Effect of murine recombinant interleukin-5 on the cell population in guinea-pig airways

¹T. Iwama, H. Nagai, H. Suda, *N. Tsuruoka and A. Koda

Department of Pharmacology, Gifu Pharmaceutical University, Gifu, Japan and *Suntory Institute for Biochemical Research, Osaka, Japan

1 An intratracheal injection of murine recombinant interleukin 5 (mrIL-5, 2–15 µg/0.25 ml/animal) induced a dose-dependent increase in the number of macrophages, eosinophils, neutrophils and epithelial cells in the bronchoalveolar lavage fluid (BALF) of guinea-pigs 24 h after administration. Bovine serum albumin (15 µg/0.25 ml/animal), used as a reference material, did not cause any change of this type.

2 The intratracheal administration of mrIL-5 at a dose of 15 µg showed a tendency to increase the number of these pulmonary inflammatory cells and epithelial cells in the BALF at 12 h with a significant increase observed at 24 h.

3 Prednisolone (20 mg kg⁻¹, i.p.) inhibited the mrIL-5-induced increase in macrophages, eosinophils, neutrophils and epithelial cells. Ketotifen (2 mg kg⁻¹, i.p.) reduced the mrIL-5-induced increase in the eosinophil, neutrophil and epithelial cell populations. The simultaneous injection of 2% disodium cromoglycate (DSCG) into the trachea prevented the mrIL-5-induced increase in the number of airway epithelial cells, without affecting changes in the other inflammatory leukocytes.

4 These results suggest that mrIL-5 is a potent inducer of lung inflammation, in terms of increased inflammatory leukocytes and epithelial cells in guinea-pig BALF. Prednisolone, DSCG and ketotifen are effective against mrIL-5-induced pulmonary inflammation, especially the desquamation of bronchial epithelial cells.

Keywords: Interleukin-5 (IL-5); airway cell populations; anti-asthmatic drugs; airway inflammation

Introduction

Airway inflammation is an important component in the pathogenesis of bronchial asthma (Barnes, 1989; Dolovich *et al.*, 1989). The incidence of inflammatory cells, such as neutrophils and eosinophils, increases in the bronchoalveolar lavage fluid (BALF) in the late phase airway response of man and animals (Barnes, 1989; Frew & Kay, 1990). Chemotactic factors, such as platelet activating factor (PAF), leukotriene B₄ (LTB₄) and complement fragments, which are thought to contribute to these events, lead to the induction of nonspecific airway hyperreactivity (Barnes, 1989). However, airway epithelial cells also play an important part in bronchial asthma, because the desquamation of these cells enhances stimulated contraction of the trachea in man and animals (Vanhoutte, 1989). Moreover, the number of airway epithelial cells present in the BALF of guinea-pigs with airway hyperreactivity induced by repeated antigen provocation, is more than in normal animals (Motojima *et al.*, 1989). Creola bodies, which are agglomerations of bronchial epithelial cells, are found in the BALF of asthma patients (Frigas & Gleich, 1986). Recently, interleukin 5 (IL-5), secreted from T lymphocytes, has been identified as a T cell-replacing factor (Takatsu *et al.*, 1980) or B cell growth factor II (Swain, 1985). This cytokine also acts as an eosinophil activating factor (Clutterbuck & Sanderson, 1988; Lopez *et al.*, 1988) and is thought to be closely related to the control of immune responses and inflammatory reactions (Frew & Kay, 1990; Szeleenyi, 1991). However, the role of IL-5 in pulmonary inflammation has not yet been fully elucidated. In this study, we have found that intratracheal administration of mrIL-5 induces an increase in the number of inflammatory leukocytes and epithelial cells in guinea-pig BALF. We also investigated the effect of various drugs, used in the treatment of human bronchial asthma, on this bronchopulmonary inflammation.

Methods

Male Hartley guinea-pigs weighing 300–400 g (Nihon SLC, Japan) were used.

