Drugs of the Future 1998, 23(10): 1083-1089

CCC: 0377-8282/98

Review Article

Eosinophilia inhibitors

Youichiro Naito*, Chikara Fukaya, Takashi Imagawa and Norifumi Nakamura

*Pharmaceutical Research Division, Yoshitomi Pharmaceutical Industries, Ltd., 2-25-1, Shodai-Ohtani, Hirakata, Osaka 573, Japan. *Correspondence*

CONTENTS

Introduction

Recently, there has been growing clinical and experimental evidence that human eosinophils (EOS) play a central role in the pathogenesis of bronchial asthma and airway hyperresponsiveness (1, 2). An increased number of EOS in circulation and bronchoalveolar lavage fluids (BALF) has been reported as a characteristic feature of chronic bronchial asthma. Furthermore, mediators released by inflammatory cells such as sulfidopeptide leukotrienes (LT), platelet activating factor (PAF), reactive oxygen species and eosinophilic cytotoxic cationic proteins can damage airway epithelial cells, which may cause airway hyperreactivity (3). Eosinophilia is also a characteristic feature of parasitic infestation and EOS are considered to be effector cells in immunity to helminths (4).

The pathogenesis of this phenomenon has not been fully elucidated, but recent research findings offer several explanations (5, 6). First, activation of allergen-specific helper ($CD4^+$) T-lymphocytes of the Th2-subset and subsequent release of cytokines including interleukin-3 (IL-3), IL-5 and granulocyte macrophage colony stimulating factor (GM-CSF) (7, 8), with IL-5 as the most likely to be specific to EOS proliferation and activation (9), links eosinophilia.

Second, the recent demonstration that $\alpha_4\beta_1$-integrin, very late activation antigen-4 (VLA-4), is expressed on EOS, suggests that the VLA-4/vascular cell adhesion molecule-1 (VCAM-1) adhesion pathway may be involved in selective eosinophilia (10). Third, a member of the 8 kd c-c group of chemokines may play an important role in EOS recruitment and degranulation (11, 12). Fourth, several inhibitors of mediators, such as LT and PAF, have been found to be effective in eosinophilia indicating that some mediators may play a role in the development of eosinophilia (6).

In this review, we describe recent explanations regarding the mechanism of eosinophilia based on studies using inhibitors, as well as the experimental results of our newly developed eosinophil inhibitor, GCC-AP0341, [5-amino-3-(4-chlorophenyl)-1-[(methylaminothiocarbonyl)]-1*H*-1,2,4-triazole] (13).

Mechanisms and inhibitors of eosinophilia

At present, no models are available for studying the complex mechanistic aspects of eosinophilia. Recently reported animal models for eosinophilia include PAF-induced (14), actively sensitized (15), Sephadex-induced (16), parasite-induced (17), IL-5-induced (18) and LTD_4-induced (19) models. This review will focus on the first three models. In the PAF-induced model, PAF alone seems to be insufficient as a basic mediator of eosinophilia. In the actively sensitized model, symptoms agreed well with those of allergic asthma but the mechanisms involved are rather complex. The Sephadex-induced model, which was developed from the parasite infection model, was found to be an effective tool for evaluation of a number of compounds. Using the model reported by Spicer *et al.* (16), we screened a variety of compounds and found one in particular to be highly active, GCC-AP0341, which is described in the latter part of this review.

PAF-induced eosinophilia

PAF is chemoattractant for both neutrophils and eosinophils *in vitro*, and induces accumulation of such cells *in vivo*. Exposure of guinea pigs to aerosols of PAF (0.01-100 μg/ml) induced a dose-dependent increased incidence of EOS in BALF at 48 h. Increased numbers of EOS were detected in BALF 1 h after exposure to PAF and the eosinophilia was inhibited by treatment with SDZ-64-412 (Fig. 1), a selective PAF antagonist, whether the compound was administered before or after inhalation of PAF. However, the fact that pulmonary airway EOS accu-

