
Effect of minocycline and doxycycline on IgE responses

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Background: We have recently found that the tetracycline minocycline suppresses inflammatory responses in serum immunoglobulin (Ig)E-positive asthmatic patients, and that IgE levels can decrease in these patients. The mechanism by which minocycline suppresses these responses is unknown.

Objective: We have now investigated the ability of the tetracyclines, minocycline and doxycycline, to regulate IgE responses of peripheral blood mononuclear cells (PBMC) obtained from serum IgE-positive asthmatic patients.

Methods: The distributions of CD3+, CD4+, CD8+, and CD19+ lymphocytes in peripheral blood of serum IgE-positive asthmatic patients and IgE-negative nonasthmatic controls, and cytokine-specific mRNA expression by their PBMC were determined by flow cytometry (reverse transcriptase-polymerase chain reaction). Serum Ig levels also were determined (nephelometry, fluoroenzymeimmunoassay, enzyme-linked immunoadsorbent assay; $n = 7/\text{group}$). PBMC ($1.5 \times 10^6/\text{mL}$) were cultured with anti-CD40 monoclonal antibody and recombinant human interleukin-4 in the presence/absence of minocycline or doxycycline (0.1 to 10 $\mu\text{g}/\text{mL}$), and IgE levels in supernatants determined on days 0, 3, and 10 (enzyme-linked immunoadsorbent assay).

Results: Asthmatic and nonasthmatic subjects had similar numbers of blood CD4+ T cells ($779/\text{mm}^3 \pm 73$ and 766 ± 115 , respectively) and CD19+ B-cells ($239/\text{mm}^3 \pm 35$ and 379 ± 95 , respectively); however, CD8+ T cell numbers were decreased in asthmatic compared with nonasthmatic subjects ($378/\text{mm}^3 \pm 66$ and 568 ± 53 , respectively; $P = 0.045$). High IgE levels were detected in supernatants of asthmatic PBMC on day 10 ($28 \text{ ng}/\text{mL} \pm 12$), whereas control IgE levels did not change ($<2.5 \text{ ng}/\text{mL}$). When either minocycline or doxycycline was included in culture, IgE production by asthmatic PBMC was strongly suppressed in dose-dependent fashion on day 10 ($>80\%$ with 10 $\mu\text{g}/\text{mL}$); control IgE did not change ($<2.5 \text{ ng}/\text{mL}$).

Conclusions: The results are consistent with the idea that the therapeutic benefits obtained by asthmatic patients from minocycline may, in part, result from IgE suppression.

Ann Allergy Asthma Immunol 2002;89:172–179.

INTRODUCTION

Human allergic asthma, a disease characterized by airway hyperresponsiveness and bronchial inflammation, is mediated by a variety of activated leukocytes, including eosinophils, mast cells,¹ CD4+ T lymphocytes,² and CD19+ B-cells.³ In asthma, as in other diseases with altered immunoglobulin (Ig)E regulation, CD4+ T cells secrete interleukin (IL)-4, a T helper cell (Th)2-type cytokine which is required for IgE production,^{3,4} and which is implicated in airway hyperresponsiveness.^{2,5} However, although a correlation between IgE and airway hyperresponsiveness has been demonstrated in allergic asthma,⁵ a cause-and-effect relationship has not as yet been established.

Current treatments which improve airway hyperresponsiveness include various anti-inflammatory agents, which reduce mucosal inflammation and asthma pathogenesis. We have previously treated allergic steroid-dependent asthmatic patients with minocycline (double-blind, placebo-controlled, crossover study), and found that oral administration of minocycline improved their symptoms, and decreased oral corticosteroid requirements.⁶

Studies of O'Dell et al^{7,8} have shown that treatment of mild and moderate rheumatoid arthritis patients with minocycline had no side effects, and minocycline seems to be an effective therapy for early rheumatoid arthritis.^{7,8} Studies of Yu et al⁹ have shown that treatment of dogs with minocycline or doxycycline reduced severity of osteoarthritis,⁹ and studies by Thong and Ferrante¹⁰ have shown that doxycycline or tetracycline inhibits the ability of mice to mount delayed-type hypersensitivity responses.¹⁰ Studies in vitro have demonstrated that treatment of human whole blood cultures with minocycline or tetracycline at physiologic doses inhibits mitotic responses to phytohemagglutinin¹¹ and inhibits inducible nitric oxide synthase expression by murine macrophages.¹²

We now report that in addition to minocycline's in vivo corticosteroid sparing effect in the treatment of asthma, minocycline, along with doxycycline, also suppresses in vitro induction of human IgE responses in a dose-dependent fashion.

