

3. Molecules

Cellular and molecular mechanisms of IL-5 synthesis in atopic diseases: A study with allergen-specific human helper T cells

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Background: Cytokines produced by helper T cells are intimately involved in chronic allergic diseases associated with eosinophilic inflammation.

Objective: We investigated the production of IL-5, a potent growth factor and chemotactic factor for eosinophils, by CD4⁺ T lymphocytes in patients with asthma.

Methods: Allergen-specific T cell clones and T cell hybridomas were established from the peripheral blood lymphocytes of patients with asthma, and the responses to various stimuli were determined.

Results: After nonspecific stimulation, IL-5 production by CD4⁺ T cells from both atopic and nonatopic subjects with asthma was significantly enhanced compared with that by cells from healthy controls. Peripheral blood mononuclear cells from atopic asthma patients both proliferated and produced IL-5 after incubation with mite allergen, suggesting that mite-specific helper T cells were involved in the eosinophilic inflammation of atopic asthma. A human IL-5 promoter/enhancer luciferase gene construct transfected into IL-5-producing T cell clones was clearly transcribed after stimulation, indicating that the 515 base pair IL-5 gene segment upstream of the coding region was sufficient to respond to activating signals in human helper T cells. The same gene segment was not transcribed in IL-5-nonproducing T cell clones, suggesting that human T cell IL-5 synthesis is regulated at the transcriptional level. Experiments with T cell hybridomas confirmed these findings and suggested that a unique transcription factor may be essential for human IL-5 gene transcription.

Conclusion: Enhanced IL-5 production by helper T cells seems to cause the eosinophilic inflammation of both atopic and nonatopic asthma. Elucidation of IL-5-specific regulatory mechanisms may facilitate the development of novel treatments for allergic diseases associated with eosinophilic inflammation. (*J Allergy Clin Immunol* 1997; 100:S56-64.)

Key words: IL-5, T cell, atopic disease, bronchial asthma

Abbreviations used

BAL:	Bronchoalveolar lavage
bp:	Base pair
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
mAb:	Monoclonal antibody
mRNA:	Messenger ribonucleic acid
PBMC:	Peripheral blood mononuclear cells
PCR:	Polymerase chain reaction
PMA:	Phorbol 12-myristate 13-acetate
SI:	Stimulation index

Persistent eosinophilic inflammation affecting the bronchial mucosa and submucosa is a prominent pathologic feature of asthma.¹⁻⁴ CD4⁺ T cells and T-cell cytokines including IL-5 have been strongly implicated in the local accumulation and activation of eosinophils.⁵⁻⁷ Bronchoalveolar lavage (BAL) fluid from atopic and nonatopic patients with asthma contains increased concentrations of IL-5⁸; also, the serum IL-5 concentration is elevated in symptomatic asthma patients, but it decreases after oral prednisolone therapy.⁹ In addition, activated T cells expressing IL-5 mRNA can be detected in the bronchial mucosa of patients with chronic asthma,¹⁰ and these cells increase after allergen challenge.¹¹ Furthermore, the extent of IL-5 mRNA expression is well correlated with the severity of eosinophilic infiltration, whereas the expression of mRNA for IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF) is not.¹² IL-5-producing T cells have also been found in the late-phase cutaneous lesions of atopic dermatitis.¹³ Thus, IL-5 seems to be one of the key mediators involved in the pathogenesis of allergic diseases characterized by eosinophilic inflammation.

Our previous study indicated that IL-5 production by CD4⁺ T cells is enhanced in both atopic and nonatopic patients with asthma.¹⁴ Glucocorticoids and immunosuppressants (FK506 and cyclosporin A) suppress *in vitro* IL-5 synthesis by peripheral blood mononuclear cells (PBMC) from asthmatic patients, and the clinical improvement of asthma after treatment with inhaled

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corticosteroids is accompanied by a definite reduction of IL-5 production by peripheral CD4⁺ T cells.¹⁵ A decrease of IL-5 production is also observed in patients with severe atopic dermatitis after treatment with topical FK506.¹⁶ These findings suggest that the inhibition of IL-5 production by activated T cells may be beneficial in patients with allergic diseases characterized by eosinophilic inflammation. In the present study, we analyzed the cytokine production profiles of PBMC obtained from atopic patients with asthma, nonatopic patients with asthma, and healthy controls. To define the cellular and molecular mechanisms involved in IL-5 production by human CD4⁺ helper T cells and to explore possible interventions to control production of this cytokine, we established allergen-specific T cell clones and T cell hybridomas from peripheral blood lymphocytes of patients with atopic asthma and analyzed IL-5 gene transcription.

MATERIALS AND METHODS

Subjects

Adult patients referred to the outpatient clinic of the Department of Medicine and Physical Therapy at the University of Tokyo were enrolled in this study. All patients were nonsmokers and fulfilled the criteria of the International Consensus Report on Diagnosis and Management of Asthma,¹⁷ and all demonstrated at least 20% improvement of their FEV₁ after β_2 -agonist inhalation. A patient was considered to be atopic if one or more skin prick tests with a range of 20 common airborne allergens gave positive results in the presence of a positive histamine control and a negative vehicle control. Patients with nonatopic asthma had negative skin test results and normal serum IgE levels. Treatment with β_2 -stimulants, anticholinergics, and theophylline was stopped at least 12 hours before blood sampling. Patients receiving systemic or inhaled corticosteroids were excluded from the study. The control subjects were healthy age- and sex-matched individuals with normal IgE levels, negative skin prick test responses and no history of allergy or asthma and were not receiving medications. The mean serum IgE concentrations of the atopic asthmatic patients, nonatopic asthmatic patients, and control subjects were 324 IU/ml (range, 184 to 498 IU/ml), 72 IU/ml (range, 19 to 21 IU/ml), and 49 IU/ml (range, 12 to 118 IU/ml), respectively. Written informed consent for participation was obtained from every subject.