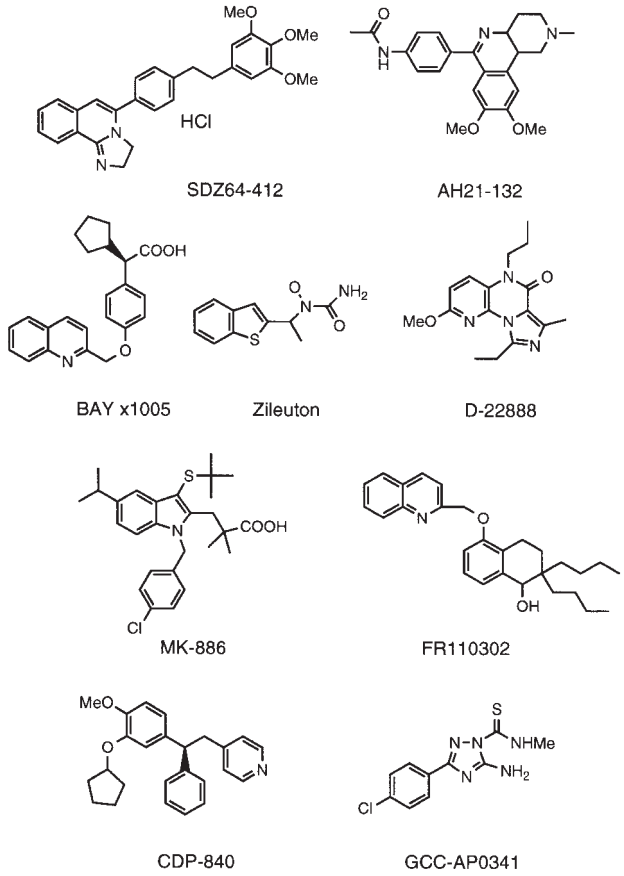

Fig. 1. Structures of eosinophil inhibitors.

mulation due to inhaled PAF (10 μg/ml) was also inhibited by prior treatment with aminophylline, cromoglycate, ketotifen, dexamathasone and AH21-132, could indicate that PAF is not a single mediator in PAF-induced eosinophilia (20).

Actively sensitized eosinophilia

As already mentioned, the symptoms of actively sensitized eosinophilia agreed well with those of allergic asthma and several attempts have been made to explain the complex mechanisms involved. Factors affecting eosinophilia include cytokines (*e.g.*, IL-3, IL-5 and GM-CSF) produced by the Th2 subset of CD4-expressing T cells (7, 8), chemotactic factors released from mast cells or basophils as a result of signaling through the high affinity IgE receptor (5), and various mediators (6).

Inhibitors reported in recent years are listed in Table I and the structures of representative compounds are shown in Figure 1. Based on these results, the mediators involved in eosinophilia are discussed. The antibody to IL-5 inhibited actively sensitized eosinophilia (21, 22) (Table I, No. 1 and 2). In addition, no eosinophilia was observed in IL-5-deficient mice after aeroallergen challenge, and reconstitution of IL-5 production with recombinant vaccinia viruses engineered to express this factor completely restored aeroallergen-induced eosinophilia (23). Sanjiv *et al.* reported that IL-5 can be detected as asthma progresses from the asymptomatic to the clinically symptomatic state in subjects with significant BALF eosinophilia (24). From these results, it appears that IL-5 plays an important role in eosinophilia. On the other hand, Nagai *et al.* reported that interferon-gamma inhibited airway eosinophilia (Table I, No. 3) possibly by the inhibition of Th2 lymphocyte-dependent eosinophilia. This result, together with the activity of IL-5, suggests that Th2 type $CD4^+$ T-lymphocyte plays an important role in eosinophilia. The immunosuppressant FK-506 (Table I, No. 4) was effective in inhibiting eosinophilia (25), while rapamycin, which is similar to FK-506, had no effect on OVA-induced eosinophil accumulation in BALF in Balb/c mice (26). Differing experimental conditions, such as strains or doses, may explain this result. As also seen in Table I (No, 5), T-cell elimination inhibits eosinophilia (27) and together with the result of FK-506 indicates that T cells may be involved in development of eosinophilia.

Chemokines are thought to play an important role in the development of eosinophilia, and studies on RANTES, monocyte chemotactic protein-3 (MCP-3)/fibroblast-induced cytokine (FIC), macrophage inflammatory protein 1α have been conducted (28). While RANTES is well known to play an important role in eosinophil recruitment and degranulation (11), no effect on development of eosinophilia has been reported (29). The chemokine antibody, anti-MCP-3/FIC, inhibited BALF eosinophilia, indicating that chemokines are involved in eosinophilia (28). The recent demonstration that VLA-4 is expressed on eosinophils and other leukocytes, but not on neutrophils, suggests that the VLA-4/VCAM-1 adhesion pathway is involved in relatively specific eosinophil migration *in vivo*. VLA-4 has been shown to play a critical role in antigen-induced airway eosinophilia (10) (Table I, No. 7). The fact that several 5-lipoxygenase and phosphodiesterase inhibitors affect eosinophilia (Table I, No. 9-13) could indicate that mediators from inflammatory cells are involved in eosinophilia (30-33).