Administration of mrIL-5

Guinea-pigs were anaesthetized by an initial injection of pentobarbitone sodium (20 mg kg⁻¹, i.p.) followed, where necessary (in about 80 to 95% of the animals), by additional doses of the drug (in increments of 10 mg kg⁻¹ up to a cumulative maximum of 70 mg kg⁻¹, i.p.) until the animal could no longer experience pain as evidenced by a cry or shudder. After the animals were sedated and restrained, the trachea was then surgically exposed and 0.25 ml sterile mrIL-5, bovine serum albumin (BSA) or 0.9% NaCl solution was injected into the trachea with a 26-G syringe. The wound was closed with sterile stitches. The lungs were lavaged 5, 12 and 24 h after the mrIL-5 challenge. In order to study the dose-dependency and the effect of drugs, the number of each cell type in the BALF was determined 24 h after mrIL-5 treatment. In the preliminary study, inhalation of human recombinant interleukin 5 (hrIL-5) (50 µg over 30 min) and an intraperitoneal injection of mrIL-5 (20 µg/kg/day for two days) did not cause a significant change in the number and population of cells in the BALF after 24 h (unpublished observations). The aerosol (spray volume 1 ml min⁻¹, mass particle diameter distribution 2.0–6.0 µm) was generated by an TUR-3200 ultrasonic nebulizer (Nihon Koden, Japan). We thus tried to investigate the effect of locally injected mrIL-5 on pulmonary inflammation in guinea-pigs.

Broncheolar lavage fluid study

Guinea-pigs were killed by the intraperitoneal injection of sodium pentobarbitone (150 mg kg⁻¹). The trachea was cannulated and the airway lumen was washed with two aliquots (5 ml) of saline containing 0.1% BSA warmed to 37°C. Typically, more than 80% of the fluid was recovered in an individual animal. In the control group, the mean percentage of recovery of fluid was 85 ± 0.1% and this was unaffected by treatment with the test drugs. Lavaged fluid from each animal was pooled in a plastic tube cooled in ice and centrifuged (150 g) at 4°C for 10 min. The cell pellet was resuspended in 2 ml saline containing 0.1% BSA. Fifty µl of cell suspension was added to 450 µl Turk's solution (1:2 dilution with saline) to count the total leukocyte numbers under microscopy

¹ Author for correspondence.

($\times 500$). In order to count differentiated cell types, the cell suspension was then diluted with saline to give a final concentration to 5×10^5 cells ml^{-1} , and then centrifuged at 400 r.p.m. for 4 min at room temperature in a Cytospin II (Shandon Southern Instruments, Sewickley, PA, U.S.A.). Thereafter the cytocentrifuged preparations were stained with May-Gruenwald and Giemsa dye solution. A total of approximately 500 differentiated leukocytes (macrophages, eosinophils, neutrophils and lymphocytes) were counted and the proportion of each cell type was calculated as a percentage of the total. The number of each leukocyte was calculated from the total leukocytes in the BALF and the percentage of the individual cell type in the smear. Simultaneously, the number of epithelial cells in the smear was also counted. Results are expressed as the total number of cells recovered in the BALF.

Drug administration

Prednisolone (20 mg kg^{-1} , i.p.) was administered 3 h, and ketotifen (2 mg kg^{-1} , i.p.) was administered 30 min before mrIL-5 treatment. DSCG was administered into the trachea mixed with the mrIL-5 solution.

Materials

Murine recombinant interleukin-5 (mrIL-5) and human recombinant interleukin 5 (hrIL-5) were synthesized at the Suntory Institute for Biochemical Research (Osaka, Japan). Ketotifen was provided by Sandoz Ltd. (Basel, Switzerland). DSCG (disodium cromoglycate) was also a gift from Fujisawa Pharmaceutical Co., Ltd. (Osaka, Japan). Prednisolone acetate (Takeda Chemical Ind. Ltd., Osaka, Japan), BSA (type IV, Sigma Chemical Co., St. Louis, U.S.A.), May-Gruenwald stain and Giemsa stain (Merk Co. Inc., N.J., U.S.A.), Turk solution (Wako Pure Chemicals, Japan) and sodium pentobarbitone (Abbot Lab, Chicago, U.S.A.) were purchased commercially. mrIL-5, DSCG and BSA were dissolved in 0.9% NaCl. Prednisolone acetate was suspended in saline containing 0.5% CMC-Na.

