

Time Course of Pharmacological Modulation of Peak Eosinophilic Airway Inflammation after Mite Challenge in Guinea Pigs: A Therapeutic Approach

Tzuen-Ren Hsiue Huan-Yao Lei Ai-Ling Hsieh Han-Yu Chang
Chang-Wen Chen

Departments of Medicine and Microbiology and Immunology, College of Medicine, National Cheng Kung University, Tainan, Taiwan

Key Words

Asthma · Cytokines · Adhesion molecules · Airway inflammation · Eosinophils · Bronchoalveolar lavage

Abstract

Background: It is well known that eosinophilic airway inflammation develops after allergen challenge in sensitized humans and animals. However, the detailed time course of suppression of early eosinophilic airway inflammation by pharmacological agents given just after challenge has not been discussed. Therefore, we aimed to evaluate the time course relationship of the suppression of peak eosinophilia by anti-cytokines and pharmacological agents given several hours after the aerosol challenge by a therapeutic approach. **Methods:** We used crude mite extract as an allergen to create a sensitization and inhalation challenge, and performed bronchoalveolar lavages (BAL) after the inhalation challenge to observe the degree of eosinophilic airway inflammation in guinea pigs. Various anti-cytokines (anti-IL-3 and anti-IL-5) and pharmacological agents (dexamethasone, theophylline, and roxithromycin) were given within several hours after the acute aerosol challenge to evaluate the suppressive effect on peak eosinophilia in BAL fluid, which occurred 24 h after the challenge. **Results:** Our

results show that anti-IL-5 and dexamethasone, given within 4 and 8 h after the inhalation challenge, respectively, inhibit the acute allergen-induced peak eosinophilia in BAL fluid. However, anti-IL-3, theophylline, and roxithromycin had no effect on peak eosinophilic airway inflammation after challenge. **Conclusion:** These observations suggest that several hours are needed to complete the process of cytokine-induced recruitment of eosinophils from the blood to the airways after acute allergen challenge. This may be the optimal time to administer anti-cytokines and dexamethasone to attenuate the subsequent eosinophilic airway inflammation after acute allergen-induced asthmatic attacks.

Introduction

Airway hyperresponsiveness is considered to be the basic pathophysiologic mechanism of asthma, and airway inflammation with eosinophils is strongly related to airway hyperresponsiveness [1–5]. Recent evidence suggests that the migration of eosinophils from the blood into airway tissue is dependent on the expression of adhesion molecules on endothelial cells. When the sensitized subject or animal inhales the allergen, the T lymphocytes will secrete many

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 1999 S. Karger AG, Basel
1018–2438/99/1194–0297 \$17.50/0

Accessible online at:
<http://BioMedNet.com/karger>

Correspondence to: Dr. Tzuen-Ren Hsiue
Department of Internal Medicine, National Cheng Kung University Hospital
No. 138 Sheng-Li Road, Tainan 704 (Taiwan)
Tel. +886 6 238 3206, Fax +886 6 276 6175

cytokines (especially IL-5). This enhances the expression of adhesion molecules, including ICAM-1 and VCAM-1, on the endothelial cell surface [6–10]. The eosinophils in the blood stream adhere to the surface of the endothelium and then migrate into the airway tissue. Previous studies have shown that pretreatment with anti-IL-5 antibodies or corticosteroids before aerosol challenge can inhibit the subsequent eosinophilic airway inflammation after the challenge. However, the therapeutic effect of anti-cytokines and pharmacological agents given just after the challenge has rarely been documented [11]. In addition, the time course of the therapeutic effect of the suppression of peak eosinophilia after allergen challenge by anti-cytokines and pharmacological agents has not yet been reported.

In this study, we used crude mite extract (CME) as an allergen to create sensitization and challenge, and performed bronchoalveolar lavage (BAL) to observe the degree and duration of eosinophilic airway inflammation in guinea pigs [12]. Various anti-cytokines and pharmacological agents were used within several hours after the challenge to evaluate the suppressive effect on peak eosinophilia which developed 24 h after challenge in BAL fluid. Our results show that anti-IL-5 and dexamethasone, given within 4 and 8 h after the inhalation challenge, respectively, could inhibit the acute allergen-induced eosinophilia in BAL fluid.

