

II. Allergy and its control

Control of IL-5 production by human helper T cells as a treatment for eosinophilic inflammation: Comparison of in vitro and in vivo effects between selective and nonselective cytokine synthesis inhibitors

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Background: Helper T cells are involved in the pathophysiologic condition of asthma, so modulation of cytokine production may be effective therapy.

Objective: We aimed to selectively control the synthesis of IL-5 by helper T cells and tested in vivo effects using a murine asthma model.

Methods: The effect of dexamethasone, FK506, cyclosporin A, and nonactin (a macrolide compound produced by *Streptomyces griseus*) on cytokine production by allergen-specific T-cell clones was determined. The effect of these agents and an anti-IL-5 neutralizing antibody on airway eosinophilic inflammation was investigated in a murine asthma model.

Results: Dexamethasone, FK506, and cyclosporin A suppressed the production of IL-2, IL-4, and IL-5 by human helper T cells, which shows a similar concentration-response relationship in each case. Cyclosporin A and dexamethasone inhibited airway eosinophilia in vivo. Nonactin suppressed IL-5 synthesis but not IL-2 or IL-4 synthesis, and it also significantly suppressed airway eosinophilia.

Conclusion: Nonactin only suppressed IL-5 synthesis and was as effective against eosinophilia as cyclosporin A and dexamethasone, which indicates that IL-5 is a reasonable therapeutic target in allergic disorders that are accompanied by eosinophilic inflammation. (*J Allergy Clin Immunol* 2000;106:S58-64.)

Key words: T cell, eosinophilic inflammation

Abbreviations used

BALF:	Bronchoalveolar lavage fluid
Der f:	<i>Dermatophagoides farinae</i>
IL-2R:	IL-2 receptor
NF-AT:	Nuclear factor of activated T cells
TcR:	T-cell receptor

Bronchial asthma is characterized by persistent eosinophilic inflammation of the bronchial mucosa.¹ Involvement of T_H cells and T-cell cytokines has been demonstrated by many investigations. IL-5 is particularly implicated in the development of eosinophilic inflammation, because it is involved in the promotion of the growth, maturation, migration, local infiltration, and activation of eosinophils.² IL-5 is the predominant eosinophil-activating cytokine in bronchoalveolar lavage fluid (BALF) obtained from patients with asthma,³ and its concentration is significantly higher in both patients with atopic and nonatopic asthma, compared with normal subjects.⁴ In addition, the serum IL-5 concentration is elevated in patients with symptomatic asthma and is decreased by oral prednisolone therapy.⁵ IL-5 production by peripheral blood T_H cells is markedly enhanced in both atopic and nonatopic asthmatics compared with healthy subjects, whereas the production of IL-2, IL-3, IL-4, IFN- γ , and GM-CSF is not significantly increased.^{6,7} Animal studies have also confirmed the essential role of IL-5. The administration of anti-IL-5-neutralizing antibody prevents the recruitment of eosinophils into the lungs and abrogates the late phase of bronchoconstriction.^{8,9} We recently reported that eosinophilic infiltration of the lungs after antigen inhalation in the absence of humoral immunity had developed in mice that had undergone adoptive transfer with IL-5-producing T_H clones, which indicates the importance of T_H cells as the source of IL-5.¹⁰ Ectopic expression of the IL-5 gene in the bronchial epithelium induces eosinophilic inflammation that histologically resembles human asthma.¹¹ Accumulating evidence sug-

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gests that IL-5 is the key cytokine involved in eosinophilic inflammation, so the control of IL-5 production by activated T cells should be a reasonable method for the management of eosinophilic inflammation.