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Funded by a SUNY Divisional Grant and by an AAUW Educational Foundation Publication Grant.

Received for publication May 17, 2001.

Accepted for publication in revised form January 29, 2002.

METHODS

Patient Specimens

Peripheral blood (40 mL total) was obtained from asthmatic patients (male and female patients, 26 to 54 years old, mean age 40 ± 14 ; $n = 7$) from the SUNY Downstate Asthma Center of Excellence, and nonasthmatic controls ($n = 7$). Asthmatic patients presented with clinically defined mild-intermittent to severe-persistent asthma, with elevated serum IgE levels (>100 IU/mL). Asthma severity was evaluated in accordance with National Institutes of Health guidelines.¹³ Airflow limitation was present in all patients at the time of evaluation. Patients gave informed consent for the use of their blood samples for an experimental study. Control, nonasthmatic subjects (male and female patients, age matched), were recruited from our hospital staff who showed no evidence of asthma as identified by history and normal total IgE levels (<100 IU/mL). The study was approved by the institutional review board of the SUNY Downstate Medical Center, Brooklyn, NY, and the procedures followed were in accordance with institutional guidelines involving human subjects.

Blood

For studies of serum Igs, blood was collected into red-top Monoject tubes (Sherwood Medical, St. Louis, MO) and allowed to clot for 30 minutes at room temperature. Tubes were rimmed and centrifuged at 1,000 rpm for 10 minutes. Sera were collected and stored at -20°C .

For studies of surface markers, blood was collected into ethylenediaminetetraacetic acid Monoject tubes (Sherwood Medical) and stored at room temperature for up to 2 hours when complete blood counts were performed (Sysmex, McGraw Park, IL); flow microfluorimetry studies (Coulter Epics XL/MCL; Beckman Coulter, Miami, FL) were performed within 3 hours.

Determination of Cell Surface Markers

Antibodies. Mouse anti-human monoclonal antibodies (mAbs) directly conjugated to fluorescein isothiocyanate CD45, Simultest CD3/CD4, Simultest CD3/CD8, Simultest CD3/CD19, and appropriately matched isotype control mAbs, were purchased from BD Biosciences (San Jose, CA), and used according to manufacturer's recommendation.

Immunofluorescence assay. Peripheral blood (100 μL) was incubated with conjugated antibodies for 10 minutes at room temperature. Erythrocytes were lysed with whole blood lysing solution (ImmunoPrep, Beckman Coulter). Flow cytometric analysis was performed on a Coulter Epics XL/MCL Flow Cytometer using System II software (Coulter) and CytoComp (Coulter). Forward and side scatter were used to identify the lymphocyte population and CD45 was used to establish an optimal lymphocyte gate. A minimum of 15,000 events were collected. The gain on the photomultiplier tube detecting fluorescence intensity was adjusted so that 99% of cells with background fluorescence staining were scored between 10^0 and 10^1 on a 4-decade log scale. Specific fluorescence was reported as the percentage of cells with relative fluorescence

intensity scored above background. Total numbers of cells were calculated from the lymphocyte count. Data are expressed as total lymphocytes/ mm^3 .

Serum Igs

Serum Igs (IgM, IgG, and IgA) were determined by nephelometry which was performed, according to manufacturer's recommendation (Beckman Array System, Beckman Coulter) at the Clinical Immunochemistry Laboratory at SUNY Downstate Medical Center. The results are expressed in mg/dL (reference ranges for healthy adult serum are IgM: 60 to 263 mg/dL; IgG: 694 to 1,618 mg/dL; IgA: 69 to 378 mg/dL). Serum IgE levels were detected by the UniCAP Total IgE fluoroenzyme immunoassay (Pharmacia & Upjohn Diagnostics, Kalamazoo, MI) which was performed according to manufacturer's recommendation. To evaluate the test results, the response for the patient's sample was compared directly to the response of the IgE calibrators. Data are expressed as IU/mL (reference range for healthy adult serum is IgE: 20 to 100 IU/mL).

