INTRODUCTION

Cell migration through a blood vessel and into inflammatory site is mediated by cell-surface receptors and their extracellular matrix ligands. Integrins - a family of heterodimeric glycoproteins composed of non-covalently associated α and β subunits play an important role in this process. By regulating cell-cell and cell-matrix interactions, they modify cell growth, migration, activation and survival (1). Integrin-mediated adhesion plays an important role in the pathogenesis of several diseases, including a number of inflammatory disorders, such as rheumatoid arthritis, inflammatory bowel disease and asthma (2). Understanding these processes may help to elucidate disease pathology and its progress. It may also suggest new therapeutic targets.

Bronchial asthma is one of the most common respiratory diseases, affecting approximately 5% of adults and up to 10% of children (3). Despite substantial progress in therapy made in the last twenty years, up to 40% of adult patients remain symptomatic, and in up to 5% the disease is "difficult to control" (4). It is probably, because pathogenesis of asthma is poorly understood and the therapeutic modalities are still focusing on the control of airway inflammation and symptomatic improvement, rather than targeting cause of the disease. Eosinophils are one of the major effector cells in chronic airway inflammation in asthma and play an important role in airway remodelling - another characteristic feature of bronchial asthma (5, 6). However, mechanisms involved in eosinophil recruitment to the lung, as well as the pathways of their activation have not been fully elucidated. T helper lymphocytes are likely pivotal in driving asthma development and progression. Adoptive transfer of Ag-primed T cells into naive mice induces eosinophilia, bronchial hyper-responsiveness and late airway response (7). Active T helper cells could be detected in the airways, even when patients are asymptomatic (8).

Among integrins, in cell trafficking the most important are those containing αi and βi subunits (9). We have previously hypothesized that also collagen receptors: α1β1 and α2β1 may be involved in cell migration to the inflammatory site in asthma. The aim of the study was to determine whether the inhibition of α1β1 or α2β1 integrins, affects transmigration of eosinophils and peripheral blood mononuclear cells (PBMC) through human microvascular endothelial cells monolayer (HMVEC) seeded on collagen IV coated wells in moderate persistent atopic asthmatics. Methods: PBMC from 9 asthmatics were separated by gradient centrifugation followed by negative magnetic separation of eosinophils. Snake venom derived anti-adhesive proteins: vipersinat and VP12 (potent selective inhibitors of α1β1 and α2β1 integrins, respectively) as well as VLO4 (a non-selective inhibitor of α5β1, α2β1 and ααβ2 - used as a positive control), were used for inhibition studies. All anti-adhesive proteins studied inhibited eosinophils, but only VLO4 affected PBMC transmigration through HMVEC. In bronchial asthma both collagen receptors α1β1 and α2β1 are likely to be involved in eosinophil transmigration to the inflammatory site. The role of α2β1 integrin has been described as a stimulator of collagen accumulation, it might be, at least in part, responsible for asthma airway remodelling.

Key words: adhesion molecule, asthma, disintegrin, eosinophil, integrin, transmigration
Collagens form a structural scaffold for all the tissues. Basement membrane of small vessels contains collagen IV, while collagen I (and III) is a major component of extracellular matrix in the lungs (10). Involvement of collagen receptors in the lymphocytic inflammatory response has been suggested in the past (12). Beneficial effects of anti-αβ1, monoclonal antibodies, have been shown in certain animal models of immunologically mediated diseases, including inflammatory bowel disease (13), arthritis (12), and allergen-induced leukocyte recruitment to the lungs associated with late airway response in sheep (14). This latter finding suggests that integrin collagen receptors may also serve as therapeutic targets in controlling inflammation in bronchial asthma. Previously we have shown that in healthy donors αβ1 is expressed on T lymphocytes, with its substantial increase in asthma patients (15). We have also reported that expression of VLA-1 and VLA-2 on blood eosinophils from asthmatic subjects is increased in comparison to healthy donors (16).

Moreover, in 2006 we have shown that αβ1 integrin specific adhesion of eosinophils from asthmatic subjects could be strongly inhibited by obtustatin and viperistatin - selective, low adhesion of eosinophils from asthmatic subjects could be 2 on blood eosinophils from asthmatic subjects is increased in (15). We have also reported that expression of VLA-1 and VLA-2 integrins affects peripheral blood neutrophils (1-4%) and lymphocytes (3-4%).