Reagents

An anti-CD3 monoclonal antibody (mAb) was obtained from Ortho (Raritan, N.J.). Fluorescein isothiocyanate-labeled anti-CD3 (Leu4), anti-CD4 (Leu2), and anti-CD8 (Leu3) mAbs were from Becton Dickinson (San Jose, Calif.). Purified Der f1 protein was provided by Torii Pharmaceutical Co. (Chiba, Japan) and recombinant Der f2 (rDer f2) protein was provided by Dr. Y. Okumura (Asahi Breweries Ltd.).¹⁸ AIM-V medium (Gibco BRL, Gaithersburg, Md.) was used for T cell cultures, whereas RPMI 1640 medium (Gibco BRL) supplemented with 10% fetal bovine serum, 100 μ g/ml streptomycin, and 100 U/ml penicillin was used for the transfection experiments.

PBMC cultures

Heparinized venous blood was collected between 9:00 and 10:00 AM and PBMC were prepared by Ficoll-paque (Pharma-

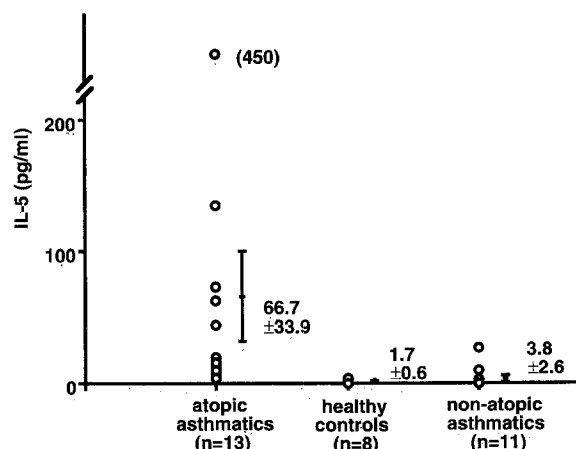


FIG. 1. IL-5 production by PBMCs in response to *D. farinae* mite allergen. PBMCs (2×10^6 /ml) were incubated with *D. farinae* mite allergen extract (1 μ g/ml) for 6 days, and the IL-5 content of the supernatant was assayed with a sandwich ELISA. Data are presented as the means of triplicate cultures.

cia, Uppsala, Sweden) density gradient centrifugation. Cells were washed and suspended in AIM-V medium at a density of 2×10^6 /ml. The PBMC were then stimulated with 20 nmol/L phorbol 12-myristate 13-acetate (PMA; Sigma, St. Louis, Mo.) and/or 1 μ mol/L ionomycin (Calbiochem, La Jolla, Calif.) for the designated periods. In some experiments, PBMC were cultured with *Dermatophagoides farinae* mite extract (Torii), Der f1, and Der f2. The supernatants were harvested and frozen at -70°C until use. For proliferation assays, cells were cultured for 6 days. Tritium-labeled-thymidine (0.5 μ Ci/well) was added for the last 16 hours. Stimulation index (SI) was calculated as the tritiated thymidine incorporation of the stimulated cultures divided by the tritiated thymidine incorporation of the unstimulated cultures.

Allergen-specific human T cell clones and T cell hybridomas

Der f2-specific human T cell clones were obtained from PBMC of donors with atopic asthma by antigenic stimulation followed by the limiting dilution method, as described previously.¹⁹ T cell hybridomas were established by fusing IL-5-producing and IL-5-nonproducing T cell clones with a hypoxanthine guanine phosphoribosyl transferase-deficient T cell line and were maintained as described previously.²⁰

Stimulation of T cell clones

T cells were harvested at least 10 days after the last antigenic stimulation, layered on Ficoll-Paque, and centrifuged. Cells at the interface were 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 (10^5 /well) were cultured in triplicate with various stimulants in 96-well round-bottomed culture plates for the designated periods. For proliferation assays, cells were cultured for 72 hours and tritiated thymidine (0.5 μ Ci/well) was added for the last 16 hours. To obtain cytoplasmic RNA, T cells (4×10^6 /well) were cultured in 24-well culture plates with various stimulants for the designated periods.