Cytokine Specific mRNA

RNA extraction and polymerase chain reaction (PCR). Total cellular RNA (2 $\mu\text{g}/\text{mL}$) was extracted from PBMC as previously described,¹⁴ using Trizol reagent (Gibco/BRL, Grand Island, NY), according to manufacturer's recommendation. Pellets were dissolved in TE buffer (10 mmol/L Tris HCl (Sigma, St. Louis, MO), pH 7.5, 1 mM EDTA (Sigma), and stored at -70°C in a Bio-Freezer (Forma Scientific, Marietta, OH). Expression of IL-2, IL-4, IL-6, IL-10, interferon (IFN)- γ , IFN- α , and ϵ -specific mRNA was determined using the Advantage One-Step reverse transcriptase-PCR kit (Clontech, Palo Alto, CA), according to manufacturer's recommendation. PCR was conducted using primer pairs specific for IL-2, IL-4, IL-6, IL-10, IFN- γ , IFN- α , and ϵ (expected band sizes: 305, 344, 628, 328, 427, 303, and 149 basepairs [bp], respectively). A β -actin primer set was used as an internal positive control. Negative controls, consisting of no mRNA, but addition of primers, were included in every experiment. The PCR amplicons were separated by electrophoresis in a 1.8% agarose (Seakem LE) gel (FMC, Rockland, ME), and visualized with ethidium bromide (Sigma).

Cell Cultures

PBMC were separated from blood on a Ficoll-Paque (Pharmacia, Piscataway, NJ) gradient (density 1.077). The PBMC were carefully removed using a transfer pipette (VWR Scientific, San Francisco, CA). Cells were washed twice in RPMI-1640 medium (Gibco/BRL), resuspended in RPMI (3 mL), and counted on a hemocytometer (Fisher Scientific, Springfield, NJ). Viability was $>98\%$, as judged by trypan blue exclusion.

Studies of IgE responses induced in vitro were carried out according to the method of Sampson and Buckley.¹⁵ Briefly, PBMC (1.5×10^6 in 1 mL) were cultured $\pm$ anti-CD40 mAb (1 $\mu\text{g}/\text{mL}$; BD Pharmingen, San Diego, CA), $\pm$ recombinant human interleukin-4 (rhIL-4; 200 U/mL; Genzyme, Boston,

MA), in the presence or absence of varying concentrations of either minocycline (Lederle Parenterals, Carolina, Puerto Rico) or doxycycline (American Pharmaceutical Partner, Schaumburg, IL), 0.1, 1.0, 10 $\mu\text{g/mL}$ for 0, 3, and 10 days at 37° C in complete medium in a humidified 4% CO₂ atmosphere. Cell viability was >98%, as judged by trypan blue exclusion, on day 10.

Antibiotics were reconstituted in sterile dH₂O, according to manufacturer's recommendations. Complete medium contained RPMI-1640 (Fisher Scientific) and was supplemented with N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffer (25 mmol/L; Fisher Scientific), penicillin (100 U/mL) (Gibco/BRL), streptomycin (100 $\mu\text{g/mL}$; Eli Lilly, Indianapolis, IN), L-glutamine (2 mM; Gibco/BRL), and 10% fetal calf serum (Gibco/BRL). The concentration of rhIL-4 in culture was within 1 log of physiologic relevance (<100 U/mL), according to bioassay data provided by supplier.

For the in vitro quantitative determination of IgE content in cell culture supernatants, solid-phase sandwich enzyme-linked immunoassay (ELISAs) were performed using IgE ELISA Test Kits (Alexon Trend, Ramsey, MN). All ELISAs were performed according to the manufacturer's recommended procedure. Specimens were analyzed in duplicate and a standard curve was derived from known concentrations of IgE. Plates were read using an automated microplate reader (Model ELx800; Bio-Tek Instruments, Winooski, VT), with a 450-nm measurement filter. Optical densities were converted to IU/mL and/or ng/mL. (1 IU = 2.4 ng/IgE protein)

Statistical Analysis

Mixed-model analyses of variance were used to compare distributions of lymphocyte subpopulations and serum Ig levels between asthmatic and nonasthmatic groups. The groups were compared on each variable. A *P* value of <0.05 was considered statistically significant for all comparisons. Statistical analyses were performed using SPSS for Windows, version 10.0 software (Chicago, IL).

