

Proliferative effects of eosinophil lysates on cultured human airway smooth muscle cells

K. Masu*, I. Ohno†, K. Suzuki‡, S. Okada†, T. Hattori† and K. Shirato*

*Department of Cardiovascular Medicine, and †Respiratory and Infectious Diseases, Tohoku University Graduate School of Medicine, and ‡Miyagi Red Cross Blood Center, Sendai, Japan

Summary

Background The hypertrophy/hyperplasia of airway smooth muscle (ASM) cells is one of the characteristic features of bronchial asthma. This structural change leads to the thickening of airway walls resulting in the amplification of airway narrowing. However, the pathogenesis of this structural change has not yet been determined. Eosinophils, which play a pathogenic role in asthma, have been demonstrated to have proliferative effects on fibroblasts and vascular smooth muscle cells.

Objective We attempted to investigate the potential of eosinophils to induce the proliferation of ASM cells.

Methods We examined the effect of lysates of eosinophils purified from peripheral blood of healthy donors on cultured human ASM cell proliferation.

Results Eosinophil lysates significantly induced ASM cell proliferation in time- and dose-dependent manners, reaching a maximum on day 6 at 50% of eosinophil lysates ($6.0 \pm 0.7 \times 10^4$ [mean $\pm$ SD]/well, $n=5$ vs. $4.5 \pm 1.1 \times 10^4$ /well, $n=5$; $P<0.05$). This proliferative activity was heat-sensitive and recovered in the soluble fraction of the eosinophil lysates. Furthermore, the molecular weight of the mitogenic activity in the soluble fraction was identified as lower than 10 kDa. The inhibitory activity to ASM cell proliferation was also found in the insoluble fraction of the lysates.

Conclusion These results indicate that circulating eosinophils store mitogenic activity for ASM cells, suggesting that eosinophils might contribute to the development of the hyperplasia of ASM cells in asthmatics through the release of the stored mitogenic activity upon stimulation at the site of inflammation.

Keywords airway smooth muscle cells, eosinophils, proliferation

Submitted 18 August 2000; revised 17 February 2001; accepted 25 June 2001

Introduction

One of the histological features of the airways of patients with bronchial asthma is hypertrophy/hyperplasia of airway smooth muscle (ASM) cells [1]. The structural change of ASM, together with subepithelial fibrosis and hypertrophy/hyperplasia of submucosal glands, leads to the thickening of airway walls, resulting in the aggravation of the chronicity and severity of bronchial asthma by amplifying airway narrowing [2]. However, the pathogenesis of hypertrophy/hyperplasia of ASM cells in bronchial asthma has not yet been determined.

Studies using cultured ASM cells from animals and human have demonstrated the induction of ASM cell proliferation by growth factors and cytokines such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF)-I, IGF-II, TGF- β_1 , endothelin, TNF- α and IL-6. ASM cells themselves are capable of synthesizing some of these factors including PDGF, TGF- β_1 , IGF-II

and IL-6, and enhancing their proliferation in an autocrine/paracrine fashion. Inflammatory mediators in asthmatic airways such as histamine, thrombin, substance P and leukotriene (LT)D₄ were shown to have the potential to promote ASM cell proliferation [3,4]. Anti-inflammatory mediators such as IL-4 [5], vasoactive intestinal peptide (VIP) [6], prostaglandin (PG)E₂ [7] and corticosteroids [8] inhibit the proliferation of ASM cells. Cell–cell interaction mediated by integrins was reported to also be important for inducing the ASM cell proliferation. Lazaar et al. reported that T lymphocytes could induce DNA synthesis of ASM cells in culture by adhering to ASM cells via integrins and CD44 [9].

Eosinophils are a prominent infiltrate in asthmatic airways and, upon activation at the site of inflammation, release inflammatory mediators including major basic protein (MBP), eosinophil cationic protein (ECP), LTs and platelet-activating factor (PAF) [10]. These mediators contribute to the development and maintenance of airway inflammation and hyper-responsiveness which are fundamental features of asthma [11–13]. ASM cells have been demonstrated to synthesize and release cytokines and chemokines such as IL-5 [14], granulocyte/macrophage-colony stimulating factor (GM-CSF) [15], eotaxin [16], RANTES [17] and monocyte chemotactic proteins (MCPs) [18] which play an

Correspondence: Kunio Shirato, Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, 1–1 Seiryomachi, Aoba-ku, Sendai 980–8574, Japan. E-mail: shirato@int1.med.tohoku.ac.jp

important role in the accumulation and activation of inflammatory cells including eosinophils in asthmatic airways.

Eosinophils have recently been reported to have the potential, without any further activation, to induce the mitogenesis of mesenchymal cells. It has been demonstrated that DNA synthesis of fibroblasts was increased under culture with extracts of eosinophils purified from peripheral blood [19,20]. Nolan et al. cultured vascular smooth muscle cells of rats with a conditioned medium of eosinophils without stimulation, in which DNA synthesis of the cells was shown to be increased by the presence of the conditioned medium in a dose-dependent manner [21].

These findings led us to hypothesize that eosinophils play a role in the induction of hypertrophy/hyperplasia of ASM cells in airways of asthmatics. To evaluate this hypothesis, we investigated the effect of lysates of eosinophils purified from peripheral blood on the proliferation of ASM cells in culture.

