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FK506 and cyclosporin A inhibit granulocyte/macrophage colony-stimulating factor production by mononuclear cells in asthma

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ABSTRACT: Bronchial asthma is associated with eosinophilic inflammation and expression of T-cell-derived cytokines, which influence eosinophilic function. FK506, a newly established immunosuppressive agent, may have potential as a therapeutic instrument for asthma because of its suppressive effect on T-cell activation.

To assess this, we compared the inhibitory effects of FK506 and cyclosporin A on production of granulocyte/macrophage colony-stimulating factor and interleukin-5 by interleukin-2- or *Dermatophagoides farinae*-stimulated mononuclear cells from patients with asthma, and their contribution to proliferation and survival of eosinophils *in vitro*.

FK506 inhibited granulocyte/macrophage colony-stimulating factor production by stimulated mononuclear cells from asthma patients at lower concentrations than cyclosporin A. Both drugs inhibited eosinophil proliferation and survival activity from mononuclear cells at comparable concentrations. Interleukin-5 production by stimulated mononuclear cells was also inhibited both by FK506 and cyclosporin A.

We conclude that both FK506 and cyclosporin A have potential for therapy of bronchial asthma.

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Bronchial asthma is characterized by airflow obstruction and an increase in airway responsiveness. Recent studies on the late asthmatic reaction (LAR) in patients with bronchial asthma have shown that airway hyperresponsiveness is induced by chronic bronchial inflammation with various inflammatory cells, particularly eosinophils and lymphocytes [1–3]. Eosinophils play important roles in bronchial epithelial injury by releasing major basic protein (MBP), eosinophil cationic protein (ECP), and eosinophil peroxidase (EPO) [4, 5]. T-lymphocytes regulate the proliferation, infiltration, activation, and survival of eosinophils by their production of various cytokines, including granulocyte/macrophage colony-stimulating factor (GM-CSF), and interleukin (IL)-3, IL-4, and IL-5 [6–9], indicating that regulation of T-cell-derived cytokine production is an essential strategy for bronchial asthma therapy.

Corticosteroids are effective agents for treatment of LAR in bronchial asthma due to their anti-inflammatory effects and inhibition of production of T-cell-derived cytokines. However, in cases of steroid-dependent bronchial asthma, nonsteroidal agents are required to suppress airway inflammation by regulating T-cell-mediated cytokine production. Cyclosporin A (CyA) is an immunosuppressive agent that mainly affects T-cell activation through inhibition of cytokine production [10]. There is encouraging evidence on the use of CyA for treatment of bronchial asthma [11, 12]. ALEXANDER *et al.* [13] reported clinical benefit from CyA for asthma symptoms and improvement of the peak flow rate in patients with severe chronic bronchial asthma who were dependent on corticosteroids. However, treatment with CyA is associated with undesirable side-effects, such as nephrotoxicity, hepatobiliary dysfunction and neurotoxicity. In this context, other immunosuppressive agents, which reportedly have more potent inhibitory effects on T-cell activation and fewer adverse effects, should be studied for treatment of bronchial asthma.

FK506, a newly established immunosuppressive agent, is structurally quite distinct from CyA but has similar mechanisms of action [14]. In the current study, we investigated the effects of FK506 on production of GM-CSF and IL-5 by mononuclear cells (MNCs) from patients with bronchial asthma and its contribution to eosinophil colony-stimulating factor (Eo-CSF) activity and enhancement of eosinophil survival in comparison with CyA.

Materials and methods

Subjects

Studies were performed in 10 patients with bronchial asthma (as defined by American Thoracic Society (ATS) criteria), whose mean age was 48±7 yrs. Their scores in a radioallergosorbent test (RAST) against *Dermatophagoides farinae* (*Df*) were ≥2 (≥2 is positive on a 0–4 scale), as measured with a Phadebas RAST kit (Pharmacia Diagnostics AB, Uppsala, Sweden). No patient had received steroid therapy. Peripheral blood was collected during periods free from asthma attack. A separate group of 10 bronchial asthmatic patients with peripheral blood eosinophils >10% was used for eosinophil survival analysis. All patients gave informed consent.