Glucocorticoids have long been the most effective agents for the treatment of bronchial asthma and atopic dermatitis.¹² Their efficacy is ascribed to multiple pharmacologic actions, 1 of which is the suppression of inflammatory cytokine production.^{13,14} We and others have reported that IL-5 synthesis by peripheral blood T_H cells was inhibited by glucocorticoids in vitro.^{6,15,16} However, a wide variety of side effects (including hypertension, diabetes, obesity, osteoporosis, cataract, and psychosis) often limit the application of these agents. The immunosuppressants FK506 and cyclosporin A have been successfully used for the treatment of severe allergic diseases in an attempt to overcome these disadvantages.^{17,18} These immunosuppressants interfere with the induction of the nuclear factor of activated T cells (NF-AT) and accordingly suppress the synthesis of various T-cell cytokines (such as IL-2, -3, -4, -5, -6, -8, and -13, and IFN- γ), which makes generalized immunosuppression inevitable.¹⁹⁻²¹

Our previous study indicated that (1) IL-5 synthesis by human T cells is regulated independently from that of other major T-cell cytokines (IL-2 and IL-4) at the transcriptional level and (2) a unique transcription factor other than AP-1, NF-AT, NF- κ B, or Oct-1 is critical for IL-5 gene regulation in human T_H cells.^{16,22,23} Agents that suppress IL-5 production and do not interfere with IL-2 or IL-4 production might be an ideal therapy for eosinophilic inflammation with minimal side effects, unlike with FK506 and cyclosporin A. During the course of screening for agents capable of inhibiting IL-5 production by human T_H cells, we have found that a chemical compound named OM-01 efficiently suppresses IL-5 production.²⁴ OM-01 has been identified as a member of the polynactin family and consists of nonactin, monactin, dinactin, trinactin, and tetranactin. Here we compared the effects of nonactin, dexamethasone, FK506, and cyclosporin A on human T-cell cytokine synthesis and analyzed their in vivo effects on experimental asthma.

METHODS

Reagents

Ovalbumin, dexamethasone, and nonactin (a macrolide compound produced by *Streptomyces griseus*) were purchased from Sigma Chemical Co (St Louis, Mo); cyclosporin A was purchased from Sandoz Japan (Tokyo, Japan). The hybridoma that generated the rat anti-mouse CD4 monoclonal antibody (GK 1.5) was obtained from the American Type Culture Collection (Rockville, Md). The antibody was purified from ascites as described previously.¹⁰ A purified rat anti-mouse IL-5 mAb (TRFK-5) was prepared as described previously.⁸ OKT3, an anti-CD3 mAb, was obtained from Ortho (Raritan, NJ). Fluorescein isothiocyanate-labeled anti-CD3 (Leu4), anti-CD4 (Leu2), and anti-CD8 (Leu3) mAbs were obtained from Becton Dickinson (San Jose, Calif). Recombinant *Dermatophagoides farinae* 2 (rDer f 2; a major house dust mite allergen) protein was provided by Dr Y. Okumura (Asahi Breweries Ltd).²⁵ AIM-V medium (Gibco BRL, Gaithersburg, Md) was used for T-cell cultures.

Allergen-specific human T_H clones

Der f 2-specific human T_H clones were obtained from peripheral blood mononuclear cells of atopic asthma donors with the use of antigenic stimulation followed by the limiting dilution method, as described previously.²⁶ Briefly, peripheral blood mononuclear cells obtained from donors with atopic asthma were stimulated with recombinant Der f 2 protein (1 μ g/mL) followed by limiting dilution. The resulting T-cell clones were expanded by antigenic stimulation, harvested at least 10 days after the last stimulation, layered onto Ficoll-Paque, and centrifuged. The interface was then recovered, washed twice, and resuspended in fresh medium. The resulting preparation usually consisted of more than 98% CD3-positive cells, as determined by flow cytometry. These cells (2×10^5 /well) were cultured in triplicate with various stimulants in 24-well culture plates for the designated periods.

Quantitation of cytokines in culture supernatants

IL-5 was measured by a sandwich ELISA using monoclonal anti-human IL-5 (D138) as the capture antibody and biotinylated purified rabbit anti-human IL-5 as the secondary antibody, as described previously.⁶ IL-2 and IL-4 were measured by specific ELISAs (DuoSet ELISA Development System, Genzyme, Cambridge, Mass) according to the manufacturer's directions.