Statistical analysis

All data were represented as mean $\pm$ s.e.mean and analysed by Dunnet's multiple range test.

Results

mrIL-5-induced leukocyte infiltration in bronchoalveolar lavage fluid

Twenty-four hours after intratracheal injection of mrIL-5 (2 – $15 \mu\text{g}/\text{animal}$), the number of macrophages, eosinophils and neutrophils in the BALF had increased in a dose-dependent manner (Table 1). mrIL-5 at a dose of $5 \mu\text{g}$ tended to increase, but not significantly, the macrophage and neutrophil level in the BALF. When $15 \mu\text{g}$ mrIL-5 was administered, a significant increase in the number of macrophages, eosinophils and neutrophils in the BALF was observed, but no significant change in the number of lymphocytes occurred. In contrast, the same dose of BSA did not affect the number of macrophages, eosinophils, neutrophils and lymphocytes (Table 1). In the time-course experiments to measure cell increase in the BALF induced by mrIL-5 ($15 \mu\text{g}$), the number of macrophages, eosinophils, neutrophils and lymphocytes increased after 5 and 12 h, but these effects were not significant (Table 2).

Effect of mrIL-5 on pulmonary epithelial cells

The intratracheal injection of mrIL-5 (2 and $5 \mu\text{g}$) exhibited a tendency to increase the number of epithelial cells in the BALF after 24 h, but the effect was not significant (Figure 1a). However, a significant increase in BALF epithelial cells was observed at 24 h following intratracheal administration of $15 \mu\text{g}$ mrIL-5. In contrast, BSA at a dose of $15 \mu\text{g}$ did not increase the epithelial cell number (Figure 1a). In the time course experiments, intratracheal administration of $15 \mu\text{g}$ mrIL-5 tended to increase the number of epithelial cells in the BALF 12 h after treatment, although no change had occurred at 5 h (Figure 1b).

Table 1 Dose-response for mrIL-5-induced changes in bronchial leukocyte populations in guinea-pigs

Dose	Leukocyte populations ($\times 10^6$)				
	Total	Macro	Eosin	Neut	Lymph
Saline	6.0 ± 1.3	4.6 ± 0.9	0.5 ± 0.2	0.5 ± 0.1	0.3 ± 0.1
BSA $15 \mu\text{g}$	6.3 ± 0.3	4.9 ± 0.2	0.5 ± 0.1	0.7 ± 0.2	0.2 ± 0.02
mrIL-5 $2 \mu\text{g}$	8.7 ± 1.7	6.5 ± 1.4	0.6 ± 0.2	1.3 ± 0.5	0.4 ± 0.03
$5 \mu\text{g}$	12.5 ± 2.4	8.1 ± 1.2	0.9 ± 0.1	3.3 ± 1.2	0.3 ± 0.1
$15 \mu\text{g}$	$17.7 \pm 4.0^*$	$8.7 \pm 1.2^*$	$1.9 \pm 0.5^*$	$6.4 \pm 2.0^*$	0.8 ± 0.2

Total = total leukocytes; Macro = macrophages; Neut = neutrophils; Eosin = eosinophils; Lymph = lymphocytes. Results are expressed as the mean $\pm$ s.e.mean of the numbers ($\times 10^6$ cells) of total leukocytes and each leukocyte type recovered from BALF of 5–8 animals 24 h after the intratracheal injection of saline, bovine serum albumin (BSA) or mrIL-5.

* $P < 0.05$, significantly different from saline response.

Table 2 Time-course for changes in bronchial leukocyte populations after mrIL-5 challenge in guinea-pigs

Time (h)	Leukocyte populations ($\times 10^6$)				
	Total	Macro	Eosin	Neut	Lymph
0	6.0 ± 0.4	5.1 ± 0.3	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.02
5	9.3 ± 1.1	6.5 ± 1.1	1.1 ± 0.3	1.5 ± 0.5	0.2 ± 0.05
12	13.8 ± 4.8	8.0 ± 1.6	1.3 ± 0.6	4.0 ± 2.4	0.5 ± 0.2
24	$17.7 \pm 4.0^*$	$8.7 \pm 1.2^*$	$1.9 \pm 0.5^*$	$6.4 \pm 2.0^*$	0.8 ± 0.2

Total = total leukocytes; Macro = macrophages; Neut = neutrophils; Eosin = eosinophils; Lymph = lymphocytes. Results are expressed as the mean $\pm$ s.e.mean of the numbers ($\times 10^6$ cells) of total leukocytes and each leukocyte type recovered from BALF of 6–8 animals after the intratracheal injection of mrIL-5 ($15 \mu\text{g}/\text{animal}$).

