prove this concept, at the same time there was no relationship between the dose of corticosteroids used and the expression of the integrins studied.

Finally, we investigated adhesive properties of eosinophils to collagen type IV using specific low molecular weight inhibitors of $\alpha_1\beta_1$ integrin isolated from viper venoms. Obtustatin and viperistatin belong to short, monomeric, KTS-containing disintegrins expressing potent and selective inhibitory activity towards $\alpha_1\beta_1$ [37, 38]. They block binding of $\alpha_1\beta_1$ integrin to collagen type IV in an adhesion assay, albeit with different potency [38]. We confirmed this dose-dependent inhibitory action studying the adhesion of eosinophils from asthmatic patients to immobilized collagen (Fig. 4). In agreement with previous observations [27], viperistatin was more potent than obtustatin in inhibiting of the $\alpha_1\beta_1$ -dependent adhesion of eosinophils to collagen type IV. The higher activity of viperistatin is attributed to the presence of arginine instead of leucine adjacent to KTS sequence in the integrin-binding loop. Thus, viperistatin seems to be an excellent template for the design of new $\alpha_1\beta_1$ integrin low molecular weight inhibitors with potential therapeutic utility. It is important to note that KTS-disintegrins are not toxic up to 5 mg/kg in a mouse experimental system *in vivo* [27], and possible low molecular weight compounds based on its structure may have limited side-effects. A similar strategy for the drug development has been applied previously. Based on the R/KGD-containing molecules of the snake venom disintegrins, two synthetic compounds, eptifibatide and tirofiban, were introduced and approved for therapy in acute coronary syndromes and the prevention of thrombosis in patients undergoing percutaneous coronary interventions [27, 39]. Caution must be applied, however, as completely unexpected complications may arise when we attempt to block leuco-

cyte adhesive functions. Cases of progressive multi-focal leucoencephalopathy during treatment with natalizumab, a monoclonal antibody blocks α_4 integrin are the most recent examples [40].

In summary, this is a first report showing the presence of collagen receptors on the eosinophil surface and their increased expression in patients with moderate persistent atopic bronchial asthma. We also show that KTS-disintegrins are effective in blocking $\alpha_1\beta_1$ integrin-dependent eosinophil interaction with collagen type IV. We speculate that the $\alpha_1\beta_1$ integrin may become an attractive pharmaceutical target in the future treatment of chronic inflammatory lung diseases.

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