RESULTS

Asthmatic Patients: Medical History and Clinical Features

All asthmatic patients (*n* = 7) exhibited mild-intermittent to severe-persistent signs or symptoms (>2 $\times$ /week) of asthma. Treatment regimens included β -agonist inhalers, inhaled corticosteroids, and if necessary, oral steroids (Table 1). Clinical profiles of asthma in this study included: positive skin prick and intradermal test(s) (tree and grass pollens, ragweed pollen, dust mite, American cockroach, *Alternaria*, *Cladosporium*, or cat antigens), and/or eczema, and elevated serum IgE (>100 IU/mL) levels. Serum IgE levels were significantly increased in asthmatic (367 IU/mL $\pm$ 51, coefficient of variation [CV] = 0.1), compared with control subjects (35 IU/mL $\pm$ 51, CV=1.0; *P* = 0.001). In contrast, serum IgM, IgG, and IgA (mg/dL) levels in asthmatic patients (128 $\pm$ 19, 1,352 $\pm$ 140, 309 $\pm$ 53 mg/dL, respectively) resembled those of controls (127 $\pm$ 19, 1,223 $\pm$ 140, 191 $\pm$ 53 mg/dL, respectively). At the time of study, none of the patients was treated with allergen immunotherapy.

Distributions of Blood Lymphocyte Subpopulations

Asthmatic and nonasthmatic subjects (*n* = 7 per group) had similar total numbers of blood CD3+CD4+ T cells (779/mm³ $\pm$ 73, CV = 0.09 and 766 $\pm$ 115, CV = 0.15, respectively) and CD19+ B-cells (239 $\pm$ 35, CV = 0.14 and 379 $\pm$ 95/mm³, CV = 0.25, respectively; Table 2). However, CD3+CD8+ T cell numbers were significantly decreased in blood of asthmatic patients (378 $\pm$ 66/mm³, CV = 0.17), compared with nonasthmatic subjects (568 $\pm$ 53/mm³, CV = 0.09; *P* = 0.045). In contrast, the CD4/CD8 ratio was not significantly different between the asthmatic and nonasthmatic groups (2.06 and 1.35, respectively).

Cytokine Expression by PBMC

On day 0, PBMC from asthmatic patients (17%) expressed IL-2-specific mRNA, compared with nonasthmatic subjects (57%). In contrast, PBMC from asthmatic patients (78%) expressed IL-10-specific mRNA compared with nonasthmatic subjects (17%).

Table 1. Asthmatic Patients: Medical History and Clinical Features

Patient	Sex (age)	Ethnicity	Asthma classification	Signs and symptoms	Pharmacotherapy	Positive skin test	Eczema history	Serum IgE (IU/mL)†
1	F (46)	Hispanic	Severe-persistent	Continual	Inhaled steroids‡	(+)	(-)	355
2	F (35)	African-American	Severe-persistent	Continual	Prednisone 10 mg po qd§	(+)	(-)	719
3	M (34)	Caucasian	Mild-intermittent	>2 $\times$ /wk	β -Agonist PRN¶	(+)	(+)	241
4	F (43)	African-American	Mild-persistent	>2 $\times$ /wk	Inhaled steroids	(+)	(-)	209
5	M (45)	Caucasian	Mild-intermittent	>2 $\times$ /wk	β -Agonist PRN	(+)	(+)	346
6	F (54)	Hispanic	Mild-persistent	>2 $\times$ /wk	Inhaled steroids	(+)	(-)	192
7	F (26)	African-American	Severe-persistent	Continual	Inhaled steroids	(+)	(-)	510

* Diagnosis and management of asthma in accordance with National Institutes of Health (NIH) guidelines, July 1997.

† Serum IgE levels of asthmatic patients (367 $\pm$ 51 IU/mL) > nonasthmatic patients (35 $\pm$ 51 IU/mL; *P* = 0.001); serum IgM, IgG, and IgA levels in asthmatic patients (128 $\pm$ 19, 1,352 $\pm$ 140, 309 $\pm$ 53 mg/dL, respectively) were similar to nonasthmatic controls (127 $\pm$ 19, 1,223 $\pm$ 140, 191 $\pm$ 53 mg/dL, respectively). Data are expressed as mg/dL or IU/mL $\pm$ pooled $\pm$ standard error (SE).

‡ Fluticasone propionate 44 μg inhalation aerosol: 2 puffs po tid: ter in die (three times daily).

§ po: per os (by mouth); qd: quaque die (once daily); PRN: pro re nata (when necessary).

