

Interleukin-5 enhances eosinophil adhesion to bronchial epithelial cells

D. SANMUGALINGHAM, E. DE VRIES, R. GAUNTLETT, F. A. SYMON, P. BRADDING and A. J. WARDLAW

Division of Respiratory Medicine, Leicester University Medical School, Glenfield Hospital, Leicester, UK

Summary

Background Eosinophil–bronchial epithelial cell interactions are thought to be central to the pathogenesis of asthma, both in terms of the epithelium as a source of pro-inflammatory mediators and as a target for eosinophil-mediated damage. We have therefore investigated adhesion interactions between these two cell types.

Objectives To determine the role of eosinophil and epithelial activation on eosinophil adhesion to bronchial epithelium and to characterize the adhesion receptors mediating eosinophil adhesion.

Methods Eosinophils were purified from human peripheral blood by immunomagnetic selection and adhesion to confluent cultures of the airway epithelial cell lines A549 and BEAS-2B was studied.

Results Stimulation of A549 cells with $\text{TNF}\alpha$, $\text{IFN}\gamma$ or a combination of 50 ng/mL of $\text{TNF}\alpha$, $\text{IFN}\gamma$ and IL-1 (cytomix) did not effect eosinophil binding despite an increase in ICAM-1 expression. Similarly stimulation of eosinophils with PAF or IL-5 had no effect on eosinophil binding to medium- or cytokine-treated A549 cells. In contrast stimulation of BEAS-2B cells with cytomix caused a significant increase in eosinophil adhesion. This was associated with an increase in expression of ICAM-1 and induced expression of VCAM-1. Treatment of eosinophils with Mn^{2+} and IL-5 but not eotaxin, RANTES or PAF also significantly enhanced eosinophil adhesion to medium-treated BEAS-2B cells. Using blocking mAbs we were able to demonstrate that the increased adhesion resulting from stimulation of eosinophils or BEAS-2B cells was in both cases mediated by a combination of CD18 and $\alpha 4$ integrins.

Conclusions This study demonstrates a selective role for IL-5 in mediating integrin-dependent eosinophil adhesion to airway epithelium and once again emphasizes the importance of this cytokine in controlling eosinophil activation in diseases such as asthma.

Keywords: adhesion, asthma, eosinophils, epithelial cells

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Introduction

The pathology of asthma is characterized by an intense mucosal inflammation with infiltration of the airways by activated eosinophils and mononuclear cells in association with epithelial desquamation [1]. Epithelial damage in asthma is thought to be primarily mediated by the highly

basic eosinophil granule proteins, such as major basic protein (MBP), which have been shown to be toxic for airway epithelium *in vitro* [2]. Indeed this observation forms one of the major pillars of the hypothesis that eosinophils and their mediators are responsible for much of the pathological and physiological abnormalities characteristic of asthma (discussed in [3]).

As well as offering a target for eosinophil-derived mediators there is good evidence for epithelial activation in asthma [4]. The bronchial epithelium is an important

Correspondence: A. Wardlaw, Department of Respiratory Medicine, Glenfield Hospital, Groby Road, Leicester LE3 9QP, UK (e-mail: aw24@le.ac.uk).

source of the eosinophil active growth factors and chemo-attractants that control eosinophil migration into the airways and their subsequent activation. For example, the epithelium in asthma synthesizes eosinophil active growth factors such as GM-CSF and chemokines such as RANTES and eotaxin, as well as expressing increased amounts of adhesion receptors, in particular ICAM-1 [5]. Eosinophil–epithelial interactions are therefore likely to be pivotal to the pathogenesis of asthma.

Epithelial activation in asthma occurs in part as a result of exposure to pro-inflammatory cytokines in particular $\text{TNF}\alpha$ and IL-1 which have been shown to activate bronchial epithelium *in vitro* (reviewed in [6]). For cells to interact they generally must first adhere, a process that occurs through members of one or more adhesion receptor families. Eosinophils express a number of adhesion receptors (reviewed in [7]). These include the integrin $\alpha 4\beta 1$ which binds to the immunoglobulin-like receptor VCAM-1 [8,9], $\alpha 4\beta 7$ which binds MAdCAM-1 [10,11] and all four members of the CD18 (β_2) leucocyte integrin family CD11a–d, which include as ligands, ICAM-1-3 and VCAM-1 [12,13]. Godding *et al.* investigated adhesion of eosinophils to A549 cells, an immortalized alveolar type 2 lung epithelial cell line, as well as commercially obtained human bronchial epithelial cells. Treatment of eosinophils with PMA increased eosinophil adhesion to both unstimulated and cytokine-stimulated epithelial cells. Interestingly no increase in adhesion of unstimulated eosinophils to $\text{TNF}\alpha$ - or IL-1-stimulated epithelial cells was observed despite enhanced expression of ICAM-1. Consistent with this observation enhanced adhesion was CD18 but not ICAM-1 dependent [14]. Infecting A549 cells with RSV also enhanced eosinophil adhesion although, unlike neutrophils, only if the eosinophils were first stimulated with PMA. Enhanced adhesion in this model was inhibited by anti-ICAM-1 as well as anti-CD18 [15]. Co-culture of eosinophils and BEAS-2B cells (an SV-40 transformed bronchial epithelial cell line) resulted in enhanced eosinophil degranulation and adhesion when the eosinophils were treated with IL-5 and the epithelial cells with $\text{TNF}\alpha$ for 16 h. Adhesion was partially blocked by anti-CD18, but not anti-ICAM-1 mAbs [16]. Recently bronchial epithelial cells were shown to express mRNA and protein for VCAM-1 and an antibody against VCAM-1, but not ICAM-1, inhibited eosinophil adhesion to $\text{TNF}\alpha$ -stimulated BEAS-2B cells [17]. This was in contrast with a previous report that could find no VCAM-1 expression on BEAS-2B cells [18]. In previous studies eosinophils have generally required stimulation with PMA for enhancement of adhesion to epithelial cells to be observed. The physiological stimuli promoting eosinophil adhesion to the epithelium have not been investigated in detail although eosinophil adhesion to unstimulated human umbilical vein endothelium (HUVE) was increased by PAF and IL-5