Reagents

The following reagents were used: recombinant human IL-2 (specific activity, 3.5×10^4 U·mg^{-1} as assayed on IL-2-dependent murine NKC3 cells; Takeda Pharmaceutical Co., Osaka, Japan); recombinant human GM-CSF (specific activity, 1.7×10^7 U·mg^{-1} protein; Genzyme Corp., Boston, MA, USA); recombinant human IL-5 (Suntory Institute for Biomedical Research, Osaka, Japan); *Df* antigen (crude product, Torii Pharmaceutical Co., Tokyo, Japan); FK506 (Fujisawa Pharmaceutical Co., Japan); and cyclosporin A (Sandoz Pharmaceutical Co., Japan). The recombinant human IL-2 and *Df* antigens used in this study did not contain levels of endotoxin detectable by the Limulus test (sensitivity limit, 0.3 ng·mL^{-1}; Seikagaku Kogyo Co., Tokyo). GM-CSF and IL-5 were used at optimal final concentrations for eosinophil proliferation assays, *i.e.* 100 U·mL^{-1} of GM-CSF and 10 ng·mL^{-1} of IL-5 [15, 16].

Isolation of peripheral blood mononuclear cells

Heparinized peripheral blood samples were obtained from patients after they had given their informed consent, and were diluted with an equal volume of phosphate buffered saline (PBS). The cells were then centrifuged at 400×g for 30 min in lymphocyte separation medium (density at 20°C 1.077–1.080 g·mL^{-1}; Organon Teknika Corp., Durham, NC, USA). The cells at the gradient interface were collected and washed twice with PBS with centrifugation at 150×g for 10 min. These cells were mainly mononuclear cells (>96%), and their viability was >98%, as judged by the trypan blue dye exclusion test.

Preparation of cell-conditioned media

MNCs were washed twice with RPMI 1640 (Nissui Pharmaceutical Co., Tokyo, Japan) and resuspended in RPMI 1640 supplemented with 10% foetal calf serum (FCS) at a final concentration of 1×10^6 cells·mL^{-1} with or without an optimal concentration of a stimulant (1 U·mL^{-1} of IL-2 or 10 µg·mL^{-1} of *Df*), and with or without FK506 at various concentrations (100 pg·mL^{-1} to 1 µg·mL^{-1}), or CyA (100 pg·mL^{-1} to 1 µg·mL^{-1}). The mixtures were incubated in triplicate in polypropylene tubes (Becton Dickinson Labware, Lincoln Park, NJ, USA) at 37°C under 5% CO_2 in air of 95% humidity. After culture for 5 days, cell-free supernatants were obtained by centrifugation at 150×g for 10 min and stored at -70°C until use.

Measurement of GM-CSF and IL-5

We examined whether FK506 and CyA inhibited production of GM-CSF and IL-5 by MNCs from patients with bronchial asthma. MNCs were incubated for 5 days with or without an optimal concentration of a stimulant (1 U·mL^{-1} of IL-2 or 10 µg·mL^{-1} of *Df*) in the presence or absence of various concentrations of FK506 and CyA.

GM-CSF was measured with a commercial enzyme linked immunosorbent assay (ELISA) kit (Factor-test; Genzyme), which specifically measures native or recombinant human GM-CSF, showing no detectable cross-reaction with human IL-1 (α or β), IL-2, IL-3, IL-4, IL-5, IL-6, granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF), or interferon-γ (IFN-γ). In this assay, the minimum detectable concentration of human GM-CSF is 4 pg·mL^{-1}. IL-5 was measured with a commercial ELISA kit (Quantikine Human IL-5 Immunoassay; R&D Systems, Minneapolis, MN, USA). This kit specifically measures native or recombinant human IL-5, with no detectable cross-reaction with human IL-1 (α or β), IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, G-CSF, M-CSF, tumour necrosis factor (TNF), or IFN-γ. The minimum detectable concentration is 7.8 pg·mL^{-1}.

