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Interferon-β augments eosinophil adhesion-inducing activity of endothelial cells

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ABSTRACT: Viral infections induce exacerbations of asthma. One of the earliest host responses to viral infections is the production of innate cytokines including type I interferons (IFNs), such as IFN-β, which may act to modify airway inflammation. The objective of the present study was to investigate whether IFN-β modifies the eosinophil adhesion-inducing activity of endothelial cells.

Human umbilical vein endothelial cells (HUVECs) were stimulated with IFN-β for 24 h in the presence or absence of tumour necrosis factor (TNF)-α. Eosinophils were isolated from the peripheral blood of healthy volunteers. The ability of the IFN-β-stimulated HUVEC monolayers to induce eosinophil adhesion was assessed according to the eosinophil peroxidase assay.

Eosinophil adhesion to HUVECs was significantly augmented by IFN-β in the presence of TNF-α but not in its absence. The augmented adhesion was inhibited by anti-α_4 integrin monoclonal antibody (mAb) or anti-β_2 integrin mAb. IFN-β significantly enhanced the expression of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 on HUVECs in the presence of TNF-α.

Interferon-β can augment the adhesiveness of endothelial cells to eosinophils, mainly through the expression of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1. This action of interferon-β may contribute to the intensification of airway inflammation in asthma that is associated with exacerbations induced by viral infections.

KEYWORDS: Asthma, endothelial cells, eosinophilic airway inflammation, viral infection

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STATEMENT OF INTEREST
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Acute respiratory infections commonly precede asthma exacerbations in both children and adults [1–3]. The majority of episodic exacerbations of asthma are induced by viral respiratory infections, in particular rhinovirus infections [4]. The mechanism by which viral respiratory infections exacerbate asthma is a complex process that may be regulated by the enhanced production of cytokines, chemokines and other classes of inflammatory molecules [4, 5]. An effective antiviral immune response requires the early clearance of viruses and the appropriate termination thereof, to minimise concomitant immunopathology and tissue damage. One of the earliest host responses to viral infections is the production of initial innate cytokines. These cytokines include type I interferons (IFNs) such as IFN-β [6, 7]. WARK *et al.* [7] recently reported that respiratory epithelial cells from asthmatics have a lower IFN-β-producing ability that is associated with a reduced ability to clear viruses. Since IFNs have a variety of pro-inflammatory actions on inflammatory cells, including eosinophils, epithelial cells and endothelial cells [8–12], it is theoretically conceivable that these cytokines may modify and aggravate the inflammatory status of airway diseases, including asthma, during or after viral infection.

Eosinophils are inflammatory cells predominantly found in the airways of asthmatic patients and are likely to contribute to the pathogenesis of asthma through the production of a variety of mediators including cysteinyl (cys) leukotriene (LT) and transforming growth factor-β [11–14]. Although neutrophils play central roles in asthma exacerbations induced by viral respiratory infections, clinical data support the involvement of eosinophils in virus-induced exacerbations and increased airway hyperresponsiveness in asthmatic patients [15–17]. In atopic asthmatics, for example, experimental infections with rhinovirus (RV)16 increased epithelial eosinophil counts; this increase appeared to persist up to convalescence [15]. In asthmatic patients with confirmed viral infection, sputum showed high eosinophilic cationic protein (ECP) levels [16]. In atopic mild asthmatics, increased airway hyperresponsiveness to histamine was correlated significantly with an increase in ECP levels and with changes in eosinophil levels in induced

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sputum after nasal administration of RV16 [17]. For eosinophils, the initial step in their participation in the airway inflammation of asthma is the adhesion of circulating eosinophils to vascular endothelial cells. It is generally accepted that this process is mainly mediated by the interaction between eosinophil integrin adhesion molecules (including α_4 integrins, such as $\alpha_4\beta_1$ (also known as CD49d, CD29 or very late activation antigen-4), and β_2 integrins, such as $\alpha_L\beta_2$ (CD11a/CD18/lymphocyte function-associated antigen-1) and $\alpha_M\beta_2$ (CD11b/CD18/macrophage-1 antigen)) and their counter ligands (vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1) on endothelial cells [18, 19].

The objective of the present study was to evaluate whether IFN-β modifies the adhesive interaction between eosinophils and endothelial cells.

MATERIAL AND METHODS

Reagents

Percoll® was obtained from Pharmacia (Uppsala, Sweden). Anti-CD16 antibody-coated magnetic beads were purchased from Miltenyl Biotec (Auburn, CA, USA). Human umbilical vein endothelial cells (HUVECs) and HuMedia EG were purchased from Kurabo Industries Ltd (Osaka, Japan). Endothelial cell growth medium was purchased from Clonetics Corporation (Palo Alto, CA, USA). Hanks' balanced salt solution (HBSS), PBS and newborn calf serum (NCS) were obtained from Life Technologies (Grand Island, NY, USA). Foetal bovine serum was purchased from ICN Biomedicals Inc. (Aurora, OH, USA). Recombinant human (rh-) IFN-α, IFN-β, IFN-γ and tumour necrosis factor (TNF)-α were purchased from R&D Systems (Minneapolis, MN, USA). Anti-α_4-integrin monoclonal antibody (mAb; clone HP2/1) was purchased from Cosmo Bio Co. Ltd (Tokyo, Japan). Anti-β_2-integrin mAb (clone L130) was purchased from Becton Dickinson (Franklin Lakes, NJ, USA). Murine immunoglobulin (Ig)G1 was purchased from ICN Biomedicals, Inc. Anti-P-selectin glycoprotein ligand (PSGL)-1 (CD162) mAb (clone PL-1) was purchased from Immunotech, a Coulter company (Marseille, France). Other reagents were purchased from Sigma-Aldrich Co. (St Louis, MO, USA) unless otherwise stated.

Preparation of HUVECs

HUVECs were prepared as previously described [20]. Briefly, HUVECs were incubated on type IV collagen-coated tissue culture flasks until confluent, transferred to collagen-coated 96-well tissue culture plates and then stimulated either with a combination of IFN-β (30–1,000 pM) and TNF-α (10 pM) or with IFN-β (30–1,000 pM) alone in 5% CO_2 at 37°C for 24 h. After incubation, the incubated mixture was decanted and the HUVECs were washed three times with HBSS. In selected experiments, HUVECs were fixed with 100 μL of 1% paraformaldehyde in PBS at room temperature for 15 min, in order to block the synthesis of mediators. After washing three times in HBSS, 200 μL of 1% glycine in HBSS were added and incubated at ambient temperature for 1 h to quench any residual paraformaldehyde. The plates were then decanted and washed three times in HBSS before use.