Materials and methods

Preparation of ASM cells

Human tracheal autopsy tissues were taken from three patients (68 years, male, acute myocardial infarction, 53 years, female, chronic renal failure and 80 years, female, acute myocardial infarction). ASM cells were obtained from these tissues as previously described with some modifications [22]. Small strips of the tracheal muscle were dissected from the epithelium and connective tissues under sterile conditions, and minced into approximately 1 mm³ pieces. After washing several times at 4°C with modified Krebs-Ringer-Henseleit (KRH) solution [(in mM) 115 NaCl (Wako Pure Chemical Industries, Osaka, Japan), 5 KCl (Wako), 2 CaCl₂ (Wako), 1 KH₂PO₄ (Wako), 1 MgSO₄ (Wako), 15 glucose (Wako), 25 *N*-2-hydroxyethyl-piperazine-*N*-2-ethanesulphonic acid (HEPES) (Wako), and 10 µg/mL phenol red (Wako), pH 7.4] containing 100 U/mL of penicillin G (Meiji Seika Co., Tokyo, Japan), 100 µg/mL of streptomycin (Meiji) and 0.5 µg/mL amphotericin B (Bristol-Myers Squibb Co., Tokyo, Japan: PSA), the minced muscle pieces were resuspended in 10 mL of KRH solution, as mentioned above except for 0.2 mM CaCl₂, containing PSA, 6400 U collagenase (Sigma Chemical Co., St Louis, MO, USA), 10 mg soybean trypsin inhibitor (Sigma) and 100 U elastase (Sigma). The suspension of muscle pieces was then stirred gently for 90 min at 37°C. The cell suspension was filtered through 100 µm nylon meshes (Cell Strainer; Becton Dickinson Labware, Franklin Lakes, NJ, USA), and centrifuged at 150 *g* for 10 min. Cells were resuspended in Ham's F-12 (Gibco BRL, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS; Cansers International Inc., Toronto, Canada) and PSA, seeded at a density of 1×10^4 /cm² in 25 cm² culture flasks (Becton Dickinson Labware) and cultured in a humidified atmosphere at 37°C in 95% air – 5% CO₂ (v/v). The medium was replaced every 2 days. After 5–7 days when the cells grew to confluence, they were subcultured by removal from flasks with 0.25% trypsin (Difco Laboratories, Detroit, MI, USA) and 0.01% EDTA (Dojindo, Kumamoto, Japan). These cell preparations were identified as ASM cells by a characteristic 'hill and valley' appearance and by immunocytochemical staining with anti- α smooth muscle actin monoclonal antibodies (Sigma) detected by the alkaline phosphatase anti-alkaline phosphatase

method as previously described [23]. Cells at passage numbers 5–10 were used for the following experiments.

Isolation of eosinophils

Eosinophils were isolated from peripheral blood of healthy donors by immunomagnetic negative selection as previously described [24] with some modifications. Donors were 35 volunteers who visited Miyagi Red Cross Blood Center (Sendai, Japan) to provide peripheral blood for blood transfusion. They did not suffer from atopic and/or infectious diseases. Four-hundred mL of blood was taken from each donor. The suspension of white blood cells anti-coagulated with 85 mM sodium citrate, 42 mM citrate and 2.2% (w/v) glucose was obtained after partially removing red blood cells, platelets and plasma. The percentage of eosinophils in the suspension was 3–7%. The suspension was mixed with 0.2 vol. of 3% dextran (Pharmacia, Uppsala, Sweden) in 0.01 M phosphate-buffered saline (PBS, pH 7.4) (Takara Shuzo Co., Otsu, Japan) and allowed to sediment for 40 min at room temperature (23–25°C). The leucocyte-rich plasma was washed twice with Hank's balanced salt solution without Ca²⁺ and Mg²⁺ (HBSS) (Life Technology Inc., Grand Island, NY, USA). The pellet suspended in HBSS was overlaid onto 1.080 g/mL of Percoll (Pharmacia) solution and centrifuged at 600 *g* for 25 min at 4°C. The second layer consisting of granulocytes and red blood cells were collected and subjected to a hypotonic shock with sterile endotoxin-free water to remove any remaining erythrocytes. Following a wash with HBSS, the granulocytes were suspended with culture medium, RPMI 1640 (Gibco) containing 10% FBS, of 3G8 cells [25] (obtained from Cancer Cell Repository, Institute of Development, Ageing and Cancer, Tohoku University, Sendai, Japan) which are B cell hybridoma cells secreting anti-CD16 mouse monoclonal IgG₁ antibodies. The suspension was gently agitated for 30 min at 4°C followed by centrifugation. The pellet was resuspended in HBSS containing immunomagnetic beads coated with sheep anti-mouse IgG (Dynabeads M-450; Dynal AS, Oslo, Norway) and gently agitated for 20 min at 4°C. The tubes containing the cell suspension were then attached to a magnet (Dynal MPC-1; Dynal AS), so that immunomagnetically labelled neutrophils were retained in the magnetic field and eosinophils were allowed to be collected as a suspension. After centrifugation, eosinophils were resuspended in Ham's F-12 medium. The purity of eosinophils, which was determined by light microscopic examination of cytopsin preparations stained by Diff-Quick (International Chemical Ltd, Osaka, Japan), was higher than 99%. The viability assessed by trypan blue dye exclusion was higher than 99%. The number of eosinophils recovered from each donor was approximately 10 million. The final concentration of eosinophils was adjusted to 10⁶/mL and stored at –20°C. The eosinophil suspension was not pooled but stored in each donor.