The objective of our study was to determine whether the inhibition of αβ1, or αβ2, integrins affects peripheral blood mononuclear cell (PBMC) and eosinophil transmigration through the human microvascular endothelial cell (HMVEC) monolayer.

MATERIAL AND METHODS

Patients

The study was conducted in a group of 9 adult atopic asthmatics. All patients suffered from stable, well-controlled moderate persistent asthma, according to GINA guidelines (3). This means all patients were stable, with well controlled symptoms while treated with low or medium dose of inhaled glucocorticosteroids and long-acting β2-agonists. Their atopic status was confirmed by a positive skin testing for at least one standard inhaled allergen (Allergopharma, Germany). Smokers and patients suffering from heart failure, diabetes mellitus, renal or hepatic diseases, as well as other chronic diseases were excluded from the study. Our study was carried out in accordance with the Declaration of Helsinki and was approved by the local Ethical Committee; all subjects gave informed consent to participate in this study.

Methods

Isolation of peripheral blood mononuclear cells and eosinophils

Peripheral blood mononuclear cells (PBMC) and eosinophils were isolated as described previously (18, 19). Briefly, 50 ml blood was collected on EDTA from the antecubital peripheral vein. Blood was diluted and layered onto 1.119 and 1.077 g/ml Histopaque gradient (Sigma Chemicals Co., St Louis MO, USA); centrifuged at 300 g by 30 min at 20°C. Both cell layers: upper - containing mononuclear cells and lower containing granulocytes, were collected, washed twice in PBS with 0.5% albumin. Upper layer (containing mainly PBMC) was incubated in RPMI 1640 (Sigma Chemicals Co., St Louis MO, USA) with 10% FCS (Sigma Chemicals Co., St Louis MO, USA) at 37°C in a humidified atmosphere of 5% CO2 for 1 hour in tissue culture flasks 25 cm2 (Corning Costar Co, Cambridge, Mass), in order to adhere and discard monocytes. Afterward, cells were washed twice with RPMI 1640.

Remaining red blood cells in a layer of granulocytes were removed by lysis with 155 mM NH4Cl and 10 mM KHCO3 buffer. Afterwards, granulocytes were incubated with cocktail of biotin-conjugated mouse mAbs against: CD2, CD14, CD16, CD19, CD56, CD123 and CD 235A (glycoporphin) and next with anti-biotin mouse mAb conjugated with magnetic microbeads (Eosinophil isolation kit, Miltenyi Biotec, Auburn CA, USA) in a manner and a concentration recommended by a product supplier. Finally, negative magnetic separation of pure eosinophils was performed with magnetic column CS and magnet-activated cell sorter system purchased from Miltenyi Biotec, Auburn CA, USA.

Purity of eosinophils was >95% (May-Grumwald-Giemsa staining) and viability >97%. Contaminating cells included neutrophils (1-4%) and lymphocytes (3-4%).

Only freshly separated eosinophils were used in further experiments.

Purification of snake venom derived anti-adhesive proteins

Snake venom disintegrins and VP12 C-lectin type protein were purified from crude venom using two steps of reverse phase HPLC. The first step of isolation was based on the preparative fractionation of crude viper venom on reverse phase HPLC using a linear gradient of increasing acetonitrile concentration. Viperstatin was eluted from C18 column in fraction 3, whereas VP12 in fraction 12. The second step of purification of viperistatin was performed using another reverse phase HPLC with "flatter" gradient of acetonitrile (20-80%) over 70 min as previously described (20). The second step of VP12 purification required the use of ion-exchange chromatography, that was performed with Mono Q column (21). Active protein fractions were lyophilized to remove acetonitrile and TFA, reconstituted in water and re-chromatographed on the same type of column with extended elution time to 120 min. Protein was lyophilized as above and stock solution (for disintegrin) was prepared in water (1-2 mg/ml) and for VP12 in 20 mM Tris pH 7.5, VLO4 was prepared from the venom of Vipera lebetina obtusa in a similar manner to viperistatin (22).

Structurally, viperistatin belongs to the disintegrin family of proteins (20), whereas VP12 is composed of two subunits VP12A and VP12B displaying amino acid sequence homology with heterodimeric C-lectin type proteins (21). The yield of purified disintegrin was approximately 12 mg per 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, Philadelphia, PA, mass spectrometry facility).