Materials and Methods

Preparation of CME

CME from purified *Dermatophagoides farinae* (Allergon AB, Uppsala, Sweden) was prepared by the method described by Haida et al. [12]. Briefly, the purified cultured mites were defatted with ether, and then 30 ml of phosphate buffer was added to 1 g of defatted mites and homogenized in an ice bath. Seventy milliliters of phosphate buffer was added, and the mixture was then stirred or rotated for 2 days, centrifuged at 200 *g* for 30 min at 4°C, and the supernatant was removed and dialyzed in distilled water for 48 h, after which it was lyophilized. The lyophilized CME was stored at –20°C until use. The protein content of the CME was about 50 v/w% as measured with a Bio-Rad protein assay kit (Bio-Rad Lab, Hercules, Calif., USA).

Guinea Pigs and Active Sensitization of Guinea Pigs to CME

Male Camm-Hartley guinea pigs, 300–360 g, from the Laboratory Animal Center of National Taiwan University were used. They were actively sensitized with an intraperitoneal injection of 100 µg CME with 4 mg Al(OH)₃ in 1 ml normal saline on days 0 and 7 [13].

Inhalation Challenge of Mite Aerosol in Mite-Sensitized Guinea Pigs

Intraperitoneally sensitized guinea pigs were challenged with mite aerosol on day 21. Animals were placed into a 10-liter clear Plexiglas box into which nebulized (DeVilbiss aerosonic 500D; DeVilbiss Co., Somerset, Pa., USA) CME solution (1 mg/ml, 5 ml) was blown at 8 liters/min. Awake animals spontaneously breathed mite aerosol

for 10 min, or until the development of severely labored breathing as evidenced by deep chest wall indrawing or cyanosis. According to the specifications of the manufacturer, the particle sizes produced ranged from 1 to 5 µm and the output of the nebulizer was 0.5 ml/min.

Bronchoalveolar Lavage

The experimental animals were anesthetized with an intraperitoneal injection of pentobarbital (75 mg/kg) and killed by exsanguination through the carotid artery. A tracheostomy was performed where 5 ml of 37°C normal saline was instilled into the trachea, allowed to dwell for 20 s, and then withdrawn. The lavage was repeated with another 5 ml, and the recovered fluid was combined with the first lavage. The recovered BAL fluid volume was measured, and the fluid was placed immediately on ice. Cells were sedimented by centrifugation at 200 *g* for 10 min at 4°C. The supernatant was discarded and the cells were resuspended in 5–10 ml of Hanks' balanced salt solution. A cell count was performed on a hemocytometer, and a differential count of more than 500 cells was performed on cells transferred to a glass slide using a Cytospin centrifuge (Shandon, Pittsburgh, Pa., USA) and stained with Liu's stain (a modified Wright stain). The counting and calculation of the cells in the BAL fluids were performed by one of the authors (A.H.) who was unaware of the status of the animals.

Study Design

To demonstrate the time course of eosinophilic airway inflammation after CME aerosol challenge in sensitized animals, we performed the inhalation challenge on day 21, and the BAL was performed at 6, 24 (day 22) and 72 h (day 24) and at 7 days (day 28) after the inhalation challenge in various actively sensitized animals. Two groups of animals were used in this experiment. Mite-sensitized and mite-challenged (M-M) animals received an intraperitoneal injection of 100 µg CME with 4 mg Al(OH)₃ on days 0 and 7, and the inhalation challenge of the CME aerosol was performed on day 21. Mite-sensitized and normal saline-challenged (M-NS) animals underwent the same procedures as the M-M animals except for using normal saline instead of CME for inhalation challenge on day 21.

To evaluate the effect of pretreatment with anti-cytokines or pharmacological agents on peak eosinophilia, which was present in BAL fluid 24 h after the challenge, M-M animals received intraperitoneal injections of either 100 or 300 µg of rat anti-mouse IL-5, IL-3, or GM-CSF monoclonal antibody in 1 ml normal saline 2 h before the CME challenge. Another group of M-M animals received dexamethasone 10 mg/kg i.p. b.i.d., dexamethasone 30 mg/kg i.p. b.i.d., theophylline 10 mg/kg per os b.i.d., theophylline 30 mg/kg per os b.i.d. roxithromycin 10 mg/kg per os b.i.d. or roxithromycin 30 mg/kg per os b.i.d. for 3 days just before the challenge. BAL was performed 24 h after the challenge.