Preparation of eosinophil lysates

Eosinophils stored at –20°C as mentioned above were subjected to freeze-thaw and sonicated at 100 W for 1 min [19]. To remove cellular debris, the lysates were centrifuged at 1200 *g* for 10 min at 4°C, and the supernatants were used as eosinophil (Eo) lysates. In some experiments, Eo lysates were further centrifuged at 100 000 *g* for 1 h at 4°C. The supernatants were collected as the soluble fraction. The pellet was resuspended in

the same volume of Ham's F-12 medium and used as the insoluble fraction. The soluble fraction was further separated to two parts with Microcon-10 (Amicon, Inc., Beverly, MA, USA) the molecular weight cut-off of which was 10kDa, according to the manufacturer's protocol. The volume of each part was adjusted with Ham's F-12 medium to that of the original soluble fraction.

Culture of ASM cells

ASM cells in Ham's F-12 containing 10% FBS and PSA were seeded at a density of 2×10^4 /mL/well in 24-well microplates (Becton Dickinson Labware) in triplicate. After post-incubation for 24h, ASM cells were further incubated with Ham's F-12 without FBS for 24h. The culture medium was then replaced with Ham's F-12 containing various concentration of FBS (2.5, 5 or 10%) with or without 50% of Eo lysates. The culture medium was changed on the 3rd day and the cell number was counted every 2 days or on the 6th day. Cell numbers were determined by counting released nuclei from the cells. The nuclei were released by gentle pipetting of the cells following the incubation with 0.1M citric acid (Wako) containing 0.1% crystal violet (Sigma) in culture wells at room temperature for 15min [26]. To examine the dose-dependent effect of Eo lysates, ASM cells were cultured with various concentration of Eo lysates [1, 2.5, 5, 7.5, 10, 25, 50 and 75%] at 2.5% FBS. The culture medium was changed on the 3rd day and the cell number was counted on the 6th day. To characterize the growth-enhancing activity of Eo lysates, ASM cells were cultured at 2.5% FBS with Eo lysates pre-incubated at 4 or 85°C for 15min, with the soluble or insoluble fraction of Eo lysates or with the solution prepared with Microcon-10 as mentioned above. The culture medium was changed on the 3rd day and the cell number was counted on the 6th day.

Culture of ASM cells with neutrophil lysates

Neutrophils were isolated from peripheral blood with dextran sedimentation and Percoll solution as mentioned above. The granulocyte population from the second layer of the Percoll density gradient was used as neutrophils. Both the purity and viability were higher than 99%. Neutrophil (N) lysates were prepared in the same way as Eo lysates. After being seeded and incubated in 24-well microplates as mentioned above, the ASM cells were cultured with 50% of N lysates at 2.5% FBS. The culture medium was changed on the 3rd day, and the cell numbers were counted on the 6th day.

Statistical analysis

Cell numbers are expressed as those in a culture well or as a percentage of control. Values are presented as mean $\pm$ SD. Mann-Whitney *U*-test was used to compare the differences between the values, except for the values in the time-course experiments in which the differences were estimated with one-way analysis of variance and Fisher's PLSD method. These analyses were performed using StatView 4.11 (Abacus Concepts, Berkeley, CA, USA) for Macintosh. The level of significance was taken at $P < 0.05$. The *n* in each figure represents the number of times an experiment was repeated using eosinophils from different donors.

Results

The effect of Eo lysates on the growth of ASM cells

The effect of Eo lysates on ASM cell proliferation was examined at 2.5, 5 or 10% FBS on day 6 (Fig. 1). The presence of 50% of Eo lysates significantly enhanced the proliferation of ASM cells at 2.5 ($179.5 \pm 18.6\%$, $n=5$; $P < 0.05$) and 5 ($157.5 \pm 4.7\%$, $n=5$; $P < 0.05$)% FBS, compared to the absence of Eo lysates. However, there was no significant difference in the number of the cells between the presence ($95 \pm 13.7\%$, $n=5$) and absence of Eo lysates at 10% of FBS. On the other hand, without FBS, 50% of Eo lysates had no mitogenic effect on ASM cells (93, 80 and 75% compared to the absence of Eo lysates).

The effect of Eo lysates was time-dependent (Fig. 2). The numbers of cells cultured with 50% of Eo lysates at 2.5% FBS were significantly higher than those without Eo lysates on days 4 and 6 ($5.9 \pm 1.1 \times 10^4$ vs. $4.5 \pm 1.1 \times 10^4$, $n=5$; $P < 0.05$ and $6.0 \pm 0.7 \times 10^4$ vs. $4.5 \pm 1.1 \times 10^4$, $n=5$; $P < 0.05$, respectively).