Transmigration assay

Human microvascular endothelial cells (HMVEC) were obtained from Cascade Biologies Inc. (Portland, Or, USA) as isolated from skin of individual adult donor and passaged as previously described (23, 24). All chemicals used for HMVEC cultures: medium 131, trypsin/EDTA solution, trypsin neutralizer solution, amphotericin/gentamycin solution, microvascular growth supplement (MVGS) and attachment factor were also obtained from Cascade Biologies Inc. (Portland, Or). HMVEC were seeded onto attachment factor coated tissue culture flasks 25 cm2 (Corning Costar Co, Cambridge, Mass, USA) and grown at 37°C in a humidified atmosphere of 5%
CO₂. Attachment factor is a sterile solution containing gelatin at 0.1 % and is used as one component in a complete culture environment for the growth of endothelial cells (25).

The medium was changed every 2 to 3 days and cells were passaged, till they reached 80-90% confluency (6-8 days); the cultures were used between 5 and 9 passages. Transmigration assay was conducted using polyethylene terephthalate (PET) track-etched membranes transwell inserts 3 µm pore size (BD Falcon Cell Culture Inserts, Franklin Lakes, NJ, USA) on 24-well tissue culture plate (BD Falcon Cell Culture Inserts, Franklin Lakes, NJ, USA). Human placenta collagen IV (BD Biosciences, Bedford, MA, USA) in a concentration of 1 µg/100 µl, diluted in 10 mM acetic acid, was used as a thin coating - 100 µl, diluted collagen IV was padded to each insert well to cover the surface evenly; incubated at 37°C for 1 hour. Afterwards, remaining volume was aspirated, surface dried and rinsed twice with medium M131; HMVEC were seeded onto inserts with density of 1×10⁵ cells in 0.7 ml of M131 with supplements per insert. Cells were then incubated at 37°C and 5% CO₂ for 1 day to develop confluence.

For transmigration assay isolated PBMC and eosinophils in a concentration of 10⁶/ml in RPMI 1640 (supplemented with 4 mM L-glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin) were incubated for 45 minutes at room temperatures with:

1. mouse IgG1 antibody recognizing keyhole limpet hemocyanin antigen (KLH), clone X40, purchased from Becton Dickinson Biosciences (San Jose, CA, USA) - working concentration 1 µg/100 µl - KLH antigen is not found on human cells and antibody serves as a negative control;
2. mouse IgG anti-human integrin α1 domain monoclonal antibody, clone F9B12 (Chemicon, Millipore Corporation, Temecula, CA, USA) - working concentration 1 µg/100 µl - this antibody reacts with I-domain (Val₁₅₁-Ala₃₆₄) of human α₁ integrin subunit (CD49a);
3. mouse IgG anti-human integrin 2 monoclonal antibody, clone P1E6 (Chemicon, Millipore Corporation, Temecula, CA, USA) - working concentration 1 µg/100 µl. Based on information given by a product supplier this antibody is suitable for attachment inhibition assay;
4. viperistatin, snake-venom derived disintegrin, potent and selective inhibitor of 11 integrin (in a concentration of 1 µg/100 µl-2.3 µM);
5. VP12, snake-venom derived lectin, potent and selective inhibitor of α5β1 integrin (in a concentration of 1 µg/100 µl-0.3 µM);
6. VLO4, snake-venom derived disintegrin, non-selective inhibitor of αβ1, αβ2, αβ3, αβ5, αβ1, αβ3, and αβ5 integrins (in a concentration of 1 µg/100 µl-0.7 µM).

Specificity and selectivity of viperistatin for αβ1 was confirmed by Kisiel et al. by ELISA and in an adhesion system of transfected cell lines (20). Viperistatin inhibited only VLA-1 (IC₅₀=0.02 nM for adhesion to collagen I and 0.08 nM for adhesion to collagen IV) and was not effective for αβ1, αβ2, αβ3, αβ5, αβ1, αβ3, and αβ5 (20).