Experimental asthma model

Specific pathogen-free male Balb/c mice, 5 to 6 weeks of age, were obtained from Japan SLC (Hamamatsu, Japan) and housed in an environmentally controlled room (temperature, 23°C $\pm$ 2°C; humidity, 55% $\pm$ 5%; lighting time, 0700 to 1900 hours) with food and water available ad libitum for 1 week before the experiment. The studies reported here adhered to the principles outlined by the Animal Welfare Act and the NIH guidelines for the care and use of animals in biomedical research. Mice were sensitized by the administration of 1 μ g of ovalbumin adsorbed to 1 mg of aluminum hydroxide gel twice at 2-week intervals. Two weeks later, dexamethasone, cyclosporin A, nonactin, or the vehicle (saline solution) was subcutaneously administered for 3 consecutive days. Thirty minutes after the last dose, mice were individually placed into 50-mL plastic tubes to inhale aerosolized ovalbumin (100 mg/mL) that was dissolved in 0.9% saline solution and delivered by a nebulizer (DeVilbiss 646; DeVilbiss Corp, Somerset, Pa) that was driven by compressed air at 18 L/min for 20 minutes. As a control, 0.9% saline solution alone was administered by the nebulizer. Dexamethasone, cyclosporin A, nonactin, and the vehicle were also administered 24 hours after the challenge. For the analysis of blocking antibodies, anti-CD4 (GK1.5, 2 mg) and anti-IL-5 (TRFK-5, 2 mg), mAbs were injected intravenously 6 days and 5 minutes before the challenge, respectively.

Bronchoalveolar lavage and histologic examination

Saline solution- or ovalbumin-challenged mice were killed with an overdose of intraperitoneal sodium pentobarbital at 48 hours after the challenge. The trachea was cannulated with a polyethylene tube, and the lungs were gently lavaged 4 times with 0.5 mL of HBSS (2 mL in total). About 1.9 mL of BALF was recovered on average and centrifuged at 1500 rpm for 5 minutes. The pellet that was obtained was immediately suspended in 250 μ L of HBSS, and the cell count in the BALF was determined by an automatic cell counter (Celltac MEK-5158; Nihon Kohden, Japan). Differential cell counts were obtained by microscopy of centrifuged preparations that were stained with May-Giemsa, with a count of 200 cells in each animal. The number of eosinophils in each sample was nor-