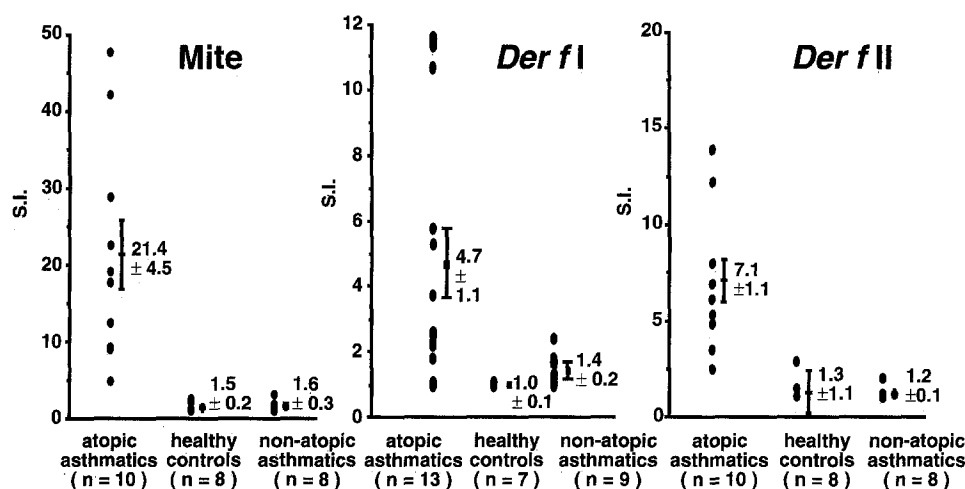


FIG. 2. Proliferative responses of PBMCs obtained from patients with atopic asthma or nonatopic asthma and healthy controls to crude mite extracts, Der f1, and Der f2. PBMCs (10^5 /well) were incubated in 96-well round-bottomed culture plates with either crude mite extract (A, $1 \mu\text{g}/\text{ml}$), Der f1 (B, $1 \mu\text{g}/\text{ml}$), or Der f2 (C, $1 \mu\text{g}/\text{ml}$) for 6 days and pulsed with tritiated thymidine ($0.5 \mu\text{Ci}/\text{well}$) for the last 16 hours. Data are presented as the mean of six wells stimulated with an allergen/the mean of six unstimulated wells. The numbers of donors included in each study are shown in parentheses.

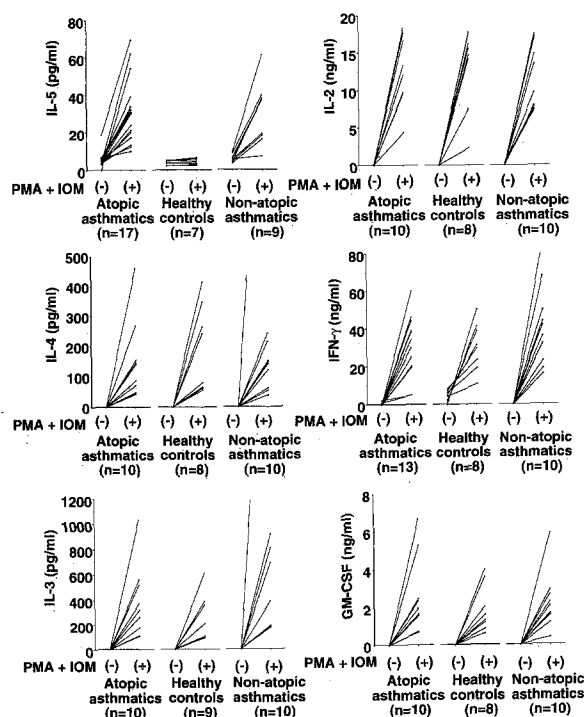


FIG. 3. Production of various cytokines by PBMCs from patients with atopic asthma or nonatopic asthma and healthy controls. PBMCs ($2 \times 10^6/\text{ml}$) were stimulated with PMA (20 nmol/L) plus ionomycin ($1 \mu\text{mol}/\text{L}$) for 24 hours and supernatants were assayed with specific ELISAs. The numbers of donors in each study are shown in parentheses. IL-5 production was significantly greater in atopic and nonatopic asthma patients than in healthy controls ($p < 0.05$). No significant differences were observed among the three groups in the production of IL-2, IL-3, IL-4, GM-CSF and interferon- γ . IOM, ionomycin.

Quantitation of cytokines in culture supernatants

IL-5 was measured with a sandwich ELISA that used monoclonal anti-human IL-5 (D138) as the capture antibody and biotinylated purified rabbit anti-human IL-5 as the second antibody, as described previously.¹⁴ IL-2, IL-3, IL-4, and GM-CSF were measured with specific ELISAs (Quantikine ELISA kits; R&D systems, Minneapolis, Minn.) according to the manufacturer's directions. Interferon- γ was also measured with a specific ELISA (Inter-Test- γ IFN-ELISA Kit; Genzyme, Cambridge, Mass.).

Reverse transcription-polymerase chain reaction assay

IL-5 gene expression was analyzed by reverse transcription-polymerase chain reaction as reported previously.¹⁴ In brief, RNA was extracted from pelleted cells essentially according to the one-step acid guanidinium isothiocyanate/phenol-chloroform extraction method of Chomczynski and Sacchi²¹ by using Isogene (Nippongene, Tokyo, Japan). Complementary DNA was synthesized from $1 \mu\text{g}$ of cytoplasmic RNA by using random primers and murine Moloney leukemia virus reverse transcriptase (Gibco BRL) according to the manufacturer's directions. The polymerase chain reaction (PCR) was performed with sets of the primers specific for human IL-5 and β -actin (Clontech, Palo Alto, Calif.), and PCR products were analyzed by 1% agarose gel electrophoresis with ethidium bromide staining.

