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Evidence that cyclosporin A and dexamethasone inhibit allergic airway eosinophilic inflammation via suppression of interleukin-5 synthesis by T cells

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1 We have recently demonstrated that airway eosinophilic inflammation can be transferred to unprimed mice by infusing interleukin (IL)-5-producing T cell clones. Using that murine model, we performed this study to delineate the mechanism of cyclosporin A and dexamethasone to inhibit allergic airway eosinophilic inflammation.

2 The ovalbumin-reactive murine T cell clones, FJ17, produced IL-2, IL-4 and IL-5 upon stimulation with relevant antigen. In FJ17-transferred mice, messenger RNA (mRNA) of IL-2 and IL-5 expressed in the lungs, the number of eosinophils in bronchoalveolar lavage fluid (BALF) was increased and the bronchial responsiveness to acetylcholine was enhanced after antigen provocation.

3 Cyclosporin A (10, 100 ng ml^{-1}) and dexamethasone (10, 100 ng ml^{-1} suppressed the production of IL-5 as well as IL-2 and IL-4 by FJ17 *in vitro*.

4 Subcutaneously administered cyclosporin A (30 mg kg^{-1}) and dexamethasone (10 mg kg^{-1}) inhibited antigen-induced mRNA expression of IL-2 and IL-5, increase of BALF eosinophils and bronchial hyperresponsiveness of FJ17-transferred mice *in vivo*. The number of BALF eosinophils was correlated with the bronchial responsiveness to acetylcholine ($r=0.672$).

5 The results clearly indicated that the suppression of IL-5 synthesis by T cells is involved in the effects of cyclosporin A and dexamethasone to inhibit allergic airway eosinophilic inflammation.

Keywords: Asthma; bronchial hyperresponsiveness; bronchoalveolar lavage; eosinophil; helper T cell; interleukin-5; cytokine

Introduction

Persistent inflammation of the bronchial mucosa has been recently recognized as the prominent pathological feature of bronchial asthma (Frigas & Gleich, 1986; Frick *et al*., 1989; Bousquet *et al*., 1990; Corrigan & Kay, 1992). Eosinophils play a central role in the mucosal inflammation, although involvement of various inflammatory cells and mediators has so far been implicated in the recruitment and activation of eosinophils.

Accumulating evidence suggests that activated T cells and T cell cytokines, including particularly interleukin (IL)-5, are crucially involved in the local infiltration and activation of eosinophils (Hamid *et al*., 1991; Okudaira *et al*., 1991; Corrigan & Kay, 1992; Kay, 1992). IL-5 promotes the terminal differentiation of committed eosinophil precursors (Sanderson *et al*., 1988) and prolongs the survival of eosinophils (Yamaguchi *et al*., 1988). CD4$^+$ T cells isolated from bronchoalveolar lavage fluid (BALF) of allergic asthmatics express elevated levels of messenger RNA (mRNA) for IL-5 (Robinson *et al*., 1993a). IL-5 production by CD4$^+$ T cells is enhanced in atopic and non-atopic asthmatics compared with normal control subjects (Mori *et al*., 1995). IL-5 concentrations in BALF and serum of asthmatic patients are increased (Walker *et al*., 1992; Corrigan *et al*., 1993). Administration of anti-IL-5 neutralizing antibody totally abrogates the induction of late phase airway eosinophilic inflammation in antigen-sensitized mice (Okudaira *et al*., 1991; Nakajima *et al*., 1992; Hamelmann *et al*., 1997a).

We have recently demonstrated that the transfer of IL-5-producing T cells is sufficient for the development of airway eosinophilic inflammation upon antigen inhalation, despite the absence of humoral immunity such as IgG, IgA and IgE antibodies (Kaminuma *et al*., 1997). Prolonged bronchial hyperresponsiveness (BHR) was induced upon antigen challenge as well. The eosinophil infiltration was dependent on IL-5 produced by the infused T cell clones, as the responses were completely suppressed by the administration of anti-IL-5 neutralizing antibody (Kaminuma *et al*., 1997). Moreover, the intensity of the eosinophil accumulation *in vivo* correlated well with the capacity of the T cell clones to produce IL-5 *in vitro*.

Involvement of several other cell types including mast cells (Bradding *et al*., 1994) and bronchial epithelial cells (Cox *et al*., 1991; Look *et al*., 1992), however, has been implicated in the eosinophilic inflammation of asthmatic patients. IgE-dependent activation of mast cells might be related to the later infiltration of eosinophils (Bradding *et al*., 1994; Coyle *et al*., 1996). It has also been reported that the IgE-mast cell pathway directly affects bronchial responsiveness (Lack *et al*., 1995; Oshiba *et al*., 1996). Allergic eosinophilic inflammation, therefore, may be a complex response involving multiple cell types and mediators.

Corticosteroids have long been the most potent therapeutic agents for asthma. Steroid treatment reduces IL-5 mRNA expression, the number of activated CD4$^+$ T cells and

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eosinophils in the bronchial mucosa (Bentrey *et al*., 1996), and more importantly improves BHR (Gauvreau *et al*., 1996). The clinical efficacy of an immunosuppressant, cyclosporin A, for the treatment of chronic asthma has recently been reported (Fukuda *et al*., 1995; Lock *et al*., 1996). The effects of cyclosporin A and corticosteroids on eosinophilic inflammation have been demonstrated using various asthma models (Nagai *et al*., 1995; De Bie *et al*., 1996; Eum *et al*., 1996). These *in vivo* effects can be ascribed to their multiple actions on various cell types determined *in vitro*. Cyclosporin A and corticosteroids inhibit proliferation and cytokine production of T cells (Gillis *et al*., 1979; Schmidt *et al*., 1994; Mori *et al*., 1995). Corticosteroids induce apoptosis of eosinophils (Druilhe *et al*., 1996) and downregulate the expression of adhesion molecules on vascular endothelial cells (Burke-Gaffney & Hellewell, 1996). In addition, both agents inhibit degranulation of mast cells (Daeron *et al*., 1982; Marone *et al*., 1992). So it has remained unsolved which actions of cyclosporin A and corticosteroids are crucial for the suppression of eosinophilic inflammation *in vivo*, as all of these *in vitro* effects may be related to the *in vivo* suppression of eosinophilic inflammation.

We have previously demonstrated that both cyclosporin A and corticosteroids suppress IL-5 production by $CD4^+$ T cells of asthmatic patients (Mori *et al*., 1995). So these agents may inhibit airway eosinophilic inflammation through the suppression of IL-5 production by $CD4^+$ T cells. To test the hypothesis, we examined the effects of the two agents on the murine model we have developed.