[19,20]. In addition eotaxin, though not RANTES, enhanced eosinophil adhesion to lung vascular endothelial cells [21].

In this study we have characterized eosinophil adhesion to A549 cells and BEAS-2B cells. Although we were unable to modulate eosinophil adhesion to A549 cells, IL-5 treatment of eosinophils and cytokine stimulation of BEAS-2B cells enhanced adhesion in a CD18- and $\alpha 4$ integrin-dependent manner. In contrast eotaxin, RANTES and PAF failed to increase eosinophil adhesion.

Methods

mAbs and cytokines

The anti-VLA-4 mAb supernatant, HP1/2, was a generous gift from Dr F. Sanchez-Madrid (Madrid, Spain). The anti-ICAM-1 mAb used was a F(ab')_2 fragment of mAb R6.5 from Dr R. Rothlein (Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT, USA) and the anti-VCAM-1 mAb, 4B9, was donated by Dr R. Lobb (Biogen, Boston, MA, USA). The anti-CD18 mAb, clone IB4, was purchased from Alexis Co. (Nottingham, UK) and isotype-matched control antibodies were purchased from Sigma (Poole, UK). IL-5, RANTES, eotaxin, $\text{TNF}\alpha$, $\text{IFN}\gamma$ and IL-1 β were all purchased from R & D (Oxford, UK).

Eosinophil isolation

Eosinophils were isolated from 100 mL blood taken from volunteers showing mild or no atopic symptoms and who were not taking medication at the time of venesection. Purification was by density gradient centrifugation and negative immunomagnetic selection using anti-CD16-coated beads and the MACS system (Milenyi Biotec, Bisley, Surrey, UK). The eosinophils obtained were routinely >98% pure and 99% viable as determined by trypan blue exclusion.

Isolated eosinophils were then labelled using ^{51}Cr (sodium chromate, Amersham, UK) for 1 h as described by Walsh *et al.* [20]. Labelled cells were then washed into Medium 199 (M199) containing 2% FCS and used directly in the adhesion assay.

Culture of epithelial cell lines

The epithelial cell lines A549 and BEAS-2B were obtained through the ECACC (Porton Down, Salisbury, UK). A549 cells were maintained in DMEM culture medium supplemented with 10% FCS in 96-well tissue culture plates. After reaching confluence the A549 monolayers were cultured for a further 18–24 h with $\text{TNF}\alpha$ (50 ng/mL) or $\text{IFN}\gamma$ (50 ng/mL). BEAS-2B cells were cultured in Small Airway Growth Medium (SAGM from Clonetics, Wokingham, Berks, UK).

Once confluent they were treated for 18–24 h with cytomix (50 ng/mL each of TNF α , IFN γ and IL-1 β).

Adhesion assay

One hundred microlitres of purified, labelled eosinophils (1.5×10^6 cells/mL) were added to the epithelial cell monolayers in 96-well plates and incubated for 30 min at 37 °C. Each condition was tested in triplicate. The wells of the plate were then filled with M199 containing 2% FCS and sealed using mylar sealing sheets (Sigma). The sealed plates were then inverted and nonadherent eosinophils removed by centrifuging at 18 g for 5 min at room temperature. The remaining adherent eosinophils were quantified using a Cobra II Auto-Gamma counter (Packard, Pangbourne, Berks, UK) and the results expressed as percentage adhesion compared with total cells added.

For the blocking antibody studies saturating concentrations of antibodies were added to either the eosinophils or the epithelial cells, depending on which antigen they recognized, for 15 min at room temperature. For the studies involving eosinophil activation eosinophils were either treated with 5 mmol/L Mn $^{2+}$ for 15 min at room temperature or 10^{-6} mol/L PAF (Bachem, Saffron Walden, UK), 10 ng/mL IL-5, 10 ng/mL RANTES or 100 ng/mL eotaxin, for 30 mins at 37 °C before adding the cells to the epithelial cells.