* $P < 0.05$, significantly different from zero time response.

Figure 1 mrIL-5-induced increase in the number of pulmonary epithelial cells in guinea-pigs: (a) indicates the dose-response relationship for the changes in bronchial epithelial cell numbers 24 h after the intratracheal injection of mrIL-5 and BSA; (b) shows the time-course for the changes in bronchial epithelial cell numbers induced by the intratracheally administered mrIL-5 (15 μ g/body); (c) illustrates the effect of anti-asthmatic drugs on the intratracheally administered mrIL-5 (15 μ g/body)-induced increase in bronchial epithelial cell numbers 24 h after challenge. V (drug vehicle) = 0.9% NaCl (saline); IL5 = mrIL-5; Ket = ketotifen; Pred = prednisolone (c). Results are expressed as the mean number of cells ($\times 10^5$) of 6–8 animals; vertical lines show s.e.mean. * $P < 0.05$, significantly different from the zero dose response (a), the zero time response (b), and the mrIL-5, no drug, response (c).

Effects of anti-asthmatic drugs on bronchoalveolar lavage fluid cellular content

As shown in Table 3 and Figure 1c, prednisolone (20 mg kg⁻¹, i.p.) inhibited the increase in macrophages, eosinophils, neutrophils and epithelial cells induced by intratracheal injection of 15 μ g mrIL-5. Ketotifen (2 mg kg⁻¹, i.p.) reduced the mrIL-5-induced increase in eosinophils, neutrophils and epithelial cells. DSCG (2%), when administered at the same time into the trachea, prevented the mrIL-5-induced increase in pulmonary epithelial cells, without affecting the number of inflammatory leukocytes.

Discussion

IL-5 was first reported as a T cell-replacing factor which differentiates B cells into antibody producing cells (Takatsu *et al.*, 1980) and as the B cell growth factor II (Swain, 1985). It is now known that IL-5 promotes the proliferation and differentiation of eosinophils (Clutterbuck & Sanderson, 1988; Lopez

Table 3 Effect of anti-asthmatic drugs on mrIL-5-induced increase in the numbers of macrophages, eosinophils and neutrophils in bronchoalveolar lavage fluid (BALF) of guinea-pigs

Treatment	Leukocyte populations ($\times 10^6$)		
	Macro	Eosin	Neut
Saline, no drug	4.6 $\pm$ 0.9	0.5 $\pm$ 0.2	0.5 $\pm$ 0.1
mrIL-5, no drug	8.7 $\pm$ 1.2	1.9 $\pm$ 0.5	6.4 $\pm$ 2.0
DSCG	8.9 $\pm$ 1.8	2.4 $\pm$ 1.1	4.3 $\pm$ 1.6
Ketotifen	7.9 $\pm$ 1.7	0.8 $\pm$ 0.3*	1.1 $\pm$ 0.5*
Prednisolone	5.0 $\pm$ 1.1*	0.6 $\pm$ 0.3*	1.0 $\pm$ 0.6*

Macro = macrophages; Neut = neutrophils; Eosin = eosinophils. Results are expressed as the mean $\pm$ s.e.mean of the numbers ($\times 10^6$ cells) of macrophages, eosinophils and neutrophils recovered from BALF of 6–8 animals, 24 h after the intratracheal injection of mrIL-5 (15 μ g/animal).

* $P < 0.05$, significantly different from 'mrIL-5, no drug' response.

et al., 1988), the production of cytotoxic T cells (Takatsu *et al.*, 1987), the expression of the IL-2 receptor on activated B cells (Takatsu *et al.*, 1987), and the production of IgA (Yokota *et al.*, 1987). These observations suggest that this cytokine is closely related to the control of immune responses and inflammatory reactions. Here we have examined mrIL-5-induced increase of inflammatory leukocytes and epithelial cells in the BALF of guinea-pigs.