Methods

Animals

Specific pathogen-free male BALB/c mice, 10 to 14 weeks of age, were obtained from Japan SLC (Hamamatsu, Japan). These animals were housed in an environmentally controlled room (temperature, 23 ± 2°C; humidity, 55 ± 5%; illumination time, from 07 00 h to 19 00 h) with food and water available *ad libitum* for 1 week prior to the experiment.

Establishment of T cell clones

Ovalbumin-reactive T cell clones (FJ17) were established from lymph node cells of immunized BALB/c mice according to the procedures previously described by Kaminuma *et al*. (1997). Briefly, inguinal lymph nodes were removed from mice 7 days after immunization with ovalbumin plus complete Freund's adjuvant. Lymph node cells (2×10^6 ml^{-1}) were cultured for 6 to 8 days in the presence of mitomycin-C-treated syngeneic spleen cells (6×10^6 ml^{-1}) and ovalbumin (100 μg ml^{-1}). Cloning by limiting dilution was performed with the remaining viable cells in the presence of spleen cells, ovalbumin and IL-2 (10 U ml^{-1}). After 2 to 3 weeks, the wells where cells survived and proliferated were transferred to macrowells along with spleen cells, antigen and IL-2. Cloned cells were also maintained by biweekly antigenic stimulation using the same culture conditions.

Stimulation of T cell clones

For stimulation experiments, T cell clones were harvested 10–14 days after the last antigenic stimulation, and purified by centrifugation over a Ficoll-Paque gradient (Davidson & Parish, 1975). To determine the profiles of cytokine production, cells (5×10^5 ml^{-1}) were suspended in AIM-V medium and cultured in 96-well culture plates with mitomycin-created spleen cells (5×10^5 ml^{-1}) with or without ovalbumin (100 μg ml^{-1}) for 24 h. The resulting supernatants were assayed for IL-2, IL-4 and IL-5 by enzyme-linked immunosorbent assay (ELISA). To examine the effects of cyclosporin A and dexamethasone, each agent was added at the start of the cultures.

Quantitation of cytokines in culture supernatants

Purified rat anti-mouse IL-2, IL-4 and IL-5 monoclonal antibodies were used as the capture antibodies, and biotinylated rat anti-mouse IL-2, IL-4 and IL-5 monoclonal antibodies as the detecting antibodies for the ELISAs. The conditions of each reaction were exactly the same as specified in the recommended protocols of the manufacturer. The minimum detectable concentrations for IL-2, IL-4 and IL-5 were 10, 10 and 20 pg ml^{-1}, respectively.

Cell transfer and challenge procedure

FJ17 (5×10^6) in 0.5 ml Hank's balanced salt solution were injected into the tail vein of normal recipient mice. Twenty-four hours after the cell transfer, mice were individually placed in 50 ml plastic tubes and made to inhale aerosolized ovalbumin (100 mg ml^{-1}) dissolved in 0.9% saline delivered by a DeVilbiss 646 nebulizer (DeVilbiss Corp., Somerset, PA, U.S.A.) driven by compressed air at 18 l min^{-1} for 20 min. As a control, 0.9% saline alone was administered by nebulizer. In some animals, cyclosporin A (3 or 30 mg kg^{-1}) or dexamethasone (1 or 10 mg kg^{-1}) was subcutaneously administered four times at 30 min before and 24 h, 48 h and 96 h after the challenge.

Messenger RNA expression

Cytokine mRNA expression in the lungs of FJ17-transferred mice was detected by reverse transcription-polymerase chain reaction (RT–PCR). Mice were anesthetized with sodium pentobarbital (5 mg, i.p.). An incision was made in the abdominal aorta to remove the whole blood. The remaining blood in the lung tissue was drained by injecting 5 ml saline via the right ventricle before the lung was removed.

RNA was extracted from lung homogenates essentially following the one-step acid guanidium isothiocyanate-phenol-chloroform extraction method of Chomczynski & Sacchi (1987) using Isogene (Nippongene, Tokyo, Japan) as reported previously (Mori *et al*., 1995). RT–PCR assay was performed using GeneAmp® RNA PCR kit (Perkin Elmer Japan, Chiba, Japan) as reported previously. cDNA was synthesized from the extracted RNA using random primers and murine Moloney leukemia virus reverse transcriptase, and was then amplified by GeneAmp® DNA polymerase using murine IL-2, IL-4, IL-5 and β-actin primers. To 50 μl (final volume) amplification solution (50 mM KCl, 10 mM Tris-HCl, pH 8.3, 0.75 mM $MgCl_2$, 0.4 mM of each deoxynucleotide triphosphate), 2 μl cDNA (corresponding to about 100 ng starting RNA material), 0.4 μM of each primer, and 2.5 U AmpliTaq DNA polymerase were added. The mixture was heated at 95°C for 1 min, followed by 35 cycles, each consisting of incubation for 30 s at 95°C, 30 s at 62°C and 90 s at 72°C. The number of cycles was titrated prior to

the experiment, at which time PCR products were amplified exponentially. The PCR products were analysed by 2% agarose gel electrophoresis in the presence of ethidium bromide. Expected sizes of PCR amplification products were 413, 357, 424 and 540 bp for IL-2, IL-4, IL-5 and β-actin, respectively.

Measurement of BHR and bronchoalveolar lavage

BHR was assessed as bronchoconstriction after infusion of acetylcholine by determining change in respiratory overflow volume, essentially following the method described by Konzett & Rössler, (1940); Kaminuma *et al.* (1997). Briefly, mice were anesthetized with sodium pentobarbital (50 mg kg^{-1}, i.p.), and their trachea and femoral vein were cannulated. The tracheal cannula was connected to a rodent ventilator (model 680–683; Harvard, South Natick, MA, U.S.A.) and then mice were mechanically ventilated at 60 strokes min^{-1} with a stroke volume of 1 ml after neuromuscular blockade with 1 mg kg^{-1} pancuronium bromide. The changes in respiratory overflow volume were measured by a differential pressure transducer (TP-602; Nihon Kohden, Tokyo, Japan) connected to a T-tube on the tracheal cannula. The increase in respiratory overflow volume provoked by acetylcholine (30–3000 μg kg $^{-1}$) is represented as a percentage of the maximal overflow volume (100%) obtained by totally occluding the tracheal cannula.