Specificity and selectivity of VP12 for αβ1 was confirmed by Staniszewska et al. (21). They proved that VP12 is a strong and specific αβ1 blocker in a cell adhesion system (IC₅₀=0.5 nM for adhesion to collagen I and 3.6 nM for adhesion to collagen IV). VLO4 contains an Arg-Gly-Asp sequence (RGD motif) and, as other such disintegrins, non selectively antagonizes inter alia α5β1, α5β3 (26), but it is also strong inhibitor of αβ1 and less potent of αβ1 integrin (22). VLO4 in our transmigration assay was used as a reference positive control. As a positive control, VLO4 could act via αβ3 and αβ5 on eosinophil and PBMC or by interaction with αβ1 on endothelial cell (22). The αβ1 integrin is a main fibronectin receptor (2). The αβ3 binds to VCAM-1 and to an alternatively spliced fibronectin domain that is distinct from the main ligand of α5β1, a fibronectin domain containing RGD sequence (2). Both these integrins were found on lymphocytes and eosinophils in the past (2, 17, 27).

Each transmigration assay was started by washing upper and lower chambers twice with RPMI 1640. Afterwards, 300 µl of cell suspension was placed into the upper chamber (above the HMVEC monolayer). The lower chamber contained 700 µl of RPMI 1640 supplemented with 10% FCS as a chemoattractant. Transmigration assay was performed at 37°C, humidified atmosphere of 5% CO₂ for 3 hours. Thereafter, cells in the lower chamber were counted in a hemocytometer. Presence of eosinophils in the lower chamber was confirmed by a cytospin followed by May-Giemsa staining.

Finally, fraction of cells that transmigrated of the cells applied to the upper chamber was calculated. All experiments were performed in duplicates.

**Serum concentration of IgE**

Serum concentration of IgE was measured using UniCAP System, Pharmacia, Sweden.

### Statistical analysis

The distribution of the obtained values was impossible to assess, because of a low number of subjects studied (n=9). For this reason for statistical purposes we used non-parametric tests usually applied for not normally distributed values. Comparison between control samples and samples with studied anti-adhesive proteins were calculated using Wilcoxon signed-rank test. We used (assumed) 5% level of significance. All statistical testing was performed by a Statistica StatSoft (Tulsa, OK, USA) software.

**RESULTS**

Clinical and laboratory characteristics of subjects studied are given in Table 1.

Results of the transmigration assay are given in Fig. 1 and 2. Fig. 1 shows the fraction of cells that transmigrated of the cells applied to the upper chamber. In Fig. 2 results are presented as a percent of transmigration inhibition (in a reference to the isotropic control).

Transmigration of PBMC in samples incubated with anti-α5 mAb, anti-α5 mAb, viperistatin and VP12 was similar to that observed in control samples (cells incubated with isotopic control only). On the contrary, VLO4 significantly diminished transmigration of PBMC through HMVEC. Transmigration of eosinophils, on the other hand, was inhibited by all tested snake venom derived anti-adhesive proteins: viperistatin, VP12 and VLO4, as well as by anti-α5 mAb as compared to cells incubated with the isotopic control. Transmigration of eosinophils incubated with anti-α5 mAb remained unchanged.

Summarizing: only VLO4 diminished PBMC transmigration through HMVEC monolayer; other anti-adhesive proteins had

### Table 1. Clinical and laboratory characteristics of the subjects studied.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female ratio</td>
<td>4/5</td>
</tr>
<tr>
<td>Age; years (mean, min-max)</td>
<td>35 (33-41)</td>
</tr>
<tr>
<td>Duration of asthma; years (mean, min-max)</td>
<td>7 (5-8)</td>
</tr>
<tr>
<td>% of predicted value (mean ± SD)</td>
<td>92 ± 15</td>
</tr>
<tr>
<td>Blood eosinophils per µl (median; interquartile range)</td>
<td>288; 64</td>
</tr>
<tr>
<td>Total IgE; IU/L (median; interquartile range)</td>
<td>150; 45</td>
</tr>
</tbody>
</table>

*Statistical analysis was performed by a Statistica StatSoft (Tulsa, OK, USA) software.*
no effect. Eosinophil movement, on the other hand, was inhibited by all the studied snake-venom derived proteins.