Plasmid constructs

Human genomic DNA isolated from HeLa cells was used as the template. The IL-5 promoter/enhancer gene (-511 to $+4$ relative to the transcription initiation site) was amplified by PCR by using a sense primer ($5'$ ATACTCGAGGGATCCTAATCAAGACCC $3'$), and an anti-sense primer ($5'$ TGCAAGCTTTGCATAGTACAAGACTGC $3'$) that were based on the DNA sequence reported by Tanabe et al.²² The 515 base pair (bp) PCR product was inserted into the *XhoI/HindIII* site

TABLE 1. Analysis of IL-5-producing and IL-5-nonproducing T-cell clones

Clone*	Stimulation	Cytokine production (pg/ml)§			Proliferation (SI)
		IL-2	IL-4	IL-5	
YA5	None	<6	<3	<1	1
	Antigen†	1200 ± 82	800 ± 49	320 ± 28	19.4
	PMA + ionomycin	1640 ± 69	740 ± 62	410 ± 29	NT
	Immobilized OKT3‡	1320 ± 71	480 ± 39	480 ± 32	NT
	IL-2	NT	<3	420 ± 49	NT
YA10	None	<6	<3	<1	1
	Antigen	1080 ± 49	1620 ± 82	<1	21.4
	PMA + ionomycin	1920 ± 52	840 ± 61	<1	NT
	Immobilized OKT3	1020 ± 32	940 ± 28	<1	NT
	IL-2	NT	<3	<1	NT

NT, Not tested; SI, stimulation index.

*T cell clones (10^5 /well) were cultured in triplicate in 96-well round-bottomed culture plates with various stimulants.

†T cells were cultured with irradiated autologous PBMC (10^5 /well) and rDer f2 (10 µg/ml).

‡Wells were preincubated overnight at 4° C with OKT3 antibody (10 µg/ml) in 0.05 mol/L carbonate/bicarbonate buffer (pH 9.6).

§Supernatants were collected after 24 hours and assayed for IL-2, IL-4, and IL-5 using specific ELISAs. Data are presented as the mean ± SEM of triplicate cultures. The minimum detectable concentration was 6 pg/ml for IL-2, 3 pg/ml for IL-4, and 1 pg/ml for IL-5.

||Cells were cultured for 72 hours and pulsed with ^{3} H-thymidine was pulsed for the last 16 hours.

of the pGL2 basic vector (Promega, Madison, Wis.) to construct pIL-5(-511)Luc. In addition, the human IL-2 promoter/enhancer gene (-480 to +2 relative to the transcription initiation site) was amplified by PCR with a sense primer (5'ATACTC-GAGCTGATAAGATTAATCTTA3') and an anti-sense primer (5'TGCAAGCTTGCAATGCTGACAAAGATA3') that was based on the DNA sequence reported by Fujita et al²³ and was inserted into the *XhoI/HindIII* site of the pGL2 basic vector to construct pIL-2(-480)Luc. Identity of the promoter/enhancer sequence with the original reported DNA sequence was confirmed by chain termination sequencing by using Sequenase 2.0 (Stratagene, La Jolla, Calif.). The pCMV-β-gal control vector (Riken DNA Bank, Tokyo, Japan) was employed as a transfection control.

Transient transfection, luciferase assay, and β-galactosidase assay

T cell clones and T cell hybridomas were transfected by electroporation with a Gene Pulser (Bio-Rad, Richmond, Calif.). In brief, 2.5×10^7 T cell clones in 500 µl of RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, and 5 ng/ml recombinant IL-2 were incubated in a 0.4 cm electroporation cuvette with 50 µg of pIL-5(-511)Luc and 10 µg of pCMV-β-gal. Electroporation was performed at 270 V and 960 µF with a mean time constant of 19 msec. 5×10^6 T cell hybridomas were transfected with 10 µg of pIL-5(-511)Luc and 10 µg of pCMV-β-gal. The transfected cells were cultured with or without stimulants for 24 hours, protein extracts were prepared, and luciferase activity was assayed with a luciferase assay system (Promega). The results were determined as relative luciferase units that were calculated with the following formula: relative luciferase unit = luciferase activity in the cell lysate/protein content (mg/ml). β-Galactosidase activity was assayed with chlorophenol red-β-D-galactopyranoside as the substrate, as described previously.¹⁹

Protein assay

Protein concentrations were determined with the bicinchoninic acid protein assay reagent (Pierce, Rockford, Ill.) according to the manufacturer's directions.

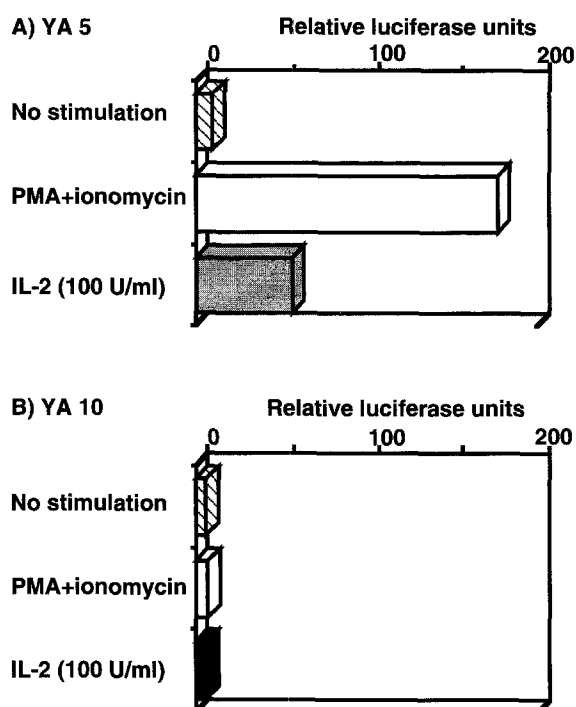


FIG. 4. Analysis of IL-5 promoter activity in T cell clones. An IL-5-producing T cell clone (YA5) and an IL-5-nonproducing T cell clone (YA10) were transiently transfected with pIL-5(-511)Luc (50 µg) plus pCMV-β-gal (10 µg) and then stimulated with either PMA (20 nmol/L) plus ionomycin (1 µmol/L) or human recombinant IL-2 (100 U/ml). After 24 hours, cell lysates were prepared and tested for luciferase and β-galactosidase activity. Values are expressed as the means of triplicate cultures.