Eosinophil isolation

Eosinophil isolation was performed according to the negative immunomagnetic bead selection method, as previously described [21]. To complete all the experiments, eosinophils were isolated from the peripheral blood of 42 healthy volunteers who were aged 20–29 yrs and had an equal sex distribution. Briefly, the heparinised blood was diluted with HBSS without Ca^{2+} and then centrifuged on Percoll® (1.090 $g \cdot mL^{-1}$; $700 \times g$ for 20 min). Plasma, mononuclear cell bands and Percoll® were removed, and red blood cells in the pellets were lysed by hypotonic shock. Granulocytes obtained were washed in 4°C HBSS supplemented with 2% NCS and then incubated with anti-CD16 antibody-coated magnetic beads at 4°C for 40 min. The cells were filtered through the steel wool column in a magnetic field (Miltenyl Biotec) to remove neutrophils bound to magnetic beads. CD16-negative eosinophils (>98% purity and >99% viability) were collected, washed and then resuspended in HBSS supplemented with 0.1% gelatin (HBSS/0.1% gelatin).

Eosinophil adhesion

Eosinophil adhesion to HUVECs was assessed by the residual eosinophil peroxidase activity of adherent eosinophils, as previously described [20–23]. Briefly, eosinophils (100 μL of 1×10^5 cells·mL^{-1} in HBSS/0.1% gelatin) were placed onto the HUVEC monolayers and then incubated at 37°C for 30 min. After five washes in 37°C HBSS, 100 μL of HBSS/0.1% gelatin were added to the reaction wells. As standards, 100 μL of serially diluted cell suspensions (1×10^3, 3×10^3, 1×10^4, 3×10^4 and 1×10^5 cells·mL^{-1}) were added to the empty wells. The o-phenylenediamine (OPD) substrate (1 mM OPD, 1 mM H_2O_2 and 0.1% Triton X-100 in Tris buffer, pH 8.0) was then added to all the wells. After incubation at room temperature for 30 min, 50 μL of 4 M H_2SO_4 were added to stop the reaction and absorbance at 490 nm was determined. The percentage of eosinophil adhesion was calculated from the log dose–response curve. Eosinophil viability after incubation, which was determined by trypan blue dye exclusion, exceeded 98%.

Determination of VCAM-1 and ICAM-1 expression on HUVECs

The expression of VCAM-1 and ICAM-1 was determined by cell ELISA, as previously reported [20, 24]. Briefly, the HUVEC monolayers were incubated in the 96-well tissue culture plates and then stimulated with either a combination of IFN-β (30–1,000 pM) and TNF-α (10 pM) or with IFN-β (30–1,000 pM) alone at 37°C for 24 h [20, 24]. Prior to the evaluation, HUVECs were washed and incubated at 37°C for 30 min with a blocking buffer (PBS containing 5% NCS and 3% nonfat dry milk). Primary antibodies (obtained from R&D Systems), *i.e.* anti-ICAM-1 mAb (clone BBIG-I1), anti-VCAM-1 mAb (clone BBIG-V1) and isotype-matched control murine IgG1, were added to the wells and the incubation was then resumed at 37°C for a further 2 h. HUVECs were washed three times in the blocking buffer and secondary antibody (peroxidase-conjugated sheep anti-murine IgG) was added to the wells. Following a 2-h incubation, cells were washed three times in PBS. The peroxidase conjugate was detected using the OPD substrate in the citrate-urea buffer according to a procedure similar to that used in the eosinophil adhesion assay. The VCAM-1 or ICAM-1 concentration in cells was expressed as absorbance at

490 nm and was reported as the actual value minus absorbance of isotype-matched control murine IgG1.

Statistical analysis

Values are expressed as mean±SEM. Paired t-tests were conducted for comparison of two groups and repeated-measures ANOVA with Scheffé's constants was used to compare more than two groups. A p-value <0.05 was considered statistically significant.

RESULTS

Effects of IFN-β on the adhesive interaction between eosinophils and endothelial cells

An initial series of experiments was conducted to determine whether IFN-β directly modifies the adhesiveness of eosinophils. Eosinophils were stimulated with rh-IFN-β (30–1,000 pM) and their adhesiveness to HUVECs was examined. The adhesiveness was not modified by IFN-β (n=5, data not shown). Subsequently, the ability of IFN-β to modify the adhesiveness of endothelial cells to eosinophils was evaluated in the presence or absence of TNF-α. HUVECs were stimulated with either IFN-β alone (30–1,000 pM) or with a combination of IFN-β (30–1,000 pM) and TNF-α (10 pM) in 5% CO_2 at 37°C for 24 h, and eosinophil adhesion to HUVECs was then examined. The percentages of eosinophil adhesion to resting and TNF-α (10 pM)-stimulated HUVECs with no IFN-β were 2.8±1.2 and 6.6±1.8, respectively; as expected, eosinophil adhesion to HUVECs was augmented by TNF-α stimulation ($p<0.01$, n=8). IFN-β (30–1,000 pM) stimulation alone did not modify the adhesiveness of HUVECs to eosinophils (n=8; fig. 1a). However, IFN-β (300–1,000 pM) stimulation significantly upregulated the eosinophil adhesion-inducing ability of HUVECs in the presence of TNF-α (adhesion of eosinophils: 6.6±1.8% by control *versus* 14.7±3.5% by 300 pM ($p<0.01$) and 14.8±3.1% by 1000 pM ($p<0.001$); n=8; fig. 1b). The IFN-β (300 pM)-augmented adhesiveness in TNF-α (10 pM)-stimulated HUVECs was not observed in the presence of anti-IFN-β mAb (adhesion of eosinophils in the presence of isotype-matched control mouse IgG1: 7.2±2.5% by control *versus* 11.4±3.5% by 300 pM IFN-β ($p<0.01$); in the presence of anti-IFN-β mAb: 7.8±3.1% by control *versus* 8.6±3.2% by 300 pM IFN-β (p=nonsignificant (NS)); n=5). Eosinophil adhesion to resting or TNF-α (10 pM)-stimulated HUVECs was not modified by anti-IFN-β mAb (data not shown).