ELISA assay

Confluent cultures of epithelial cells were washed with PBS and fixed with 5% formyl saline at room temperature for 10 min. After further washing with PBS the primary antibody was added (100 μ L of supernatant per well) and allowed to bind for 60 min at 37 °C. Unbound antibody was then removed by washing and rabbit antimouse peroxidase-conjugated antibody (Dako; 50 μ L/well diluted 1 : 500 from stock in PBS) was added for 30 min at 37 °C. To further amplify the signal swine antirabbit peroxidase (Dako, Ely, Cambridgeshire, UK) was added under the same conditions. OPD and H $_2$ O $_2$ were then added to each well in citrate buffer for 15 min at room temperature in the dark and the reaction was stopped by adding 3 mol/L H $_2$ SO $_4$. The reaction was read in a spectrophotometer at 490 nm and the results expressed in optical density units (OD).

Flow cytometry

We used indirect immunofluorescence followed by flow cytometry to assess adhesion molecule expression by cytokine-treated and untreated BEAS-2B cells. The epithelial monolayers were washed with PBS and detached using Cell Dissociation Solution (Sigma). The resulting cell suspension was washed into PBS, 1% BSA (staining buffer) and incubated with mAb or isotype-matched control for 15 min

at 4 °C. The cells were then washed once in staining buffer and incubated for a further 15 min, at 4 °C, with FITC-labelled F(ab') $_2$ rabbit-antimouse Ig (Dako). After a final wash the cells were analysed using a FACScan flow cytometer (Becton Dickinson, Oxford, UK).

Statistics

For the adhesion experiments each data point per experiment was the mean of triplicates. Data was expressed as the mean and standard error of the mean (SEM) calculated from a variable number of different experiments using different eosinophil donors. Statistical comparisons were undertaken using a paired Student *t*-test. *P* < 0.05 was regarded as significant.

Results

Eosinophil adhesion to A549 cells

We initially investigated eosinophil adhesion to A549 cells stimulated with TNF α or IFN γ . Both of these cytokines markedly increased expression of ICAM-1 (Fig. 1a,b). No increase in VCAM-1 expression was observed with either cytokine (data not shown). Despite this observation concentrations of TNF α or IFN γ that were optimal for enhancement of ICAM-1 expression failed to significantly increase adhesion of eosinophils to A549 cells above background levels. In addition stimulation of eosinophils with PAF failed to increase adhesion to either medium-treated or cytokine-treated A549 cells (Fig. 2a,b). Furthermore, a mixture of 50 ng/mL each of IL-1, IFN γ and TNF α (cytomix) with or without prior stimulation of the eosinophils with IL-5, failed to modulate adhesion (Fig. 2c).

Eosinophil adhesion to BEAS-2B cells

The inability to modulate eosinophil adhesion to A549 cells led us to investigate eosinophil adhesion to the bronchial epithelial cell line BEAS-2B. To obtain maximum stimulation of these cells we used cytomix. We also investigated whether treating eosinophils with manganese (Mn $^{2+}$) to maximally upregulate integrin function would effect eosinophil adhesion to BEAS-2B cells. Cytomix increased ICAM-1 expression on BEAS-2B cells and in addition induced expression of VCAM-1 (Fig. 3). Cytomix treatment of BEAS-2B cells significantly enhanced adhesion of unstimulated eosinophils from a background adhesion of 10.3 ($\pm$ 1.3) to 17.2% ($\pm$ 2.7) *P* < 0.05. Treatment of eosinophils with Mn $^{2+}$ caused a similar degree of enhancement of adhesion to medium-treated BEAS-2B cells increasing from 10.3 to 16.3% ($\pm$ 1.6) *P* < 0.05. The combination of Mn $^{2+}$ and cytomix resulted in a further significant enhancement to 24.4% ($\pm$ 2.2) *P* < 0.05 (Fig. 4).