In the present study, the injection of mrIL-5 into the trachea of guinea-pigs induced a dose-dependent accumulation of macrophages, eosinophils, neutrophils and lung epithelial cells in the BALF after 24 h. However, in our preliminary study, neither the inhalation of hrIL-5 nor the intraperitoneal injection of mrIL-5 caused any change in guinea-pig BALF cell counts and populations after 24 h. In addition, intratracheally administered BSA did not cause any change. These results indicate that IL-5 formed in the airway lumen may be important in the induction of airway inflammation.

Airway inflammation is an important pathogenic symptom in asthma patients. A number of animal models of airway inflammation have been described. Antigen challenge elicits the pulmonary recruitment of eosinophils and neutrophils in guinea-pigs, the eosinophilia, but not the neutrophilia, being inhibited by a LTB₄ antagonist (Richards *et al.*, 1989) and a PAF antagonist (Coyle *et al.*, 1988). Endotoxin-induced eosinophilia and neutrophilia are reduced by a PAF antagonist (Rylander *et al.*, 1988), and the inhalation of PAF produces a selective accumulation of eosinophils in the airways of guinea-pigs (Coyle *et al.*, 1988; Sanjar *et al.*, 1990a). Granulocyte macrophage colony stimulating factor (GM-CSF), IL-3 and tumour necrosis factor (TNF) also stimulate pulmonary eosinophil accumulation in guinea-pigs (Kings *et al.*, 1990). Furthermore, GM-CSF enhances the PAF-induced pulmonary eosinophilic response, whereas IL-3 suppresses it (Sanjar *et al.*, 1990c). This effect of IL-3 may be explained by an induced blood neutrophilia, since infected animals with high blood or bronchial neutrophils do not exhibit an eosinophilia in response to PAF (Sanjar *et al.*, 1990c). In our study, intratracheally administered mrIL-5 clearly induced the accumulation of macrophages, eosinophils and neutrophils in the BALF. These observations demonstrate that IL-5 shares a common effect with PAF, LTB₄, GM-CSF, IL-3 and TNF.

The glucocorticoid, prednisolone, reduced the mrIL-5-induced increase in the macrophage, eosinophil and neutrophil populations of BALF in guinea-pigs. Ketotifen inhibited the increase in eosinophils and neutrophils. DSCG tended partially to inhibit the pulmonary neutrophilia, without affecting pulmonary macrophage and eosinophil accumulation. Others have studied the effects of anti-asthmatic drugs on pulmonary eosinophilia and neutrophilia with similar results. Thus, DSCG was found to inhibit antigen-induced BALF neutrophilia in guinea-pigs (Hutson *et al.*, 1988), while ketotifen and dexamethasone prevented respiratory antigen-induced eosinophilia and neutrophilia (Sanjar *et al.*, 1990b) and PAF-induced eosinophilia (Sanjar *et al.*, 1990a). On the other hand, the previous finding that DSCG inhibits PAF-induced eosinophilia (Sanjar *et al.*, 1990a) contradicts our results. However, Hutson *et al.* (1988) reported that DSCG given after antigen challenge modifies eosinophilia but not when given beforehand. Whether the inhibitory effects of these anti-asthmatic drugs on BALF inflammatory leukocyte increases are based on inhibition of cell chemotaxis, cell proliferation, cell differentiation or other mechanisms remains to be determined.