We also investigated whether peak eosinophilia can be blocked by giving anti-cytokines or pharmacological agents at different time intervals after the inhalation challenge from a therapeutic point of view. Anti-IL-5 or anti-IL-3 (300 µg) in 1 ml normal saline was given intraperitoneally to various M-M animals at various intervals, i.e. 2, 4, 8, or 12 h after the challenge. Another group of M-M animals received dexamethasone 30 mg/kg i.p., theophylline 30 mg/kg per os, or roxithromycin 30 mg/kg per os at 2, 4, 8 or 12 h after the challenge. All these treated animals had BAL 24 h after the inhalation challenge.

Table 1. Total and differential counts in BAL fluids after aerosol challenges in mite-sensitized guinea pigs ($\times 10^6$ cells)

Procedure	Total cells	Eosinophils	Neutrophils	Lymphocytes	Macrophages
6 h					
M-NS	11.69 $\pm$ 1.12	1.34 $\pm$ 0.26	0.21 $\pm$ 0.08	0.59 $\pm$ 0.21	11.12 $\pm$ 0.98
M-M	14.98 $\pm$ 2.04	1.30 $\pm$ 0.27	1.68 $\pm$ 0.31 ^a	0.59 $\pm$ 0.19	11.41 $\pm$ 1.38
24 h					
M-NS	10.36 $\pm$ 0.84	1.15 $\pm$ 0.13	0.12 $\pm$ 0.03	0.87 $\pm$ 0.21	8.21 $\pm$ 0.78
M-M	29.57 $\pm$ 4.40 ^a	12.78 $\pm$ 2.64 ^a	1.18 $\pm$ 0.27 ^b	0.84 $\pm$ 0.25	14.77 $\pm$ 2.37 ^b
3 days					
M-NS	11.98 $\pm$ 1.23	1.26 $\pm$ 0.16	0.11 $\pm$ 0.03	0.68 $\pm$ 0.16	9.93 $\pm$ 1.43
M-M	22.78 $\pm$ 2.19 ^a	10.05 $\pm$ 2.11 ^a	0.12 $\pm$ 0.03	0.88 $\pm$ 0.22	11.73 $\pm$ 1.98
7 days					
M-NS	11.88 $\pm$ 1.19	1.24 $\pm$ 0.11	0.10 $\pm$ 0.02	0.78 $\pm$ 0.24	9.76 $\pm$ 1.03
M-M	19.77 $\pm$ 1.68 ^a	8.21 $\pm$ 1.47 ^a	0.08 $\pm$ 0.03	0.54 $\pm$ 0.12	10.93 $\pm$ 1.74

Values are means $\pm$ SEM. There were 6–8 animals in each group. ^a $p < 0.01$ as compared with the M-NS group; ^b $p < 0.05$ as compared with the M-NS group.

Drugs

The following materials and reagents were used: rat anti-mouse IL-5, rat anti-mouse IL-3, rat anti-mouse GM-CSF, and isotype rat IgG1 (Pharmagene, San Diego, Calif., USA), dexamethasone and theophylline (Sigma Chemical Co., St. Louis, Mo., USA), and roxithromycin (Hoechst Marion Roussel, Germany).

Statistics

All data are presented as means $\pm$ SEM. A one-way ANOVA with the Newman-Keuls multiple comparisons procedure or Student's *t* test was used for comparison of the data. $p < 0.05$ was considered to be significantly different.

Results

Total and Differential Counts in BAL Fluid after CME Aerosol Challenge

The total white cell and differential counts of BAL fluid at different time intervals after the CME inhalation challenge in actively sensitized guinea pigs are shown in table 1. Total white cells and eosinophils were significantly increased at 24 h, 3 and 7 days after the challenge in the M-M but not in the M-NS animals. Neutrophils were also significantly increased in M-M animals at 6 and 24 h after the challenge.