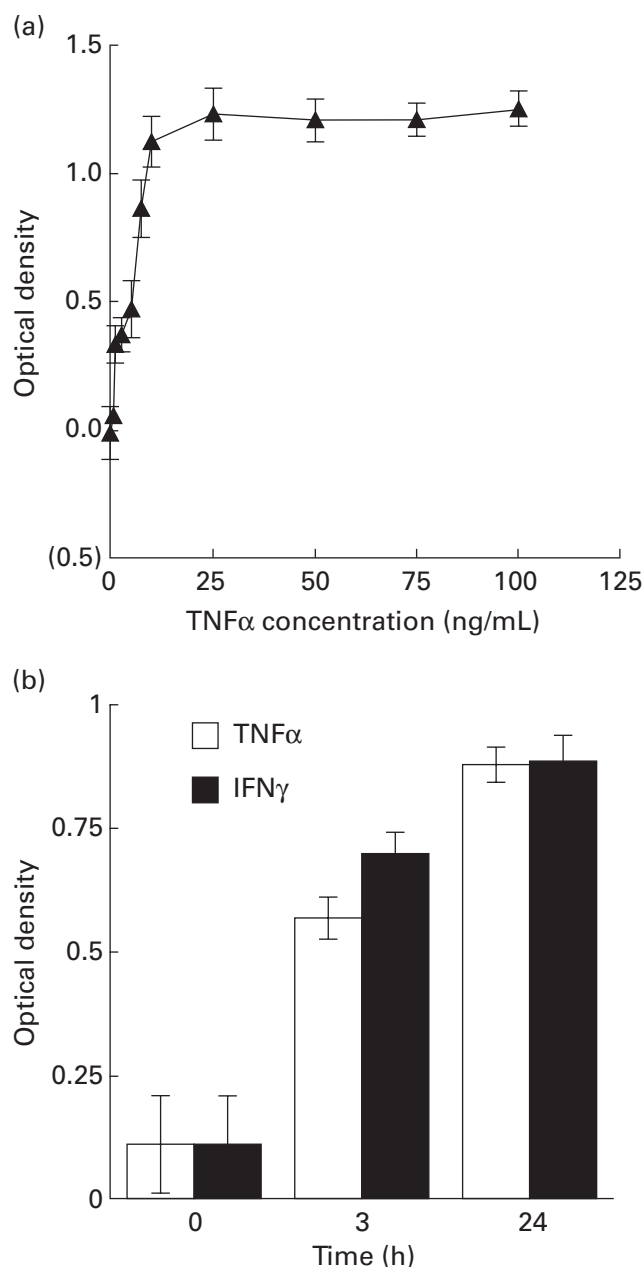


Fig. 1. Expression of ICAM-1 on A549 cells. Expression was determined using an ELISA assay as detailed in the Methods section and results expressed as optical density units. Low levels of background expression of ICAM-1 were observed in all experiments. Expression was markedly increased in (a) dose- (TNF α) and (b) time- (TNF α and IFN γ) dependent fashion ($n = 3$).

Effect of IL-5 and chemoattractants on eosinophil adhesion to BEAS-2B cells

As stimulation of integrin function on eosinophils enhanced adhesion we investigated which physiological mediators

would mimic the effects of Mn²⁺ and upregulate eosinophil adhesion. IL-5 significantly increased eosinophil adhesion to medium-treated BEAS-2B cells from a background of 12.4 (± 2.4) to 20.1% (± 2.1) $P < 0.05$. IL-5 treatment of eosinophils also resulted in a small enhancement of adhesion over and above that seen with cytomix treatment of epithelial cells, although this failed to reach significance. RANTES, eotaxin and PAF had no effect on eosinophil adhesion to either medium-treated or cytomix-treated BEAS-2B cells (Fig. 5). The concentrations of chemokines and PAF were chosen because they gave optimal chemotactic activity in a Boyden chamber assay (data not shown).

Adhesion receptors mediating eosinophil adhesion to BEAS-2B cells

We then investigated the adhesion receptors mediating the enhanced adhesion of eosinophils to BEAS-2B cells. As discussed above, cytomix treatment of BEAS-2B cells induced just under a twofold increase in eosinophil adhesion which in this set of experiments was from 17.0 (± 2.7) to 26.7% (± 2.9). This increase was completely and significantly inhibited by antibodies against either CD18 and $\alpha 4$ (Fig. 6a). In contrast no significant inhibition was observed using mAbs against either VCAM-1 or ICAM-1. Again as noted above, IL-5 induced an increase in eosinophil adhesion to unstimulated BEAS-2B cells from 17.0 to 28.1% (± 4.0) with a further small increase induced by cytomix treatment from 26.7 to 32.6% (± 3.6). Antibodies against CD18 or $\alpha 4$ brought the level of adhesion of IL-5-stimulated eosinophils down to background with both medium- and cytomix-treated epithelial cells. Again antibodies against either VCAM-1 or ICAM-1 had no effect (Fig. 6b).

Discussion

We initially started our investigations with A549 cells as they were a widely used model for investigating the cell biology of airway epithelium and there were previous studies of eosinophil adhesion to A549 cells in the literature. We found considerable variability in the background adhesion of unstimulated eosinophils to unstimulated A549 cells ranging from 5 to 23% (data not shown). This was not obviously related to the status of the eosinophil donor, most of whom were either normal donors or had only mild allergic symptoms. We do not therefore believe this variability can be explained by different degrees of *in vivo* eosinophil activation. Our adhesion assay uses centrifugation to remove nonadherent cells which reduces the variability seen with pipette washing in which the vigour of the pipetting step will effect background adhesion. The reason for the variability in baseline adhesion to A549 cells is therefore unclear.