Measurement of Eo-CSF activity

Eo-CSF activity was measured by a liquid-suspension culture method using human bone marrow cells, which were prepared as described previously [17]. Briefly, heparinized bone marrow aspirates were obtained from different healthy volunteers, that is, from those used as controls in this study. The bone marrow cell suspension was diluted 1:2 with Dulbecco's modified Eagle medium (DMEM, Nissui) and centrifuged at 400×g for 20 min in lymphocyte separation medium. Cells from the interface of the gradient, including progenitor cells, were collected and washed twice with DMEM. These cells were allowed to adhere to culture dishes by two incubations at 37°C in DMEM supplemented with 10% FCS for 1 h each time. Nonadherent cells were then harvested, washed with DMEM and suspended at a final concentration of 1×10^5 cells·mL^{-1} in DMEM containing 10% FCS and 10% cell culture medium obtained as outlined above. Aliquots of the suspension were incubated in triplicate in polypropylene tubes at 37°C

under 5% CO_2 in air of 95% humidity. Half the supernatant was replaced at 3 day intervals by fresh medium containing 10% cell culture medium. After culture for 14 days, the cells were resuspended and counted in a Coulter counter (Coulter Electronics, Inc., Hialeah, FL, USA), and differential cells counts were made on cytocentrifuge preparations stained with May-Grünwald Giemsa. Cell viability was assessed by the trypan blue dye exclusion test. The number of viable eosinophils in the culture media on day 14 of culture was expressed as the total number of cells harvested × eosinophils as a percentage of the total cells harvested × viable cells (%)/10,000 (number of bone marrow cells cultured). Eo-CSF activity in the culture media was expressed as the number of eosinophils as a percentage of those from a positive control, which had been cultured with optimal concentrations of IL-5 (10 ng·mL^{-1}) and GM-CSF (100 U·mL^{-1}).

Measurement of stimulation of eosinophil survival

The eosinophils used for survival assays were obtained from the peripheral blood of 10 patients with bronchial asthma who had differential counts showing more than 10% eosinophils. These patients were different from the patients with bronchial asthma described above, and they participated in this study by donating eosinophils only after their informed consent had been obtained. Eosinophils were separated by the dextran sedimentation method [18], and by centrifugation on a discontinuous gradient of Percoll-Hypaque with densities of 1.085, 1.095, and 1.100 g·mL^{-1} as described previously [19]. Cells obtained from the interface between the layers with densities of 1.095 and 1.100 g·mL^{-1} were used as the eosinophil fraction. These eosinophils were "normodense eosinophils", and they showed the same content of eosinophilic cationic protein (ECP) measured by radioimmunoassay (RIA) (Pharmacia ECP RIA; Pharmacia) as eosinophils from normal volunteers and from the first group of study patients. This eosinophil fraction was used for eosinophil survival assays. The purity of the eosinophils was 87%, and more than 95% were viable, as judged by the trypan blue exclusion test. The cells were resuspended at a final concentration of 1×10^5 cells·mL^{-1} in DMEM containing 10% FCS and 10% MNC culture medium obtained as outlined above. Aliquots of the suspension were incubated in triplicate in polypropylene tubes at 37°C under an atmosphere of 95% humidity of 5% CO_2 in air. Cells were harvested daily for 1 week and counted with a Coulter counter. Differential cell counts were made on preparations obtained by cytocentrifugation and stained with May-Grünwald Giemsa. The viability of eosinophils was calculated as 100 × total number of eosinophils harvested × viable eosinophils (%)/original number of eosinophils seeded × viable eosinophils (%).

Statistical analysis

Data are presented as mean±SEM for the 10 patients with bronchial asthma. Values were compared by one-way analysis of variance (ANOVA), and were considered significant at a p-value of less than 0.05.