Sephadex-induced eosinophilia

This model was developed from a parasite-infected model of eosinophilia and provides some insight into the mechanistic aspects of eosinophilia (34). In 1969, Basten *et al.* reported on eosinophilia caused by parasite infection in rats (17). The EOS response to inoculation by the intravenous route is shown in Figure 2. The blood EOS level remained within the normal range for the first 48 h, but then the counts rose rapidly, reaching a mean peak value of approximately 650 cells/mm^3 on the 6th day. Thereafter, the counts fell back to the normal range by the 14th day. On the other hand, they also reported that animals given homogenate samples of larvae did not develop an EOS response. This finding suggests that the

Table I: Inhibitors of actively sensitized eosinophilia.

No.	Type of inhibitor	Compound	Comments	Ref.
1	Cytokine antibody	anti-IL-5 (TRFK-5)	An i.p. injection of 2 mg/kg of anti-IL-5 24 h before and 3 days after OVA challenge reduced the BALF EOS count in BALB/c mice.	21
2	Cytokine antibody	anti-IL-5 (TRFK-5)	An i.v. injection of 0.3 mg/kg of anti-IL-5 60 min before Ascaris challenge reduced the BALF EOS count for up to 3 months in monkeys.	22
3	Cytokine	IFN-γ	I.v. injection of 40-4000 U/animal for 10 days during OVA challenge reduced BALF EOS counts in BALB/c mice.	26
4	Immunosuppressant	FK-506	An s.c. injection of FK-506 (2 mg/kg) 1 h before and 5 h after the four OVA challenges reduced the BALF EOS count in mice.	25
5	T cell elimination	anti-CD3 monoclonal antibody (mAb)	An i.v. injection of anti-CD3 mAb (100 μg/dose) on each of the two days preceding secondary challenge with OVA reduced the BALF EOS count in mice via inhibition of eotaxin.	27
6	Chemokine antibody	anti-MCP3/FIC	An intrabronchial injection of 0.05 ml of polyclonal anti-MCP3/FIC 4 h before OVA challenge reduced the BALF EOS count in BALF/c mice.	28
7	Adhesion molecule inhibitor	Anti-VLA-4 mAb	An i.v. injection of 1 mg/body of anti-VLA-4 2 h before OVA challenge reduced the BALF EOS count in guinea pigs.	10
8	LT inhibitor (FLAP) inhibitor	BAY x1005	Oral administration of 30 mg/kg of BAY x1005 1 h before every inhalation of OVA reduced the BALF EOS count in guinea pigs.	45
9	LT inhibitor (FLAP inhibitor)	MK-886	I.p. administration of MK-886 (3 mg/kg) 30 min before each intranasal dose of OVA reduced the BALF EOS count in BALB/c mice.	30
10	5-LO inhibitor	Zileuton	I.p. administration of zileuton (35 mg/kg) 30 min before each intranasal dose of OVA reduced the BALF EOS count in BALB/c mice.	30
11	PDE-IV inhibitor	AH21-132	Subcutaneous administration of AH 21-132 for 7 days before antigen challenge reduced the BALF EOS count in guinea pigs but did not diminish AHR.	31
12	PDE-IV inhibitor	D-22888	P.o. administration of D-22888 (30 mg/kg) for 7 days before OVA challenge reduced the BALF EOS count in guinea pigs.	32
13	PDE-IV inhibitor	CDP-840	I.p. administration of MK-886 (0.1 mg/kg) 2 h before and 6 h and 24 h after OVA challenge reduced the BALF EOS count in guinea pigs.	33

parasites were embolized to the lungs where they were trapped because of their size, and that EOS production is induced under certain circumstances as a consequence of interactions between intact parasites and various host cells in blood.

Walls *et al.* reported a model of developed eosinophilia evoked by injections of Sephadex beads in a manner resembling the result of injection of parasitic larvae (34). The results obtained by injection of G200 superfine Sephadex beads were similar to those obtained with parasite in that the rise began after the 2nd day, peaked on the 6th or 7th day and had returned to within the normal range by the 10th day. In this examination, the EOS counts rose at least 5-fold, the neutrophil count did not exceed twice the resting level and mononuclear cell numbers remained within the normal range. Fragmentation of Sephadex beads to traverse the pulmonary circulation significantly diminished the eosinophilia, similar to the

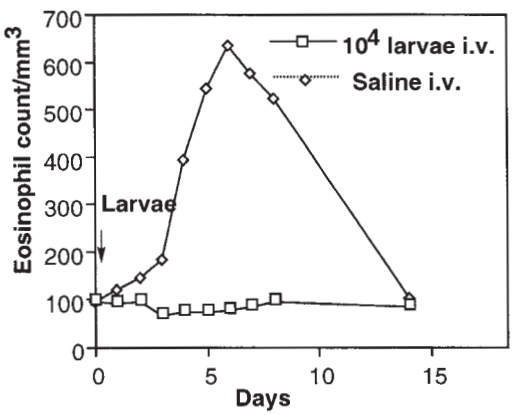

Fig. 2. Eosinophil response to innoculation of *Trichinella larvae* by the i.v. route. The broad arrow indicates the time at which the i.v. injection was given (17).