Airway epithelial cells play an important role in modulating bronchial smooth muscle tone. The airway epithelium is capable of releasing relaxant factors and epithelial destruction leads to airway hyperreactivity (Barnes, 1989; Vanhoutte, 1989). It has been observed that there are more respiratory epithelial cells in the BALF of guinea-pigs with airways rendered hyperreactive by antigen challenge than in normal animals (Motojima *et al.*, 1989). It has also been reported that clumps of airway epithelial cells, called Creola bodies (Naylor, 1985), are found in the sputum of asthmatics (Frigas & Gleich,

1986). In addition, epithelial desquamation is related to increased bronchial hyperresponsiveness following antigen challenge in asthma patients (Kushima *et al.*, 1990). In our experiments, mrIL-5 induced an increase in the number of epithelial cells in the BALF of guinea-pigs. Prednisolone attenuated the mrIL-5-induced respiratory epithelial desquamation, as shown by a smaller increase in the number of macrophages, eosinophils and neutrophils. Ketotifen inhibited epithelial cell desquamation and prevented the pulmonary eosinophilia and neutrophilia induced by mrIL-5. These results suggest that the inhibitory effect of prednisolone and ketotifen on mrIL-5-induced epithelial cell desquamation may be related to that

inhibition of the increase in BALF leukocytes, especially that of eosinophils and neutrophils. However, it is interesting that DSCG suppressed mrIL-5-induced epithelial cell desquamation without significantly affecting pulmonary leukocyte accumulation. At present, the mechanism by which DSCG inhibits the desquamation of epithelial cells is unclear and further studies are required e.g. investigation of the blockade by DSCG of direct epithelial damage by IL-5; epithelial damage by IL-5-induced active oxygen species and toxic proteins from macrophages, neutrophils and eosinophils; epithelial damage by IL-5-induced cytotoxic T cell activation, or a combination of these.