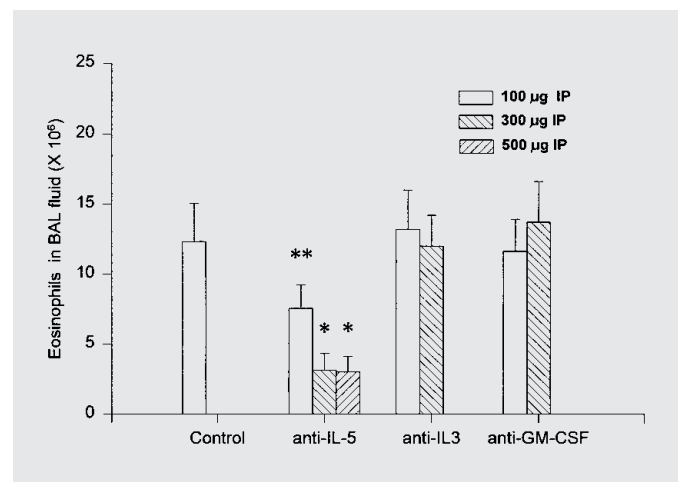


Fig. 1. Effect of intraperitoneal pretreatment with anti-cytokines, given 2 h before the challenge, on peak eosinophilia in BAL fluid 24 h after the challenge in mite-sensitized guinea pigs ($n = 5-6$). Control animals received 300 μ g of isotype rat IgG1 as a control antibody. * $p < 0.01$, ** $p < 0.05$ as compared with the control animals.

Effect of Pretreatment with Anti-Cytokines or Pharmacological Agents before Challenge on Peak Eosinophilia in BAL Fluid

The eosinophil count in BAL fluids 24 h after the inhalation challenge with pretreatment with anti-cytokines is shown in figure 1. Pretreatment with 100 or 300 μ g of

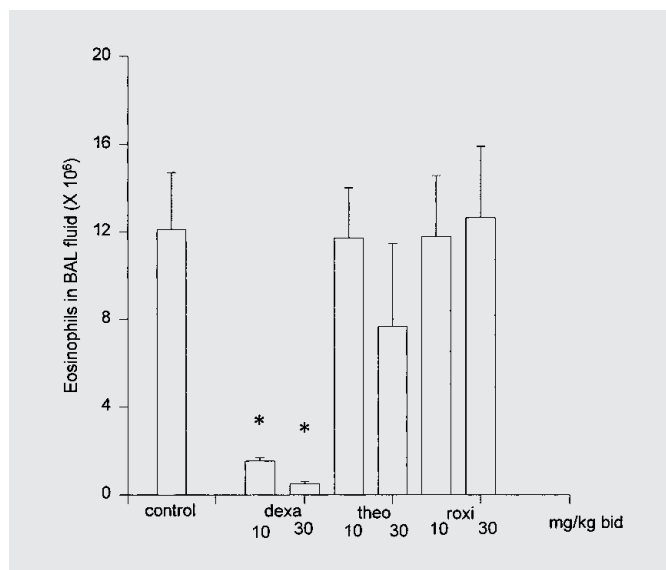


Fig. 2. Effect of pretreatment with pharmacological agents, given before the challenge, on peak eosinophilia in BAL fluid 24 h after the challenge in mite-sensitized guinea pigs (n = 6–8). Control animals received no drugs. *p < 0.01 as compared with the control animals. dexa = dexamethasone; theo = theophylline; roxi = roxithromycin.

anti-IL-5 instead of anti-IL-3 or anti-GM-CSF 2 h before the challenge caused a dose-dependent inhibition of the peak eosinophilia in BAL fluids. In addition, pretreatment with 500 µg of anti-IL-5 did not increase the degree of inhibition of peak eosinophilia. The effects of pretreatment with various pharmacological agents on peak eosinophilia are shown in figure 2. Only dexamethasone inhibited peak eosinophilia. Theophylline and roxithromycin did not have this effect.

Effects of Anti-Cytokines and Pharmacological Agents Given at Different Intervals after the Challenge on Peak Eosinophilia in BAL Fluid

The effects of 300 µg anti-IL-5 given at different intervals before and after the inhalation challenge are shown in figure 3a. Anti-IL-5 given 2 h before and 2 and 4 h after the challenge caused a significant decrease in eosinophilia in BAL fluid 24 h after the challenge. However, anti-IL-5 given at 8 h did not have this effect. The effects of anti-IL-3 given at different intervals before and after the challenge are shown in figure 3b. Anti-IL-3, given at any interval, did not decrease eosinophilia in BAL fluid.