The effect of Eo lysates was also dose-dependent (Fig. 3). The numbers of cells cultured with 10, 25, 50 or 75% of Eo lysates at 2.5% FBS on day 6 were significantly higher than those without Eo lysates ($135.4 \pm 26.8\%$, $n=8$; $P < 0.05$, $137.3 \pm 27.1\%$, $n=7$; $P < 0.05$, $170.3 \pm 27.1\%$, $n=8$; $P < 0.001$ or $147.2 \pm 29.9\%$, $n=6$; $P < 0.01$, respectively). The numbers of cells cultured with 1, 2.5, 5 or 7.5% of Eo lysates at 2.5% FBS on day 6 were not significantly different from those without Eo lysates.

Characterization of the growth-enhancing activity of Eo lysates

To examine the heat-stability of the growth-enhancing activity of Eo lysates, ASM cells were cultured with 50% of Eo lysates pretreated with or without heating. In contrast to non-treated Eo lysates ($150 \pm 29.1\%$, $n=4$), heat-treated Eo lysates induced no significant increase in the number of cells at 2.5% FBS on day 6 ($100 \pm 13.1\%$, $n=4$), compared to the number of cells cultured without Eo lysates (Fig. 4).

To further characterize the activity of Eo lysates, ASM cells were cultured with the soluble or insoluble fraction of Eo

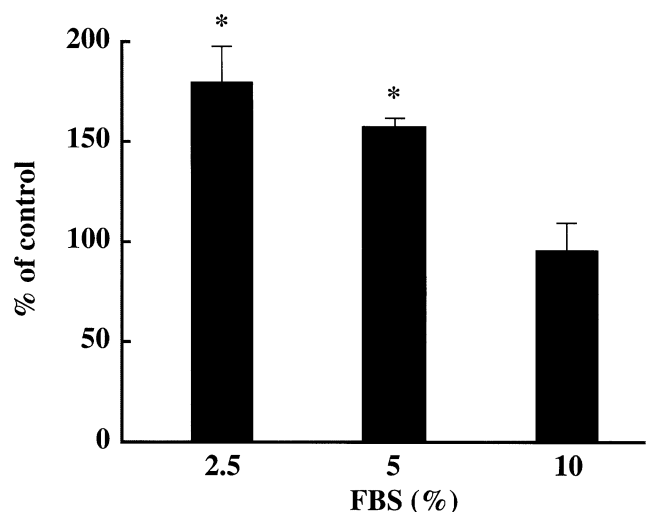


Fig. 1. The effect of Eo lysates on ASM cell proliferation at the different concentrations of FBS. ASM cells were cultured at 2.5, 5 or 10% FBS with or without 50% of Eo lysates ($n=5$). The number of cells was counted and expressed as percentages of that without Eo lysates as a control. * $P < 0.05$.

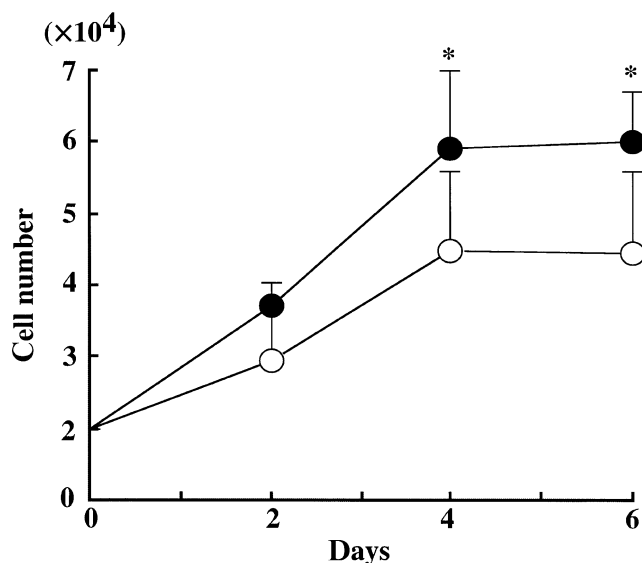


Fig. 2. The time-dependent effect of Eo lysates on ASM cell proliferation. ASM cells were cultured at 2.5% FBS with (closed circles) or without (open circles) 50% of Eo lysates ($n=5$). The number of cells was counted and expressed as indicated in Materials and methods. * $P < 0.05$ compared to the number of cells cultured without Eo lysates.

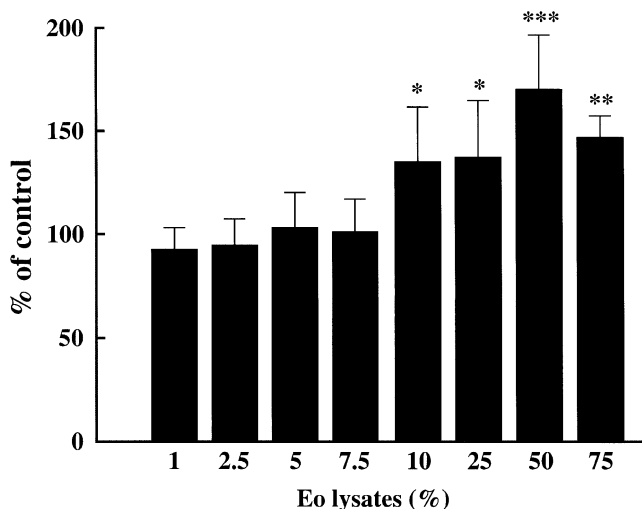


Fig. 3. The dose-dependent effect of Eo lysates on ASM cell proliferation. ASM cells were cultured at 2.5% FBS with or without various concentrations of Eo lysates ($n=6-8$). The number of cells was counted and expressed as percentages of that without Eo lysates as a control. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