Results

Inhibitory effects of FK506 and CyA on production of GM-CSF and IL-5 by MNCs

The concentrations of GM-CSF in culture supernatants of bronchial asthmatic patient MNCs stimulated with IL-2 (1 U·mL^{-1}) or *Df* (10 µg·mL^{-1}) were significantly higher than those in medium without stimulants (56.8±15.6 and 22.5±5.3 *versus* 8.9±3.4 pg·mL^{-1}; n=10; $p<0.05$ and $p<0.05$, respectively) (figs 1 and 2). FK506 and CyA decreased GM-CSF production by IL-2-stimulated MNCs in a concentration-dependent manner. Significant inhibition of GM-CSF production by FK506 was observed at concentrations of 10 ng·mL^{-1} or more. Significant inhibition of GM-CSF production by CyA was also observed at concentrations of 100 ng·mL^{-1} or more (fig. 1). Similarly, FK506 and CyA decreased GM-CSF production by *Df*-stimulated MNCs from bronchial asthmatic patients in a concentration-dependent manner, with significant inhibition by FK506 at a concentration of 10 ng·mL^{-1} or more and by CyA at a concentration of 100 ng·mL^{-1} or more.

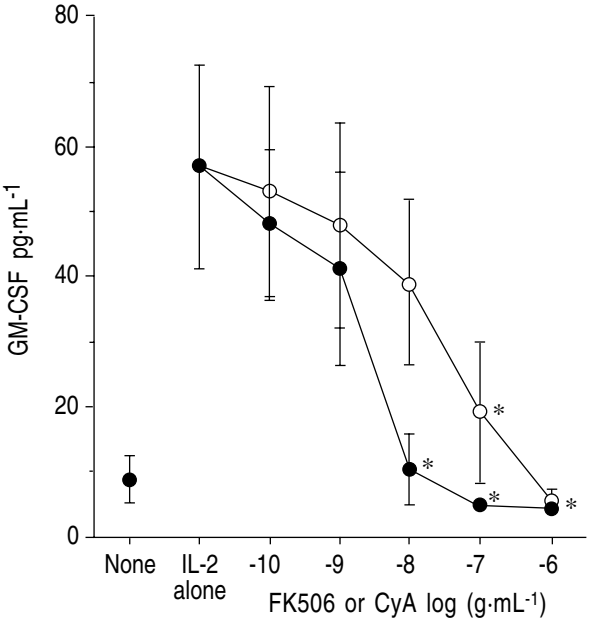

Fig. 1. – Effect of FK506 (—●—) and cyclosporin A (CyA) (—○—) on release of GM-CSF by peripheral mononuclear cells from patients with bronchial asthma. MNCs were incubated in the presence of IL-2 (1 U·mL^{-1}) without or with FK506 (100 pg·mL^{-1} to 1 µg·mL^{-1}) or CyA (100 pg·mL^{-1} to 1 µg·mL^{-1}) and cell-free supernatants were harvested on day 5 of culture. The GM-CSF concentration in the supernatant was determined by ELISA as described under Materials and Methods. Values are presented as mean±SEM of those for 10 patients with bronchial asthma. *: significant differences ($p<0.05$) from the control value (with IL-2 but without FK506 and CyA). GM-CSF: granulocyte/macrophage colony-stimulating factor; MNCs: mononuclear cells; IL-2: interleukin-2; ELISA: enzyme-linked immunosorbent assay.