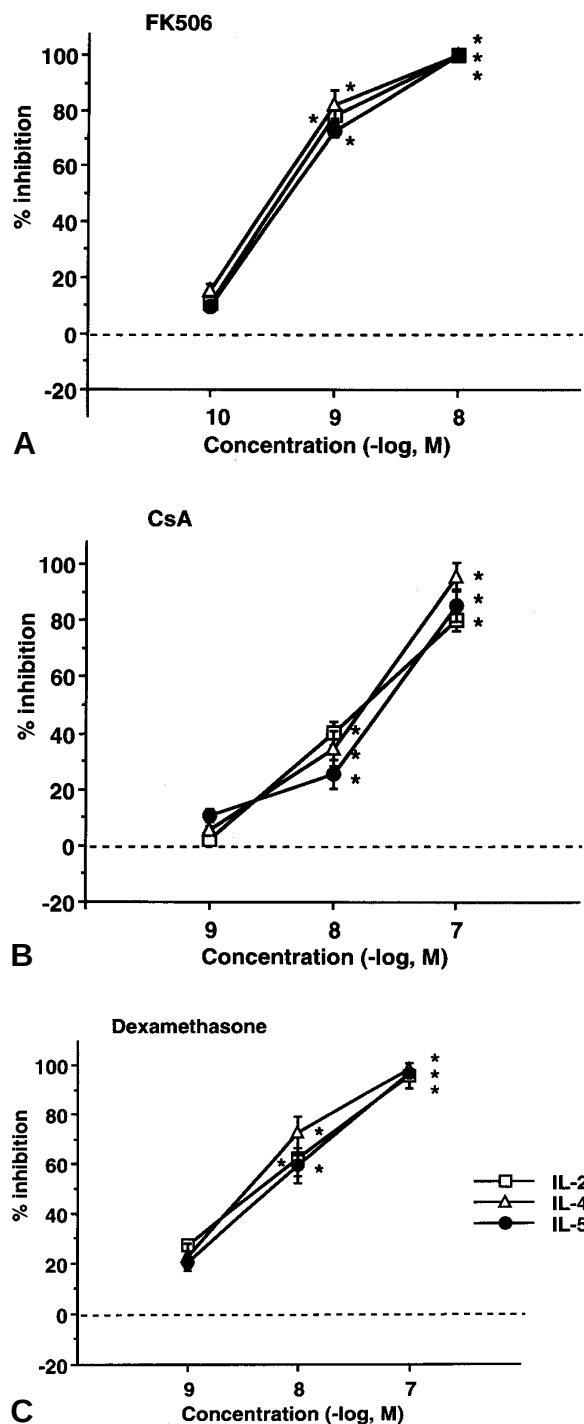


FIG 1. IL-2, IL-4, and IL-5 production by human T_H clones is suppressed by (A) FK506, (B) cyclosporin A (CsA), and (C) dexamethasone with a similar concentration-response relationship. Der f 2-specific human T-cell clones (2×10^6 cells) were stimulated by immobilized anti-CD3 for 24 hours. Varying concentrations of FK506, cyclosporin A, and dexamethasone were added at the start of some cultures. IL-2 (□), IL-4 (△), and IL-5 (●) concentrations in the supernatants were assayed by sandwich ELISA. Data are from 3 independent experiments with the use of different T_H clones (HK1, HK12, and YA15), each of which assessed triplicate cultures. The mean $\pm$ SEM of the percent inhibition is shown. * $P < .05$ as compared with the culture not containing immunosuppressive agents.

malized against that obtained from the ovalbumin-challenged positive control animals (100%).

Statistical analysis

Statistical analysis was performed with the Student *t* test, and a probability value of less than .05 was considered statistically significant. Results are presented as the mean $\pm$ SEM.

RESULTS

FK506, cyclosporin A, and dexamethasone suppressed IL-2, IL-4, and IL-5 synthesis by human T_H cells in a similar concentration-response relationship

The first experiment was performed to determine the effect of FK506, cyclosporin A, and dexamethasone on IL-2, IL-4, and IL-5 synthesis by the allergen-specific T_H clones. Der f 2-specific T_H clones were established from the peripheral blood lymphocytes of patients with atopic asthma, as described previously.^{26,27} All the T-cell clones that were established were of the T_{H0} phenotype, producing both IL-2 and IL-4. T-cell clones were studied at least 10 days after the last antigenic stimulation. As described earlier, T cells obtained from the interface of the Ficoll-Paque density gradient consisted of more than 98% pure CD3⁺CD4⁺ cells. When these cells were washed 3 times, resuspended in fresh medium, and stimulated with immobilized anti-CD3 mAb, the production of IL-2, IL-4, and IL-5 by 3 different T-cell clones (HK1, HK12, and YA15) was clearly induced. Production of these cytokines was suppressed by the 3 test agents in a dose-dependent manner. The percent inhibition at each concentration of FK506, cyclosporin A, and dexamethasone is shown in Fig 1. Cell viability was determined by the trypan blue dye exclusion test and was not changed significantly at 24 hours after the addition of the agents, excluding cytotoxicity at the concentrations used (data not shown). The median effective dose for the suppression of cytokine synthesis was approximately 0.3 nmol/L for FK506, 30 nmol/L for cyclosporin A, and 3 nmol/L for dexamethasone and was not significantly different among the 3 cytokines.