After the measurement of BHR, bronchoalveolar lavage was performed in the same animals. The trachea was re-cannulated with a polyethylene tube through which the lungs were gently lavaged with 0.5 ml Hank's balanced salt solution four times (2 ml total). On average, 1.9 ml BALF was recovered and then centrifuged at 1500 r.p.m. for 5 min. The pellet obtained was immediately suspended in 250 μl Hank's balanced salt solution and total cell numbers in BALF were counted by automatic cell counter (Celltack MEK-5158; Nihon Kohden, Japan). Differential cell counts were made by microscopic examination of centrifuged preparations stained with May-Giemsa, counting 200 cells in each animal.

Reagents

Ovalbumin, dexamethasone and mitomycin-C were purchased from Sigma (St. Louis, MO, U.S.A.); cyclosporin A was from Sandoz Japan (Tokyo, Japan). Purified and biotinylated rat anti-mouse IL-2, IL-4 and IL-5 monoclonal antibodies and recombinant murine IL-2, IL-4 and IL-5 were purchased from Pharmingen (San Diego, CA, U.S.A.). Murine IL-2, IL-4, IL-5 and β-actin primer were from CLONTECH Laboratories, Inc. (Palo Alto, CA, U.S.A.). Other reagents were as follows: complete Freund's adjuvant (Difco Laboratories, Detroit, MI, U.S.A.), Ficoll-Paque (Pharmacia, Uppsala, Sweden), AIM-V medium (Gibco BRL, Gaithersburg, MD, U.S.A.), pentobarbital sodium (Tokyo Kasei, Tokyo, Japan), acetylcholine chloride (Nacalai Tesque, Kyoto, Japan), pancuronium bromide (Sankyo, Tokyo, Japan), tween 80 (Katayama Chemistry, Osaka, Japan).

Statistical analysis

Results were expressed as mean $\pm$ s.e.mean. Statistical analysis was performed using Student's *t*-test for comparison between two groups and one-way analysis of variance with Bonferroni's method for three groups or more. Values of $P<0.05$ were considered to be statistically significant.

Results

Effects of cyclosporin A and dexamethasone on antigen-induced IL-2, IL-4 and IL-5 production by murine T cell clones in vitro

The first experiment was carried out to confirm the effects of cyclosporin A and dexamethasone on the cytokine production of T cell clones *in vitro*. As shown in Table 1, the T cell clone, FJ17, produced IL-2, IL-4 and IL-5 upon antigen stimulation. Both cyclosporin A and dexamethasone inhibited IL-2, IL-4 and IL-5 production in a dose-dependent manner. We have confirmed that the number of FJ17 did not significantly change after 24 h culture in the presence of antigen (data not shown), suggesting that the reduced levels of cytokines was not merely a consequence of inhibition of FJ17 proliferation.

Effects of cyclosporin A and dexamethasone on antigen-induced IL-2, IL-4 and IL-5 mRNA expression by murine T cell clones in lung specimens

We next determined the effects of cyclosporin A and dexamethasone on cytokine synthesis by T cells *in vivo*. Lungs were removed from FJ17-transferred mice 9 h after antigen provocation. Then cytokine mRNA expression in the lungs were analysed by RT–PCR. Preliminary experiments indicated that IL-2 and IL-5 mRNA became detectable at 3 h and peaked at 9 h after antigen inhalation (data not shown). As shown in Figure 1, significant mRNA expression for IL-2 and IL-5 was detected upon antigen challenge. Cyclosporin A (30 mg kg^{-1}) and dexamethasone (10 mg kg^{-1}) clearly suppressed IL-2 and IL-5 mRNA expression. IL-4 mRNA was detectable neither before nor after antigen challenge.

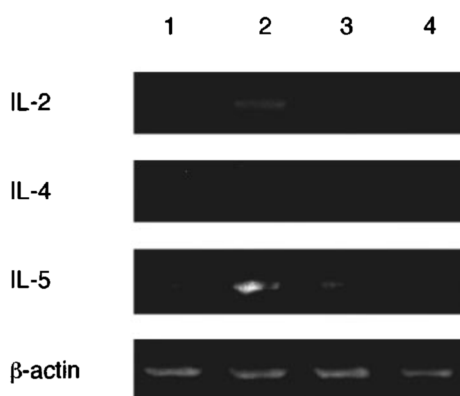

Figure 1 Effects of cyclosporin A and dexamethasone on antigen-induced cytokine mRNA expression in the lungs of T cell clone-transferred mice. FJ17 (5×10^{6}) was transferred to normal mice by intravenous injection. After 24 h, these animals were challenged with inhaled ovalbumin (100 mg ml^{-1}) for 20 min. Lung tissue was obtained 9 h after antigen provocation. RNA was extracted, reverse transcribed and amplified by PCR using specific primers. The expected size of PCR amplification products was 413, 357, 424 and 540 bp for IL-2, IL-4, IL-5 and β-actin, respectively. Each test compound was administered 30 min before antigen provocation.

Table 1 Effects of cyclosporin A and dexamethasone on antigen-induced IL-2, IL-4 and IL-5 production by murine T cell clones

Stimulation	*Agents*	*Dose* (ng ml^{-1})	*Cytokine production* (pg ml^{-1}) *IL-2*	*IL-4*	*IL-5*
–			1.0±0.0*	2.2±1.4*	368.8±114.1*
Ovalbumin			625.8±96.0	107.9±2.5	2499.0±87.0
	Cyclosporin A	1	166.8±47.1*	85.2±8.5*	2562.8±187.8
		10	13.2±1.5*	9.4±3.4*	1191.8±175.5*
		100	6.0±0.3*	1.0±0.0*	484.5±61.4*
	Dexamethasone	1	273.2±63.9*	102.8±3.8	2309.5±171.4
		10	72.8±4.9*	67.6±4.2*	662.3±79.4*
		100	40.1±1.6*	37.0±2.6*	373.0±53.9*

FJ17 (5×10^5 ml^{-1}) was cultured with mitomycin-C-treated spleen cells (5×10^5 ml^{-1}) with or without ovalbumin (100 μg ml^{-1}) for 24 h. Various concentrations of cyclsporin A and dexamethasone were added at the start of the ovalbumin-stimulated cultures. The production of IL-2, IL-4 and IL-5 with or without cyclosporin A or dexamethasone are shown ($n=4$). Data are expressed as the mean±s.e.mean. *$P<0.05$, compared with ovalbumin-treated FJ17 (Bonferroni's test).