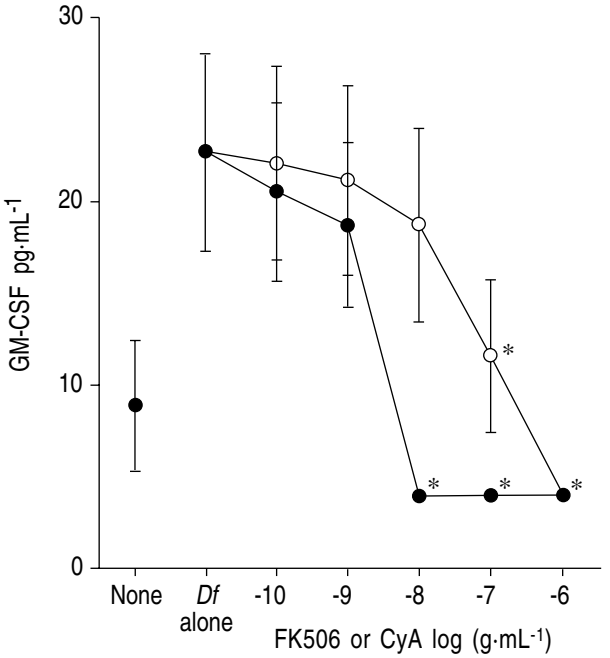

Fig. 2. – Effect of FK506 (—●—) and cyclosporin A (CyA) (—○—) on release of GM-CSF by peripheral mononuclear cells from patients with bronchial asthma. MNCs were incubated in the presence of *Dermatophagoides farinae* (*Df*) (10 μg·mL^{-1}) without or with FK506 (100 pg·mL^{-1} to 1 μg·mL^{-1}) or CyA (100 pg·mL^{-1} to 1 μg·mL^{-1}) and cell-free supernatants were harvested on day 5 of culture. The GM-CSF concentration in the supernatant was determined by ELISA as described under Materials and Methods. Values are presented as mean±SEM of those for 10 patients with bronchial asthma. *: significant differences ($p<0.05$) from the control value (with *Df* but without FK506 and CyA). For abbreviations see legend to figure 1.

Table 1. – Effects of FK506 and cyclosporin A on IL-5 production (pg·mL^{-1}) by mononuclear cells from patients with bronchial asthma (n=10)

Stimulant	Immunosuppressive agent		
	None	FK506 10 ng·mL^{-1}	CyA 100 ng·mL^{-1}
None	15.2±4.7	7.8±0*	7.8±0*
IL-2	107.8±21.4	36.9±7.6*	51.1±13.5*
Df	124.7±40.5	64.5±23.7*	80.1±29.6*

Values are presented as mean±SEM of IL-5 production (pg·mL^{-1}). *: significant difference from control ($p<0.05$). *Df*: *Dermatophagoides farinae*; IL-5: interleukin-5; CyA: cyclosporin A; IL-2: interleukin-2.

In a parallel experiment, we also examined the effects of FK506 and CyA on IL-5 production by MNCs stimulated with IL-2 or *Df*. Results are given in table 1. The concentrations of IL-5 in culture supernatants of bronchial asthmatic patient MNCs stimulated with IL-2 (1 U·mL^{-1}) or *Df* (10 μg·mL^{-1}) were significantly higher than those cultured in medium alone (107.8±21.4 and 124.7±40.5 *versus* 15.2±4.7 pg·mL^{-1}, respectively). FK506 (10 ng·mL^{-1}) and CyA (100 ng·mL^{-1}) significantly inhibited IL-5 production by IL-2- or *Df*-stimulated MNCs.

Table 2. – Effects of FK506 and cyclosporin A on release of eosinophil colony-stimulating factor (Eo-CSF) activity^{+} by mononuclear cells from patients with bronchial asthma (n=10)

Stimulant	Immunosuppressive agent		
	None	FK506 10 ng·mL^{-1}	CyA 100 ng·mL^{-1}
None	31±10	14±6*	19±7*
IL-2	142±8	113±11*	92±7*
Df	112±24	27±5*	63±3*

$^{+}$: %Eo-CSF activity was calculated as described in the Materials and Methods. Values are presented as mean±SEM of %Eo-CSF activity. *: significant difference from control ($p<0.05$). For abbreviations see legend to table 1.

Effects of FK506 and CyA on Eo-CSF release from MNCs

Culture media of unstimulated MNCs from bronchial asthmatic patients induced slightly, but significantly, more eosinophil proliferation in bone marrow cultures obtained from normal volunteers than that in medium alone (%Eo-CSF activity 31±10 *versus* 7±3%). IL-2 (1 U·mL^{-1}) and *Df* (10 μg·mL^{-1}) stimulated the release of Eo-CSF activity from MNCs (142±8 and 112±24 *versus* 31±10%) (table 2). FK506 (10 ng·mL^{-1}) significantly inhibited the release of Eo-CSF from unstimulated or IL-2- or *Df*-stimulated MNCs (14±6 *vs* 31±10%, 113±11 *vs* 142±8%, and 27±5 *vs* 112±24%, respectively). Similarly, CyA (100 ng·mL^{-1}) significantly inhibited the release of Eo-CSF from unstimulated or IL-2- or *Df*-stimulated MNCs (19±7 *vs* 31±10%, 92±7 *vs* 142±8%, and 63±3 *vs* 112±4%, respectively). Direct addition of FK506 or CyA to bone marrow cultures did not inhibit eosinophil proliferation induced by culture media of MNCs (data not shown).