Nonactin selectively inhibited IL-5 production by human T_H clones

We have reported that nonactin, a polynactin family compound, selectively inhibits IL-5 production by human T cells.²⁴ To determine whether nonactin acts directly on T cells or by way of other cells, its effect on cytokine synthesis by human T_H clones was determined. A representative result is shown in Fig 2. Nonactin significantly suppressed IL-5 production at concentrations of 10 nmol/L and higher, whereas IL-2 or IL-4 production was not affected, excluding nonspecific toxicity as the mode of action.

Effect of cyclosporin A, dexamethasone, and nonactin on experimental asthma

We previously reported that cyclosporin A and dexamethasone inhibited airway eosinophilic inflammation

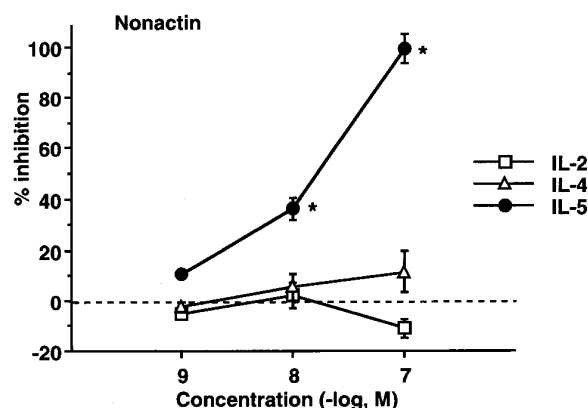


FIG 2. Nonactin is a selective inhibitor of IL-5 production. Der f 2-specific human T-cell clones (2×10^6 cells) were stimulated by immobilized anti-CD3 for 24 hours. Varying concentrations of nonactin were added at the start of some cultures. IL-2 ($\square$), IL-4 (Δ), and IL-5 ($\bullet$) concentrations in the supernatants were assayed by sandwich ELISA. Data are from 3 independent experiments with the use of different T_H clones (HK1, HK12, and YA15), each of which assessed triplicate cultures. The mean $\pm$ SEM of the percent inhibition is shown. * $P < .05$ as compared with the culture not containing nonactin.

through the suppression of IL-5 synthesis by T cells in a study that used adoptive transfer of T_H clones.²⁸ The effect of these agents was next confirmed with the use of sensitized animals and was compared with that of an anti-CD4 mAb and an anti-IL-5 neutralizing antibody. Balb/c mice were immunized with ovalbumin absorbed in alum and were challenged by the inhalation of ovalbumin. Late-phase eosinophil infiltration into the lungs was assessed with the use of BALF obtained 48 hours after challenge. As shown in Fig 3, eosinophilic infiltration was clearly induced by antigen inhalation, whereas saline solution challenge scarcely induced eosinophilia. The administration of the anti-CD4 or anti-IL-5 antibodies clearly abrogated eosinophilic infiltration, which indicates the essential role of T_H cells and IL-5 in this model, as previously reported by others.^{29,30} The administration of dexamethasone at 3 mg/kg, but not 0.3 mg/kg, significantly inhibited eosinophilic infiltration into the lung. The administration of nonactin at either 3 or 30 mg/kg inhibited airway eosinophilia as effectively as 30 mg/kg cyclosporin A, whereas 3 mg/kg cyclosporin A was not effective. The effect of nonactin was equivalent to that of cyclosporin A, which suggests that IL-5 is a relevant target for the downregulation of eosinophilia by immunosuppressants.