Table 2 Effects of cyclosporin A and dexamethasone on antigen-induced airway eosinophilia and bronchial hyperresponsiveness of T cell clone-tranferred mice

Challenge	*Agents*	*Dose* (mg kg^{-1})	*Eosinophil number* ($\times10^3$ BALF^{-1})	*Bronchoconstriction to acetylcholine* (300 μg kg^{-1}, i.v.)
Saline			0.0±0.0*	22.0±5.9*
Ovlbumin			391.3±88.7	67.4±9.8
	Cyclosporin A	3	475.6±30.5	64.8±7.2
		30	21.9±6.0*	26.3±3.3*
	Dexamethasone	1	115.8±18.3*	51.0±11.5
		10	2.1±0.9*	24.1±2.7*

T cell clones (FJ17, 5×10^6) were transferred to unprimed mice by intravenous injection. After 24 h, these animals were challenged with inhaled ovalbumin (100 mg ml^{-1}) for 20 min. Eosinophil number in BALF and bronchial responsiveness to acetylcholine (300 μg kg^{-1}) 192 h after the antigen challenge are shown ($n=4-5$). Cyclosporin A and dexamethansone were administered four times at 30 min before 24, 48 and 96 h after the challenge by subcutenous injection. *$P<0.05$, compared with antigen-challenged mice (Bonferroni's test).

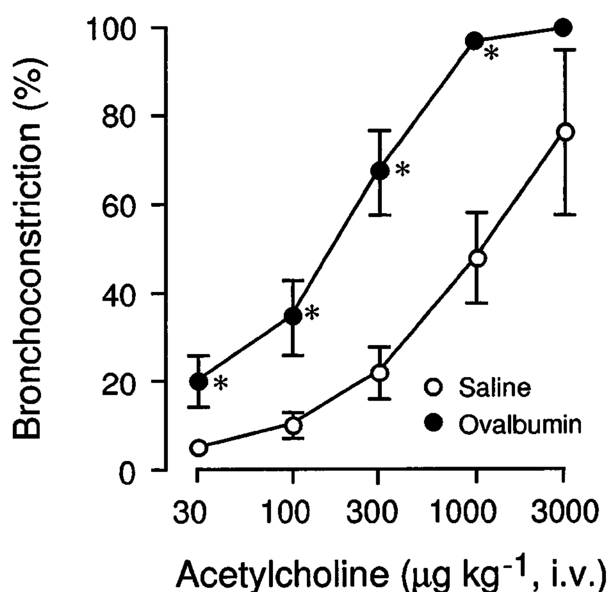

Figure 2 Antigen-induced bronchial hyperresponsiveness of T cell clone-transferred mice. FJ17 (5×10^6) was transferred to normal mice by intravenous injection. After 24 h, these animals were challenged with inhaled ovalbumin (100 mg ml^{-1}) for 20 min. Bronchial responsiveness to acetylcholine (30–3000 μg/kg, i.v.) was measured 192 h after antigen challenge. Data are presented as the mean± s.e.mean. ($n=4-5$). *$P<0.05$, compared with saline-challenged mice (Bonferroni's test).

Effects of cyclosporin A and dexamethasone on antigen-induced airway eosinophilia in T cell clone-transferred mice

As we previously reported, massive eosinophil infiltration was induced upon antigen inhalation in the T cell clone-transferred mice (Kaminuma *et al*., 1997). The effects of cyclosporin A and dexamethasone on eosinophil infiltration into the airway of FJ17-transferred mice were next examined. Preliminary experiments indicated that maximum increase in BALF eosinophils was obtained at 192 h after the challenge (data not shown). As shown in Table 2, the eosinophil number in BALF was significantly reduced by treatment with cyclosporin A or dexamethasone in a dose-dependent manner.

Effects of cyclosporin A and dexamethasone on antigen-induced BHR in T cell clone-transferred mice

Significant augmentation of bronchial responsiveness to acetylcholine was observed upon antigen challenge (Figure 2), in parallel with the increase of BALF eosinophils (Table 2). Significant BHR was noted following injection of 30–1000 μg kg^{-1} acetylcholine. There were no significant differences in baseline of respiratory overflow volume (data not shown). Cyclosporin A at the dose of 3 mg kg^{-1} was ineffective, but significantly suppressed BHR at 30 mg kg^{-1}. Dexamethasone at the dose of 10 mg kg^{-1} also effectively prevented BHR. Both agents did not affect bronchial

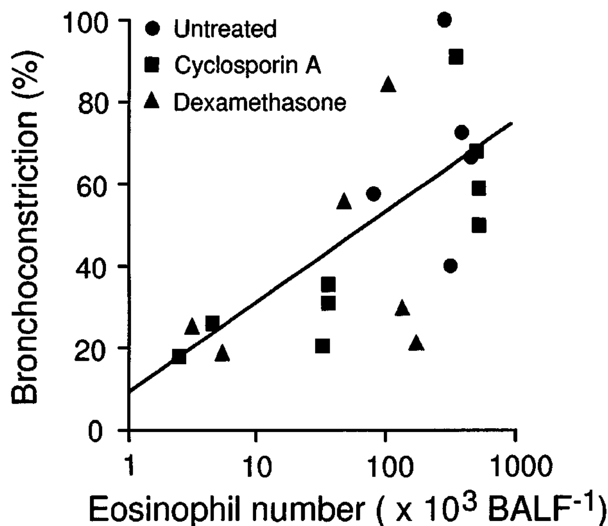

Figure 3 Relationship between airway eosinophilia and BHR of T cell clone-transferred mice. The number of BALF eosinophils and the bronchial responsiveness to acetylcholine (300 μg kg^{-1}) after antigen provocation are shown for each animal that was untreated, or treated with cyclosporin A or dexamethasone. All data are presented in Table 2 ($n=20$).

responsiveness to acetylcholine of saline-challenged mice (data not shown).

Correlation between airway eosinophilia and BHR induced upon antigen inhalation in T cell clone-transferred mice

Accumulating evidence suggests that BHR of asthmatic patients is related to the eosinophilic inflammation of the bronchial mucosa (Wardlaw *et al.*, 1988; Robinson *et al.*, 1993a; 1993b). Several investigators, however, reported that BHR induced after antigen provocation in some animal models was independent from eosinophilic accumulation (Martin *et al.*, 1993; Hessel *et al.*, 1995). So, we examined the possible relationship between the number of eosinophils recovered in BALF and the airway responsiveness to acetylcholine of FJ17-transferred mice induced after antigen provocation. As shown in Figure 3, the number of BALF eosinophils strongly correlated with the magnitude of BHR ($r=0.672$, $n=20$, $P<0.01$).

Discussion

Our present study clearly demonstrated that airway eosinophilic inflammation was induced by the transferred T cell clone upon antigen inhalation, and was suppressed by the administration of cyclosporin A or dexamethasone (Table 2). These agents not only suppressed IL-5 production by the T cell clones *in vitro* (Table 1), but also inhibited IL-5 gene expression induced in the antigen-challenged lung tissues (Figure 1). The attenuation of eosinophil infiltration into the lung was accompanied by a reduction in the development of BHR (Table 2, Figure 3).