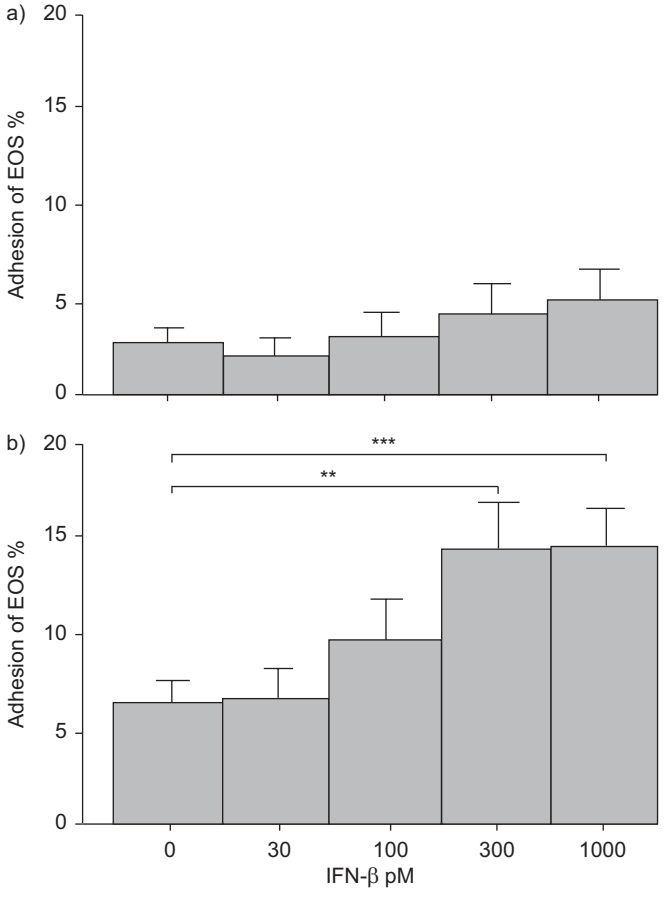

FIGURE 1. Effects of interferon (IFN)-β on the eosinophil (EOS) adhesion-inducing ability of human umbilical vein endothelial cells in the a) absence or b) presence of tumour necrosis factor-α (10 pM). For each bar, the mean±SEM of eight experiments using EOS from different donors is shown. **: $p<0.01$; ***: $p<0.001$.

The effects of incubation time of IFN-β on the enhanced adhesiveness of TNF-α (10 pM)-stimulated HUVECs were then evaluated. TNF-α (10 pM)-stimulated HUVECs were incubated in the presence or absence of IFN-β (300 pM) for a range of incubation times (1–24 h) and then washed, and HUVEC adhesiveness to eosinophils was evaluated. The present study revealed that the IFN-β (300 pM)-augmented adhesiveness of HUVECs to eosinophils appeared at 4 h and lasted until 24 h of incubation time ($p<0.05$ for comparison of each time-point with the previous one; n=5; fig. 2).

To examine whether HUVECs by themselves generate IFN-β, HUVECs were cultured in the presence or absence of TNF-α (10 pM) for 24 h and then the supernanatants were evaluated by ELISA. IFN-β protein was not detected in the supernatants of these HUVECs.

Effects of anti-adhesion molecule antibodies on the eosinophil adhesion augmented by IFN-β

To identify eosinophil adhesion molecules that are involved in the IFN-β-augmented adhesion of eosinophils to HUVECs, eosinophils were pretreated with either anti-β_2 integrin mAb (clone L130, mouse IgG1, 3 $\mu g \cdot mL^{-1}$), anti-α_4 integrin mAb (clone HP2/1, mouse IgG1, 3 $\mu g \cdot mL^{-1}$), anti-PSGL-1 (CD162) mAb (clone PL-1, mouse IgG1, 3 $\mu g \cdot mL^{-1}$) or isotype-matched control murine IgG1 (3 $\mu g \cdot mL^{-1}$) at ambient temperature for 15 min. Subsequently, eosinophil adhesion to HUVECs was stimulated with a combination of IFN-β (300 pM) plus TNF-α (10 pM) or with TNF-α (10 pM) alone. IFN-β (300 pM) stimulation significantly augmented adhesiveness of HUVECs to eosinophils when eosinophils were pretreated with murine IgG1 or anti-PSGL-1 mAb ($p<0.01$; n=5; fig. 3). In contrast, the effects of IFN-β (300 pM) on the augmented adhesion of eosinophils to HUVECs were significantly inhibited by either anti-β_2 integrin mAb or anti-α_4 integrin mAb (fig. 3), suggesting roles for the counter ligands of these integrins, namely ICAM-1 and VCAM-1. When anti-β_2 integrin mAb was present, the augmentation of eosinophil adhesion to HUVECs by IFN-β (300 pM) stimulation was modest but remained significant (1.8±0.5% by TNF-α (10 pM) alone *versus*

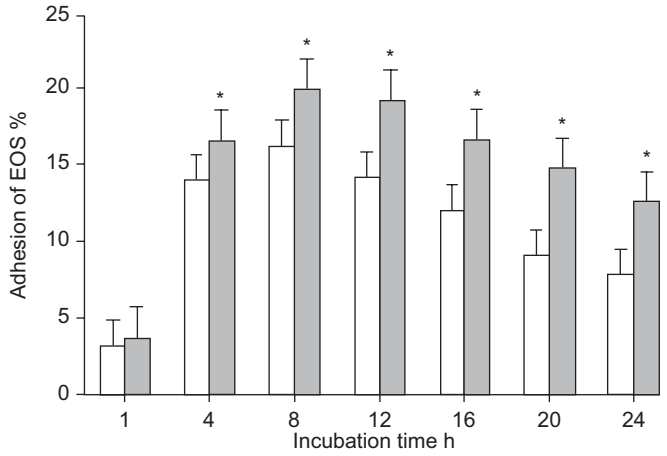

FIGURE 2. Effects of incubation time (1–24 h) of tumour necrosis factor-α (10 pM) in the presence (■) or absence (□) of interferon-β (300 pM) on the eosinophil (EOS) adhesion-inducing ability of human umbilical vein endothelial cells. For each bar, the mean±SEM of five experiments using EOS from different donors is shown. *: $p<0.05$ for comparison with previous time-point.