The effects of dexamethasone are shown in figure 4a. We found that, like anti-IL-5, dexamethasone inhibited eosinophilia when the drug was given within 8 h after the inhalation challenge. However theophylline or roxithro-

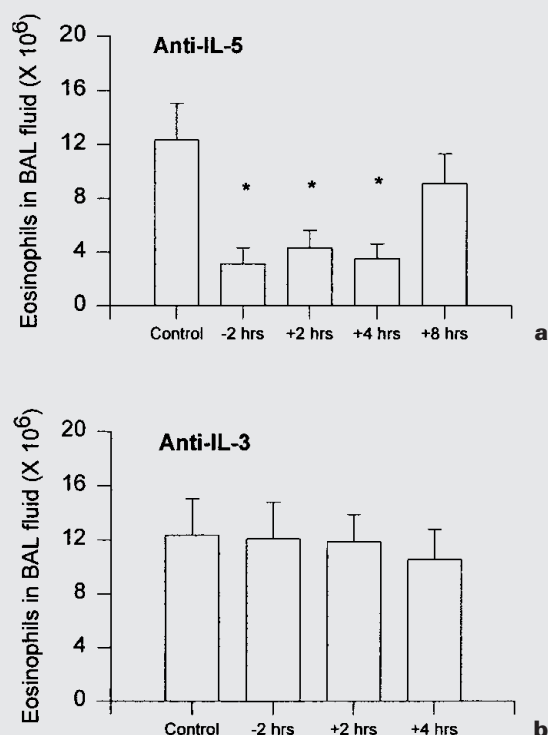


Fig. 3. Effect of anti-IL-5 300 µg i.p. (a) and anti-IL-3 300 µg (b), given at various time intervals after the challenge, on peak eosinophilia in BAL fluid 24 h after the challenge (n = 5–6). Control animals received 300 µg of isotype rat IgG1 as a control antibody 2 h before challenge. Anti-IL-5, given within 4 h after the challenge, attenuated the eosinophilia in BAL fluid; however, anti-IL-3 had no effect. *p < 0.01 as compared with the control animals.

mycin given within 8 h after the challenge did not have any effect on eosinophilia in BAL fluid (fig. 4b, c).

Discussion

Bronchial mucosal eosinophilic inflammation is recognized as one of the main events associated with bronchial hyperresponsiveness and asthma [1–5]. It is now well-established that cooperation between eosinophils and T lymphocytes is mandatory for the pathogenesis of asthma [14]. In this study, aerosol challenge of intraperitoneally mite-sensitized animals caused eosinophilia in BAL fluid, which peaked 24 h after the aerosol challenge. Eosinophilia was generally sustained for at least 1 week, and subsided after 2 weeks [13].

The pathogenesis of the peak eosinophilia in BAL fluid after the challenge is strongly related to cytokines released from Th2 cells during the inhalation challenge [15–17]. Previous studies [18–23] have already demonstrated that pretreatment with anti-IL-5 or cytokine-suppressing drugs such as dexamethasone and theophylline before the challenge can inhibit the peak eosinophilia after the challenge in ovalbumin-induced asthma models. In this study, we also found that pretreatment with dexamethasone and anti-IL-5 2 h before the challenge inhibited peak eosinophilia in BAL fluid in mite-induced allergic airway inflammation in guinea pigs. However, other anti-cytokines such as anti-IL-3 and anti-GM-CSF did not have any inhibitory effect. Because theophylline and roxithromycin have been demonstrated to have anti-inflammatory and anti-cytokine effects [19–21, 24], we also evaluated the possible suppressive effects of these agents on eosinophilic airway inflammation. We found that pretreatment with a high dose of theophylline had no effect. This result was different from previous studies [19–21] in which theophylline reduced peak eosinophilia 24 h after challenge in ovalbumin-sensitized guinea pigs. This discrepancy may be related to the different allergen and method used. We also found that roxithromycin did not have a suppressive effect on airway eosinophilia in our model.

The time course of the inhalation challenge, expression of adhesion molecules, and eosinophilic airway inflammation have not been fully explored before. Allergen challenge can cause secretion of cytokines (especially IL-5) from T lymphocytes which results in pulmonary eosinophilia [15–17]. The mechanism of IL-5-induced eosinophilia is not totally clear, but previous reports have demonstrated that IL-5-induced expression of adhesion molecules on eosinophils and endothelial cells [25–27] is a major component. Our previous report indicated that the expression of adhesion molecules in endothelial cells develops until 6 h after the inhalation challenge in sensitized mice [28]. From these findings and the therapeutic point of view, we speculate that there is a golden period in treating acute allergic airway inflammation. If we can give anti-cytokines or cytokine-suppressive drugs before full expression of the adhesion molecules on the endothelial cell surface, we can decrease peak eosinophilic airway inflammation and subsequent epithelial damage and airway hyperresponsiveness. We found that anti-IL-5 given within 4 h after the inhalation challenge significantly inhibited eosinophilic airway inflammation 24 h after the challenge. However, when anti-IL-5 was given more than 8 h after the challenge, the peak eosinophilic airway inflammation did not decrease. From this observation and previous studies, a possible explanation