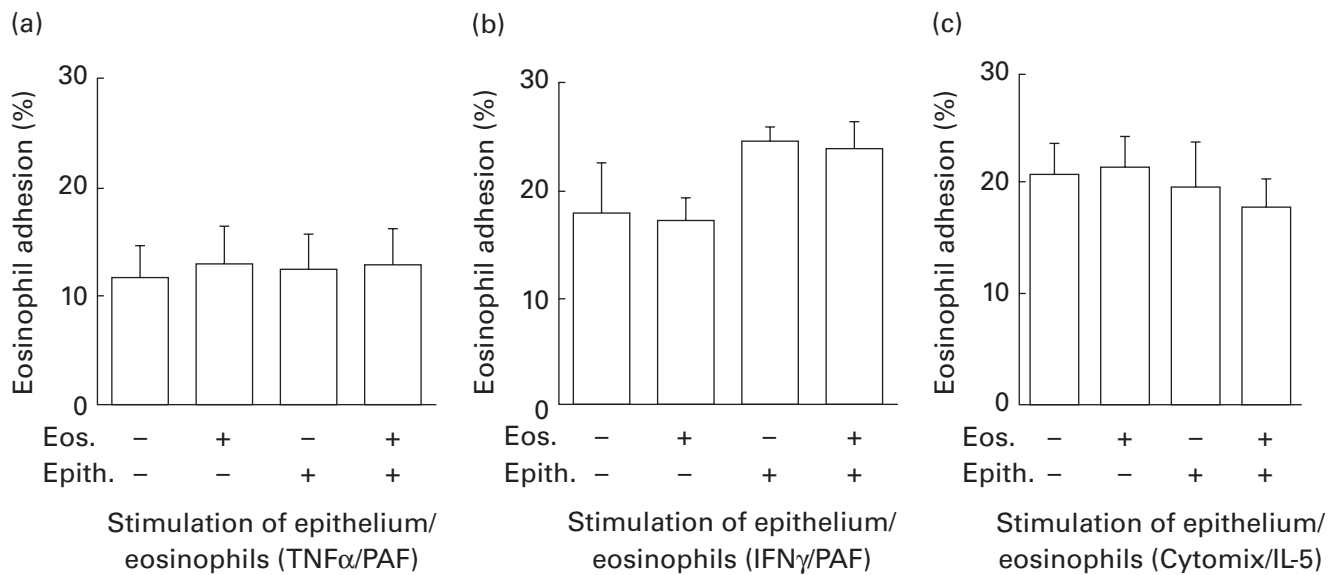


Fig. 2. Eosinophil adhesion to A549 cells. Background adhesion of untreated eosinophils to medium-treated A549 cells ranged between 5 and 23% over a series of experiments. Stimulation of eosinophils with PAF (10^{-6} mol/L) and A549 cells with either (a) TNF α ($n = 7$) or (b) IFN γ ($n = 3$) had no consistent effect on adhesion. (c) Similarly stimulation of the A549 cells with a mixture of TNF α , IFN γ and IL-1 (cytomix) with or without prior stimulation of the eosinophils with IL-5 for 30 min has no effect on adhesion ($n = 3$). A549 cells were treated with 50 ng/mL of each cytokine, or cytomix for 18–24 h. Each data point is the mean of triplicate wells and the results are expressed as mean ($\pm$ SEM) of three or seven separate experiments as indicated above.

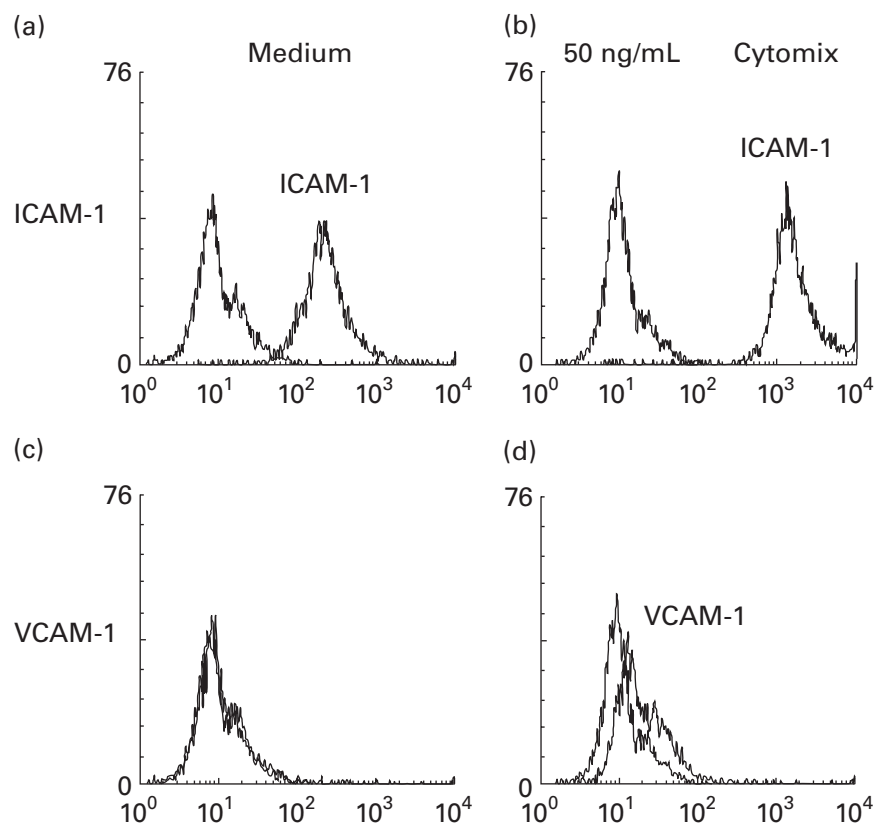


Fig. 3. Expression of ICAM-1 and VCAM-1 by BEAS-2B cells using flow cytometry. BEAS-2B cells cultured with medium or cytomix for 24 h were detached, stained with the appropriate antibody and analysed by FACS as described in Methods. ICAM-1 but not VCAM-1 expression was observed on medium-treated cells. ICAM-1 expression was markedly increased and a modest induction of VCAM-1 was observed after cytomix treatment. The control fluorescence represents mean fluorescence of epithelial cells stained with an isotype control first antibody and the FITC F(ab) ^{2} second antibody.