DISCUSSION

The present study clearly indicated that nonactin inhibits the synthesis of IL-5, but not IL-2 or IL-4, by human T_H cells, whereas FK506, cyclosporin A, and dexamethasone suppressed the synthesis of all 3 cytokines with an identical concentration-response relationship. All of these agents inhibited airway eosinophilic infiltration

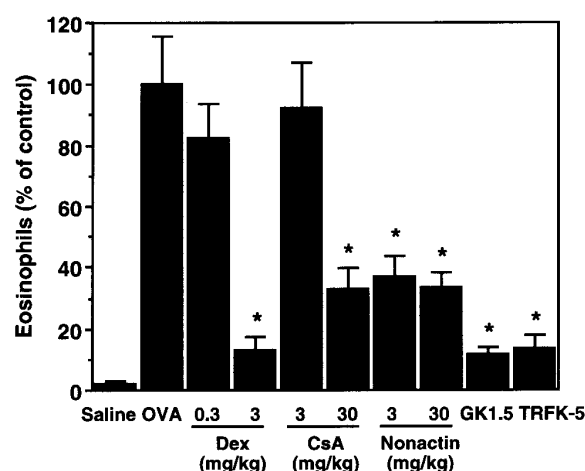


FIG 3. In vivo effect of cyclosporin A (CsA), dexamethasone (Dex), and nonactin on experimental asthma. Ovalbumin-sensitized Balb/c mice were challenged by inhalation of ovalbumin (OVA) solution on day 0. Dexamethasone, cyclosporin A, nonactin, or vehicle was subcutaneously administered on days -2, -1, 0, and 1. Anti-CD4 (GK1.5, 2 mg) and anti-IL-5 (TRFK-5, 2 mg) antibodies were injected intravenously 6 days and 5 minutes before challenge, respectively. BALF was obtained 48 hours after challenge, and eosinophils were counted. Eosinophil numbers were normalized against those obtained from ovalbumin-challenged positive controls (100%). Data are presented as the mean $\pm$ SEM (n = 5-7). * $P < .05$ as compared with the ovalbumin-challenged control.

in a murine asthma model, which indicates that inhibitors of IL-5 synthesis downregulate eosinophilia in vivo. Thus, blocking IL-5 synthesis seems an effective way to treat allergic inflammation.

The importance of persistent eosinophilic inflammation in the pathogenesis of bronchial asthma has been well established.³¹⁻³³ IL-5 is a T-cell cytokine that is crucially involved in the promotion of the local infiltration and activation of eosinophils,^{2,34} although a network of cytokines produced by various cell types is also implicated in the process. Several animal studies have revealed that neutralization of a single cytokine, IL-5, with an anti-IL-5 mAb inhibits airway infiltration by eosinophils and prevents late-phase bronchoconstriction after antigen inhalation, clearly demonstrating that IL-5 is essential for the airway eosinophilic response.^{8,9,35,36} In humans, the number of CD4⁺ T cells that express IL-5 messenger RNA is increased in the bronchial mucosa of patients with symptomatic asthma, shows a correlation with the number of activated eosinophils, and increases further on allergen inhalation challenge.³⁷⁻³⁹ IL-5 is the predominant eosinophil-activating cytokine in BALF obtained from patients with asthma,³ and its concentration is increased in patients with atopic and nonatopic asthma.⁴ The serum IL-5 concentration is elevated in patients with symptomatic asthma and shows a decrease after oral prednisolone therapy.⁵ In addition, the extent of IL-5 mRNA expression is correlated with the degree of eosinophilic infiltration, whereas the expression of IL-3