lysates. The number of cells cultured with the soluble fraction at 2.5% FBS on day 6 ($119.4 \pm 8.6\%$, $n=8$; $P < 0.05$), which was similar to that with unfractionated Eo lysates ($117.6 \pm 10.4\%$, $n=8$), was significantly increased compared to that without Eo lysates. In contrast, the number of cells cultured with the insoluble fraction ($69.0 \pm 18.0\%$, $n=8$; $P < 0.01$) was significantly decreased compared to that without Eo lysates (Fig. 5). The growth-enhancing activity in the soluble fraction of Eo lysates ($139.7 \pm 16.8\%$, $n=5$) was recovered in factors in which the molecular weight was lower than 10 kDa ($148.8 \pm 24.1\%$, $n=5$), but not in those higher than 10 kDa ($104.4 \pm 27.7\%$, $n=5$) (Fig. 6).

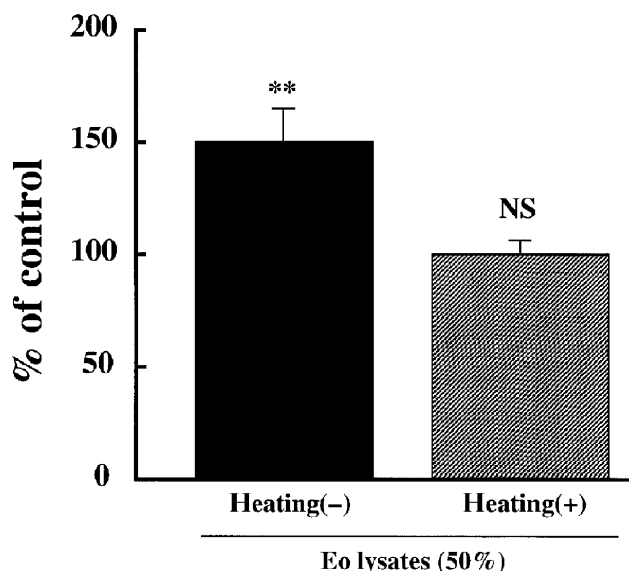


Fig. 4. The effect of heat treatment on the growth-enhancing activity of Eo lysates. ASM cells were cultured at 2.5% FBS in the absence or presence of 50% of Eo lysates pretreated with (hatched column) or without (closed column) heating ($n=4$). The number of cells was counted and expressed as percentages of that without Eo lysates as a control. ** $P < 0.01$, NS; not significant.

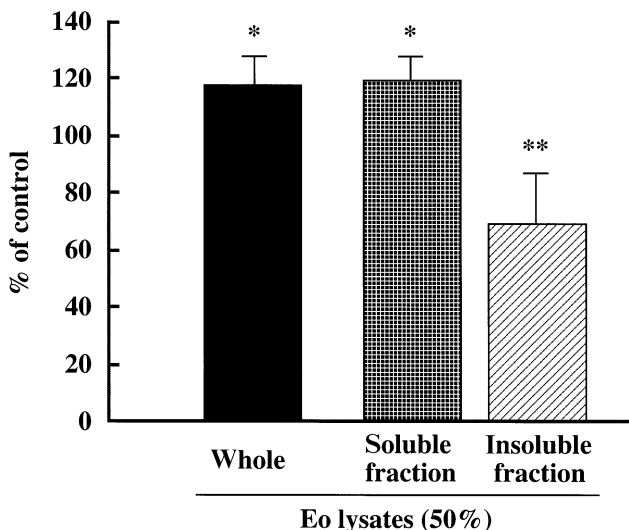


Fig. 5. The effect of soluble and insoluble fractions of Eo lysates on ASM cell proliferation. ASM cells were cultured at 2.5% FBS in the absence or presence of unfractionated (closed column) or fractionated (soluble (checked column) and insoluble (hatched column) fraction) Eo lysates ($n=8$). The number of cells was counted and expressed as percentages of that without Eo lysates as a control. * $P < 0.05$, ** $P < 0.01$.

The effect of N lysates on the growth of ASM cells

The effect of N lysates on ASM cell proliferation was examined at 2.5% FBS on day 6. There was no significant difference in the number of cells between in the presence ($111.0 \pm 11.3\%$, $n=4$) and in the absence (as a control) of 50% of N lysates.