We have reported that cyclosporin A, FK506 and dexamethasone inhibit IL-5 synthesis by human helper T cells (Mori *et al.*, 1995). Cyclosporin A and FK506 interact with their cytoplasmic ligands, cyclophilins and FK-binding proteins (FKBP), respectively. The drug-receptor complexes then inhibit Ca^{2+}-calmodulin-dependent phosphatase, calcineurin, whose activity is essential for the nuclear translocation of nuclear factor of activated T cells (NF-AT) (Schreiber & Crabtree, 1992). Dexamethasone interacts with the glucocorticoid receptor and further forms a complex with a transcription factor, AP-1, to interfere with the transactivation mediated by AP-1 (Weinberger *et al.*, 1985; Vacca *et al.*, 1992). We confirmed that cyclosporin A and dexamethasone suppressed IL-2 and IL-4 production as well as IL-5 production by murine T cells *in vitro* (Table 1).

The effect of cyclosporin A and dexamethasone on cytokine synthesis *in vivo* was next determined. mRNA for IL-5 as well as IL-2 was clearly detectable in the lung tissue of FJ17-transferred mice upon antigen challenge. The expression of IL-5 and IL-2 mRNA in the lung tissue is thought to reflect activation of the infused T cell clones, as neither IL-5 nor IL-2 mRNA was detectable in the lung specimens obtained from ovalbumin-challenged mice without transfer of cloned T cells (data not shown). Failure in detecting IL-4 mRNA might have been due to the relatively low sensitivity of our method compared to the detection of IL-2 and IL-5 mRNA. IL-5 mRNA expressed in the lung tissue of FJ17-transferred mice was clearly downregulated by treatment with cyclosporin A or dexamethasone (Figure 1), indicating that these agents suppressed IL-5 synthesis of the T cell clones *in vivo* as well. It has been reported that steroid treatment reduces the amount of IL-5 production in the bronchial mucosa (Bentrey *et al.*, 1996) and serum IL-5 concentration of asthmatic patients (Corrigan *et al.*, 1993). Eosinophil accumulation as well as the increase in IL-5 concentration of BALF upon antigen provocation were inhibited by the administration of dexamethasone (Nagai *et al.*, 1995; Eum *et al.*, 1996), although the source of IL-5 and the target cell types of steroid action were not identified in these studies. Our present findings are consistent with those previous studies and further indicate the *in vivo* suppression of IL-5 synthesis by dexamethasone at the level of gene expression. In addition, this is the first report that demonstrates the suppression of cytokine mRNA expression by cyclosporin A *in vivo*.

Administration of cyclosporin A and dexamethasone clearly inhibited the accumulation of eosinophils in the lungs and the development of BHR (Table 2). Eosinophil infiltration into the lungs of T cell clone-transferred mice upon antigen inhalation was completely suppressed by anti-IL-5 neutralizing antibody (Kaminuma *et al.*, 1997). It was, therefore, assumed that the inhibition of eosinophil accumulation by cyclosporin A and dexamethasone might be due to the suppression of IL-5 production by the infused T cell clones, although there is another possibility that the effect of cyclosporin A and dexamethasone on IL-2 and IL-4 production may also be relevant for the suppression of eosinophil accumulation, as these agents suppress synthesis of various cytokines other than IL-5.

It was reported that airway eosinophilia as well as BHR was suppressed by dexamethasone, employing an asthma model in which mice were actively immunized with relevant antigens (Eum *et al.*, 1996). Inhibition of eosinophil infiltration and BHR in sensitized mice by cyclosporin A and another immunosuppressant, FK506, was also reported (Nagai *et al.*, 1995). These effects are supported by the finding that dexamethasone, cyclosporin A and FK506 attenuated IL-5 production in the airway (Nagai *et al.*, 1995; Eum *et al.*, 1996). The mice employed in their studies were primed with antigens, and accordingly generated antigen-specific immunoglobulins such as IgE, IgG and IgA, besides primed T cells. Cytokines, chemokines and chemical mediators produced by mast cells

and airway epithelial cells during the immediate allergic response may also participate in the development of late phase eosinophil infiltration (Hunter *et al*., 1985; Bradding *et al*., 1994). Activation of T cells through the IgE-CD23 pathway is implicated in the development of eosinophilic inflammation (Coyle *et al*., 1996). Cyclosporin A and corticosteroids inhibit mast cell degranulation (Daeron *et al*., 1982; Marone *et al*., 1992). Therefore, the principal mechanism of cyclosporin A and dexamethasone in inhibiting eosinophilic inflammation have thus far remained unclear. As the mice employed in our study were devoid of antigen-specific IgE, IgG and IgA antibodies, eosinophilic inflammation was solely dependent on the transferred T cells. Our present findings, therefore, strongly suggest that the suppression of eosinophilic inflammation in asthmatic patients by corticosteroids and cyclosporin A is mediated, at least in part, by the inhibition of IL-5 production by activated $CD4^+$ T cells.

BHR has been known as a hallmark of bronchial asthma (Arm & Lee, 1992). The magnitude of BHR correlates with the severity of asthma symptoms, although the mechanism of how BHR develops is yet to be clarified (Juniper *et al*., 1981). There are some studies indicating that the development of BHR is independent of eosinophilic inflammation (Barnes, 1986; Goldie *et al*., 1986; Corry *et al*., 1996; Hessel *et al*., 1997). A very conventional theory, β-blockade theory, states that subsensitivity of β-adrenoceptors on airway smooth muscle results in an autonomic imbalance favoring cholinergic predominance and airway smooth muscle contraction (Goldie *et al*., 1986). Increased sensitivity to agents such as histamine and acetylcholine of the smooth muscle itself have also been proposed to account for BHR (Barnes, 1986). Hamelmann and coworkers reported that IgE was required as a second signal for the development of BHR in sensitized mice (Hamelmann *et al*., 1997b). IgE-mediated mast cell activation induced BHR without significant eosinophilic inflammation (Martin *et al*., 1993; Hessel *et al*., 1995). It was also reported that T cells directly affected bronchial responsiveness (Garssen *et al*., 1994).