Table 2. Distributions of Lymphocyte Subpopulations in Peripheral Blood of Asthmatic and Nonasthmatic Human Subjects

Subject	Fluorescent Cells						CD4/CD8
	Lymphocytes						
	CD3+CD4+		CD3+CD8+		CD19+		
	mm ³	(%)	mm ³	(%)	mm ³	(%)	
Asthmatic	779 ± 73	45 ± 3	378 ± 66†	21 ± 3	239 ± 35	14 ± 2	2.06
Nonasthmatic	766 ± 115	36 ± 3	568 ± 53†	28 ± 3	379 ± 95	17 ± 3	1.35

* The distributions of lymphocyte subpopulations in peripheral blood of asthmatic (n = 7) and nonasthmatic (n = 7) subjects were determined by flow microfluorimetry (Coulter Epics XL/MCL). Data are expressed as mean total cells/mm³ or mean percentage (%) of positive cells ± SE.

† Asthmatic < nonasthmatic (P = 0.045).

matic subjects (29%). Neither group expressed IFN- γ mRNA. However, IL-4, IL-6, and IFN- α , and ϵ -specific (data not shown) mRNA were similarly expressed by PBMC of both groups (Table 3).

Effect of Minocycline or Doxycycline on IgE Responses Induced in Vitro

When PBMC from either asthmatic or nonasthmatic subjects were cultured with anti-CD40 mAb and rhIL-4, virtually no IgE was detected in culture supernatants on days 0 and 3 (<2.5 ng/mL; data not shown). In contrast, when asthmatic PBMC (5 of 7 patients) were cultured with anti-CD40 mAb and rh-IL-4, high levels of IgE were detected in supernatants on day 10 (28 ng/mL ± 12; Table 4); IgE levels from 2 of 7 asthmatic patients, and all nonasthmatic patients did not in-

Table 3. Summary of Cytokine Production by PBMC from Asthmatic and Nonasthmatic Subjects

Subject	Cytokines					IFN- α
	Th-1		Th-2			
	IL-2	IFN- γ	IL-4	IL-6	IL-10	
Asthmatic						
1	—	—	+	—	+	+
2	—	—	+	+	+	+
3	+	—	+	+	+	+
4	—	—	—	+	—	+
5	nt	—	nt	nt	+	+
6	—	—	+	+	+	+
7	—	—	+	+	—	+
Nonasthmatic						
1	—	—	+	—	—	+
2	—	—	+	+	—	+
3	+	—	—	—	+	+
4	+	—	+	+	+	+
5	—	+	—	—	—	+
6	+	—	—	—	—	+
7	+	—	—	+	—	+

* Unfractionated PBMC from asthmatic and nonasthmatic subjects were evaluated for the presence (+) or absence (—) of Th1-type (IL-2, IFN- γ) cytokines, Th2-type (IL-4, IL-6, IL-10) cytokines, and IFN- α . Expression of cytokine-specific mRNA production was determined by Advantage One-Step RT-PCR (Clontech), as described in methods. nt: not tested.

Table 4. Doxycycline or Minocycline Inhibits IgE Production by Stimulated PBMC

Additions	IgE (ng/mL)	% Inhibition
Anti-CD40 + rhIL-4	28 ± 12	0
Anti-CD40 + rhIL-4 + 0.1 μ g doxycycline	20 ± 9	29
Anti-CD40 + rhIL-4 + 1.0 μ g doxycycline	14 ± 7	50
Anti-CD40 + rhIL-4 + 10.0 μ g doxycycline	5 ± 2	83
Anti-CD40 + rhIL-4 + 0.1 μ g minocycline	16 ± 5	43
Anti-CD40 + rhIL-4 + 1.0 μ g minocycline	18 ± 7	36
Anti-CD40 + rhIL-4 + 10.0 μ g minocycline	5 ± 2	83

* Supernatants were collected from 10-day cultured PBMC (1.5×10^6 /mL) stimulated with anti-CD40 mAb (1 μ g/mL) plus rhIL-4 (200 U/mL) with or without varying concentrations (0.1, 1.0, 10 μ g/mL, respectively) of doxycycline or minocycline. The supernatants were collected and IgE levels were measured by IgE ELISA Test Kit (Alexon Trend). Results are from five experiments, repeated three times, using PBMC obtained from five asthmatic subjects. Data are expressed as ng/mL ± SE. % Inhibition was calculated as follows: % Inhibition = 1 - (IgE production in each treatment group/IgE production of PBMC stimulated with anti-CD40 mAb plus rhIL-4 without treatment) $\times$ 100 (%).

crease (<2.5 ng/mL). No increases in IgE were detected when PBMC from either asthmatic or nonasthmatic subjects were cultured with either anti-CD40 mAb or rhIL-4 alone (<2.5 ng/mL), or with either anti-CD40 mAb or rhIL-4 alone in the presence of either minocycline or doxycycline (<2.5 ng/mL; data not shown).