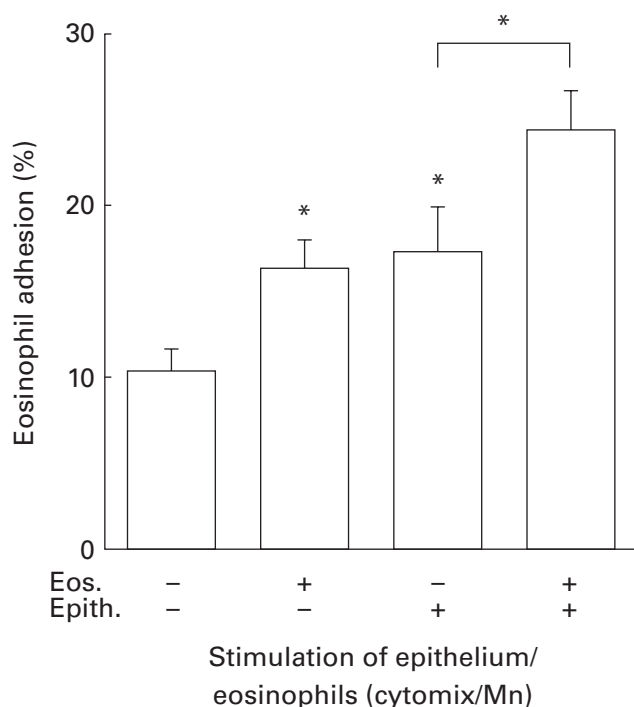


Fig. 4. Adhesion of eosinophils to BEAS-2B cells: effect of cytomix and Mn^{2+} . Background adhesion of untreated eosinophils to medium-treated BEAS-2B cells was consistently in the region of 10%. Eosinophil adhesion to medium-treated BEAS-2B cells was significantly increased by pretreatment of eosinophils with 5 mmol/L Mn^{2+} for 15 min at room temperature. An increase of similar magnitude over background adhesion was observed when unstimulated eosinophils were allowed to adhere to BEAS-2B cells treated with cytomix for 18–24 h. A further significant increase in adhesion to cytomix-treated cells was observed when the eosinophils were treated with Mn^{2+} (* $P < 0.05$). Each data point is the mean of triplicate wells and the results are expressed as mean ($\pm$ SEM) of seven separate experiments.

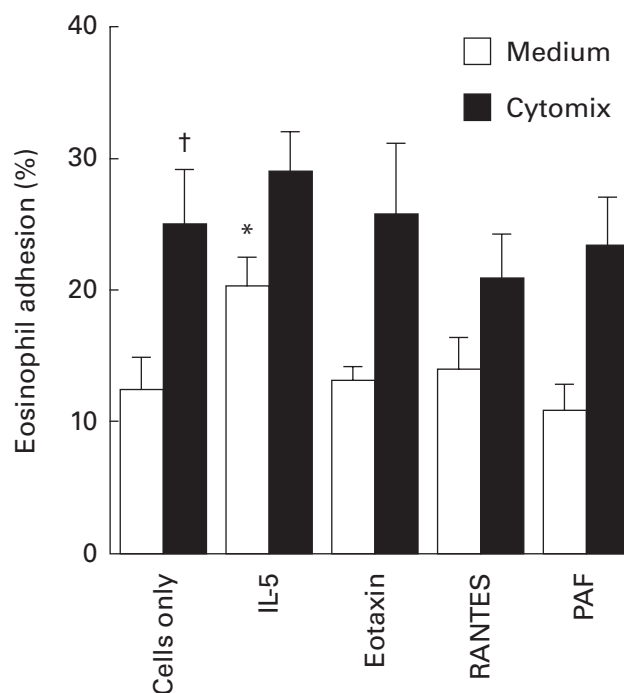


Fig. 5. Effect of stimulating eosinophils on adhesion to medium or cytomix-treated BEAS-2B cells. As shown in Fig. 4 cytomix treatment of BEAS-2B cells resulted in a significant increase in adhesion of unstimulated eosinophils († $P < 0.05$). Stimulation of eosinophils with IL-5 resulted in an increase in adhesion to unstimulated BEAS-2B cells (* $P < 0.05$). A small additional increase in adhesion of IL-5-treated eosinophils to cytomix-treated BEAS-2B cells was observed but this failed to reach significance. Eotaxin, RANTES and PAF had no effect on adhesion of eosinophils to either medium or cytomix-treated epithelial cells. Eosinophil stimulation was undertaken as detailed in the methods section. Each data point is the mean of triplicate wells and the results are expressed as mean ($\pm$ SEM) of six separate experiments.

We were unable to modulate adhesion to A549 cells either by treating eosinophils with PAF or IL-5, both reliable eosinophil activators which have been shown to enhance integrin-mediated eosinophil adhesion to vascular endothelium [19,20], or by stimulating the epithelial cells with either $TNF\alpha$, $IFN\gamma$ or cytomix. This was despite a marked increase in ICAM-1 expression. This is consistent with the work of Godding *et al.* [14] who found only small changes in eosinophil adhesion to A549 cells unless eosinophils were treated with the non-physiological stimulus PMA.