On the contrary, quite a few investigators have indicated that BHR of asthmatic patients is a consequence of eosinophilic inflammation of the bronchial mucosa (Barnes, 1989; Bjornsson *et al*., 1996; Busse & Sedgwick, 1992; Corrigan & Kay, 1992; Mehlhop *et al*., 1997). Eosinophils release toxic granule proteins such as major basic protein (MBP), eosinophil peroxydase, eosinophil cationic protein and eosinophil-derived neurotoxin (Frigas & Gleich, 1986; Gleich & Adolphson, 1986). These granule proteins are localized on the damaged respiratory epithelium of asthmatic patients (Filley *et al*., 1982). Toxic concentrations of MBP are detected in sputum of chronic asthmatics (Frigas *et al*., 1981). Direct instillation of MBP into the lungs increased bronchial reactivity to methacholine (Gleich & Adolphson, 1993). In addition, the loss of airway epithelium results in increased airway hyperresponsiveness to non-specific stimuli (Beasley *et al*., 1989). BHR has recently been considered by many investigators as a consequence of inflammation involving various cell types including eosinophils as the principal cell type. Our present study showed that the reduction in BHR after treatment with cyclosporin A or dexamethasone correlated well with the reduction in eosinophil infiltration, consistent with the recent concept that BHR is a consequence of eosinophilic inflammation.

In conclusion, cyclosporin A and dexamethasone suppressed IL-5-synthesis by T cell clones *in vitro* and *in vivo*, inhibited airway eosinophilia upon antigen inhalation of T cell clone-transferred mice, and prevented BHR. These results directly indicate that the suppression of IL-5 synthesis by T cells is involved in the mechanisms of cyclosporin A and dexamethasone to inhibit allergic eosinophilic inflammation. Agents which downregulate IL-5 production seem to have potential to control airway eosinophilic inflammation in allergic disorders.

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References

ARM, J.P. & LEE, T.H. (1992). The pathobiology of bronchial asthma. *Adv. Immunol.,* **51,** 323–382.

BARNES, P.J. (1986). Asthma as an axon reflex. *Lancet,* **1,** 242–245.

BARNES, P.J. (1989). New concepts in the pathogenesis of bronchial hyperresponsiveness and asthma. *J. Allergy Clin. Immunol.,* **83,** 1013–1026.

BEASLEY, R., ROCHE, W.R., ROBERTS, J.A. & HOLGATE, S.T. (1989). Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am. Rev. Respir. Dis.,* **139,** 806–817.

BENTREY, A.M., HAMID, Q., ROBINSON, D.S., SCHOTMAN, E., MENG, Q., ASSOUFI, B., KAY, A.B. & DURHAM, S.R. (1996). Prednisolone treatment in asthma. *Am. J. Respir. Crit. Care Med.,* **153,** 551–556.

BJORNSSON, E., JANSON, C., HAKANSSON, L., ENANDER, I., VENGE, P. & BOMAN, G. (1996). Eosinophil peroxydase: a new serum marker of atopy and bronchial hyper-responsiveness. *Respir. Med.,* **90,** 39–46.

BOUSQUET, J., CHANEZ, P., LACOSTE, J.Y., BARNEON, G., GHAVANIAN, N., ENANDER, I., VENGE, P., AHLSTEDT, S., SIMONY LAFONTAINE, J., GODARD, P. & MICHEL, F.B. (1990). Eosinophilic inflammation in asthma. *N. Engl. J. Med.,* **323,** 1033–1039.

BRADDING, P., ROBERTS, J.A., BRITTEN, K.M., MONTEFORT, S., DJUKANOVIC, R., MUELLER, R., HEUSSER, C.H., HOWARTH, P.H. & HOLGATE, S.T. (1994). Interleukin-4, -5 and -6 and tumor necrosis factor-α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am. J. Respir. Cell Mol. Biol.,* **10,** 471–480.

BURKE-GAFFNEY, A. & HELLEWELL, P.G. (1996). Regulation of ICAM-1 by dexamethasone in a human vascular endothelial cell line EAhy926. *Am. J. Physiol.,* **270,** C552–C561.

BUSSE, W.W. & SEDGWICK, J.B. (1992). Eosinophils in asthma. *Ann. Allergy,* **68,** 286–290.

CHOMCZYNSKI, P. & SACCHI, N. (1987). Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.,* **162,** 156–159.

CORRIGAN, C.J., HACZKU, A., GEMOU ENGESAETH, V., DOI, S., KIKUCHI, Y., TAKATSU, K., DURHAM, S.R. & KAY, A.B. (1993). CD4 T-lymphocyte activation in asthma is accompanied by increased serum concentrations of interleukin-5. Effect of glucocorticoid therapy. *Am. Rev. Respir. Dis.,* **147,** 540–547.

CORRIGAN, C.J. & KAY, A.B. (1992). T cells and eosinophils in the pathogenesis of asthma. *Immunol. Today,* **13,** 501–507.

CORRY, D.B., FOLKESSON, H.G., WARNOCK, M.L., ERLE, D.J., MATTHAY, M.A., WIENER KRONISH, J.P. & LOCKSLEY, R.M. (1996). Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperreactivity. *J. Exp. Med.,* **183,** 109–117.

COX, G., OHTOSHI, T., VANCHERI, C., DENBURG, J.A., DOLOVICH, J., GAULDIE, J. & JORDANA, M. (1991). Promotion of eosinophil survival by human bronchial epithelial cells and its modulation by steroids. *Am. J. Respir. Cell Mol. Biol.,* **4,** 525–531.

COYLE, A.J., WAGNER, K., BERTRAND, C., TSUYUKI, S., BEWS, J. & HEUSSER, C. (1996). Central role of immunoglobulin (Ig) E in the induction of lung eosinophil infiltration and T helper 2 cell cytokine production: inhibition by a non-anaphylactogenic anti-IgE antibody. *J. Exp. Med.,* **183,** 1303–1320.

DAERON, M., STERK, A.R., HIRATA, F. & ISHIZAKA, T. (1982). Biochemical analysis of glucocorticosteroid-induced inhibition of IgE-mediated histamine release from mouse mast cells. *J. Immunol.,* **129,** 1212–1218.

DAVIDSON, W.F. & PARISH, C.R. (1975). A procedure for removing red cells and dead cells from lymphoid cell suspensions. *J. Immunol. Methods,* **7,** 291–300.

DE BIE, J.J., HESSEL, E.M., VAN ARK, I., VAN ESCH, B., HOFMAN, G., NIJKAMP, F.P. & VAN OOSTERHOUT, A.J.M. (1996). Effect of dexamethasone and endogenous corticosterone on airway hyperresponsiveness and eosinophilia in the mouse. *Br. J. Pharmacol.,* **119,** 1484–1490.

DRUILHE, A., CAI, Z., HAILE, S., CHOUAIB, S. & PRETOLANI, M. (1996). Fas-mediated apoptosis in cultured human eosinophils. *Blood,* **87,** 2822–2830.