References

- BARNES, P.J. (1989). New concepts in the pathogenesis of bronchial hyperresponsiveness and asthma. *J. Allergy Clin. Immunol.*, **83**, 1013–1026.
- COYLE, A.J., URWIN, S.C., PAGE, C.P., TOUVEY, C., VILLAIN, B. & BRAQUET, P. (1988). The effect of selective PAF antagonist BN 52021 on PAF- and antigen-induced bronchial hyper-reactivity and eosinophil accumulation. *Eur. J. Pharmacol.*, **148**, 51–58.
- CLUTTERBUCK, E.J. & SANDERSON, C.J. (1988). Human eosinophil hematopoiesis studied *in vitro* by means of murine eosinophil differentiation factor (IL5): production of functionally active eosinophils from normal human bone marrow. *Blood*, **71**, 646–651.
- DOLOVICH, J., HARGREAVE, F.E., JORDANA, M. & DENBURG, J. (1989). Late-phase airway reaction and inflammation. *J. Allergy Clin. Immunol.*, **83**, 521–524.
- FREW, A.J. & KAY, A.B. (1990). Eosinophils and T-lymphocytes in late-phase allergic reactions. *J. Allergy Clin. Immunol.*, **85**, 533–539.
- FRIGAS, E. & GLEICH, G.J. (1986). The eosinophil and the pathophysiology of asthma. *J. Allergy Clin. Immunol.*, **77**, 527–537.
- HUTSON, P.A., HOLGATE, S.T. & CHURCH, M.K. (1988). The effect of cromolyn sodium and albuterol on early and late phase bronchoconstriction and airway leukocyte infiltration after allergen challenge of nonanaesthetized guinea pigs. *Am. Rev. Respir. Dis.*, **138**, 1157–1163.
- KINGS, M.A., CHAPMAN, I., KRISTERSSEN, A., SANJAR, S. & MORLEY, J. (1990). Human recombinant lymphokines and cytokines induce pulmonary eosinophilia in the guinea pig which is inhibited by ketotifen and AH 21-132. *Int. Arch. Allergy Appl. Immunol.*, **91**, 354–361.
- KUSHIMA, A., MOTOJIMA, S., YAMAI, T. & MAKINO, S. (1990). The participation of epithelial desquamation in the increase of bronchial hyperresponsiveness after antigen challenge in patients with bronchial asthma. *Jpn. J. Allergol.*, **39**, 1581–1589.
- LOPEZ, A.F., SANDERSON, C.J., GAMBLE, J.R., CAMPBELL, H.D., YOUNG, I.G. & VADAS, M.A. (1988). Recombinant human interleukin 5 is a selective activator of human eosinophil function. *J. Exp. Med.*, **167**, 219–224.
- MOTOJIMA, S., TERASI, Y., MAKINO, S., LOEGERING, D.A. & GLEICH, G.J. (1989). The relationship between eosinophils and respiratory epithelial cells in bronchoalveolar lavage fluid (BALF) and airway responsiveness in a guinea pig model of bronchial asthma. *Am. Rev. Respir. Dis.*, **139** (Suppl.), A480.
- NAYLOR, B. (1985). Creola bodies. Their “discovery” and significance. *Cytotechnol. Bull.*, **22**, 33–34.
- RICHARDS, I.M., GRIFFIN, R.L., OOSTVEEN, J.A., MORRIS, J., WISHKA, D.G. & DUNN, C.J. (1989). Effect of the selective leukotriene B₄ antagonist U-75302 on antigen-induced bronchopulmonary eosinophilia in sensitized guinea pigs. *Am. Rev. Respir. Dis.*, **140**, 1712–1716.
- RYLANDER, R., BEIJER, L., LANTZ, R.C., BURRELL, R. & SEDIVY, P. (1988). Modulation of pulmonary inflammation after endotoxin inhalation with a platelet-activating factor antagonist (48740 RP). *Int. Arch. Allergy Appl. Immunol.*, **86**, 303–307.
- SANJAR, S., AOKI, S., BOUBEKEUR, K., CHAPMAN, I.D., SMITH, D., KINGS, M.A. & MORLEY, J. (1990a). Eosinophil accumulation in pulmonary airways of guinea pigs induced by exposure to an aerosol platelet-activating factor: effect of anti-asthma drugs. *Br. J. Pharmacol.*, **99**, 267–272.
- SANJAR, S., AOKI, S., KRISTERSSEN, A., SMITH, D. & MORLEY, J. (1990b). Antigen challenge induces pulmonary airway eosinophil accumulation and airway hyperreactivity in sensitized guinea-pigs: the effect of anti-asthma drugs. *Br. J. Pharmacol.*, **99**, 679–686.
- SANJAR, S., SMITH, D., KINGS, M.A. & MORLEY, J. (1990c). Pretreatment with rh-GM-CSF, but not rh-IL3, enhances PAF-induced eosinophil accumulation in guinea-pig airways. *Br. J. Pharmacol.*, **100**, 399–400.
- SWAIN, S.L. (1985). Role of BCGF_{II} in the differentiation to antibody secretion of normal and tumor B cells. *J. Immunol.*, **134**, 3934–3943.
- SZELENYI, I. (1991). Tomorrow's asthma therapy—are antiasthmatics in the nineties anti-inflammatory drugs? *Agents Actions*, **32**, 24–33.
- TAKATSU, K., KIKUCHI, Y., TAKAHASHI, T., HONJO, T., MATSUMOTO, M., HARADA, N., YAMAGUCHI, N. & TOMINAGA, A. (1987). Interleukin 5, a T-cell-derived B-cell differentiation factor also induces cytotoxic T lymphocytes. *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 4234–4238.
- TAKATSU, K., TANAKA, K., TOMINAGA, A., KUMAHARA, Y. & HAMAOKA, T. (1980). Antigen-induced T cell-replacing factor (TRF) III. Establishment of T cell hybrid clone continuously producing TRF and functional analysis of released TRF. *J. Immunol.*, **125**, 2646–2653.
- VANHOUTTE, P.M. (1989). Epithelium-derived relaxing factor(s) and bronchial reactivity. *J. Allergy Clin. Immunol.*, **83**, 855–861.
- YOKOTA, T., COFFMAN, R.L., HAGIWARA, H., RENNICK, D.M., TAKEBE, Y., YOKOTA, K., GENNELL, L., SHRADER, B., YANG, G., MEYERSON, P., LUH, J., HOY, P., PENE, J., BRIERE, F., SPITS, H., BANCHEREAU, J., VRIES, J.D., LEE, F.D., ARAI, N. & ARAI, K. (1987). Isolation and characterization of lymphokine cDNA clones encoding mouse and human IgA-enhancing factor and eosinophil colony-stimulating factor activities: Relationship to interleukin 5. *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 7388–7392.

(Received May 30, 1991)

Revised August 19, 1991

Accepted August 27, 1991