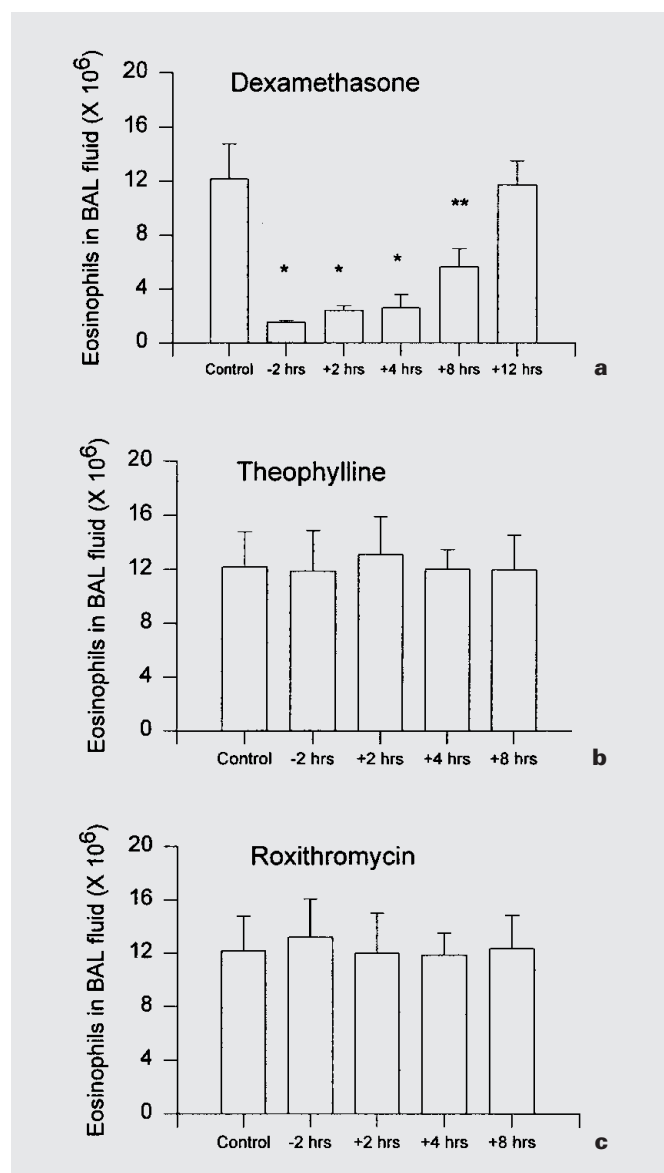


Fig. 4. Effects of dexamethasone 30 mg/kg i.p. (a), theophylline 30 mg/kg per os (b), and roxithromycin 30 mg/kg per os (c), given at different time intervals after the challenge, on peak eosinophilia in BAL fluid 24 h after the challenge (n = 6). Control animals received no medication. Dexamethasone, given within 8 h after the challenge, inhibited peak eosinophilia in BAL fluid. Theophylline and roxithromycin had no effect. *p < 0.01, **p < 0.05 as compared with the control animals.

tion is that the full expression of the adhesion molecules for the eosinophils on the endothelial surface develops several hours after the inhalation challenge. Anti-IL-5 given within 4 h after the challenge can stop the progress of the IL-5-induced expression of adhesion molecules. After 8 h, it is possible that IL-5-induced expression of the adhesion

molecules has already fully developed; therefore, anti-IL-5 given after this time does not further decrease adhesion molecules and eosinophilic airway inflammation. This speculation needs further study.

The cytokine-suppressive drug dexamethasone given within 8 h after the inhalation challenge can also inhibit eosinophilic airway inflammation. Dexamethasone possibly inhibits eosinophilic airway inflammation by its suppressive effect on cytokine (including IL-5) production from Th2 lymphocytes and an enhanced apoptosis effect [29]. The inhibition of eosinophilia by dexamethasone was more complete and more prolonged even at 8 h after inhalation challenge. These observations suggest that a high dose of corticosteroids is a strong inhibitor of cytokine production, and enhanced apoptosis may be another important mechanism in the suppressive effect of allergic eosinophilic inflammation by corticosteroids. From the therapeutic point of view, our observations demonstrate that the optimal time to give anti-IL-5 or corticosteroids to decrease the degree of

peak eosinophilic airway inflammation, possible subsequent airway inflammation or hyperresponsiveness after an allergen-induced acute asthmatic attack is within 4–8 h after the acute attack.