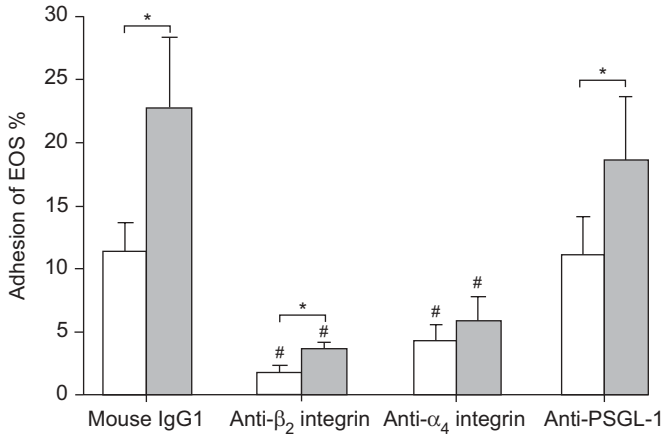

FIGURE 3. Effects of anti-adhesion molecule antibodies on eosinophil (EOS) adhesion to human umbilical vein endothelial cells stimulated with tumour necrosis factor-α (10 pM) in the presence (■) or absence (□) of interferon (IFN)-β (300 pM). For each bar, the mean±SEM of seven experiments using EOS from different donors is shown. Ig: immunoglobulin; PSGL: P-selectin glycoprotein ligand. *: $p<0.05$ *versus* no IFN-β; #: $p<0.05$ *versus* murine IgG1.

3.6±0.8% by TNF-α (10 pM) plus IFN-β (300 pM); p=0.03; n=7; fig. 3). Conversely, the augmented adhesiveness of HUVECs to eosinophils by IFN-β (300 pM) stimulation was not significant when eosinophils were pretreated with anti-α_4 integrin mAb (4.4±1.1% by TNF-α (10 pM) alone *versus* 5.9±1.4% by TNF-α (10 pM) plus IFN-β (300 pM); p>0.05; n=7).

Effects of IFN-β on the expression of endothelial cell adhesion molecules

Whether IFN-β modifies the expression of ICAM-1 and VCAM-1 on HUVECs was next evaluated. HUVECs were stimulated with IFN-β (30–1,000 pM) in the presence or absence of TNF-α (10 pM) for 24 h; the expression of ICAM-1 and VCAM-1 was then determined by cell ELISA. In the absence of TNF-α, IFN-β (30–1,000 pM) modified the expression of neither VCAM-1 (fig. 4a) nor ICAM-1 (fig. 4b). Conversely, IFN-β (300–1,000 pM) significantly upregulated the expression of VCAM-1 on HUVECs stimulated with TNF-α (10 pM) (absorbance optical density (OD): 0.026±0.003 by TNF-α alone *versus* 0.072±0.012 by TNF-α plus IFN-β (300 pM); p=0.03; n=6; fig. 4a). Similarly, IFN-β (30–1,000 pM) significantly upregulated the expression of ICAM-1 on HUVECs stimulated with TNF-α (OD: 0.492±0.023 by TNF-α alone *versus* 0.614±0.019 by TNF-α (10 pM) plus IFN-β (30 pM); p<0.01; n=6; fig. 4b). Finally, the effects of IFN-β on the expression of VCAM-1 and ICAM-1 on TNF-α-stimulated HUVECs were also confirmed by flow cytometric analysis. Following stimulation with 300 pM IFN-β for 24 h, the expression of both VCAM-1 and ICAM-1 on HUVECs stimulated with TNF-α were enhanced (mean fluorescence index for VCAM-1: 24.4±8.5 by medium control *versus* 42.7±16.5 by IFN-β (p<0.01); for ICAM-1: 708.7±108.2 by medium control *versus* 1,450.7±144.5 by IFN-β (p<0.01); n=4).

Mechanism of IFN-β-augmented adhesion of eosinophils to endothelial cells

IFN-β may trigger the production of pro-inflammatory mediators from endothelial cells and, hence, may augment eosinophil adhesiveness. To corroborate this hypothesis, HUVECs were stimulated with or without IFN-β (300 pM) in the presence of TNF-α (10 pM) for 24 h, washed, and then fixed with 1% paraformaldehyde. Following washing, HUVECs were used to evaluate eosinophil adhesiveness. Even following the fixation, HUVEC adhesiveness to eosinophils augmented by the addition of IFN-β was significant (10.3±5.1% by TNF-α alone *versus* 14.2±5.3% by TNF-α plus IFN-β; p<0.05; n=6). Eosinophils may be activated by the interaction with VCAM-1 or ICAM-1 on HUVECs that were stimulated with IFN-β, and may thus augment their adhesiveness in an autocrine/paracrine fashion. For example, eosinophils are capable of producing cysLT, which can enhance the adhesiveness of eosinophils themselves. To assess this possibility, eosinophils were pretreated with a cysLT receptor antagonist, montelukast (1 μM), and eosinophil adhesiveness to HUVECs stimulated with or without IFN-β in the presence of TNF-α was evaluated. The cysLT antagonist did not attenuate eosinophil adhesiveness to HUVECs stimulated with or without IFN-β in the presence of TNF-α (without IFN-β (TNF-α alone): 6.6±2.1 *versus* 7.5±1.5% for control *versus* montelukast (p=NS); with IFN-β: 10.5±1.5 *versus* 11.9±2.1% for control *versus* montelukast (p=NS); n=4; data not shown).

Effects of a variety of IFNs on endothelial cell adhesiveness to eosinophils

To evaluate whether the adhesiveness of endothelial cells to eosinophils is modified by other classes of IFNs, HUVECs were treated with IFN-α, a type I IFN, or with IFN-γ, a type II IFN, in the presence or absence of TNF-α (10 pM) at 37°C for 24 h. The ability to induce increased eosinophil adhesion of HUVECs was thus examined. None of the IFNs (α, β or γ) at 300 pM modified HUVEC adhesiveness to eosinophils in the absence of TNF-α (10 pM). However, IFN-α, IFN-β and IFN-γ (all 300 pM) significantly augmented the eosinophil adhesion-inducing ability of HUVECs in the presence of TNF-α (10 pM; 5.2±0.3% by control *versus* 12.7±2.5% by IFN-α (p<0.05),