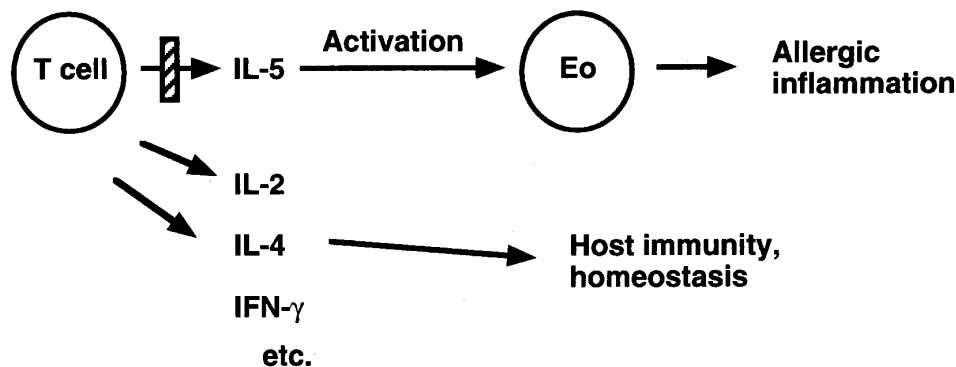


FIG 4. Treatment of atopic diseases with IL-5 synthesis inhibitors. Glucocorticoids are the currently available antiinflammatory agents for asthma therapy, although FK506 and cyclosporin A are now undergoing clinical trials. These 3 agents suppress IL-2, IL-4, and IL-5 synthesis in a nonselective manner (Fig 1). Nonactin does not affect IL-2 or IL-4 synthesis but still downregulates airway eosinophilia (Eo) as effectively as cyclosporin A (Figs 2 and 3). Selective inhibition of IL-5 synthesis (*hatched rectangle*) seems to be a promising treatment for eosinophilic inflammation because of the lack of generalized immunosuppression.

and GM-CSF mRNA is not.⁴⁰ Furthermore, we have shown that IL-5 production by CD4⁺ T cells of patients with asthma is significantly enhanced compared with that of healthy control subjects.⁶ T cells that produce IL-3, GM-CSF, and IL-5 have also been demonstrated to have a role in the late-phase cutaneous reaction in patients with atopic dermatitis after antigen challenge.⁴¹ Thus, there is accumulating evidence that IL-5 is the key cytokine involved in allergic disorders that feature eosinophilic inflammation, so regulation of IL-5 synthesis by T cells seems an effective therapy.

Glucocorticoids have long been considered the most effective treatment for eosinophilic disorders, including chronic asthma.¹² The efficacy of glucocorticoids is ascribed to multiple pharmacologic actions, 1 of which is the suppression of inflammatory cytokine production.^{13,14} In fact, we and others have reported that IL-5 synthesis by peripheral T_H cells was inhibited by glucocorticoids in vitro.^{6,15,16} Clinical improvement of patients with asthma who are treated with inhaled corticosteroids is accompanied by a clear reduction of IL-5 production.⁶ However, glucocorticoids have a wide range of actions not only on the immune system but also on various other tissues and organs, leading to multiple side effects that include hypertension, diabetes mellitus, and osteoporosis. Immunosuppressants such as FK506 and cyclosporin A can inhibit IL-5 production by CD4⁺ T cells in vitro^{6,23} and have recently been shown to be highly effective for the treatment of severe atopic dermatitis.^{18,42} IL-5 production by peripheral blood T_H cells is enhanced in patients with dermatitis before treatment and is downregulated after treatment with FK506 ointment.^{43,44} These agents have a more restricted target specificity compared with glucocorticoids but still interfere with too many T-cell functions, thereby causing generalized immunosuppression. Our present finding that FK506 and cyclosporin A both suppressed the synthesis of 3 cytokines (Fig 1) clearly demonstrates that agents that interfere with NF-AT transcription factors will equally inhibit IL-2, IL-4, and IL-5. The effect of dexamethasone

on cytokine synthesis has been partly explained by a molecular interaction between the glucocorticoid receptor and a transcription factor, AP-1. Our present finding of nonselective suppression of IL-2, IL-4, and IL-5 synthesis by dexamethasone suggests that AP-1 antagonists also lack selectivity in terms of the inhibition of cytokine synthesis. Therefore, a treatment that achieved more selective regulation of cytokine synthesis was explored.