Statistical analysis

Statistical analysis was performed with Student's *t* test, and a *p* value less than 0.05 was considered statistically significant. Results are presented as the mean ± standard error of the mean.

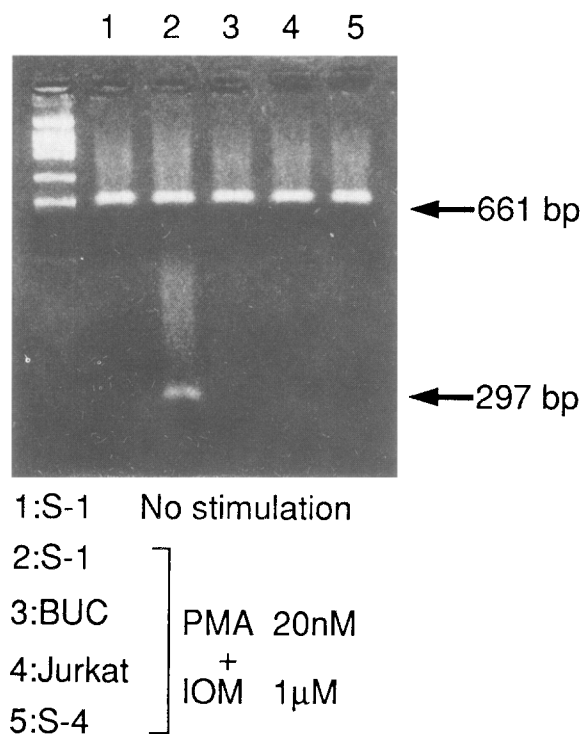


FIG. 5. IL-5 mRNA expression by human T cell hybridomas, (S-1 and S-4), fusion partner cells (BUC), and Jurkat cells. Cells (2×10^6 /well) were incubated in 24-well culture plates for 6 hours. PMA (20 nmol/L) and ionomycin (1 μ mol/L) were added from the start of culture. Cytoplasmic RNA was extracted, reverse transcribed, and amplified by PCR with IL-5 and β -actin primers sets. The 297 and 661 bp products correspond to the expected size of the IL-5 and β -actin amplification products, respectively.

RESULTS

D. farinae mite extract-induced IL-5 production by PBMC

The first experiment was carried out to examine in vitro IL-5 production by PBMC from patients with atopic asthma after specific antigen challenge. All atopic patients included in this study showed sensitivity to house dust mites. PBMC from patients with atopic or nonatopic asthma and healthy controls were incubated with *D. farinae* mite extract, and the resulting supernatants were assayed for IL-5. Significant IL-5 production was detected on day 3 and reached a maximum on day 6 after stimulation of PBMC from patients with atopic asthma (data not shown). The optimal concentration of *D. farinae* mite extract was 1 μ g/ml in the atopic subjects. Fig. 1 shows that PBMC from atopic asthma patients produced significantly more IL-5 in response to stimulation with mite extract when compared to cells from nonatopic asthma patients and healthy controls. Similar results were obtained when mite extract was added to cultures at 0.1 or 10 μ g/ml (data not shown). IL-5 production was totally abolished by the depletion of CD4⁺ cells from PBMC with anti-CD4 mAb-coated magnetic beads (Dyna, N.Y.), suggesting that helper T cells that recognized mite allergens were present in the

PBMC of atopic asthma patients and produced IL-5 after antigenic stimulation.

Proliferative response of PBMC

The proliferative response of PBMC obtained from patients with atopic or nonatopic asthma and healthy controls was examined next. PBMC were incubated with *D. farinae* mite extract, Der f1, or Der f2 for 6 days, and the proliferative response was measured by pulsing with tritiated thymidine for the last 16 hours of culture. Antigen-induced proliferation of PBMC from patients with atopic asthma was significantly enhanced, compared with that of cells from nonatopic asthma patients and healthy controls (Fig. 2). This proliferative response was abolished by the addition of anti-CD4 mAb, which indicated that the response was dependent on CD4⁺ (helper) T cells (data not shown).

Cytokine production by PBMC

Preliminary experiments indicated that combined stimulation with PMA and ionomycin was required for IL-5 production, suggesting that both protein kinase C activation and Ca²⁺ influx are necessary for IL-5 synthesis by T cells, as is the case for IL-2 synthesis. Accordingly, cytokine production by PBMC obtained from patients with atopic or nonatopic asthma and controls was compared after stimulation with PMA plus ionomycin. Supernatants were collected after 24 hours of culture and assayed for IL-2, IL-3, IL-4, IL-5, interferon- γ , and GM-CSF. As shown in Fig. 3, there were no significant differences among the three groups in the production of any cytokine except IL-5. However, significantly more IL-5 production was observed in cultures of cells from atopic or nonatopic asthma than in cultures of cells from healthy controls (37.6 ± 7.1 pg/ml, 25.7 ± 5.5 pg/ml, and 3.6 ± 0.4 pg/ml, respectively; $p < 0.05$). These results indicated that T cells producing IL-5 after non-specific stimulation were present in the peripheral blood of atopic and nonatopic asthma patients, but not in healthy controls.