**DISCUSSION**

In this study we investigated for the first time an effect of $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrin inhibition on eosinophil and PBMC transmigration through HMVEC monolayer seeded on collagen IV coated wells. In the past we have shown that in some asthma patients both collagen receptors: $\alpha_1\beta_1$ and $\alpha_2\beta_1$ could be found on eosinophils. However, based on the available literature, the presence of both collagen receptors on eosinophils is debatable. In 1993 Georas with co-workers reported that blood eosinophils of healthy donors and mildly allergic subjects were found to consist of only the $\alpha_4\beta_1$ and $\alpha_6$ integrins (28). No expression of the $\alpha_1$, $\alpha_2$, $\alpha_3$, $\alpha_5$, or $\beta_4$ subunits was detected. This information was afterwards included in many review publications (29, 30). In the same year, however, Kuijpers et al. showed VLA-2, -4, -5, and -6 mediated eosinophil migration through filters covered with HUVEC (17). They also confirmed the presence of VLA-2 and VLA-5, but not VLA-1, on eosinophil surface by flow cytometry. There is also a slight possibility that VLA-2 detected in our experiments comes from blood platelets adherent to eosinophils. Platelets are known to bind to eosinophils to a varying degree (31). It has been shown that in bronchial asthma eosinophil-associated P-selectin might probably derive from activated platelets and in turn activate eosinophils (32). Recently, Laidlaw et al. have shown that platelets adherent to leukocytes reveal higher expression of several adhesion markers than nonadherent platelets (33). Remarkable adherence of platelets to eosinophils suggests that platelets could contribute substantially to the eosinophil pathology and their accumulation in the tissue by priming them for adhesion and prolonging their survival (33). However, platelets express abundantly the $\alpha_2$ subunit (34), and not the $\alpha_1$. For this reason, $\alpha_1$ expression on isolated eosinophils could not be related to platelet "satelliteism", but to eosinophils themselves. Interestingly, in our hands VLA-1 on blood eosinophils has been mainly shown in asthma (16). It could be then hypothesized that eosinophil priming, or activation is required for VLA-1 expression. Bates et al. using oligonucleotide microarrays could not show VLA-1 or VLA-2 expression after eosinophil stimulation (35). However, VLAs appear on the cell surface in a delayed manner (10), so the detection time after stimulation might be crucial. Alternatively, integrins may require a different signal for their expression than that used by Bates et al. in their experiments (35).

Experiments performed in this study confirmed an involvement of both $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins in eosinophil, but not PBMC transmigration through the HMVEC monolayer. Accordingly, all snake-venom derived disintegrins, as well as the anti-$\alpha_1$ I domain mAb, inhibited eosinophil transmigration. On the contrary, anti-$\alpha_2$ mAb, was not effective. The discrepancy between the efficacy of both used $\alpha_2\beta_1$ integrin antagonists: anti-$\alpha_2$ mAb and VP12, could be explained by the different accessibility to the active site of cell surface antigens, with anti-$\alpha_2$ mAb being a much larger molecule.
Based on our results, it could be hypothesized in functional terms that in bronchial asthma the $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins possibly mediate eosinophil adhesion to and transmigration through airway blood vessels, as it has been described for other types of leukocytes (2). Our findings may also explain why a specific antibody against $\alpha_1\beta_1$, blocked the recruitment of eosinophils in a sheep model of bronchial asthma (14). Of course, preferential recruitment and accumulation of eosinophils within the bronchial mucosa also involves other adhesive molecules, as documented by the successful application of antagonists to the $\alpha_4\beta_1$ and $\alpha_L\beta_2$ integrins in asthmatic subjects, and in experimental models of bronchial asthma (36, 37, 38).

In contrast the inhibition of both collagen receptors has no impact on transmigration of PBMC, despite the documented presence of the $\alpha_1\beta_1$ integrin on CD4 and CD8 T lymphocytes (15). However, PBMC are a heterogeneous population. Beside T cells, PBMC contain monocytes, NK, B lymphocytes and dendritic cells. On the other hand, the biological function of the $\alpha_1\beta_1$ integrin on T cells may differ from that on the eosinophils. The main ligands for the $\alpha_1\beta_1$ integrin are collagen I, II, III, IV, laminin and fibronectin (39). The $\alpha_2\beta_1$ integrin has recently been described as a stimulator of collagen and fibronectin accumulation in the airways, so in bronchial asthma it could rather be involved in progression of airway remodeling (39).