Table II: Inhibitors of Sephadex-induced eosinophilia.

No.	Type of inhibitor	Compound	Comments	Ref.
1	Cytokine antibody	anti-IL-5 (TRFK-5)	An i.v. injection of 1 mg/kg of anti-IL-5 15 min before the injection of Sephadex completely reduced the BALF EOS count in guinea pigs.	35
2	Adhesion molecule antibody	anti-VLA-4 anti-CD18	An i.v. injection of 3 mg/kg of anti-VLA-4 and 1 mg/kg of anti-CD18 before the injection of Sephadex completely reduced the BALF EOS counr in guinea pigs.	35
3	5-LO inhibitor	FR110302	Sephadex injection on day 1 and p.o. amdinistration of FR110302 (10 mg/kg) on days 1-3 d.i.d. and 1 h before measurement on day 4 reduced the BALF EOS count in rats.	37
4	5-LO inhibitor	Zileuton	P.O. administrations of zileuton (30 mg/kg) 2 h prior to Sephadex injection on day 1 and further administrations of the drug every 12 h thereafter through the 4 days time-course reduced the BALF EOS count in rats.	38
5	5-LO inhibitor (FLAP inhibitor)	BAY x1005	P.o. administration of BAY x1005 (19 mg/kg) 2 h prior to Sephadex injection on day 1 and further administrations of the drug every 12 h thereafter through the 4 days time-course reduced the BALF EOS count in rats.	38
6		GCC-AP0341	I.p. administrations of GCC-AP0341 (3 mg/kg) 30 min before each i.v. injection of Sephadex on days 0, 3 and 5 reduced the BALF EOS count in rats.	13

case of *Trichinella larvae*. An injection of inert particles of similar size, polystyrene beads, caused no detectable rise in circulation, indicating that the antigen is trapped in the lungs.

Using these Sephadex models, several inhibitors have been discovered (Table II, Fig. 1). As shown in Table II, anti IL-5 (TRFK-5) was effective in the Sephadex-induced eosinophilia model (35). A similar result was obtained with the parasite-induced model (36), indicating that in both models IL-5 may evoke eosinophilia. Adhesion molecules, VLA-4 and CD-18, also play an important role in this model (Table II, No. 2) (35), as well as inhibitors of 5-lipoxygenase (Table II, No. 3-5) (37, 38). These results, together with those for the actively sensi-

