

Effect of AA-2414, a thromboxane A₂ receptor antagonist, on airway inflammation in subjects with asthma

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Background: Asthma is a chronic inflammatory disease of the airways. The chemokines are potent chemoattractants for eosinophils and other types of cells associated with allergic inflammation. AA-2414, a new thromboxane A₂ receptor antagonist, reduces bronchial hyperresponsiveness in asthmatic subjects, but its mechanism of action is unclear.

Objective: We tested the hypothesis that the beneficial effects of AA-2414 in asthma result from reduction in the number of inflammatory cells infiltrating the airway associated with inhibition of chemokine release.

Methods: We studied bronchial biopsy specimens from 31 asthmatic subjects before and after oral treatment with AA-2414 (80 mg/day) or matched placebo for 4 months in a double-blind manner. Biopsy specimens were examined by immunohistochemistry. Each subject recorded symptom score and peak expiratory flow (PEF). Lung function and bronchial responsiveness to methacholine were measured before and after treatment.

Results: After treatment, significant improvements in symptom score ($P < .05$), PEF ($P < .01$), diurnal variation of PEF ($P < .01$), and bronchial responsiveness ($P < .01$) were observed in the AA-2414 group compared with the placebo group. These improvements were accompanied by a significant decrease in the number of submucosal EG2⁺ eosinophils ($P < .05$). There was also a reduction in the number of cells expressing RANTES ($P < .05$) and macrophage inflammatory protein (MIP)-1 α ($P < .05$) in the epithelium and of cells expressing monocyte chemoattractant protein-3 ($P < .01$), RANTES ($P < .05$), MIP-1 α ($P < .01$), and eotaxin ($P < .01$) in the submucosa in the AA-2414 treatment group. A significant correlation was found between the number of EG2⁺ eosinophils and numbers of monocyte chemoattractant protein-3⁺ ($r_s = 0.52$, $P < .005$), MIP-1 α ⁺ ($r_s = 0.34$, $P < .05$), and eotaxin⁺ cells ($r_s = 0.47$, $P < .01$) in the submucosa. There was a significant negative correlation between the increase in bronchial responsiveness and the change in number of submucosal EG2⁺ cells ($r_s = -0.65$, $P < .001$).

Conclusions: These findings suggest that AA-2414 treatment of patients with asthma may inhibit activated eosinophil infiltration in part by modulating the expression of chemokines in bronchial tissues. (*J Allergy Clin Immunol* 1999;103:1054-61.)

Key words: Thromboxane A₂, airway inflammation, chemokines, bronchial hyperresponsiveness

Bronchial asthma is a chronic inflammatory disorder characterized by increased infiltration of eosinophils, T lymphocytes, and mast cells into the bronchial mucosa.¹⁻⁴ It is believed that eosinophils play a crucial role in the development of chronic bronchial asthma. Thromboxane A₂ (TXA₂) is a chemical mediator and a product of arachidonate metabolism.⁵ TXA₂ has been considered an important mediator in the pathophysiology of asthma because of its potent bronchoconstrictive activity,⁶ and it is believed to be involved not only in late asthmatic responses⁷ but also in airway hyperresponsiveness.⁸ A topically administered TXA₂ mimetic, U-46619, caused eosinophil infiltration in the conjunctival epithelium of guinea pigs.⁹ Takami et al¹⁰ recently found excellent correlations among sephadex-induced eosinophilia in rat lung, TXA₂ release, and nonspecific airway hyperresponsiveness. Moreover, there is accumulating evidence suggesting that TXA₂ is an immunomodulator. Antigen-presenting cells, such as macrophages and dendritic cells in the spleen and thymus, also express TX synthetase.¹¹ On the other hand, TX receptor mRNA is abundantly expressed in lung, thymic, and splenic T cells.¹² Thus antigen-presenting cells express TXA₂ synthetase, whereas T cells express TX receptor, suggesting that TX receptor is involved in, and modulates, T-cell responses. CS-518, a TXA₂ synthetase inhibitor, inhibited not only antigen-induced eosinophil accumulation in bronchoalveolar lavage fluid (BALF) but also eosinophil activation of actively sensitized guinea pigs.¹³ DP-1904, a TXA₂ synthetase inhibitor, attenuated platelet-activating factor or IgG-induced eosinophil cationic protein release from human eosinophils.¹⁴ Recently, it was reported that either TXA₂ synthetase inhibitor or TXA₂ receptor antagonist significantly inhibited BALF eosinophilia by modulation of suppression of cytokine production in antigen-sensitized mice.¹⁵ Moreover, this effect was stronger with TXA₂ receptor antagonist than with TXA₂ synthetase inhibitor.