A549 cells are derived from an alveolar cell carcinoma and lack tight junctions. We therefore investigated eosinophil adhesion to another widely used model of airway epithelium, BEAS-2B cells. Background adhesion to these cells was much less variable than A549 cells being in the region of 10%. We observed a consistent enhancement of

eosinophil adhesion to cytomix-treated BEAS-2B cells. We used this combination of cytokines because it had been previously found to give more reliable stimulation of chemokine release from epithelial cells than each cytokine used alone [22]. We also found that Mn^{2+} which upregulates the binding function of both β_1 and β_2 integrins on a variety of cell types including eosinophils [23], enhanced eosinophil adhesion to BEAS-2B cells. The effect of Mn^{2+} suggested that mediators which modulate integrin function on eosinophils may increase their adhesion to BEAS-2B cells. Consistent with this hypothesis, we found that IL-5 significantly enhanced eosinophil adhesion to unstimulated BEAS-2B cells and that this enhanced adhesion was mediated through CD18 and $\alpha 4$ integrins. As well as being a growth and survival factor for eosinophils [24], IL-5 has a potent effect on a range of mature eosinophil functions including

enhancing eosinophil adhesion to unstimulated HUVE in a CD18-dependent manner [20] or (reviewed in [3]). IL-5 appears of crucial importance to the development of the airway eosinophilia and tissue damage that characterizes certain animal models of pulmonary inflammation [25], and is generated in the human airway in asthma [26]. The ability

of IL-5 to stimulate peripheral blood-derived eosinophils to bind to bronchial epithelium is therefore of considerable physiological relevance. Eosinophils are relatively poor phagocytes preferring to secrete their mediators onto a large non-phagocytosible particle such as the larvae of helminthic parasites. It has been suggested that the eosinophil may be inappropriately recognizing the epithelium as a helminthic target with the combination of IL-5 stimulation of eosinophils and cytokine stimulation of epithelium being sufficient to opsonize the epithelial cells and trigger eosinophil degranulation. This possibility was supported by the observations of Takafuji *et al.* who reported that IL-5 enhanced eosinophil degranulation and adhesion when these cells were co-cultured with cytokine-treated BEAS-2B cells for 16 h [16]. In contrast with our results they only observed enhanced adhesion when both the eosinophils were stimulated with IL-5 and the BEAS-2B cells with $\text{TNF}\alpha$. The differences with our results are possibly due to the 30 minute time-point we used for the adhesion assay and our use of cytomix rather than just $\text{TNF}\alpha$.

Previous studies have consistently pointed to a role for CD18 integrins in mediating eosinophil adhesion to bronchial epithelium. This is consistent with the important function of the β_2 leucocyte integrins in a number of eosinophil adhesion interactions including homotypic aggregation [27], binding to vascular endothelium [28] and adhesion to helminthic parasites [29]. The counter-structure on epithelial cells recognized by the CD18 integrins in our study is unclear. We found no consistent inhibition of adhesion using F(ab')_2 fragments of R6.5, a monoclonal antibody that blocks both LFA-1- and Mac-1-mediated binding to ICAM-1. Other studies have also reported ICAM-1-independent eosinophil adhesion to BEAS-2B cells [16,17] and studies of neutrophil adhesion to epithelium have identified a CD18-dependent/ICAM-1-independent pathway [30]. It seems likely that an alternative ligand for CD18 exists on BEAS-2B cells. In a recently reported study investigating eosinophil adhesion to