Discussion

This is the first report demonstrating that eosinophils have an ability to enhance the proliferation of human ASM cells. There

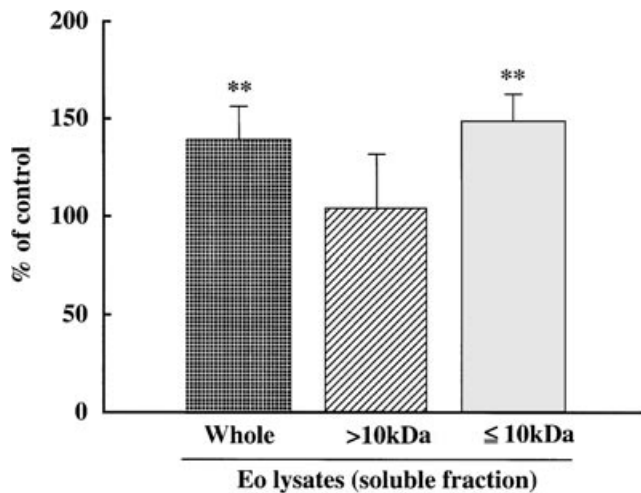


Fig. 6. The molecular weight of the growth-enhancing activity in the soluble fraction of Eo lysates. ASM cells were cultured in the absence or presence (checked column) of soluble fraction. The soluble fraction was further separated based on molecular weight (higher than 10kDa: hatched column or lower than 10kDa: open column). The number of cells was counted and expressed as percentages of that without Eo lysates as a control. ** $P < 0.01$.

has been some evidence showing that eosinophils have mitogenic activity for mesenchymal cells. Pincus et al. demonstrated that lysates of eosinophils from normal subjects induced the proliferation of fibroblasts, suggesting the localization of the mitogenic activity in the granule compartment [19]. It has been reported that a conditioned medium of eosinophils cultured without any stimulation had a stimulatory effect on the proliferation of fibroblasts [20] and DNA synthesis in vascular smooth muscle cells [21]. However, the factors responsible for these activities in eosinophils have not been identified yet.

In this study, the contamination in the purified eosinophil population was neutrophils, constituting less than 1%. However, the growth-enhancing activity of Eo lysates was unlikely to be due to the neutrophil contamination because N lysates had no effect on the proliferation of ASM cells. The activity of Eo lysates was abolished by heat treatment. Furthermore, the proliferative activity in unfractionated lysates was recovered in the soluble fraction. Together with the results of the further characterization based on molecular weight, our findings suggest that eosinophil-derived mitogenic factor(s) are likely to be stored, heat-labile soluble protein(s) with a molecular weight lower than 10kDa. Since Eo lysates did not induce the ASM cell proliferation in the absence of FBS, the activity in the lysates could be due to a comitogen. It is also assumed that, because they are comitogenic, Eo lysates were not observed to further increase the number of ASM cells in the presence of 10% FBS in which ASM cells grew confluent. Since the procedures to prepare Eo lysates in this study were the same as in a previous study by Pincus et al. [19], our preparation would include eosinophilic granule protein, which could be recovered in the soluble fraction. MBP, ECP, eosinophil-derived neurotoxin (EDN) and eosinophil peroxidase (EPO), major components in eosinophilic granules [27], have not been reported to have mitogenic activities for ASM cells. β -hexosaminidase, which is one of the molecules stored in the granules, was reported to be increased in bronchoalveolar lavage fluids of asthmatics after antigen challenge [28]. This molecule has been shown to

induce the proliferation of ASM cells *in vitro* [29]. However, β -hexosaminidase is present in not only eosinophils but also neutrophils [30] the lysates of which did not enhance ASM cell proliferation. Therefore, the mitogenic activity of eosinophils does not seem to be due to β -hexosaminidase. IL-6 is preformed and stored in the specific granules of eosinophils [31,32] and has the potential to induce ASM cell proliferation [33]. However, its molecular weight is higher than 10kDa [34]. Eosinophils are capable to synthesize PDGF-BB [23], TGF- β_1 [35,36] and TNF- α [37] which have the potential to induce ASM cell proliferation [3,4]. However, PDGF-BB is a full mitogen, able to induce the proliferation of ASM cells in the absence of FBS [38], and the molecular weight of these molecules is higher than 10kDa [39–41]. Therefore, they are unlikely to be candidate molecules for the activity in Eo lysates. It would be interesting to further characterize the mitogenic activity of Eo lysates by focusing on granule proteins. Lazaar et al. reported that T lymphocytes induced the proliferation of airway smooth muscle cells in culture through the adherence to VCAM-1 and ICAM-1 on ASM cells via VLA-4 and LFA-1, respectively [9]. These integrins are also expressed on eosinophils [42]. Therefore, it is possible that these integrins recovered in the soluble fraction induced the proliferation of ASM cells in the present study.

The fractionation of Eo lysates also made apparent the presence of an anti-proliferative activity in eosinophils. IL-4 [5] and PGE₂ [7] have been shown to inhibit ASM cell proliferation. Although circulating eosinophils have been demonstrated to store IL-4 in the secondary granules [43], the anti-proliferative activity in eosinophils was not further characterized in this study.