In conclusion, we found that anti-IL-5 given within 4 h and dexamethasone given within 8 h after the inhalation challenge can attenuate the acute allergen-induced peak eosinophilia in BAL fluid in guinea pigs. These findings suggest that several hours are needed to complete the process of cytokine-induced recruiting of eosinophils from the blood to the airways after acute allergen challenge. This period is very important from the therapeutic point of view, especially for patients with an evident environmental allergen-induced acute asthmatic attack.

Acknowledgement

This work was supported by the National Science Council grant NSC 84-2331-B-006-099 M03 of the Government of Taiwan.

References

- Bousquet J, Chanez P, Lacoste JY, Barneon G, Ghavanian N, Enander I, Venge P, Ahlstedt S, Simony-Lafontaine J, Godard P: Eosinophilic inflammation in asthma. *N Engl J Med* 1990; 323:1033–1039.
- Barnes PJ: New concepts in the pathogenesis of bronchial hyperresponsiveness and asthma. *J Allergy Clin Immunol* 1989;83:1013–1026.
- Cockcroft DW: Therapy for airway inflammation in asthma. *J Allergy Clin Immunol* 1991; 87:914–919.
- Walker C, Kaegi HK, Braun P, Blaser K: Activated T cells and eosinophilia in bronchoalveolar lavages from subjects with asthma correlated with disease severity. *J Allergy Clin Immunol* 1991;88:935–942.
- Rossi GA, Crimi E, Lantero S, Gianiorio P, Oddera S, Crimi P, Brusasco V: Late-phase asthmatic reaction to inhaled allergen is associated with early recruitment of eosinophils in the airways. *Am Rev Respir Dis* 1991;144: 379–383.
- Gavett SH, Chen X, Finkelman F, Wills-Karp M: Depletion of murine CD4⁺ T lymphocytes prevents antigen-induced airway hyperreactivity and pulmonary eosinophilia. *Am J Respir Cell Mol Biol* 1994;10:587–593.
- Nakajima H, Iwamoto I, Tomoe S, Matsumura R, Tomioka H, Takatsu K, Yoshida S: CD4⁺ T-lymphocytes and interleukin-5 mediate antigen-induced eosinophilic infiltration into the mouse trachea. *Am Rev Respir Dis* 1992;146: 374–377.
- Barnes PJ: Cytokines as mediators of chronic asthma. *Am J Respir Crit Care Med* 1994;150: s42–s49.
- Dobrina A, Menegazzi R, Carlos TM, Nardon E, Craner R, Zacchi T, Harlan JM, Patriarc P: Mechanisms of eosinophil adherence to cultured vascular endothelial cells. Eosinophils bind to the cytokine-induced ligand vascular cell adhesion molecule-1 via the very late activation antigen-4 integrin receptor. *J Clin Invest* 1991;88:20–26.
- Weller PF, Rand TH, Goelz SE, Chi-Rosso G, Lobb RR: Human eosinophil adherence to vascular endothelium mediated by binding to vascular cell adhesion molecules 1 and endothelial leukocyte adhesion molecule 1. *Proc Natl Acad Sci USA* 1991;88:7430–7433.
- Chand N, Harrison JE, Rooney SM, Nolan KW, DeVine CL, Jakubicki RG, Pillar J, Diamantis W, Sofia RD: Allergic bronchial eosinophilia: A therapeutic approach for the selection of potential bronchial anti-inflammatory drugs. *Allergy* 1993;48:624–626.
- Haida M, Okudaria H, Ogita T: Allergens of the house dust mite *Dermatophagoides farinae* – immunochemical studies of four allergic fractions. *J Allergy Clin Immunol* 1985;75: 686–692.
- Hsiue TR, Ley HY, Hsieh AL, Wang TY, Chang HY, Chen CR: Mite-induced allergic airway inflammation in guinea pigs. *Int Arch Allergy Immunol* 1997;112:295–302.
- Robinson DS, Durham SR, Kay AB: Cytokines in asthma. *Thorax* 1993;48:845–853.
- Hamid Q, Azzawi M, Ying S, Moqbel R, Wardlaw AJ, Corrigan CJ, Bradley B, Durham SR, Collins JV, Jeffery PJ, Quint DJ, Kay AB: Expression of mRNA for interleukin-5 in mucosal bronchial biopsies from asthma. *J Clin Invest* 1991;87:1541–1546.
- Broide DH, Paine MM, Firestein GS: Eosinophils express interleukin-5 and granulocyte macrophage-colony-stimulating factor mRNA at sites of allergic inflammation in asthmatics. *J Clin Invest* 1992;90:1414–1424.
- Tanaka Y, Delaporte E, Dubucquoi S, Gounni AS, Porchet E, Capron A, Capron M: Interleukin-5 messenger RNA and immunoreactive protein expression by activated eosinophils in lesional atopic dermatitis skin. *J Invest Dermatol* 1994;103:589–592.
- Elwood W, Lotvall JO, Barnes PJ, Chung KF: Effect of dexamethasone and cyclosporin A on allergen-induced airway hyperresponsiveness and inflammatory cell responses in sensitized Brown-Norway rats. *Am Rev Respir Dis* 1992; 145:1289–1294.
- Matsumoto T, Ashida Y, Tsukuda R: Pharmacological modulation of immediate and late airway response and leukocyte infiltration in the guinea pig. *J Pharmacol Exp Ther* 1994; 269:1236–1244.
- Chand N, Harrison JE, Rooney SM, De Vine CL, Jakubicki RG, Pillar J, Diamantis W, Sofia RD: Allergic bronchial eosinophilia: A therapeutic approach for the selection of potential bronchial anti-inflammatory drugs. *Allergy* 1993;48:624–626.
- Tarayre JP, Aliaga M, Barbara M, Tisseyre N, Vieu S, Tisne-Versailles J: Pharmacological modulation of a model of bronchial inflammation after aerosol-induced active anaphylactic shock in conscious guinea pigs. *Int J Immunopharmacol* 1991;13:349–356.