Our previous study demonstrated that human allergen-specific T_H clones produced IL-5 after activation that was not only mediated through the T-cell receptor (TcR) but also the IL-2 receptor (IL-2R).^{26,45} TcR signaling induced the transcription of IL-2, IL-4, and IL-5 genes, whereas IL-2R signaling only induced IL-5 gene transcription, which indicates that the regulation of the IL-5 gene differed from that of the IL-2 and IL-4 genes. NF-AT, AP-1, and NF-κB were induced by TcR stimulation, but not after IL-2R stimulation, whereas the IL-5 protein production, gene expression, and promoter activity induced by TcR and IL-2R stimulation were quite comparable. These findings suggest that some undefined transcription factor(s) distinct from AP-1, NF-AT, or NF-κB is involved in the activation of the IL-5 gene. In addition, stimulated T-cell hybridomas that are derived from the fusion of IL-5-producing T-cell clones with a T-cell tumor line expressed IL-5 mRNA and transcribed pIL-5(-511)Luc, in which the human IL-5 promoter/enhancer region (-511 to +4 relative to the transcription initiation site) was connected to the luciferase reporter gene, whereas other T-cell hybridomas that were derived from IL-5-nonproducing T-cell clones did not,^{22,46} which suggests that IL-5 synthesis is positively regulated through the approximately 500-bp promoter/enhancer region of the human IL-5 gene. IL-2 and IL-4 gene transcription and NF-AT, AP-1, NF-κB, and Oct-1 binding were not essentially different between IL-5 gene-expressing and nonexpressing hybridomas. The results indicate that IL-5 gene transcription is differentially regulated from that of IL-2 and IL-4 by a unique transcription factor (tentative-

ly named NF-IL-5). An agent capable of blocking NF-IL-5 activity might selectively suppress IL-5 synthesis without affecting IL-2 or IL-4 synthesis.

Our present finding that nonactin inhibited IL-5 synthesis but not IL-2 or IL-4 synthesis (Fig 2) is consistent with the concept that IL-5 synthesis by human T_H cells is regulated independently from that of other major T-cell cytokines by a unique transcription factor and further supports the notion that IL-5 synthesis can be modulated separately from other T-cell functions. OM-01, a member of polynactin family, inhibited the transcription of both endogenous IL-5 gene and pIL-5(-511)Luc but did not affect NF-AT, AP-1, NF-κB, and Oct-1 binding (unpublished observation). Identification of the target molecule of OM-01 is now underway, and this transcription factor might be responsible for the enhanced activation of IL-5 gene in eosinophilic disorders such as asthma and atopic dermatitis.

The in vivo effects of dexamethasone, cyclosporin A, and nonactin were next determined with the use of a murine asthma model. Eosinophilic infiltration was induced by antigen inhalation, whereas saline solution challenge scarcely induced eosinophilia (Fig 3). The administration of anti-CD4 and anti-IL-5 antibodies blocked eosinophilic infiltration, which indicates the essential role of T_H cells and IL-5 in this model. These effects of dexamethasone and cyclosporin A on murine asthma were consistent with the reports of others.⁴⁷⁻⁴⁹ The administration of either 3 or 30 mg/kg nonactin inhibited eosinophilic infiltration into the lungs as effectively as 30 mg/kg of cyclosporin A. Dexamethasone (3 mg/kg) caused more profound suppression of eosinophilia, possibly because of its effects on cell types of non-T lineage (such as eosinophils and endothelial cells). Bronchial hyperresponsiveness, another characteristic feature of asthma, is also prevented by nonactin (data not shown). Our finding that nonactin inhibits IL-5 synthesis, but not IL-2 or IL-4 synthesis, and downregulates airway eosinophilia as effectively as cyclosporin A (which inhibits IL-2, IL-4, and IL-5) clearly indicates that suppression of IL-5 synthesis by T cells can be a target for asthma therapy with efficacy in vivo (Fig 4).

In summary, our findings indicate that IL-5 synthesis by human T cells can be selectively controlled, because its transcriptional regulation is distinct from the regulation of other major T-cell cytokines (IL-2 and IL-4). Elucidation of the critical transcription factor involved in IL-5 gene dysregulation in atopic diseases would improve our understanding of the pathogenesis of allergic inflammation and could facilitate the future development of selective inhibitors for pinpoint control of eosinophilic inflammation.

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