The pathogenesis of hyperplasia of ASM cells in asthmatics has not been determined yet. This study was performed *in vitro* with cultured ASM cells and Eo lysates which were prepared from circulating eosinophils. Increased numbers of eosinophils have been recognized in smooth muscle in asthmatics [44]. Activated and degranulated eosinophils are observed in asthmatic airways [12,13]. Therefore, it is possible that, in asthmatics, eosinophils migrate from the circulation to the smooth muscle area and then release the stored mitogenic activity for ASM cells upon stimulation with cytokines and inflammatory mediators such as GM-CSF and PAF. Eosinophils have also been demonstrated to express PDGF-BB [23] and TGF- β_1 [45] in asthmatic airways. Eosinophils in circulation have been shown not to store but to synthesize these molecules upon stimulation with IL-5 or GM-CSF [46]. Accordingly, together with the present study, it is suggested that eosinophils contribute to the development of hyperplasia of ASM cells through the release of both the stored and newly synthesized mitogenic factors such as PDGF-BB and TGF- β_1 at the site of inflammation. This possibility is supported by the observation that massive eosinophilic infiltration of airways and interstitial spaces in *Toxocara canis*-infected lungs of rats was accompanied by smooth muscle hyperplasia in blood vessels and airways [47].

Further studies will be needed to characterize the mitogenic activity in Eo lysates and to clarify the mechanisms by which Eo lysates induce the proliferation of ASM cells. It would also be of interest to investigate the mitogenic activity in eosinophils from asthmatic/allergic donors, which was not determined in this study.

Acknowledgements

We thank B. Bell for reading the manuscript.