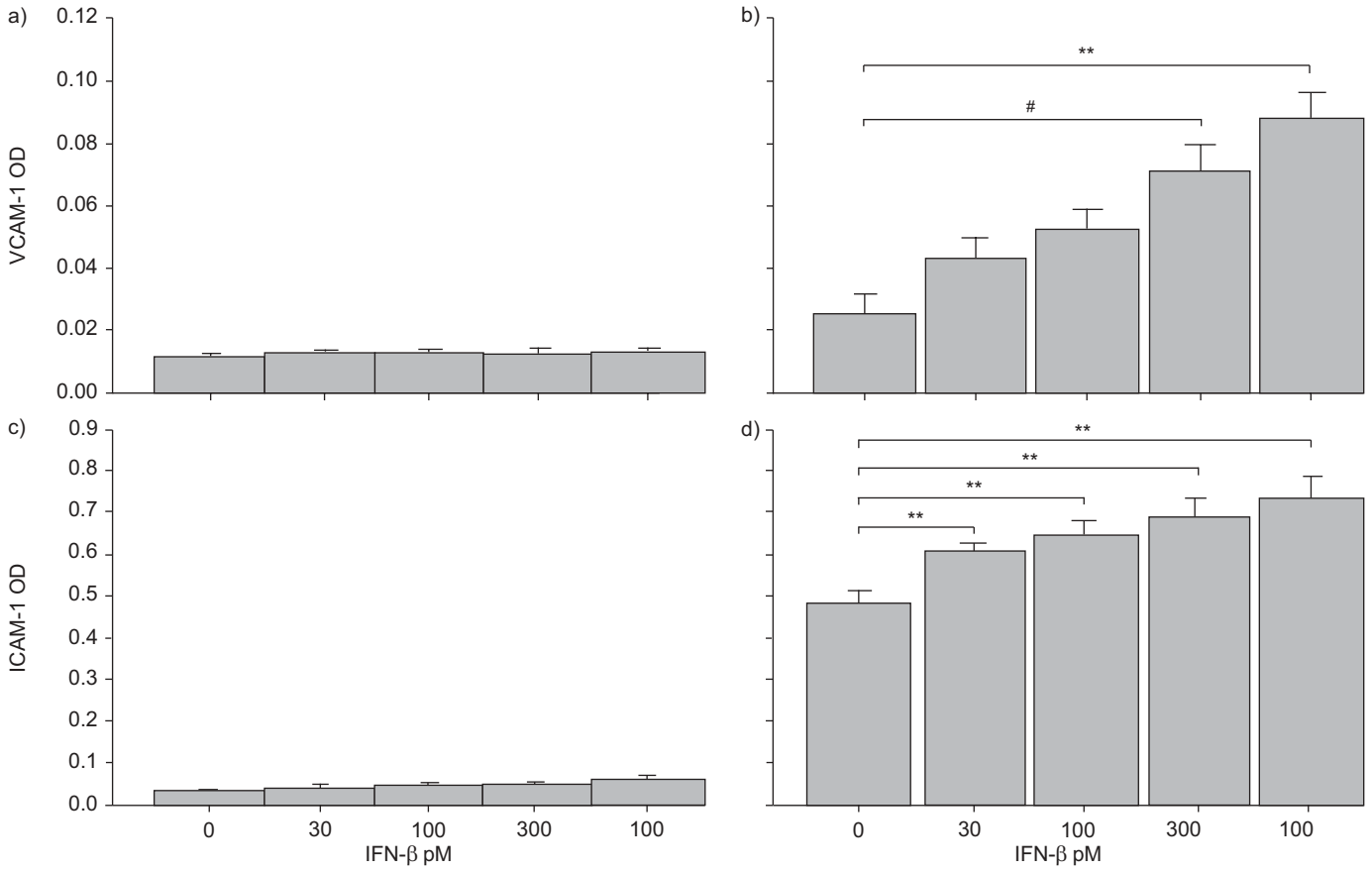

FIGURE 4. Effects of interferon (IFN)-β on the expression of vascular cell adhesion molecule (VCAM)-1 (a and b) and intercellular adhesion molecule (ICAM)-1 (c and d) on human umbilical vein endothelial cells in the absence (a and c) or presence (b and d) of tumour necrosis factor-α (10 pM). For each bar, the mean±SEM of six different experiments is shown. OD: optical density. **: $p<0.01$; #: $p=0.03$.

13.7±2.8% by IFN-β ($p<0.05$) and 12.1±2.1% by IFN-γ ($p<0.05$); n=4; fig. 5b). There was no significant difference between the three IFN-treated groups. Finally, IFN-α, IFN-β and IFN-γ (all 300 pM) significantly augmented the expression of VCAM-1 and ICAM-1 on HUVECs in the presence of TNF-α (10 pM) and there was no significant difference between the three groups ($p<0.01$; n=4; fig. 5c–f).

DISCUSSION

In the present study, evidence was provided that the IFN family, including IFN-β, augments the adhesiveness of endothelial cells to eosinophils, which may be a novel regulatory mechanism for eosinophilic inflammation in the airways of patients with asthma. Although IFN-β by itself did not directly modify eosinophil adhesiveness, it was observed that endothelial cell stimulation with IFN-β augments the eosinophil adhesion-inducing ability of HUVECs in the presence of TNF-α. The neutralising effects of mAbs on IFN-β demonstrated that the augmented adhesiveness of endothelial cells to eosinophils was mediated specifically by this cytokine. The effect of IFN-β appears to involve the expression of adhesion molecules on endothelial cells, a conclusion that can be drawn from the following results. First, the adhesiveness of eosinophils augmented by IFN-β stimulation was blocked by anti-α_4 integrin or anti-β_2 integrin antibody. Secondly, IFN-β significantly augmented the expression of VCAM-1 and ICAM-1. Although both endothelial cells and eosinophils could produce mediators which may modify eosinophil adhesiveness, the effects of IFN-β were observed in fixed HUVECs or eosinophils treated with inhibitors for representative eosinophil-derived mediators. Therefore, the current authors speculate that IFN-β mainly enhances the expression of VCAM-1 and ICAM-1, and that the effect also confers greater adhesiveness to eosinophils. Finally, the present study provided evidence that not only IFN-β but also IFN-α and IFN-γ augment the adhesiveness of endothelial cells to eosinophils. Collectively, the current results suggest a potentially important biological effect of IFNs in the development of adhesive interaction between eosinophils and endothelial cells when IFNs and TNF-α are overproduced in the airways.