When either minocycline or doxycycline were included in cultures with asthmatic PBMC and anti-CD40 mAb and rhIL-4, the high levels of IgE obtained on day 10 in the absence of these tetracyclines were strongly suppressed, in dose-dependent fashion (to >80% with 10 μ g/mL; Table 4). There was no change in IgE levels when either minocycline or doxycycline was included in cultures of nonasthmatic PBMC with anti-CD40 mAb and rhIL-4 (<2.5 ng/mL; Fig 1).

DISCUSSION

The present studies have demonstrated the ability of the tetracyclines, minocycline and doxycycline, to suppress anti-CD40 mAb and rhIL-4 mediated induction of IgE responses by PBMC obtained from asthmatic serum IgE+ atopic donors (asthmatic donors). Previous studies of asthmatic pa-

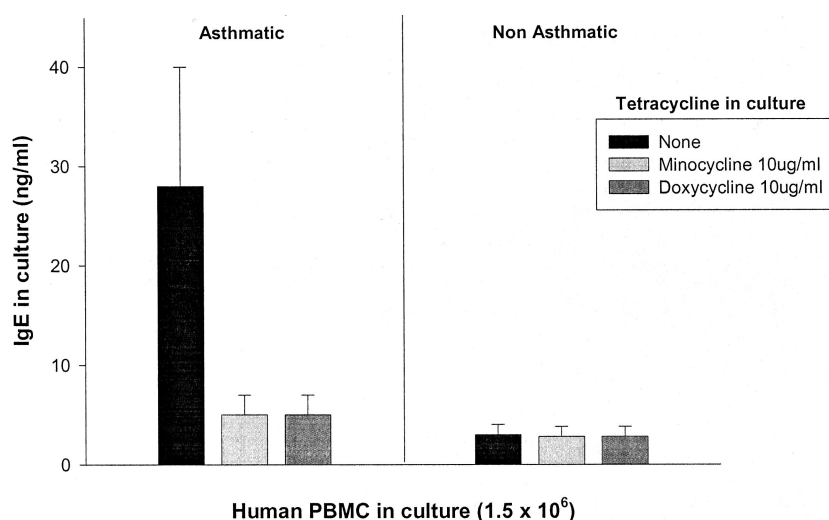


Figure 1. IgE production in vitro by PBMC from asthmatic and nonasthmatic subjects after tetracycline treatment. Human PBMC (1.5×10^6 /mL) were obtained from asthmatic and nonasthmatic subjects (see Methods) and cultured $\pm$ anti-CD40 mAb ($1 \mu\text{g}/\text{mL}$), $\pm$ rhIL-4 ($200 \text{ U}/\text{mL}$), in the presence or absence of minocycline ($10 \mu\text{g}/\text{mL}$) or doxycycline ($10 \mu\text{g}/\text{mL}$) for 10 days. High levels of IgE obtained on day 10 in the absence of tetracyclines were strongly suppressed when tetracyclines were included in cultures of PBMC from asthmatic patients. There was no change in IgE levels when tetracyclines were included in cultures of PBMC from nonasthmatic subjects. Results are from 12 experiments, repeated three times, using PBMC obtained from asthmatic subjects ($n = 5$) and nonasthmatic subjects ($n = 7$). Data are expressed as $\text{ng}/\text{mL} \pm$ standard error (SE).

tients treated with minocycline were notable for improvement of their asthmatic symptoms.⁶

In the present studies, anti-CD40/rhIL-4 induced five- to 10-fold higher IgE levels in culture supernatants of PBMC obtained from the asthmatic donors compared with PBMC of nonasthmatic, nonatopic serum IgE negative donors (nonasthmatic donors). This was true despite the fact that there were similar numbers of both CD19+ B-cells and CD4+ T cells in cultures of both donor groups (Table 2) on day 0. However, PBMC of the asthmatic donors contained 20 to 30% fewer CD8+ T cells than those of the nonasthmatic donors, with a concomitant increase in other cells in PBMC, which have not as yet been identified. Further studies will characterize these cells, which may include γ/δ T cells and natural killer cells. It has been reported that γ/δ T cells downregulate airway responsiveness to allergen challenge, possibly by controlling the "repair" response of the airway epithelium to α/β T cell-mediated damage.¹⁶