EUM, S.Y., CREMINON, C., HAILE, S., LEFORT, J. & VARGAFTIG, B.B. (1996). Inhibition of airways inflammation by dexamethasone is followed by reduced bronchial hyperreactivity in BP2 mice. *Clin. Exp. Allergy,* **26,** 971–979.

FILLEY, W.F., HOLLEY, K.E., KEPHART, G.M. & GLEICH, G.J. (1982). Identification by immunofluorescence of eosinophil granule major basic protein in lung tissues of patients with bronchial asthma. *Lancet,* **2,** 11–16.

FRICK, W.E., SEDGWICK, J.B. & BUSSE, W.W. (1989). The appearance of hypodense eosinophils in antigen-dependent late phase asthma. *Am. Rev. Respir. Dis.,* **139,** 1401–1406.

FRIGAS, E. & GLEICH, G.J. (1986). The eosinophil and the pathophysiology of asthma. *J. Allergy Clin. Immunol.,* **77,** 527–537.

FRIGAS, E., LOEGERINGS, D.A., SOLLEY, G.O., FALLOW, G.M. & GLEICH, G.J. (1981). Elevated level of the eosinophil granule major basic protein in the sputum of patients with bronchial asthma. *Mayo Clin. Proc.,* **56,** 345–353.

FUKUDA, T., ASAKAWA, J., MOTIJIMA, S. & MAKINO, S. (1995). Cyclosporin A reduces T lymphocyte activity and improves airway hyperresponsiveness in corticosteroid-dependent chronic severe asthma. *Ann. Allergy Asthma Immunol.,* **75,** 65–72.

GARSSEN, J., NIJKAMP, F.P., VAN VUGT, E., VAN DER VLIET, H. & VAN LOVEREN, H. (1994). T cell-derived antigen binding molecules play a role in the induction of airway hyperresponsiveness. *Am. J. Respir Crit. Care Med.,* **150,** 1528–1538.

GAUVREAU, G.M., DOCTOR, J., WATSON, R.M., JORDANA, M. & O'BYRNE, P.M. (1996). Effects of inhaled budesonide on allergen-induced airway responses and airway inflammation. *Am. J. Respir. Crit. Care Med.,* **154,** 1267–1271.

GILLIS, S., CRABTREE, G.R. & SMITH, K.A. (1979). Glucocorticoid-induced inhibition of T cell growth factor production. *J. Immunol.,* **123,** 1624–1631.

GLEICH, G.J. & ADOLPHSON, C. (1993). Bronchial hyperreactivity and eosinophil granule proteins. *Agents Actions Suppl.,* **43,** 223–230.

GLEICH, G.J. & ADOLPHSON, C.R. (1986). The eosinophilic leukocyte: structure and function. *Adv. Immunol.,* **39,** 177–253.

GOLDIE, R.G., SPINA, D., HENRY, P.J., LULICH, K.M. & PATERSON, J.W. (1986). *In vitro* responsiveness of human asthmatic bronchus to carbachol, histamine, β-adrenoceptor agonists and theophylline. *Br. J. Clin. Pharmacol.,* **22,** 669–676.

HAMELMANN, E., OSHIBA, A. & LOADER, G.L. (1997a). Anti-interleukin-5 antibody prevents airway hyperresponsiveness in a murine model of airway sensitization. *Am. J. Respir. Crit. Care Med.,* **155,** 819–825.

HAMELMANN, E., VELLA, A.T., OSHIBA, A., KAPPLER, J.W., MARRACK, P. & GELFAND, E.W. (1997b). Allergic airway sensitization induces T cell activation but not airway hyperresponsiveness in B-cell deficient mice. *Proc. Natl. Acad. Sci. USA,* **94,** 1350–1355.

HAMID, Q., AZZAWI, M., YING, S., MOQBEL, R., WARDLAW, A.J., CORRIGAN, C.J., BRADLEY, B., DURHAM, S.R., COLLINS, J.V., JEFFERY, P.K., QUINT, J. & KAY, A.B. (1991). Expression of mRNA for interleukin-5 in mucosa bronchial biopsies from asthma. *J. Clin. Invest.,* **87,** 1541–1546.

HESSEL, E.M., VAN OOSTERHOUT, A.J., HOFSTRA, C.L., DE BIE, J.J., GARSSEN, J., VAN LOVEREN, H., VERHEYEN, A.K., SAVELKOUL, H.F. & NIJKAMP, F.P. (1995). Bronchoconstriction and airway hyperresponsiveness after ovalbumin inhalation in sensitized mice. *Eur. J. Pharmacol.,* **293,** 401–412.

HESSEL, E.M., VAN OOOSTERHOUT, A.J.M., ARK, I.V., ESCH, B.V., HOFMAN, G., LOVEREN, H.V., SAVELKOUL, F.J. & NIJKAMP, F.P. (1997). Development of airway hyperresponsiveness is dependent on interferon-γ and independent of eosinophil infiltration. *Am. J. Respir. Cell Mol. Biol.,* **16,** 325–334.

HUNTER, J.A.., FINKBEINER, W.E., NADEL, J.A., GOETZL, E.J. & HOLTZMAN, M.J. (1985). Predominant generation of 15-lipoxygenase metabolites of arachidonic acid by epithelial cells from human trachea. *Proc. Natl. Acad. Sci. USA,* **82,** 4633–4637.

JUNIPER, E.F., FRITH, P.A. & HARGREAVE, F.E. (1981). Airway responsiveness to histamine and methacholine: relationship to minimum treatment to control symptoms of asthma. *Thorax,* **36,** 575–579.

KAMINUMA, O., MORI, A., OGAWA, K., NAKATA, A., KIKKAWA, H., NAITO, K., SUKO, M. & OKUDAIRA, H. (1997). Successful transfer of late phase eosinophil infiltration in the lung by infusion of helper T cell clones. *Am. J. Respir. Cell Mol. Biol.,* **16,** 448–454.

KAY, A.B. (1992). ''Helper'' (CD4+) T cells and eosinophils in allergy and asthma. *Am. J. Respir. Dis.,* **145,** S22–S26.

KONZETT, H. & RÖSSLER, R. (1940). Versuchsanordnung zu untersuchungen an der bronchialmuskulatur. *Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmacol.,* **195,** 71–74.

LACK, G., OSHIBA, A., BRADLEY, K.L., LOADER, J.E., AMRAN, D., LARSEN, G.L. & GELFAND, E.W. (1995). Transfer of immediate hypersensitivity and airway hyperresponsiveness by IgE-positive B cells. *Am. J. Respir. Crit. Care Med.,* **152,** 1765–1773.