- 22 Lapa e Silva JR, Ruffie C, Vargaftig BB, Pretolani M: Modulation of the bronchial inflammation in sensitized guinea pigs by FK506, nedocromil sodium and dexamethasone. *Eur Respir J* 1995;8:1321–1327.
- 23 Ghand N, Harrison JE, Sofia RD: Effects of PAF-antagonists on aeroallergen-induced bronchial eosinophilia in guinea pigs: A therapeutic approach. *Res Commun Mol Pathol Pharmacol* 1994;1:75–81.
- 24 Konno S, Adachi M, Asano K, Okamoto K, Takahashi T: Anti-allergic activity of roxithromycin: Inhibition of interleukin-5 production from mouse T lymphocytes. *Life Sci* 1993;52:PL25–30.
- 25 Zangrilli JG, Shaver JR, Cirelli RA, Cho SK, Garlisi CG, Falcone A, Cuss FM, Fish JE, Peters SP: sVCAM-1 levels after segmental antigen challenge correlate with eosinophil influx, IL-4 and IL-5 production, and the late phase response. *Am J Respir Crit Care Med* 1995;151:1346–1353.
- 26 Chihara J, Kurachi D, Hayashi N, Yamamoto T, Higashimoto I, Kakazu T, Nakajima S: Induction of expression of adhesion molecules on an eosinophilic cell line (Eo1-1) by the supernatant of lymphocytes stimulated with specific allergen from asthmatic patients. *Int Arch Allergy Immunol* 1994;104:54–56.
- 27 Neeley SP, Hamann KJ, White SR, Baranowski SL, Burch RA, Leff AR: Selective regulation of expression of surface adhesion molecules Mac-1, L-selectin, and VLA-4 on human eosinophil and neutrophils. *Am J Respir Cell Mol Biol* 1993;8:633–639.
- 28 Yu CK, Lee SC, Wang JY, Hsiue TR, Lei HY: Early-type hypersensitivity-associated airway inflammation and eosinophilia induced by *Dermatophagoides farinae* in sensitized mice. *J Immunol* 1996;156:1923–1930.
- 29 Adachi T, Motojima S, Hirata A, Fukuda T, Kihara N, Kosaku A, Hideki O, Makino S: Eosinophil apoptosis caused by theophylline, glucocorticoids, and macrolides after stimulation with IL-5. *J Allergy Clin Immunol* 1996;98:S207–215.

Copyright: S. Karger AG, Basel 1999. Reproduced with the permission of S. Karger AG, Basel. Further reproduction or distribution (electronic or otherwise) is prohibited without permission from the copyright holder.