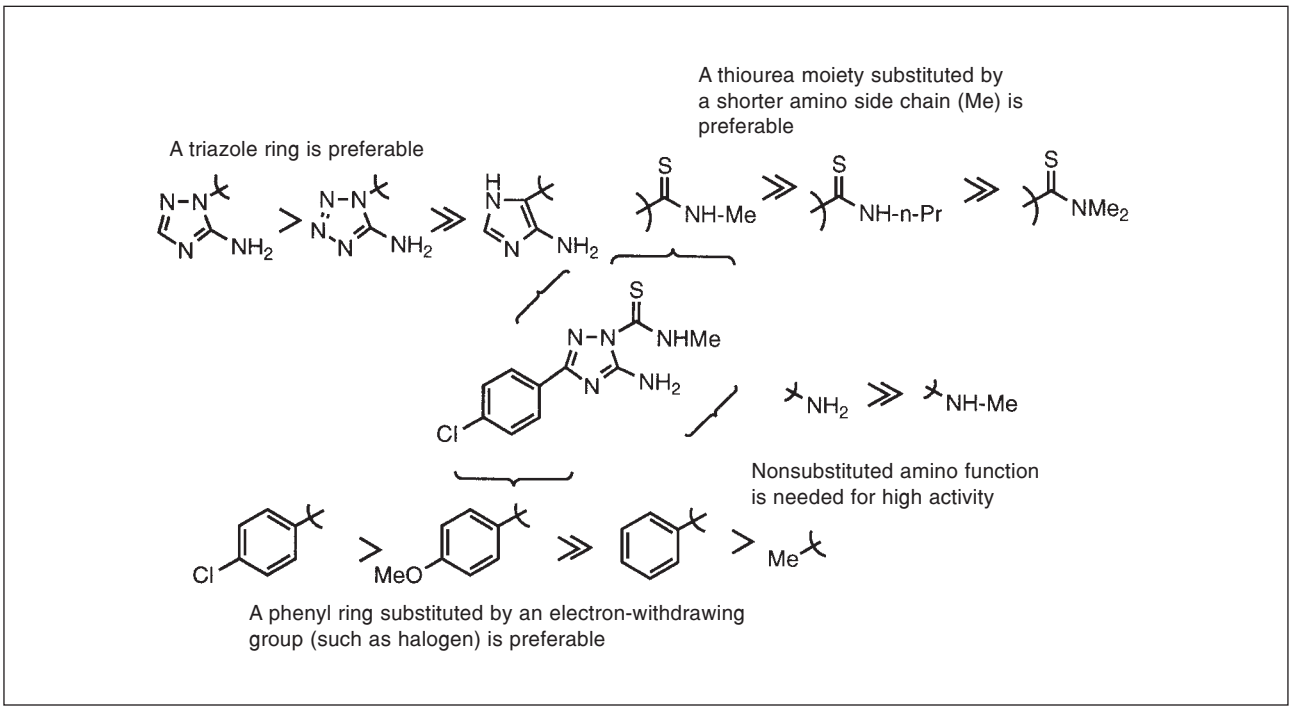

Fig. 3. Structure-activity relationship of GCC-AP0341 in the Sephadex-induced eosinophilia model.

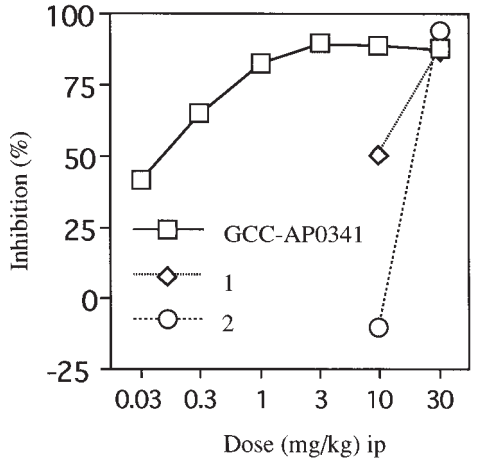

Fig. 4. Dose-response examination of GCC-AP0341, **1** and **2** in the eosinophilia model.

tized eosinophilia model (Table I), indicate that quite similar factors (*e.g.*, IL-5, adhesion molecules and 5-lipoxygenase products) play a role in the development of eosinophilia.

Evaluation of GCC-AP0341 in the Sephadex model

Since inhibitors of eosinophilia may prove to be novel agents for the treatment of asthma, we initiated a program to modify various compounds using the Sephadex model (16), in which eosinophilia was induced in the airway through i.v. injection of Sephadex particles on days 0, 2 and 5. Inhibitory activity was determined by counting the number of EOS and total cells in the BALF under a microscope on day 7. Using this model, we screened a variety of compounds and found one to be highly effective, GCC-AP0341 (13).

The structure-activity relationships of GCC-AP0341 and its dose-response examination are shown in Figures 3 and 4, respectively. The structure of GCC-AP0341

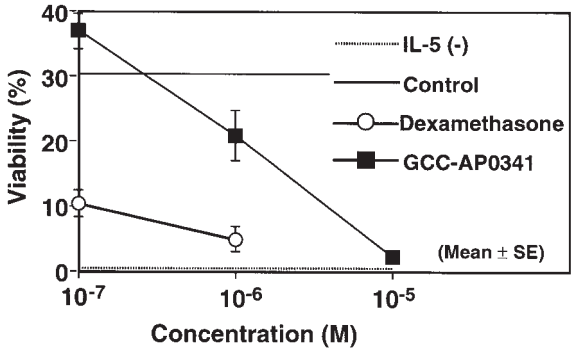

Fig. 5. Effect of GCC-AP0341 and dexamethasone on IL-5 (10 U/ml)-stimulated human eosinophil survival. Values are the mean ± SE (n = 4) (13).

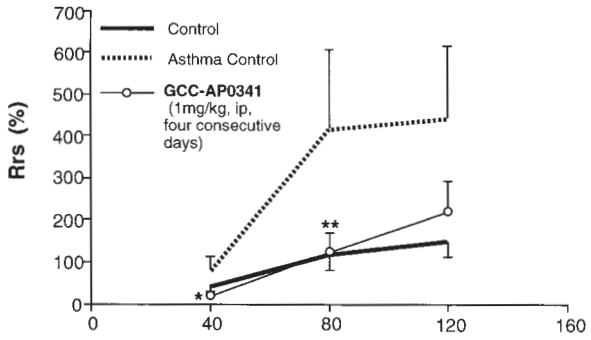

Fig. 6. Effect of GCC-AP0341 on ascaris-induced hyperresponsiveness in guinea pigs. Values are mean ± SE (n = 8) (13). Statistical analysis was carried out by Bonferroni's method. *$p < 0.05$ *vs.* asthma control. **$p < 0.01$ *vs.* asthma control.