Regulation of IL-5 synthesis by human helper T cell clones

Our previous studies indicated that the overproduction of IL-5 by PBMC was decreased in patients responding to treatment with glucocorticoids and FK506, suggesting that IL-5 synthesis was an effective target for the therapy of allergic diseases associated with eosinophilic inflammation.^{14, 15} To investigate the cellular and molecular mechanisms involved in IL-5 synthesis, T cell clones specific for Der f2, a major house dust mite allergen, were established from PBMC of donors with atopic asthmatic by antigenic stimulation followed by limiting dilution techniques. Cytokine production was then analyzed after antigenic stimulation and after stimulation with immobilized anti-CD3 mAb (OKT3) or PMA plus ionomycin. More than 60 clones were tested, and all of them produced both IL-2 and IL-4 (T_{H0} phenotype). Two representative clones were employed

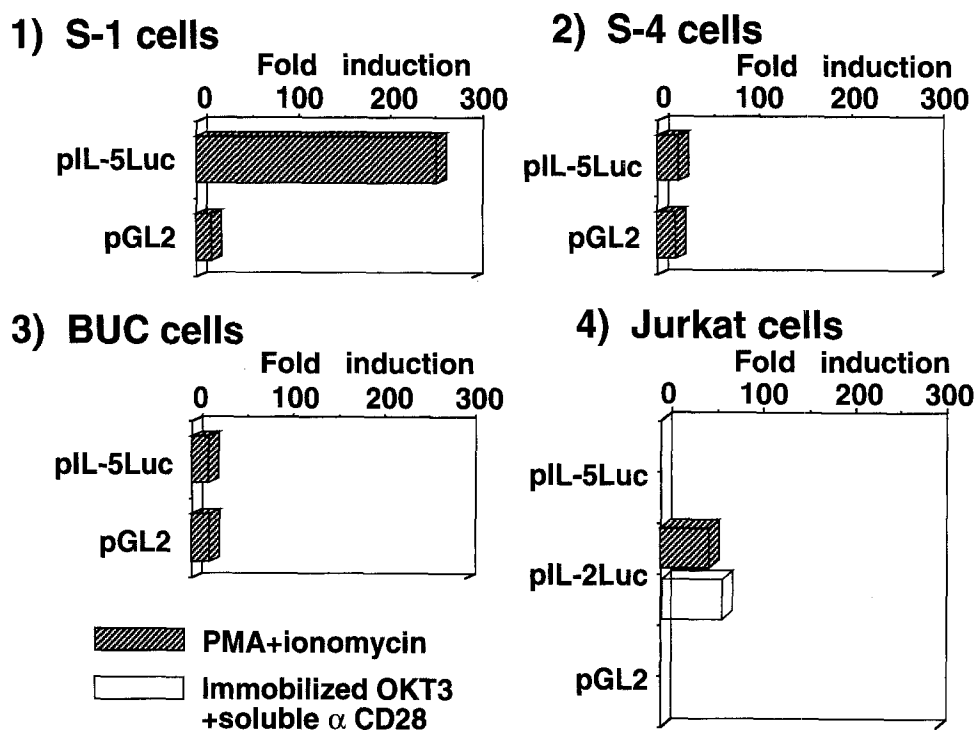


FIG. 6. Analysis of IL-5 and IL-2 promoter activity of T cell hybridomas, BUC cells, and Jurkat cells. IL-5 gene-expressing T cell hybridomas (S-1), IL-5 gene-nonexpressing T cell hybridomas (S-4), BUC cells, and Jurkat cells were transiently transfected with pIL-5(-511)Luc or pIL-2(-470)Luc and then stimulated with PMA (20 nmol/L) plus ionomycin (1 μ mol/L). Jurkat cells were also stimulated with immobilized OKT3 (10 μ g/ml) plus soluble anti-CD28 antibody (1 μ g/ml). After 24 hours, cell lysates were prepared and tested for luciferase and β -galactosidase activity. Values are expressed as the means of triplicate cultures.

for the transfection analysis (Table I). Clone YA5 produced IL-2, IL-4, and IL-5 upon stimulation, whereas YA10 did not produce IL-5.

To analyze the mechanisms of human IL-5 gene transcription, a human IL-5 promoter/enhancer-luciferase reporter gene plasmid (pIL-5[-511]Luc, containing -511 to +4 of the human IL-5 gene segment relative to the transcription start site) was constructed and transfected into these human helper T cell clones. A representative result is shown in Fig. 4. The IL-5-producing T cell clone, YA5, clearly transcribed pIL-5(-511)Luc upon stimulation with PMA plus ionomycin or IL-2 (100 U/ml), indicating that the approximately 500 bp human IL-5 promoter/enhancer was sufficient to mediate activation-dependent transcriptional activity in human T cells. In contrast, the IL-5-nonproducing clone, YA10, did not transcribe pIL-5(-511)Luc. The transfection efficiency was determined by β -galactosidase activity derived from the cotransfected CMV- β -gal control plasmid and was comparable between each clone (A574/ μ g protein was 0.462 for YA5 versus 0.470 for YA10), suggesting that IL-5 synthesis might be regulated at the transcriptional level. Similar results were obtained in additional experiments using two IL-5-producing T cell clones and three IL-5-nonproducing T cell clones (data not shown).