The IFN family of cytokines has essential roles in immunity. There is evidence that the IFN family modifies either the expression of adhesion molecules or the proliferation of endothelial cells. The present study is the first to verify that IFNs actually augment the adhesiveness of endothelial cells to eosinophils. This observation is in agreement with previous studies on the interaction between IFN-β and human vascular endothelial cells. For example, MILLER *et al.* [25] reported that IFN-β, but not IFN-γ, modestly enhances the expression of

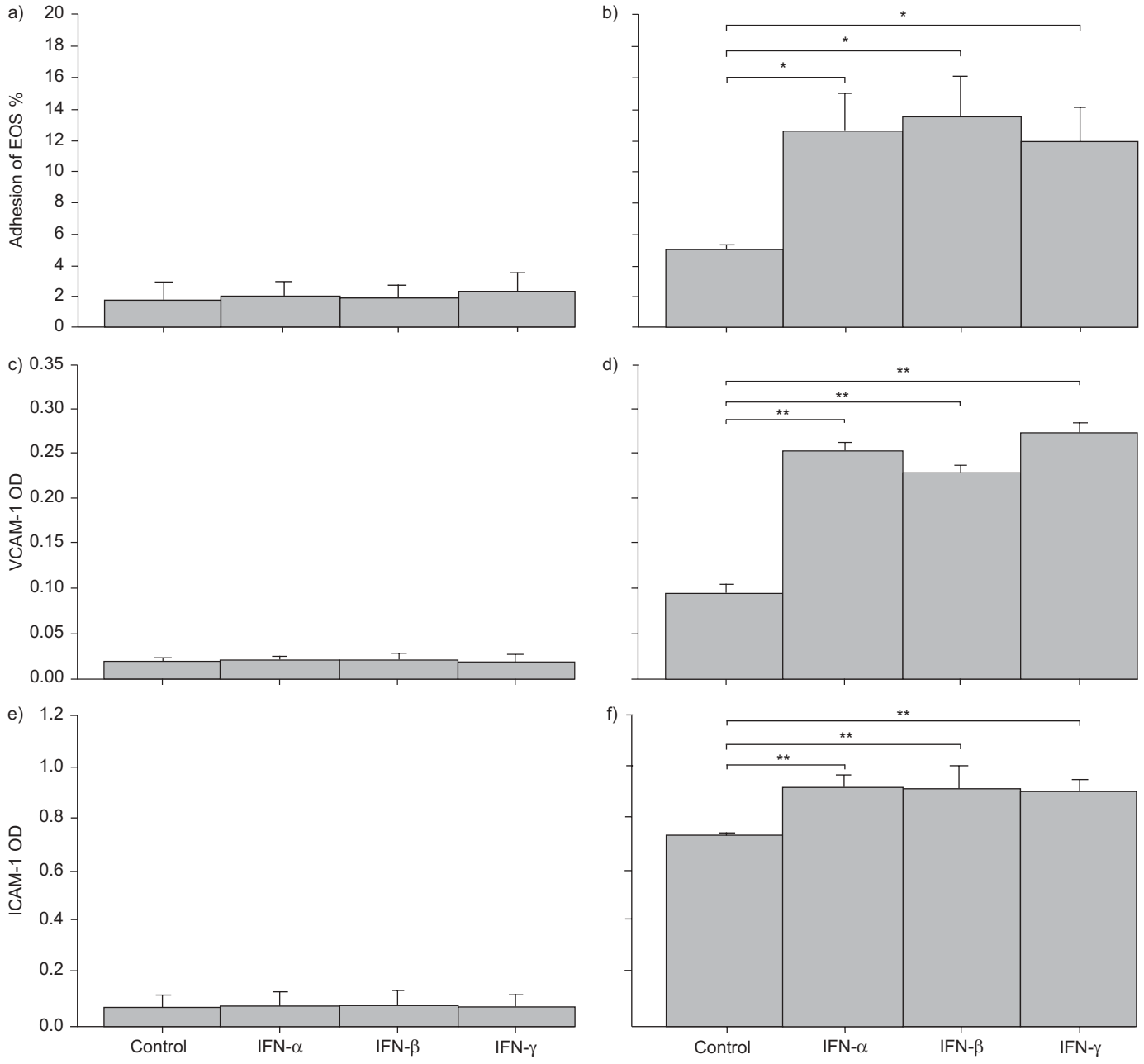

FIGURE 5. Effects of interferon (IFN)-α, IFN-β and IFN-γ (all 300 pM) on the eosinophil (EOS) adhesion-inducing ability (a and b) and the expression of vascular cell adhesion molecule (VCAM)-1 (c and d) and intercellular adhesion molecule (ICAM)-1 (e and f) of human umbilical vein endothelial cells in the absence (a, c and e) or presence (b, d and f) of tumour necrosis factor-α (10 pM). For each bar, the mean ± SEM of four different experiments is shown. OD: optical density. *: $p<0.05$; **: $p<0.01$.

ICAM-1. Similarly, LECHLEITNER *et al.* [11] reported that IFN-α and IFN-γ enhance the TNF-α-induced transcription of VCAM-1 mRNA and the protein expression in human endothelial cells at the transcriptional level *via* the nuclear IFN-related factor-1-dependent pathway. KITAYAMA *et al.* [26] reported that the culture supernatants of HUVEC stimulated with TNF-α and IFN-γ induced eosinophil chemotaxis and eosinophil adhesion to ICAM-1 and VCAM-1 mainly *via* generation of CCR3 ligands. More recently, GOMEZ and REICH [27] provided evidence that IFNs can stimulate the proliferation of primary human endothelial cells and that the effect may be attributed to the activation of signal transducer and activator of transcription (STAT)3 and STAT5. These reports and the present observations suggest that the IFN family augments either activation status or adhesiveness of endothelial cells and, in turn, contributes to the development of eosinophilic inflammation in the airways of patients with allergic diseases such as asthma.

For eosinophils to adhere and then migrate across endothelial cells, endothelial cell adhesion molecules are required. In the present study, it was observed that the IFN-β-augmented adhesiveness of HUVECs to eosinophils in the presence of TNF-α was inhibited by either anti-α_4 integrin mAb or anti-β_2

integrin mAb (mAbs against counter ligands for VCAM-1 and ICAM-1, respectively). Although the expression of both VCAM-1 and ICAM-1 was augmented by IFN-β in the presence of TNF-α, the effects of IFN-β (300–1,000 pM) on VCAM-1 were consistent with its effects on HUVEC adhesiveness to eosinophils (figs 1 and 4a). Meanwhile, the enhanced expression of ICAM-1 was observed with lower concentrations of IFN-β ($\geqslant$30 pM; fig. 4b). The ability to induce eosinophil spontaneous adhesion is more potent with VCAM-1 than with ICAM-1 [28]. From this point of view, the current results demonstrated that the IFN-β-augmented adhesion of eosinophils to HUVECs was still observed following treatment with anti-β_2 integrin mAb. Conversely, the augmented adhesion of eosinophils was abrogated by anti-α_4 integrin mAb. Therefore, it can be speculated that VCAM-1 takes precedence in the induction of the enhanced eosinophil adhesion in this system.