(Fig. 3) is the most preferable one among those we examined and modification of each substituent on the triazole ring results in a drastic reduction of the activity. The dose-response studies of GCC-AP0341 and its derivatives 1 (5-amino-1-[(methylaminothiocarbonyl)]-1*H*-1,2,4-triazole) and 2 (5-amino-3-(4-cyanophenyl)-1-[(methylaminothiocarbonyl)]-1*H*-1,2,4-triazole (Fig. 4) revealed that GCC-AP0341 is highly effective and 4-chlorophenyl moiety plays an important role in exerting highly potent activity. Oral administration of GCC-AP0341 also potently suppressed eosinophilia (ID_{50}= 0.3 mg/kg). The mechanism by which GCC-AP0341 acts has been studied extensively in our laboratory. No or only very weak activity has been observed on the binding of PAF (39, 40) and LTD_4 (41), as well as the inhibition of 5-lipoxygenase (42) (our unpublished results).

To investigate the activity for IL-5, we examined the effect of GCC-AP0341 on IL-5-mediated biological events. We detected an effect on IL-5-stimulated human eosinophil survival (43) with an IC_{50} of 2 μM (Fig. 5). This result demonstrates that GCC-AP0341 reduced eosinophilia via acting on the activity of IL-5.

The efficacy of GCC-AP0341 against bronchial asthma, an eosinophilia affecting disease, was evaluated in a model of antigen (ascaris)-induced hyperresponsiveness in guinea pigs, using the modified methods of sensitization reported by Inoue *et al.* (44). Airway hyperresponsiveness was found to be inhibited at a dose of 1 mg/kg (i.p.) (Fig. 6). It should be noted that inhibitors of Sephadex-induced eosinophilia potently inhibit allergen-induced airway hyperresponsiveness by actively sensitizing eosinophilia. This indicates the presence of a factor that controls both actively and Sephadex-induced eosinophilia.

Conclusions

Explanations of eosinophilia based on the study of inhibitors, including one only recently developed, have been described. The mechanisms involved in eosinophilia

are complex and not easily defined. We speculate that mediators such as IL-5 and LT products play an important role in eosinophilia, and that inhibitors of eosinophilia could prove to be potent agents for the treatment of allergic diseases such as asthma.

References

1. Gleich, G.J. *The eosinophil and bronchial asthma.* J Allergy Clin Immunol 1990, 85: 422-36.

2. Reed, C.E. *The importance of eosinophils in the immunology of asthma and allergic disease.* Ann Allergy 1994, 72: 376-80.

3. Bochner, B.S., Undem, B.J., Lichtenstein, L.M. *Immunological aspects of allergic asthma.* Annu Rev Immunol 1994, 12: 295-335.

4. Hagan, P., Wilkins, H.A., Blumenthal, U.J., Hayes, R.J., Greenwood, B.M. *Eosinophilia and resistance to Schistosoma haematobium in man.* Parasite Immunol 1985, 7: 625-31.

5. Desreumaux, P., Capron, M. *Eosinophils in allergic reactions.* Curr Opin Immunol 1996, 8: 790-5.

6. Teixeira, M.M., Williams, T.J., Hellewell, P.G. *Mechanisms and pharmacological manipulation of eosinophil accumulation in vivo.* Trends Pharmacol Sci 1995, 16: 418-23.

7. Coorigan, C.J., Kay, A.B. *T cells and eosinophils in the pathogenesis of asthma.* Immunol Today 1992, 13: 501-7.

8. Kay A.B. *"Helper" ($CD4^+$) T cells and eosinophils in allergy and asthma.* Am Rev Respir Dis 1992, 145: s22-6.

9. Van Oosterhout, A.J.M., Van Der Poel, A., Koenderman, L., Roos, D., Nijikamp, F.P. *Interleukin-5 potentiates sulfidepeptide leukotriene production by human eosinophils.* Mediat Inflamm 1994, 3: 53-5.

10. Sagara, H., Matsuda, H., Wada, N. et al. *A monoclonal antibody against very late activation antigen-4 inhibits eosinophil accumulation and late asthmatic response in a guinea pig model of asthma.* Int Arch Allergy Immunol 1997, 112: 287-94.