In our experiments HMVEC were seeded in collagen IV coated inserts. Although $\alpha_1\beta_1$ preferentially binds to collagen IV while $\alpha_2\beta_1$ to collagen I (11), they both interact with various ECM proteins. In this respect $\alpha_1\beta_1$ could bind to: collagen I, II, III, IV, laminin and fibronectin; and $\alpha_2\beta_1$ to: collagen I, II, IV, laminin and tenascin (39). So, both integrins could adhere to collagen IV. Previously, we have shown that peripheral blood eosinophils of asthmatic patients adhere to collagen IV (16). Moreover, this interaction was inhibited in a dose-dependent manner by obtustatin and viperistatin, snake-venom derived disintegrins, selective inhibitors of $\alpha_1\beta_1$ integrin (16). However, based on literature, eosinophil adhesion to collagen is also controversial. Barthel and colleagues reported that blood eosinophils did not adhere to fibronectin, laminin or collagen type I (40). On the other hand, Tourkin et al showed that, in fact, eosinophils from normal donors did not adhere to fibronectin, laminin and collagen types I and IV, but these cells in vitro activated by IL-5, or isolated from blood of patients with eosinophilia, did it in a satisfactory manner (41). Recently El-Shazly et al. also confirmed increased adhesion of CD244 agonist-stimulated eosinophils to collagen IV (42). Finally, our experimental model does not allow to indicate, whether integrin inhibition leads to the alteration of eosinophil or HMVEC, or and cross-talk between these cells. It is also worth noting that in vitro cultured HMVEC secrete various ECM proteins e.g. fibronectin, laminin and collagens to the culture medium. Some of these proteins associate with the cell surface and possibly interact with leukocytes during their transmigration (43, 44). The $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins are also abundantly expressed on the microvascular endothelial cells (45, 46).
Until now anti-integrin treatment has been widely used only for the prevention of platelet thrombus formation in cardiovascular medicine. Based on the R/KGD-containing sequence of the snake venom disintegrins, two synthetic compounds, epifibatide and tirofiban, were introduced as an adjuvant therapy in patients undergoing percutaneous coronary interventions (47). In asthma anti-integrin experimental treatment was focused mainly on VLA-4 antagonists (48). However, other anti-inflammatory therapeutic approaches, such as Th2 cytokine profile inhibition, or lung tissue protection against oxidative stress, are also being considered, at least in animal asthma model (49, 50, 51). It could be envisioned that based on disintegrin structure anti-inflammatory integrin antagonists targeting multiple cell types, could also be effective. Viperistatin and VP12 seem to be an excellent template for the design of new αβ1 and αβ2 integrin low molecular weight inhibitors with potential therapeutic utility. It is important to mention in this respect that up to 5 mg/kg, KTS-disintegrins (such as viperistatin) are not toxic in a mouse experimental system in vivo (20). It is tempting to speculate that such anti-adhesive snake-venom derived disintegrins, could be used as an anti-inflammatory therapy in the future, especially in eosinophil dependent diseases. These cells are involved not only in asthma, but probably are also important in the pathology of chronic obstructive pulmonary disease, at least in its initial course (52).

However, before such therapeutic option a comprehensive understanding of integrin/ligand interactions is required to specifically limit the inflammatory response without adversely affecting other physiologic functions of integrins.

Our study has also certain limitations. Culturing HMVEC primary cell line purchased from Cascade Biologics we were not able to harvest enough microvascular cells, to perform transmigration assays in both intended groups: asthma and healthy controls. For this reason we conducted our experiments in asthmatics only. This is also the reason why our study was carried out in only 9 asthma patients.

In summary, this is the first report showing an experimental evidence of possible αβ1 and αβ2 integrin involvement in eosinophil transmigration through a human microvascular endothelial cell monolayer, seeded in collagen IV coated wells. We have shown that both, viperistatin (KTS-disintegrin) and VP12 (snake-venom derived lectin) are effective in blocking the αβ1 and αβ2 integrin-dependent eosinophil transmigration. It could be speculated that both of these integrins may become an attractive target in the future treatment of chronic inflammatory diseases with eosinophil involvement, such as asthma. Any possible functional role of αβ1 integrin on T lymphocytes, on the other hand, remains to be established.

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