Effects of FK506 and CyA on release of eosinophil survival activity from MNCs

Even the culture media of unstimulated MNCs significantly prolonged eosinophil survival compared to medium alone (21±1 *vs* 0%). IL-2 (1 U·mL^{-1}) and *Df* (10 μg·mL^{-1}) enhanced the release of this stimulating activity for eosinophil survival from MNCs (68±4 and 45±4 *vs* 21±1%) (table 3). FK506 at 10 ng·mL^{-1} significantly inhibited the release of the activity from unstimulated or IL-2- or *Df*-stimulated MNCs (10±3 *vs* 21±1%, 34±5 *vs* 68±4%, and 19±6 *vs* 45±4%, respectively). Similarly, CyA at 100 ng·mL^{-1} significantly inhibited the release of the activity from unstimulated or IL-2- or *Df*-stimulated MNCs (14±6 *vs* 21±1%, 43±3 *vs* 68±4%, and 34±7 *vs* 45±4%, respectively). Addition of either FK506 or CyA alone to the cultures did not inhibit eosinophil survival induced by culture media of MNCs (data not shown).

Table 3. – Effects of FK506 and cyclosporin A on release of eosinophil survival activity+ by mononuclear cells from patients with bronchial asthma (n=10)

Stimulant	Immunosuppressive agent		
	None	FK506 10 ng·mL^{-1}	CyA 100 ng·mL^{-1}
None	21±1	10±3*	14±6*
IL-2	68±4	34±5*	43±3*
Df	45±4	19±6*	34±7*

+: eosinophil survival activity was calculated as described in the Materials and Methods. Values are presented as mean±SEM of eosinophil survival activity. *: significant difference from control ($p<0.05$). For abbreviations see legend to table 1.

Discussion

In the present study, we have demonstrated that FK506 and CyA inhibit production of GM-CSF and IL-5 by MNCs from patients with bronchial asthma in response to IL-2 or *Df in vitro*. This inhibition by FK506 and CyA is accompanied by a decrease in the release of Eo-CSF activity and an activity required for eosinophil survival.

We have previously reported that the culture supernatants of IL-2- and *Df*-stimulated MNCs from patients with bronchial asthma who were sensitized with *Df* contained an activity which stimulated proliferation and survival of eosinophils, whilst those of MNCs from normal volunteers did not. Eosinophil proliferation and survival activity was inhibited mainly by anti-GM-CSF and anti-IL-5 antibodies, and partially by anti-IL-3 antibody [20, 21]. Thus, cytokines, such as GM-CSF, IL-5 and IL-3, which are produced by allergen-specific T-cells, may be significantly involved in the pathogenesis of bronchial asthma. This finding extended a recent observation by MORI *et al*. [22] showing that FK506 and CyA suppress IL-5 synthesis *in vitro* and *in vivo* by MNCs from patients with atopic disease. The present study clearly demonstrated that FK506 and CyA inhibit the production of GM-CSF and IL-5 by IL-2- or *Df*-stimulated MNCs from patients with bronchial asthma. This effect is probably responsible for the decreased release from MNCs of Eo-CSF activity and the activity needed for eosinophil survival. Since eosinophils play a central role as effector cells in the pathogenesis of bronchial asthma, inhibition of their proliferation and function in bone marrow and survival in airways may be beneficial for the treatment of bronchial asthma.

The maximum concentrations of FK506 and CyA in peripheral blood during therapeutic administration after organ transplantation (*per os*, 0.3 and 10 mg·kg^{-1} *q.d.*, respectively) have been reported as >40 ng·mL^{-1} for FK506 and 1 µg·mL^{-1} for CyA [23, 24]. In the current study, FK506 significantly inhibited GM-CSF production at concentrations ≥10 ng·mL^{-1} and CyA was inhibitory at concentrations ≥0.1 µg·mL^{-1}. In the assays of Eo-CSF and the eosinophil survival activity produced by MNCs, FK506 also significantly inhibited these activities at lower concentrations than CyA. Thus, clinically achievable concentrations of FK506 and CyA would be sufficient to inhibit eosinophilic inflammation *in vivo*.