11. Zhang, L., Redington, A.E., Holgate, S.T. *RANTES: A novel mediator of allergic inflammation.* Clin Exp Allergy 1994, 24: 899-904.

12. Resnick, M.B., Weller, P.F. *Mechanisms of eosinophil recruitment.* Am J Respir Cell Mol Biol 1993, 8: 349-55.

13. Naito, Y., Akahoshi, F., Takeda, S. et al. *Synthesis and pharmacological activity of triazole derivatives inhibiting eosinophilia.* J Med Chem 1996, 39: 3019-29.

14. Sanjar, S., Aoki, S., Boubekeur, K. et al. *Inhibition of PAF-induced eosinophil accumulation in pulmonary airways of guinea pigs by anti-asthma drugs.* Jpn J Pharmacol 1989, 51: 167-72.

15. Yamada, N., Ohgaki, M., Muramatsu, M. *Antigen-induced airway hyperresponsiveness is associated with infiltration of eosinophils in lung tissue, but not with bronchoalveolar lavage eosinophilia or neutrophilia.* Int Arch Allergy Immunol 1994, 103: 73-8.

16. Spicer, B.A., Baker, R.C., Hatt, P.A., Laycock, S.M., Smith, H. *The effects of drugs on Sephadex-induced eosinophilia and lung hyperreponsiveness in the rat.* Br J Pharmacol 1990, 101: 821-8.

17. Basten, A., Boyer, M., Beeson, P.B. *Mechanism of eosinophilia I. Factors affecting the eosinophil response of rats to Trichinella spiralis.* J Exp Med 1970, 131: 1271-87.

18. Kraneveld, A.D., Ark, I.V., Linde, H.J.V.D., Fattah, D., Nijkamp, F.P., Oosterhout, A.J.M.V. *Antibody to very late activation antigen 4 prevents interleukin-5-induced airway hyperresponsiveness and eosinophil infiltration in the airways of guinea pigs.* J Allergy Clin Immunol 1997, 100: 242-50.

19. Underwood, D.C., Osborn, R.R., Newsholme, S.J., Torphy, T.J., Hay, D.W.P. *Persistent airway eosinophilia after leukotriene (LT) D_4 administration in the guinea pig.* Am J Respir Crit Care Med 1996, 154: 850-7.

20. Sanjar, S., Aoki, S., Boubekeur, K. et al. *Eosinophil accumulation in pulmonary airways of guinea-pigs induced by exposure to an aerosol of platelet-activating factor: Effect of anti-asthma drugs.* Br J Pharmacol 1990, 99: 267-72.

21. Hogan, S.P., Koskinen, A., Foster, P.S. *Interleukin-5 and eosinophils induce airway damage and bronchial hyperreactivity during allergic airway inflammation in BALB/C mice.* Immunol Cell Biol 1997, 75: 284-8.

22. Mauser, P.J., Pitman, A.M., Fernandez, X. et al. *Effects of an antibody to interleukin-5 in a monkey model of asthma.* Am J Respir Crit Care Med 1995, 152: 467-72.

23. Foster, P.S., Hogan, S.P., Ramsay, A.J., Matthaei, K.I., Young, I.G. *Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model.* J Exp Med 1996, 183: 195-201.

24. Sanjiv Sur, S., Gleich, G.J., Swanson, M.C., Bartemes, K.R., Broide, D.H. *Eosinophilic inflammation is associated with elevation of interleukin-5 in the airways of patients with spontaneous symptomatic asthma.* J Allergy Clin Immunol 1995, 96: 661-8.

25. Eum, S.Y., Zuany-Amorim, C., Lefort, J., Pretolani, M., Vargaftig, B.B. *Inhibition by the immunosuppressive agent FK-506 of antigen-induced airways eosinophilia and bronchial hyperreactivity in mice.* Br J Pharmacol 1997, 120: 130-6.

26. Nagai, H., Maeda, Y., Tanaka, H. *The effect of anti-IL-4 monoclonal antibody, rapamycin and interferon-γ on airway hyperreactivity to acetylcholine in mice.* Clin Exp Allergy 1997, 27: 218-24.

27. MacLean, J.A., Ownbey, R., Luster, A.D. *T cell-dependent regulation of eotaxin in antigen-induced pulmonary eosinophilia.* J Exp Med 1996, 184: 1461-9.

28. Stafford, S., Li, H., Forsythe, P.A., Ryan, M., Bravo, R., Alam, R. *Monocyte chemotacitic protein-3 (MCP-3)/fibroblast-induced cytokine (FIC) in eosinophilic inflammation of the airways and the inhibitory effects of an anti-MCP-3/FIC antibody.* J Immunol 1997, 158: 4953-60.