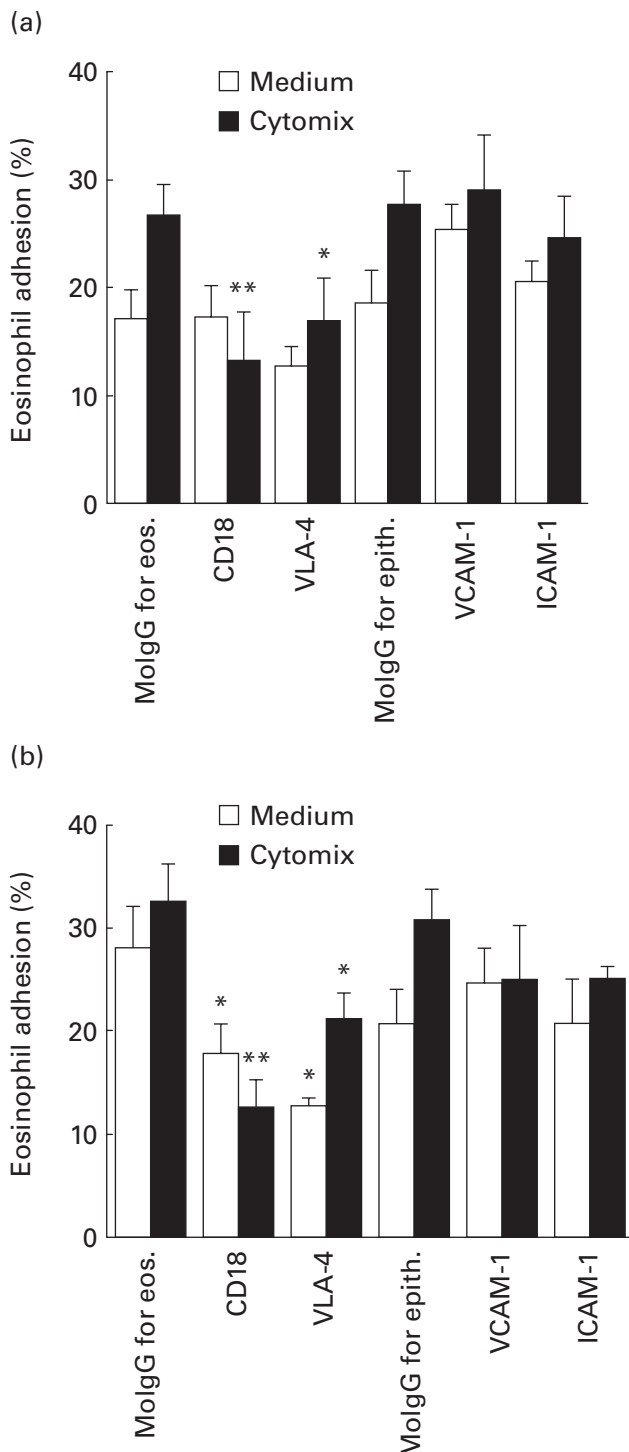


Fig. 6. Antibody inhibition of eosinophil adhesion (a) with and (b) without IL-5. Adhesion of unstimulated eosinophils to medium-treated BEAS-2B cells was in the region of 17% with the mouse control Ab. Cytomix treatment resulted in an increase to 28% adhesion. This increase was completely inhibited by the anti-CD18 mAb IB4 ($P < 0.01$) and the anti- $\alpha 4$ mAb HP1/2 ($P < 0.05$). The anti-VCAM-1 mAb 4B9 and the anti-ICAM-1 mAb R6.5 had no effect (a). When eosinophils were treated with IL-5 there was an increase in adhesion to both medium-treated and to a lesser extent cytomix-treated BEAS-2B cells. Levels of adhesion were returned to baseline values by treatment with either anti-CD18 or anti-VLA-4. Again anti-VCAM-1 and anti-ICAM-1 mAbs had no significant effect. Each data point is the mean of triplicate wells and the results are expressed as mean ($\pm$ SEM) of between three and eight separate experiments.

commercially obtained human bronchial epithelial cells, Burke-Gaffney and Hellewell [31] were able to inhibit eosinophil adhesion to cytokine-stimulated epithelium using an anti-ICAM-1 mAb. This difference may reflect the different origin of the epithelial cells used. The anti- $\alpha 4$ mAb HP1/2 inhibited eosinophil adhesion mediated both by IL-5 and cytomix. Atsuta *et al.* [17] recently demonstrated that TNF α treatment of BEAS-2B cells induced expression of VCAM-1 and we have confirmed this observation using cytomix. The role of $\alpha 4$, a ligand for VCAM-1, in eosinophil adhesion to cytomix-treated BEAS-2B cells is consistent with this observation, although surprisingly we were unable to inhibit adhesion using the well-established blocking anti-VCAM-1 mAb 4B9. Atsuta *et al.* were able to inhibit adhesion using an anti-VCAM-1 mAb. The difference with our results may be due to their use of a F(ab')₂ fragment of anti-VCAM-1 which we do not have available. We also observed inhibition of IL-5-induced adhesion to unstimulated BEAS-2B cells with HP1/2 although we were unable to detect, using flow cytometry, any VCAM-1 expression on resting cells. It is possible that very low level expression, below the limits of our flow cytometer was sufficient to support binding.

Like Burke-Gaffney and Hellewell [31] we were unable to modulate adhesion with RANTES or PAF despite their highly effective chemotactic activity [32,33]. However, unlike these authors we were unable to see any effect with eotaxin, although this chemokine is also an effective chemo-attractant at the concentrations used [34]. The differences between our findings could again be due to the different cell type studied.

We have not studied primary cultures of bronchial epithelial cells. Explant cultures derived from lung resection specimens do not passage well and are therefore difficult to use in adhesion assays. They also rapidly undergo squamous differentiation. Because of these problems most studies of leucocyte adhesion have used commercially available cells derived from human bronchial epithelium as a substitute for primary cultures. However, these cells have very different growth characteristics to explant cultures and do readily passage. Exactly how close these commercially derived cells are to true primary cultures of bronchial epithelial cells is not clear.

In summary, therefore, we have demonstrated that eosinophil adhesion to a human bronchial epithelial cell line was significantly enhanced by activating the epithelial cells with a mixture of pro-inflammatory cytokines, or by stimulating eosinophils with the growth factor IL-5, though not with either lipid or chemokine chemoattractants. The enhanced adhesion was mediated by the CD18 and $\alpha 4$ integrins binding to as-yet undetermined counter-structures on the epithelium. This study provides further evidence for the pivotal role of IL-5 in eosinophil function in asthma.

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