LOCK, S.H., KAY, A.B. & BARNES, N.C. (1996). Double-blind, placebo-controlled study of cyclosporin A as a corticosteroid-sparing agent in corticosteroid-dependent asthma. *Am. J. Respir. Crit. Care Med.,* **153,** 509–514.

LOOK, D.C., RAPP, S.R., KELLER, B.T. & HOLTZMANN, M.J. (1992). Selective induction of intracellular adhesion molecule-1 by interferon-γ in human airway epithelial cells. *Am. J. Physiol.,* **263,** L79–L87.

MARONE, G., DE PAULIS, A., CASOLARO, V., CICARELI, A., SPADARO, G. & CIRILLO, R. (1992). In vitro and in vivo characterization of the anti-inflammatory effects of cyclosporin A. *Int. Arch. Allergy Immunol.,* **99,** 279–283.

MARTIN, T.R., TAKEISHI, T., KATZ, H.R., AUSTEN, F., DRAZEN, J.M. & GALLI, S.J. (1993). Mast cell activation enhances airway responsiveness to methacholine in the mouse. *J. Clin. Invest.,* **91,** 1176–1182.

MEHLHOP, P.D., VANDERIJN, M., GOLDBERG, A.B., BREWER, J.P., KURUP, V.P., MARTIN, T.R. & OETTGEN, H.C. (1997). Allergen-induced bronchial hyperreactivity and eosinophilic inflammation occur in the absence of IgE in a mouse model of asthma. *Proc. Natl. Acad. Sci. USA,* **94,** 1344–1349.

MORI, A., SUKO, M., NISHIZAKI, Y., KAMINUMA, O., KOBAYASHI, S., MATSUZAKI, G., YAMAMOTO, K., ITO, K., TSURUOKA, N. & OKUDAIRA, H. (1995). IL-5 production by CD4+ T cells of asthmatic patients is suppressed by glucocorticoids and the immunosuppressants FK 506 and cyclosporin A. *Int. Immunol.,* **7,** 449–457.

NAGAI, H., YAMAGUCHI, S., TANAKA, H. & INAGAKI, N. (1995). Effect of some immunosuppressors on allergic bronchial inflammation and airway hyperresponsiveness in mice. *Int. Arch. Allergy Immunol.,* **108,** 189–195.

NAKAJIMA, H., IWAMOTO, I., TOMOE, S., MATSUMURA, R., TOMIOKA, H., TAKATSU, K. & YOSHIDA, S. (1992). $CD4^+$ T-lymphocytes and interleukin-5 mediate antigen-induced eosinophil infiltration into the mouse trachea. *Am. Rev. Respir. Dis.,* **146,** 374–377.

OKUDAIRA, H., NOGAMI, M., MATSUZAKI, G., DOHI, M., SUKO, M., KASUYA, S. & TAKATSU, K. (1991). T-cell-dependent accumulation of eosinophils in the lung and its inhibition by monoclonal anti-interleukin-5. *Int. Arch. Allergy Appl. Immunol.,* **94,** 171–173.

OSHIBA, A., HAMELMANN, E., TAKEDA, K., BRADLEY, K.L., LOADER, J.E., LARSEN, G.L. & GELFAND, E.W. (1996). Passive transfer of immediate hypersensitivity and airway hyperresponsiveness by allergen-specific immunoglobulin (Ig) E and IgG1 in mice. *J. Clin. Invest.,* **97,** 1398 – 1408.

ROBINSON, D., HAMID, Q., BENTLEY, A., YING, S., KAY, A.B. & DURHAM, S.R. (1993a). Activation of $CD4^+$ T cells, increased T_{H2}-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma. *J. Allergy Clin. Immunol.,* **92,** 313 – 324.

ROBINSON, D.S., YING, S., BENTLEY, A.M., MENG, Q., NORTH, J., DURHAM, S.R., KAY, A.B. & HAMID, . (1993b). Relationships among numbers of bronchoalveolar lavage cells expressing messenger ribonucleic acid for cytokines, asthma symptoms, and airway methacholine responsiveness in atopic asthma. *J. Allergy Clin. Immunol.,* **92,** 397 – 403.

SANDERSON, C.J., CAMPBELL, H.D. & YOUNG, I.G. (1988). Molecular and cellular biology of eosinophil differentiation factor (interleukin-5) and its effects on human and mouse B cells. *Immunol. Rev.,* **102,** 29 – 50.

SCHMIDT, J., FLEIBNER, S., HEIMANN-WEITSCHAT, I., LINDSTAEDT, R. & SZELENYI, I. (1994). The effect of different corticosteroids and cyclosporin A on interleukin-4 and interleukin-5 release from murine TH2-type T-cells. *Eur. J. Pharmacol.,* **260,** 247 – 250.

SCHREIBER, S.L. & CRABTREE, G.R. (1992). The mechanism of action of cyclosporin A and FK 506. *Immunol. Today,* **13,** 136 – 142.

VACCA, A., FELLI, M.P., FARINA, A.R., MARTINOTTI, S., MARODER, M., SCREPANTI, I., MECO, D., PETRANGELI, E., FRATI, L. & GULINO, A. (1992). Glucocorticoid receptor-mediated suppression of the interleukin 2 gene expression through impairment of the cooperativity between nuclear factor of activated T cells and AP-1 enhancer elements. *J. Exp. Med.,* **175,** 637 – 646.

WALKER, C., BODE E., BOER, L., HANSEL, T.T., BLASER, K. & VIRCHOW, J.C. (1992). Allergic and nonallergic asthmatics have distinct patterns of T-cell activtion and cytokine production in peripheral blood and bronchoalveolar lavage. *Am. Rev. Respir. Dis.,* **146,** 109 – 115.

WARDLAW, A.J., DUNNETTE, S., GLEICH, G.J., COLLINS, J.V. & KAY, A.B. (1988)., Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma. *Am. J. Respir. Dis.,* **137,** 62 – 69.

WEINBERGER, C., HOLLENBERG, S.M., ONG, E.S., HARMON, J.M., BROWER, S.T., CIDLOWSKI, J., THOMPSON, E.B., ROSENFELD, N.G. & EVANS, R.H. (1985). Identification of human glucocorticoid receptor complementary DNA clones by epitope selection. *Science,* **228,** 740 – 742.

YAMAGUCHI, Y., HAYASHI, Y., SUGAMA, Y., MIURA, Y., KARASAWA, T., KITAMURA, S., TORISU, M., MITA, S., TOMINAGA, A. & TAKATSU, K. (1988). Highly purified murine interleukin 5 (IL-5) stimulates eosinophil function and prolongs in vitro survival. IL-5 as an eosinophil chemotactic factor. *J. Exp. Med.,* **167,** 1737 – 1742.

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