29. Sur, S., Kita, H., Gleich, G.J., Chenier, T.C., Hunt, L.W. *Eosinophil recruitment is associated with IL-5, but not with RANTES, twenty-four hours after allergen challenge.* J Allergy Clin Immunol 1996, 97: 1272-8.

30. Henderson, W.R. Jr., Lewis, D.B., Albert, R.K. et al. *The importance of leukotrienes in airway inflammation in a mouse model of asthma.* J Exp Med 1996, 184: 1483-94.

31. Sanjar, S., Aoki, A., Kristersson, A., Smith, D., Morley, J. *Antigen challenge induces pulmonary airway eosinophil accumulation and airway hyperreactivity in sesitized guinea-pigs: The effect of antiasthmatic drugs.* Br J Pharmacol 1990, 99: 679-86.

32. Poppe, H., Marx, D., Hofgen, H., Szekenyi, I. *Effect of the selective phophodiesterase type 4 inhibitor D-22888 in comparison with other anti-asthma drugs on allergen-induced eosinophiliain actively sensitized guinea-pig airways.* Arch Pharmacol 1996, 354 (Suppl.): 48.

33. Hughes, B., Howat, D., Lisle, H. et al. *The inhibition of antigen-induced eosinophilia and bronchoconstriction by CDP840, a novel stereo-selective inhibitor of phosphodiesterase type 4.* Br J Pharmacol 1996, 118: 1183-91.

34. Walls, R.S., Beeson, P.B. *Mechanism of eosinophilia IX. Induction of eosinophilia in rats by certain forms of dextran.* Proc Soc Exp Biol Med 1972, 140: 689-93.

35. Das, A.M., Williams, T.J., Lobb, R., Nourshargh, S. *Lung eosinophilia is dependent on IL-5 and the adhesion molecules CD18 and VLA-4, in a guinea-pig model.* Immunology 1995, 84: 41-6.

36. Coffman, R.L., Seymour, B.W.P., Hudak, S., Jackson, J., Rennick, D. *Antibody to interleukin-5 inhibits helminth-induced eosinophilia in mice.* Science 1989, 245: 308-10.

37. Asano, M., Inamura, N., Nakahara, K. et al. *A 5-lipoxygenase inhibitor, FR110302, suppresses airway hyperresponsiveness and lung eosinophilia induced by Sephadex particles in rats.* Agents Actions 1992, 36: 215-21.

38. Namovic, M.T., Walsh, R.E., Goodfellow, C., Harris, R.R., Carter, G.W., Bell, R.L. *Pharmacological modulation of eosinophil influx into the lungs of Brown Norway rats.* Eur J Pharmacol 1996, 315: 81-8.

39. Tahraoui, L., Floch, A., Cavero, I. *Functional validation of platelet-activating factor receptor sites characterized biochemically by a specific and reproducible [^{3}H]-platelet-activating factor binding in human platelets.* J Pharmacol Exp Ther 1990, 252: 1221-7.

40. Hwang, S.B., Lam, M.H., Shen, T.Y. *Specific binding sites for platelet activating factor in human lung tissues.* Biochem Biophys Res Commun 1985, 128: 972-9.

41. Weichman, B.M., Wasserman, M.A., Holden, D.A. et al. *Antagonism of the pulmonary effects of the peptidoleukotrienes by a leukotriene D_4 analog.* J Pharmacol Exp Ther 1983, 227: 700-5.

42. Sugiura, M., Naito, Y., Yamaura, Y., Fukaya, C., Yokoyama, K. *Inhibitory activities and inhibition specificities of caffeic acid derivatives and related compounds toward 5-lipoxygenase.* Chem Pharm Bull 1989, 37: 1039-43.

43. Wallen, N., Kita, H., Weiler, D., Gleich, G.J. *Glucocorticoids inhibit cytokine-mediated eosinophil survival.* J Immunol 1991, 147: 3490-5.

44. Inoue, H., Aratani, H., Takishima, T., Kagoshima, M., Iwahisa, Y., Terasawa, M. *Effect of a potent PAF antagonist, Y-24180, on an allergen-induced late asthmatic response in guinea-pigs.* J Lipid Mediat 1992, 5: 173-6.

45. Nagai, H., Takeda, H., Uno, T., Tanaka, H., Matsuo, A. *Effect of a novel leukotoriene synthesis inhibitor, BAYx1005, on the antigen- and LPS-induced airway hyperresponsiveness in guinea pigs.* Prostaglandins 1996, 51: 139-48.