Viral respiratory infections can cause bronchial hyperresponsiveness and exacerbate asthma. In general, neutrophils play major roles in asthma exacerbations induced by viral infections. However, eosinophilic inflammation can also be enhanced under certain conditions. Clinical data support the possible involvement of eosinophils in virus-induced exacerbations and increased airway hyperresponsiveness in asthmatic patients: experimental infections with RV16 led to increases in eosinophils and ECP levels in the airways and to airway hyperresponsiveness in atopic asthmatics [15–17]. In infants with respiratory syncytial virus (RSV) bronchiolitis, ECP and LTC_4 levels in upper airway secretions are significantly associated [29]. In a guinea pig model of asthma, both airway responsiveness and eosinophil accumulation in the airways increased after a respiratory infection with parainfluenza-3 virus [30]. In mice, RSV infection, which induces an immune response dominated by IFN-γ, resulted in airway hyperresponsiveness and eosinophil influx into the airways [31].

WARK *et al.* [7] reported that respiratory epithelial cells from asthmatics produce lower levels of IFN-β. They demonstrated that both impaired apoptosis and increased virus replication in infected asthmatic cells are recovered by exogenous IFN-β, suggesting a possible use for type I IFNs in the treatment of virus-induced asthma exacerbations. O'SULLIVAN *et al.* [32] reported that treatment with inhalational corticosteroids reduced the number of cells expressing IFN-β in the lamina propria of bronchial biopsy specimens obtained from mild asthmatics. These findings suggest that endothelial cells in the airways of asthma patients may be exposed to relatively lower concentrations of IFN-β in asthma. In the present study, however, the authors focused on a pro-inflammatory aspect of IFN-β and a possible role of this cytokine in asthma exacerbation. Despite its important role in anti-viral immunity, IFN-β may enhance airway inflammation *via* an enhancement of eosinophil adherence to endothelial cells. This effect of IFN-β may be important in a variety of clinical conditions seen with asthma. For example, in cases of cigarette smoking, production of IFN-β by the airway leukocytes from corticosteroid-treated asthmatics is enhanced [33]. RÖDEL *et al.* [34] demonstrated that *Chlamydia pneumoniae* induced the production of IFN-β in bronchial and vascular smooth muscle cells in the presence of TNF-α. In this context, TLIBA *et al.* [35] reported that a combination of TNF-α and IFN-β acts synergistically to induce CD38 mRNA and protein expression in human smooth muscle cells. These two studies are consistent with the present study with respect to the interaction between IFN-β and TNF-α. At present, there is not enough clinical evidence that IFN-β is actually involved in asthma exacerbation. However, the current authors speculate that such an effect of IFN-β on the interaction between endothelial cells and eosinophils may be one mechanism for the enhanced airway inflammation seen with asthma exacerbation.

The current results provide new insights into the mechanisms that regulate eosinophilic inflammation in the airways of asthmatic patients, especially those with viral infections. When activated, a variety of cells, including T-helper type 1 cells, epithelial cells and natural killer cells, are capable of producing IFNs at the sites of airway inflammation. Therefore, endothelial cells are likely to be exposed to IFNs, which enhance their interaction with eosinophils at least partially through the enhancement of VCAM-1 or ICAM-1 expression. Interaction with VCAM-1 or ICAM-1 may enhance the effector functions of eosinophils, *e.g.* the release of radical oxygen species and the production of cysLTs [25, 27]. The exposure of eosinophils to these products in an autocrine and/or paracrine fashion possibly modifies their functions. For example, a chemotactic response and the interaction between ICAM-1 and eosinophils would be augmented by newly produced cysLTs [21]. An oxygen metabolite, hydrogen peroxide, further augments eosinophil adhesiveness to ICAM-1 [22]. Hence, the present study demonstrated that IFN-β and other IFNs can augment the adhesive interaction between eosinophils and endothelial cells, with the resultant modification of other adhesion-dependent effector functions of eosinophils through their interaction with either VCAM-1 or ICAM-1. These changes may contribute to the eventual manifestation of airway inflammation in asthmatic patients. Understanding the clinical relevance of the effects mediated by IFN-β may have important implications in designing therapeutic strategies for asthmatic patients with viral respiratory infection-induced exacerbations.

Conclusion

Interferon-β can augment the eosinophil adhesion-inducing activity of endothelial cells in the presence of tumour necrosis factor-α, mainly through the enhancement of vascular cell adhesion molecule-1 or intercellular adhesion molecule-1 expression. This action of interferon-β could, in turn, potentially contribute to the intensification of airway inflammation in asthmatic patients that is associated with exacerbations induced by viral infections.

REFERENCES

1 Osur SL. Viral respiratory infections in association with asthma and sinusitis: a review. *Ann Allergy Asthma Immunol* 2002; 89: 553–560.
2 Johnston SL, Pattemore PK, Sanderson G, *et al.* Community study of role of viral infections in exacerbations of asthma in 9–11 year old children. *BMJ* 1995; 310: 1225–1229.
3 Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. *BMJ* 1993; 307: 982–986.
4 Friedlander SL, Busse WW. The role of rhinovirus in asthma exacerbations. *J Allergy Clin Immunol* 2005; 116: 267–273.