The specific antigen *Df* was recently found to stimulate the production of GM-CSF and IL-5 by T-cells from the peripheral blood of patients with bronchial asthma who were sensitized with *Df*, but not that by T-cells from normal volunteers [20, 21], indicating that activated T-cells are present in the peripheral blood of patients with bronchial asthma as well as in the airways. It is likely that activated T-cells are not only recruited from the peripheral circulation into airways but also return or overflow from the airways into the peripheral circulation. Thus, the present findings regarding circulating blood MNCs suggest that FK506 and CyA may act to inhibit the local production of cytokines by stimulated T-cells, which is responsible for the pathogenesis of bronchial asthma.

Of particular interest was the previous finding that GM-CSF production by MNCs from patients with bronchial asthma treated with corticosteroids is significantly lower than that by MNCs from patients with bronchial asthma not treated with corticosteroids, and that GM-CSF production by MNCs *in vitro* is decreased by addition of corticosteroids [20]. These findings support the therapeutic effect of steroids for bronchial asthma through inhibition of eosinophilic inflammation. However, the mechanism by which corticosteroids inhibit cytokine production is known to be different from that of FK506 and CyA [25, 26]. Consistent with this, CORRIGAN *et al*. [27] have shown that the phytohaemagglutinin (PHA)-responsiveness of T-cells from patients with steroid-dependent bronchial asthma is inhibited by CyA but not by dexamethasone. Together with the present findings, these observations indicate that FK506 and CyA may be beneficial for dose reduction or withdrawal of steroids in patients with steroid-dependent bronchial asthma.

Therefore, both FK506 and CyA have potential benefits for the treatment of patients with steroid-dependent bronchial asthma. Clinical trials of FK506 for bronchial asthma should be undertaken in the near future.

References

1. De Monchy JGR, Kauffman HF, Venge P, *et al*. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. *Am Rev Respir Dis* 1985; 131: 373–376.
2. Frew AJ, Kay AB. Eosinophils and T-lymphocytes in late-phase allergic reactions. *J Allergy Clin Immunol* 1990; 85: 533–538.
3. Bousquet J, Chanez P, Lacoste JY, *et al*. Eosinophilic inflammation in asthma. *N Engl J Med* 1990; 323: 1033–1039.
4. Motojima S, Frigas E, Loegering DA, Gleich GJ. Toxicity of eosinophil cationic proteins for guinea-pig tracheal epithelium *in vitro*. *Am Rev Respir Dis* 1989; 139: 801–805.
5. Fredens K, Dahl R, Venge P. The Gordon phenomenon induced by the eosinophil cationic protein and eosinophil protein X. *J Allergy Clin Immunol* 1982; 70: 361–366.
6. Hamid Q, Azzawi M, Ying S, *et al*. Expression of mRNA