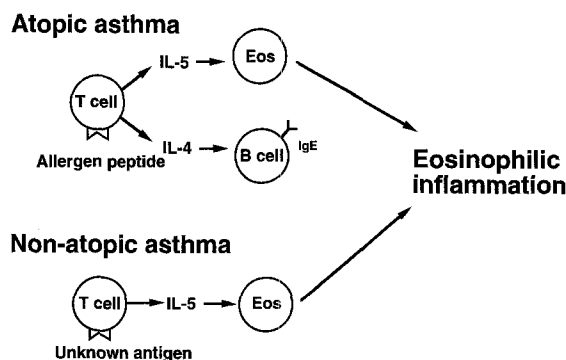


FIG. 7. Association of eosinophilic inflammation in atopic and nonatopic asthma with enhanced IL-5 production by helper T cells. *Eos*, eosinophils.

IL-5 transcriptional activity of IL-5 gene-expressing T cell hybridomas

In order to further analyze the human IL-5 promoter/enhancer gene, the T cell clones YA5 and YA10 were fused with a human HGPRT-deficient transformed T cell line. IL-5 messenger RNA (mRNA) was clearly induced after stimulation of the hybridoma derived from the YA5 clones (S-1 cells) with PMA plus ionomycin but not after stimulation of the hybridoma derived from the YA10 clone (S-4 cells). In addition, Jurkat cells that

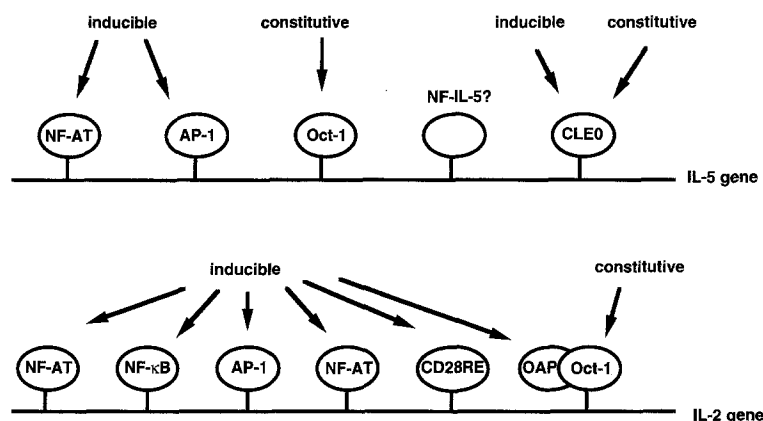


FIG. 8. Possible involvement of an unidentified transcription factor in IL-5 synthesis by human T cells.

were used as a control did not express IL-5 mRNA (Fig. 5).

An approximately 250-fold increase in luciferase activity was induced in S-1 cells transfected with pIL-5(-511)Luc after stimulation with PMA plus ionomycin (Fig. 6). In contrast, S-4 cells or BUC cells (fusion partner cells) showed only a 30-fold increase, which was comparable to the response observed when S-1, S-4, or BUC cells were transfected with a pGL2 basic vector containing no promoter segment. Jurkat cells, which produce IL-2 and IL-4 but not IL-5 upon stimulation (our unpublished results), did not show any luciferase activity when stimulated after transfection with pIL-5(-511)Luc. In contrast, Jurkat cells did transcribe pIL-2(-470)Luc, which contained the human IL-2 promoter (-470 to +2), after stimulation either with PMA plus ionomycin or with immobilized anti-CD3 mAb plus soluble anti-CD28 mAb. The finding that gene transcription mediated by the approximately 500 bp human IL-5 promoter/enhancer segment was only initiated in the IL-5 gene-expressing hybridomas and not in the IL-5 gene-nonexpressing hybridomas or Jurkat cells closely reflected the transcriptional regulation data obtained with T cell clones.

DISCUSSION

PBMC obtained from atopic asthma patients showed significantly greater proliferation and IL-5 production in response to mite allergens compared with cells obtained from nonatopic asthma patients and healthy controls. Anti-CD4 mAb abrogated these responses, which suggests that helper T cells that respond to allergens may play a role in the development of eosinophilic inflammation in atopic asthma. IL-5 production in response to nonspecific stimulation of T cells was investigated next. It was clearly demonstrated that peripheral blood CD4⁺ T cells of both atopic and nonatopic patients with asthma produced significantly higher amounts of IL-5 in response to nonspecific stimulation, when compared to cells of healthy controls. It was previously demonstrated that CD4⁺ T cells are the principal source of IL-5 in

PBMC.¹⁴ The production of IL-3 and GM-CSF, other T cell cytokines acting on eosinophils, did not differ significantly among the three groups, a finding consistent with the report by Ohnishi et al that IL-5 is the predominant eosinophil-activating cytokine involved in the asthmatic response.²⁴ Our finding that T cells producing IL-5 after stimulation with PMA plus ionomycin exist in the peripheral blood of both atopic and nonatopic asthma patients (but not in healthy subjects) suggests that so-called nonatopic patients with asthma are actually hypersensitive at the T cell level.