5 Message SD, Johnston SL. The immunology of virus infection in asthma. *Eur Respir J* 2001; 18: 1013–1025.
6 Tsutsumi H, Takeuchi R, Ohsaki M, Seki K, Chiba S. Respiratory syncytial virus infection of human respiratory epithelial cells enhances inducible nitric oxide synthase gene expression. *J Leukoc Biol* 1999; 66: 99–104.
7 Wark PA, Johnston SL, Bucchieri F, *et al.* Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med* 2005; 201: 937–947.
8 Valerius T, Repp R, Kalden JR, Platzer E. Effects of IFN on human eosinophils in comparison with other cytokines. A novel class of eosinophil activators with delayed onset of action. *J Immunol* 1990; 145: 2950–2958.
9 Saito H, Hayakawa T, Yui Y, Shida T. Effect of human interferon on different functions of human neutrophils and eosinophils. *Int Arch Allergy Appl Immunol* 1987; 82: 133–140.
10 Konno S, Grindle KA, Lee WM, *et al.* Interferon-γ enhances rhinovirus-induced RANTES secretion by airway epithelial cells. *Am J Respir Cell Mol Biol* 2002; 26: 594–601.
11 Lechleitner S, Gille J, Johnson DR, Petzelbauer P. Interferon enhances tumor necrosis factor-induced vascular cell adhesion molecule 1 (CD106) expression in human endothelial cells by an interferon-related factor 1-dependent pathway. *J Exp Med* 1998; 187: 2023–2030.
12 Gleich GJ. Mechanisms of eosinophil-associated inflammation. *J Allergy Clin Immunol* 2000; 105: 651–663.
13 Cowburn AS, Sladek K, Soja J, *et al.* Overexpression of leukotriene C4 synthase in bronchial biopsies from patients with aspirin-intolerant asthma. *J Clin Invest* 1998; 101: 834–846.
14 Seymour ML, Rak S, Aberg D, *et al.* Leukotriene and prostanoid pathway enzymes in bronchial biopsies of seasonal allergic asthmatics. *Am J Respir Crit Care Med* 2001; 164: 2051–2056.
15 Fraenkel DJ, Bardin PG, Sanderson G, Lampe F, Johnston SL, Holgate ST. Lower airways inflammation during rhinovirus colds in normal and in asthmatic subjects. *Am J Respir Crit Care Med* 1995; 151: 879–886.
16 Pizzichini MM, Pizzichini E, Efthimiadis A, *et al.* Asthma and natural colds. Inflammatory indices in induced sputum: a feasibility study. *Am J Respir Crit Care Med* 1998; 158: 1178–1184.
17 Grünberg K, Smits HH, Timmers MC, *et al.* Experimental rhinovirus 16 infection. Effects on cell differentials and soluble markers in sputum in asthmatic subjects. *Am J Respir Crit Care Med* 1997; 156: 609–616.
18 Bochner BS, Schleimer RP. The role of adhesion molecules in human eosinophil and basophil recruitment. *J Allergy Clin Immunol* 1994; 94: 427–438.
19 Yamamoto H, Nagata M. Regulatory mechanisms of eosinophil adhesion to and transmigration across endothelial cells by α_4 and β_2 integrins. *Int Arch Allergy Immunol* 1999; 120: Suppl. 1, 24–26.
20 Nagata M, Sedgwick JB, Vrtis R, Busse WW. Endothelial cells upregulate eosinophil superoxide generation *via* VCAM-1 expression. *Clin Exp Allergy* 1999; 29: 550–561.
21 Nagata M, Saito K, Tsuchiya K, Sakamoto Y. Leukotriene D4 upregulates eosinophil adhesion *via* the cysteinyl leukotriene 1 receptor. *J Allergy Clin Immunol* 2002; 109: 676–680.
22 Nagata M, Yamamoto H, Shibasaki M, Sakamoto Y, Matsuo H. Hydrogen peroxide augments eosinophil adhesion *via* β_2 integrin. *Immunology* 2000; 101: 412–418.
23 Nagata M, Sedgwick JB, Bates ME, Kita H, Busse WW. Eosinophil adhesion to vascular cell adhesion molecule-1 activates superoxide anion generation. *J Immunol* 1995; 155: 2194–2202.
24 Choo JH, Nagata M, Sutani A, Kikuchi I, Sakamoto Y. Theophylline attenuates the adhesion of eosinophils to endothelial cells. *Int Arch Allergy Immunol* 2003; 131: Suppl. 1, 40–45.
25 Miller A, Lanir N, Shapiro S, *et al.* Immunoregulatory effects of interferon-β and interacting cytokines on human vascular endothelial cells. Implications for multiple sclerosis autoimmune diseases. *J Neuroimmunol* 1996; 64: 151–161.
26 Kitayama J, Mackay CR, Ponath PD, Springer TA. The C-C chemokine receptor CCR3 participates in stimulation of eosinophil arrest on inflammatory endothelium in shear flow. *J Clin Invest* 1998; 101: 2017–2024.
27 Gomez D, Reich NC. Stimulation of primary human endothelial cell proliferation by IFN. *J Immunol* 2003; 170: 5373–5381.
28 Nagata M, Sedgwick JB, Kita H, Busse WW. Granulocyte macrophage colony-stimulating factor augments ICAM-1 and VCAM-1 activation of eosinophil function. *Am J Respir Cell Mol Biol* 1998; 19: 158–166.
29 Dimova-Yaneva D, Russell D, Main M, Brooker RJ, Helms PJ. Eosinophil activation and cysteinyl leukotriene production in infants with respiratory syncytial virus bronchiolitis. *Clin Exp Allergy* 2004; 34: 555–558.
30 Scheerens J, Folkerts G, Van Der Linde H, *et al.* Eotaxin levels and eosinophils in guinea pig broncho-alveolar lavage fluid are increased at the onset of a viral respiratory infection. *Clin Exp Allergy* 1999; 29: Suppl. 2, 74–77.
31 Schwarze J, Cieslewicz G, Hamelmann E, *et al.* IL-5 and eosinophils are essential for the development of airway hyperresponsiveness following acute respiratory syncytial virus infection. *J Immunol* 1999; 162: 2997–3004.
32 O'Sullivan S, Cormican L, Burke CM, Poulter LW. Fluticasone induces T cell apoptosis in the bronchial wall of mild to moderate asthmatics. *Thorax* 2004; 59: 657–661.
33 Liebhart J, Cembrzynska-Nowak M, Kulczak A, Siemieniec I. Diverse production of interferons α, β and γ by airway leukocytes of asthmatics with regard to cigarette smoking and corticosteroid treatment. *J Interferon Cytokine Res* 2007; 27: 463–470.
34 Rödel J, Assefa S, Prochnau D, *et al.* Interferon-β induction by *Chlamydia pneumoniae* in human smooth muscle cells. *FEMS Immunol Med Microbiol* 2001; 32: 9–15.
35 Tliba O, Panettieri RA Jr, Tliba S, Walseth TF, Amrani Y. Tumor necrosis factor-α differentially regulates the expression of proinflammatory genes in human airway smooth muscle cells by activation of interferon-β-dependent CD38 pathway. *Mol Pharmacol* 2004; 66: 322–329.