for interleukin-5 in mucosal bronchial biopsies from asthma. *J Clin Invest* 1991; 87: 1541–1546.
7. Robinson DS, Hamid Q, Ying S, *et al.* Predominant Th2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992; 326: 298–304.
8. Bentley AM, Meng Q, Robinson DS, Hamid Q, Kay AB, Durham SR. Increases in activated T-lymphocytes, eosinophils, and cytokine mRNA expression for interleukin-5 and granulocyte/macrophage colony-stimulating factor in bronchial biopsies after allergen inhalation challenge in atopic asthmatics. *Am J Respir Cell Mol Biol* 1993; 8: 35–42.
9. Walker C, Bode E, Boer L, Hansel TT, Blaser K, Virchow JJ. Allergic and nonallergic asthmatics have distinct patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage. *Am Rev Respir Dis* 1992; 146: 109–115.
10. Kronke M, Leonard WJ, Depper JM, *et al.* Cyclosporin A inhibits T-cell growth factor gene expression at the level of mRNA transcription. *Proc Natl Acad Sci USA* 1984; 81: 5214–5218.
11. Finnerty NA, Timothy JS. Effect of cyclosporin on corticosteroid-dependent asthma. *J Allergy Clin Immunol* 1991; 87: 297.
12. Szczeklil A, Nizankowska E, Dworski R, Domagala B, Pinis G. Cyclosporin for steroid-dependent asthma. *Allergy* 1991; 46: 312–315.
13. Alexander AG, Barnes NC, Kay AB. Trial of cyclosporin in corticosteroid-dependent chronic severe asthma. *Lancet* 1992; 339: 324–328.
14. Thomson AW. FK506: How much potential? *Immunol Today* 1989; 10: 6–9.
15. Sonoda Y, Arai N, Ogawa M. Humoral regulation of eosinophilopoiesis *in vitro*: analysis of the targets of interleukin-3, granulocyte-macrophage colony-stimulating factor (GM-CSF), and interleukin-5. *Leukemia* 1989; 3: 14–18.
16. Enokihara H, Furusawa S, Nakakubo H, *et al.* T cells from eosinophilic patients produce interleukin-5 with interleukin-2 stimulation. *Blood* 1989; 73: 1809–1813.
17. Strath M, Clutterback EJ, Sanderson CJ. Production of human and murine eosinophils *in vitro* and assay for eosinophil differentiation factors. *Animal Cell Culture* 1990; 5: 361–378.
18. Boyum A. Isolation of mononuclear cells and granulocytes from human blood: isolation of mononuclear cells by one centrifugation and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand J Clin Lab Invest* 1968; 27 (Suppl. 97): 77–89.
19. Frick WE, Sedgwick JB, Busse WW. The appearance of hypodense eosinophils in antigen-dependent late phase asthma. *Am Rev Respir Dis* 1989; 139: 1401–1406.
20. Nakamura Y, Ozaki T, Kamei T, *et al.* Increased granulocyte/macrophage colony-stimulating factor production by mononuclear cells from peripheral blood of patients with bronchial asthma. *Am Rev Respir Dis* 1993; 147: 87–91.
21. Kamei T, Ozaki T, Kawaji K, *et al.* Production of interleukin-5 and granulocyte/macrophage colony-stimulating factor by T-cells of patients with bronchial asthma in response to *Dermatophagoides farinae* and its relation to eosinophil colony-stimulating factor. *Am J Respir Cell Mol Biol* 1993; 9: 378–385.
22. Mori A, Suko M, Nishizaki Y, et al. Regulation of interleukin-5 production by peripheral blood mononuclear cells from atopic patients with FK506, cyclosporin A and glucocorticoid. *Int Arch Allergy Appl Immunology* 1994; 104 (Suppl. 1): 32–35.
23. Nashan B, Bleck J, Wonigeit K, *et al.* Effect of the application form of cyclosporin on blood levels: comparison of oral solution and capsules. *Transplant Proc* 1988; 20 (Suppl. 2): 637–639.
24. Japanese FK506 study group. Japanese study of FK506 on kidney transplantation: the benefit of monitoring the whole blood FK506 concentration. *Transplant Proc* 1991; 23: 3085–3088.
25. Liu J, Farmer JJ, Lane WS, Friedman J, Weissman I, Schreiber SL. Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. *Cell* 1991; 66: 807–815.
26. Paliogianni F, Raptis A, Ahuja SS, Najjar MS, Boumpas TD. Negative transcriptional regulation of human interleukin 2 (IL-2) gene by glucocorticoids through interference with nuclear transcription factors AP-1 and NF-AT. *J Clin Invest* 1993; 91: 1481–1489.
27. Corrigan CJ, Brown PH, Barnes NC, Tsai JJ, Frew AJ, Kay AB. Glucocorticoid resistance in chronic asthma: peripheral blood T-lymphocyte activation and comparison of the T-lymphocyte inhibitory effects of glucocorticoids and cyclosporin A. *Am Rev Respir Dis* 1991; 144: 1026–1032.