In asthma, both CD4 and CD8 T cell subsets are activated and their numbers are increased in the airways compared with nonasthmatic subjects.¹⁷⁻¹⁹ Studies by Schwarze et al²⁰ have reported that CD8 T cells are critical in mediating respiratory syncytial virus-induced development of lung eosinophilia and airway hyperresponsiveness after allergic airway sensitization in mice. In animal models, it has been reported that CD8+ T cells can attenuate allergic responses including IgE synthesis.¹ CD8+ T cells may provide suppressor function by exerting their various cytokines or through regulation of inflammatory mediators. Our results are the first to suggest that asthmatic patients may exhibit reduced CD8+ T cell suppressor function, thus leading to the perpetuation of CD4+ T cell-mediated inflammation and pro inflammatory (Th2) cytokines. Studies of McMenamin and Holt²¹ demonstrated that the natural immune response to inhaled protein antigens, especially in rats expressing the low IgE responder phenotype, includes a major histocompatibility complex class

I-restricted CD8+ T cell component, which is associated with active suppression of IgE antibody production.

PBMC obtained from asthmatic donors also differed from nonasthmatic donors with respect to expression of constitutive IL-2 and IL-10 mRNA, and in the levels of IgE they secreted in culture. IL-2 mRNA, which was expressed by PBMC of nonasthmatic donors (57%) was expressed by fewer asthmatic patients (17%), whereas IL-10 specific mRNA was expressed in the majority of asthmatic patients (78%), compared with nonasthmatic donors (29%), implicating the possibility of a reduced Th1-type response and/or an increased Th2-type response in asthmatic patients. In general, constitutive cytokine-specific mRNA expression for IL-4, IL-6, IFN- γ , IFN- α , and ϵ (not shown) by PBMC was similar for both groups (Table 3). It is well known that Th2-type cytokines play an important role in the immunologic processes of allergic asthma.³ However, the differences in processing and expression of these mRNA transcripts is unknown.

In the present study, the substantially higher levels of IgE secreted by PBMC of the asthmatic group in response to the nonspecific stimulus, anti-CD40/rhIL-4, suggest that different regulatory cells such as CD4+ Th2 cells,²² and macrophages^{23,24} may be present in the asthmatic compared with the nonasthmatic PBMC. Although it is well recognized that Th2-type cytokines (IL-4, IL-6, IL-10, respectively) favor IgE responses, and these asthmatic patients had high levels of serum IgE at the time at which their PBMC were obtained (Table 1), there were no detectable differences in mRNA expression of Th2 versus Th1-type cytokines by PBMC of the asthmatic versus nonasthmatic donors, except for IL-2 and IL-10 mRNA expression. However, other cytokines may have been secreted in vitro to account for the dramatic increase of IgE production by PBMC of the asthmatic group.

The significantly increased IgE production by asthmatic compared with nonasthmatic PBMC can not simply be attrib-

uted to increased numbers of either CD19+ B-cells or CD4+ T cells, as similar numbers were in PBMC of both groups. However, the possibility exists that PBMC of the asthmatic donors who produced IgE *in vivo* contained high numbers of IgE "memory" CD19+ B-cells, compared with CD19+ B-cells in PBMC of the nonasthmatic group. In this regard, it should be mentioned that PBMC of both groups expressed ϵ -specific mRNA (data not shown), but the exact numbers of CD19+ B-cells expressing ϵ mRNA have not been quantified. With respect to IL-4 as a potential helper cytokine, it should also be mentioned that optimal concentrations of rhIL-4 were used; this does not rule out the possibility that additional or other cytokines (eg, IL-13) were secreted by asthmatic PBMC in culture, or even that their CD19+ B-cells were more sensitive to the effects of IL-4 or other agents in culture.

In vitro and *in vivo* studies, in both animal and human models, have implicated IL-4 as an important inflammatory mediator in asthma.²⁵ IL-4 is also required for induction of IgE responses *in vitro*.²⁶ However, studies of Herrick et al²⁷ have shown that *in vitro* induction of hapten-specific memory IgE responses by spleen cells of benzylpenicilloyl-keyhole limpet hemocyanin (BPO-KLH)-sensitized mice was partially IL-4-independent. In their studies, the remaining 50% of the memory IgE response required IFN- α . Thus, another cytokine(s) may play a role(s) in induction of memory IgE responses.