References

- Arm JP, Lee TH. The pathobiology of bronchial asthma. *Adv Immunol* 1992; 51:323–82.
- Wiggs BR, Bosken C, Pare PD, Hogg JC. A model of airway narrowing in asthma and in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1992; 145:1251–8.
- Hirst SJ. Airway smooth muscle cell culture: application to studies of airway wall remodeling and phenotype plasticity in asthma. *Eur Respir J* 1996; 9:808–20.
- Knox AJ, Pang L, Johnson S, Hamad A. Airway smooth muscle function in asthma. *Clin Exp Allergy* 2000; 30:606–14.
- Hawker KM, Johnson PRA, Hughes JM, Black JL. Interleukin-4 inhibits mitogen-induced proliferation of human airway smooth muscle cells in culture. *Am J Physiol* 1998; 275:L469–77.
- Maruno K, Absood A, Said SI. VIP inhibits basal and histamine-stimulated proliferation of human airway smooth muscle cells. *Am J Physiol* 1995; 268:L1047–51.
- Johnson PRA, Armour CL, Carey D, Black JL. Heparin and PGE₂ inhibit DNA synthesis in human airway smooth muscle cells in culture. *Am J Physiol* 1995; 269:L514–9.
- Stewart AG, Fernandes D, Tomlinson PR. The effect of glucocorticoids on proliferation of human cultured airway smooth muscle. *Br J Pharmacol* 1995; 116:3219–26.
- Lazaar BAL, Albelda SM, Pilewski JM, Brennan B, Pure E, Panettieri RA. T lymphocytes adhere to airway smooth muscle cells via integrins and CD44 and induce smooth muscle cell DNA synthesis. *J Exp Med* 1994; 180:807–16.
- Gleich GJ. The eosinophil and bronchial asthma: current understanding. *J Allergy Clin Immunol* 1990; 85:422–36.
- Bousquet J, Chané P, Lacoste JY et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990; 323:1033–9.
- Kay AB. Asthma and inflammation. *J Allergy Clin Immunol* 1991; 87:893–910.
- Busse WW, William FC, Sedgwick JD. Mechanism of airway inflammation in asthma. *Am Rev Respir Dis* 1993; 147:S20–4.
- Hakonarson H, Maskeri N, Carter C, Grunstein MM. Regulation of TH1- and TH2-type cytokine expression and action in atopic asthmatic sensitized airway smooth muscle. *J Clin Invest* 1999; 103:1077–87.
- Saunders MA, Mitchell JA, Seldon PM et al. Release of GM-CSF by human cultured airway smooth muscle cells: suppression by dexamethasone. *Br J Pharmacol* 1997; 120:545–6.
- Ghaffar O, Hamid Q, Renzi PM et al. Constitutive and cytokine-stimulated expression of eotaxin by human airway smooth muscle cells. *Am J Respir Crit Care Med* 1999; 159:1933–42.
- John M, Hirst SJ, Jose PJ et al. Human airway smooth muscle cells express and release RANTES in response to T helper 1 cytokines: regulation by T helper 2 cytokines and corticosteroids. *J Immunol* 1997; 158:1841–7.
- Pype JL, Dupont LJ, Menten P et al. Expression of monocyte chemotactic protein (MCP)-1, MCP-2, and MCP-3 by human airway smooth-muscle cells. *Am J Respir Cell Mol Biol* 1999; 21:528–36.
- Pincus SH, Ramesh KS, Wyler DJ. Eosinophil stimulate fibroblast DNA synthesis. *Blood* 1987; 70:572–4.
- Shock A, Rabe KF, Dent G et al. Eosinophils adhere to and stimulate replication of lung fibroblasts 'in vitro'. *Clin Exp Immunol* 1991; 86:185–90.
- Nolan CR, Saenz KP, Thomas CA, Murphy KD. Role of the eosinophil in chronic vascular rejection of renal allografts. *Am J Kidney Dis* 1995; 26:634–42.
- Hall IP, Kotlikoff M. Use of cultured airway myocytes for study of airway smooth muscle. *Am J Physiol* 1995; 268:L1–11.
- Ohno I, Nitta Y, Yamauchi K et al. Eosinophils as a potential source of platelet-derived growth factor B-chain in nasal polyposis and bronchial asthma. *Am J Respir Cell Mol Biol* 1995; 13:639–47.
- Hansel TT, De Vires IJM, Iff T et al. An improved immunological procedure for the isolation of highly purified human blood eosinophils. *J Immunol Meth* 1991; 145:105–10.
- Glennie MJ, McBride HM, Worth AT, Stevenson GT. Preparation and performance of bispecific F(ab')₂ antibody containing thioether-linked Fab'γ fragments. *J Immunol* 1987; 139:2367–75.
- Absher M, Makrides W, Shapiro P, Evans JN. Hyperoxia inhibits proliferation of cultured rat tracheal smooth muscle cells. *Am J Physiol* 1994; 267:L101–5.
- Giembycz MA, Lindsay MA. Pharmacology of the eosinophil. 1999; 51:213–339.
- Tonnel AB, Joseph M, Gosset PH, Fourmer E, Capron A. Stimulation of alveolar macrophages in asthmatic patients after local provocation test. *Lancet* 1983; i:1406–8.
- Lew DB, Rattazzi MC. Mitogenic effect of lysosomal hydrolases on bovine tracheal myocytes in culture. *J Clin Invest* 1991; 88:1969–75.
- Davies P, Allison AC. Secretion of macrophage enzymes in relation to the pathogenesis of chronic inflammation. In: Nelson DS, eds *Immunology of the Macrophages*. New York: Academic Press Inc., 1976; 427–61.
- Hamid Q, Barkans J, Meng Q et al. Human eosinophils synthesize and secrete interleukin-6 *in vitro*. *Blood* 1992; 80:1496–501.
- Lacy P, Levi Schaffer F, Mahmudi-Azer S et al. Intracellular localization of interleukin-6 in eosinophils from atopic asthmatics and effects of interferon γ. *Blood* 1998; 91:2508–16.
- De S, Zelazny ET, Souhrada JF, Souhrada M. Interleukin 1β and interleukin 6 induce hyperplasia and hypertrophy of cultured guinea pig airway smooth muscle cells. *J Appl Physiol* 1995; 78:1555–63.
- Le J, Vilcek J. Biology of disease. Interleukin 6: a multifunctional cytokine regulating immune reactions and the acute phase protein response. *Laboratory Invest* 1989; 61:588–602.
- Wong DTW, Elvov A, Matossian K et al. Eosinophil from patients with eosinophilia express transforming growth factor β₁. *Blood* 1991; 78:2702–7.
- Ohno I, Lea RG, Flanders KC et al. Eosinophils in chronically inflamed human upper airway tissues express transforming growth factor β1 gene (TGFβ1). *J Clin Invest* 1992; 89:1662–8.
- Finotto S, Ohno I, Marshall JS et al. TNF-α production by eosinophils in upper airways inflammation (nasal polyposis). *J Immunol* 1994; 153:2278–89.
- Hirst SJ, Barnes PJ, Twort CHC. PDGF isoform-induced proliferation and receptor expression in human cultured airway smooth muscle cells. *Am J Physiol* 1996; 270:L415–28.
- Ross R, Raines EW, Bowen-Pope DF. The biology of platelet-derived growth factor. *Cell* 1986; 46:155–69.
- Barnard JA, Ryons RM, Moses HL. The cell biology of transforming growth factor β. *Biochim Biophys Acta* 1990; 1032:79–87.
- Pennica D, Nedwin GE, Hayflick JS et al. Human tumor necrosis factor: Precursor structure, expression and homology to lymphotoxin. *Nature* 1984; 312:724–9.
- Resnick MB, Weller PF. Mechanisms of eosinophil recruitment. *Am J Respir Cell Mol Biol* 1993; 8:349–55.
- Moqbel R, Ying S, Barkans J, Newman TM et al. Identification of messenger RNA for IL-4 in human eosinophils with granule

- localization and release of the translated product. *J Immunol* 1995; 155:4939–47.
- 44 Hamid Q, Song Y, Kotsimbos C et al. Respiratory pathophysiologic responses. Inflammation of small airways in asthma. *J Allergy Clin Immunol* 1997; 100:44–51.
- 45 Ohno I, Nitta Y, Yamauchi K et al. Transforming growth factor β 1 (TGF β 1) gene expression by eosinophils in asthmatic airway inflammation. *Am J Respir Cell Mol Biol* 1996; 15:404–9.
- 46 Brach MA, Scott C, Kientopf M, Herrman F. Expression of the transforming growth factor alpha gene by human eosinophils is regulated by interleukin-3, interleukin-5 and granulocyte-macrophage colony stimulating factor. *Eur J Immunol* 1994; 24:646–50.
- 47 Yoshikawa S, Kayes SG, Martin SL, Parker JC. Eosinophilia-induced vascular and airway remodeling and hyperresponsiveness in rat lungs. *J Appl Physiol* 1996; 81:1279–87.