Nonatopic asthma patients account for approximately 10% to 30% of the asthmatic population.²⁵ Both types of asthma are characterized by episodic bronchoconstriction and by the development of bronchial hyperresponsiveness. Infiltration of activated T cells and eosinophils, deposition of toxic granule proteins released from eosinophils, and destruction of the bronchial epithelium are common pathologic features observed in both atopic and nonatopic asthma. In addition, activated T cells producing IL-5 are increased in the bronchial mucosa in both types of asthma.^{1, 4, 5, 8, 26, 27} Our present findings, together with these data, suggest that enhanced IL-5 production by peripheral helper T cells may be related to the development of eosinophilic inflammation, which is a common feature of both atopic and nonatopic asthma (Fig. 7).

We previously reported that IL-5 production by PBMCs obtained from symptomatic asthma patients is downregulated in patients responding to inhaled corticosteroids.¹⁴ IL-5 production by PBMCs is also enhanced in atopic dermatitis patients and is downregulated after topical FK506 treatment.^{16, 28} In contrast, IL-2 and IL-4 production by PBMCs is far less affected by these treatments when compared with IL-5 production.^{14, 16} These findings suggest that regulation of IL-5 synthesis by T cells might be an effective treatment for chronic allergic disorders characterized by eosinophilic inflammation. Accordingly, we explored possible means of suppressing IL-5 production by studying human T cell clones and hybridomas.

Cytokine genes, including the IL-5 gene, remain quiescent in resting T cells. After antigenic stimulation, various activation signals are transduced via membrane receptors, and these initiate the transcription of several cytokine genes.²⁹ The various signal-transducing molecules involved in these processes include tyrosine kinases, protein kinase C, protein kinase A, calmodulin, and phosphatases. IL-2 synthesis is mainly controlled at the transcriptional level, and transcription of the human IL-2 gene is controlled primarily by an approximately 300 bp segment located upstream of the transcription initiation site.³⁰

The finding that pIL-5(-511)Luc was effectively transcribed by IL-5-producing clones, but not by IL-5-nonproducing clones, suggests that transcriptional control is important in IL-5 synthesis, as well as in IL-2 synthesis. Cells unable to transcribe pIL-5(-511)Luc may lack a transcription factor (tentatively named NF-IL-5) that is expressed in IL-5-producing T cell clones, or they may express a repressor factor that actively interferes with IL-5 gene transcription. Our present finding that a T cell hybridoma derived from fusion between an IL-5-producing T cell clone and a T cell tumor line expressed IL-5 mRNA and transcribed pIL-5(-511)Luc upon stimulation, whereas another T cell hybridoma derived from an IL-5-nonproducing T cell clone did not, makes the former possibility more likely. It has been demonstrated that all five transcription factors, including Oct/OAP₄₀, AP-1, NF- κ B, CD28 responsive element binding factor, and NF-AT, are required for full transcription of the IL-2 gene in activated Jurkat cells.³¹ In the present study, electrophoretic mobility shift analysis revealed that the nuclear factors NF-AT, AP-1, and NF- κ B were induced upon activation, whereas Oct-1 was constitutively expressed in both IL-5 gene-expressing and nonexpressing T cells (data not shown). This result further suggests the possible involvement of a unique transcription factor (NF-IL-5) in IL-5 gene transcription (Fig. 8).

FK506 and cyclosporin A exert an immunosuppressive effect through binding to their respective cellular ligands, FK-binding protein and cyclophilin.³² These complexes inhibit the Ca²⁺-calmodulin-dependent phosphatase, calcineurin, which is essential for the nuclear translocation of NF-AT that is required for initiation of IL-2 gene transcription.³³ On the other hand, glucocorticoids and their receptor form a complex that competitively inhibits the binding of AP-1 to the enhancer element (TPA-responsive element) of cytokine genes.^{34,35} Both AP-1 and NF-AT play a critical role in IL-2 gene transcription.³⁴ Our previous finding that glucocorticoids, FK506, and cyclosporin A suppress human IL-5 production and gene expression suggests the importance of AP-1 and NF-AT in IL-5 gene transcription.¹⁴ However, the possible action of NF-IL-5 may lead to downregulation of IL-5 gene transcription separately from that of other cytokine genes such as the IL-2 and IL-4 genes.

We recently found a substance (OM-01) that sup-

pressed IL-5 synthesis by T cells without any effect on IL-2 and IL-4 synthesis.³⁶ We showed that OM-01 inhibited IL-5 gene expression and also suppressed the induction of luciferase activity mediated by an approximately 2 kb human IL-5 promoter/enhancer gene segment, whereas neither IL-2 nor IL-4 promoter/enhancer activity was affected.³⁶ These results strongly suggest that gene transcription of IL-5 can be regulated differentially from that of IL-2 or IL-4 and further support the existence of a distinct transcription factor (NF-IL-5) essential for IL-5 gene transcription.

Glucocorticoids have a wide range of pharmacologic actions on various tissues and organs other than the immune system and can cause multiple severe side effects, such as diabetes, hypertension, osteoporosis, psychosis, immunosuppression, and cataract. FK506 and cyclosporin A have more restricted actions compared to glucocorticoids, but they still interfere with too many T cell functions, thereby causing generalized immunosuppression. Our findings suggest that an agent capable of interfering with NF-IL-5 activity may selectively suppress IL-5 production without influencing IL-2 or IL-4 production. Such an agent would be able to control eosinophilic inflammation without causing generalized immunosuppression.

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