Another Th2-type cytokine, IL-13, similar to IL-4, can induce IgE synthesis by human B-cells.²⁶ IL-13 induces IgE synthesis by PBMC as well as by purified B-cells obtained from PBMC in the presence of activated CD4+ T cell clones.²⁸ In addition, IL-13 induces B-cell proliferation and differentiation into IgE-secreting cells in the presence of the ligand for CD40,²⁹ and contributes to the induction of IgE switching and the maintenance of ongoing IgE synthesis *in vivo*.²⁸

Studies of Smith-Norowitz et al^{30,31} have demonstrated that *in vitro* induction of ragweed-specific memory IgE responses by PBMC obtained from atopic (ragweed-sensitized) donors required CD8+CD60+ T cells. Several cytokines, including IL-2, IL-4, IL-10, IL-12, IFN- α , and IFN- γ , were necessary in ragweed-specific memory IgE responses induced *in vitro*, as inclusion of antibodies to any of these cytokines in culture with PBMC of ragweed-sensitized donors in the presence of ragweed antigen, strongly suppressed IgE responses.^{30,31} It should also be noted that studies by Vukmanovic-Stejić and Vyas³² have shown that subpopulations of CD8+ T cell clones secrete IL-4, in addition to IFN- γ , thus providing "help" for IgE production.³²

The present studies have clearly demonstrated that minocycline and doxycycline suppress anti-CD40/rhIL-4 mediated induction of IgE responses by PBMC of asthmatic donors, in dose-dependent fashion, but had no effect on the lower IgE responses elicited by these agents from PBMC of the nonasthmatic donors (Fig 1). The mechanism(s) by which minocycline mediated suppression of anti-CD40/rhIL-4 induced

IgE responses in cultured human cells is under investigation. Because minocycline also suppressed *in vivo* IgE responses of the asthmatic patients from whom the PBMC were obtained (R. Joks, SUNY Downstate Medical Center, Brooklyn, NY, unpublished studies, 2000), it could be that similar mechanisms are in operation *in vivo* and *in vitro*.

One possible mechanism of action of tetracycline induced suppression may be that they interfere with the activities of adhesion/receptor molecules expressed on endothelial cells and/or leukocytes, thus inhibiting the biologic activities of other potent inflammatory mediators released by lymphocytes, such as IL-4. It could also be, that tetracyclines may inhibit the recruitment of inflammatory cells into the airways in response to allergen, thus serving as a protective mechanism to limit tissue damage. The clinical significance of understanding this system is that it is unique with respect to the regulatory mechanism controlling the IgE response(s), which is still not fully understood.

CONCLUSION

In the present studies, minocycline and doxycycline suppressed *in vitro* induction of IgE responses. Other agents previously shown to suppress IgE responses both *in vivo* and/or *in vitro* include: the neuropeptide substance P, which inhibits IgE production both *in vitro* and *in vivo* in mice sensitized to BPO-KLH³³; the bacterial cell wall products muramyl dipeptide and murabutide, which were fed to mice sensitized to this allergen, induce a dose-dependent, transient decrease in IgE specific to BPO-KLH³⁴; various suppressor factors, such as the mAb rhuMab-E25, which decreases both serum IgE levels as well as expression of basophil high-affinity IgE receptor Fc ϵ RI *in vivo*³⁵; and cytokines such as IL-8, which inhibits IgE production,³⁶ transforming growth factor- β , which acts directly on B-cells, and targets ϵ germ-line transcript expression,³⁷ IFN- α , IFN- γ , and IL-12, which also inhibit IgE secretion.³⁷⁻³⁹ The mechanism by which these agents accomplish their effects involves neural and endocrine pathways, with no clear mechanism as yet identified, either *in vivo* or *in vitro*. The mechanism by which minocycline-and/or doxycycline-suppressed IgE responses *in vitro* and *in vivo* remains to be elucidated. The present studies represent a model system in which the mechanism of tetracycline suppression can be identified.

ACKNOWLEDGMENTS

The authors thank Dr. R. Margolis, PhD, Computer Center, SUNY Downstate Medical Center, Brooklyn, NY, for his statistical computations. This work was supported by a SUNY Divisional grant and an AAUW Foundation Educational Research Publication grant.

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