AA-2414 ([$\pm$]-7-[3,5,6-Trimethyl-1,4-benzoquinon-2-yl]-7-phenylheptanoic acid), a novel TXA₂ receptor antagonist, is a benzoquinone derivative.¹⁶ AA-2414 has been shown to inhibit bronchoconstriction induced by a TXA₂ mimetic, U-46619, in guinea pigs.¹⁷ It was recently reported that AA-2414 inhibited IL-4 and IL-5 production in splenic mononuclear cells in mice.¹⁸ Furthermore, in a clinical study, Fujimura et al¹⁹ reported the finding of sig-

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Abbreviations used

BALF:	Bronchoalveolar lavage fluid
Dmin:	Minimum cumulative dose
MCP:	Monocyte chemotactic protein
MIP:	Macrophage inflammatory protein
PEF:	Peak expiratory flow
Rrs:	Respiratory resistance
TXA ₂ :	Thromboxane A ₂

nificant improvement of bronchial airway response to methacholine after administration of AA-2414.

The past few years have seen the discovery of a group of cytokines with similarities in structure, the principal activities of which appear to include chemoattraction and activation of a wide range of leukocytes, including granulocytes, monocytes, and T lymphocytes. For this reason, they have been termed *chemotactic cytokines* or *chemokines*.²⁰ Because both eosinophils and TXA₂ appear to play critical roles in the pathogenesis of bronchial asthma, the question arises whether TXA₂ regulates the chemotaxis or function of eosinophils.

We hypothesized that the improvement by AA-2414 of bronchial hyperresponsiveness in asthmatic subjects results in part from downregulation of release of chemokines from many types of inflammatory cells. This study was designed to examine the effects of treatment with AA-2414 on the expression of chemokines and on the number of inflammatory cells in asthmatic mucosa.

METHODS

Subjects

Thirty-one nonsmoking volunteers with mild-to-moderate asthma (age range, 16 to 54 years) were recruited for this study. All patients satisfied the American Thoracic Society Criteria for asthma.²¹ All had documented reversible airflow obstruction (a 20% improvement in peak expiratory flow [PEF] or FEV₁ either spontaneously or in response to inhaled β_2 -agonists) and methacholine airway hyperresponsiveness. None had received inhaled or oral corticosteroids or any other anti-inflammatory drugs, such as sodium cromoglycate or nedocromil sodium, in the previous 4 months. Only inhaled salbutamol was used as required as a bronchodilator. Patients showed no signs of upper or lower respiratory tract disease for at least 2 weeks before entering the study. The study was approved by the Ethics Committee of Toho University School of Medicine, and signed informed consent was obtained from each patient.

Study design

Patients were randomly assigned to one of 2 parallel treatment groups. Each patient received a 4-month course of oral administration of either AA-2414 (80 mg/day; Takeda Chemical Industries Ltd, Osaka, Japan) or matched placebo every evening. Skin prick tests to common aeroallergens were performed, and blood was taken for measurement of total and specific IgE (Phadebas RAST, Uppsala, Sweden). A diary card tracking asthma symptoms, medication requirements, and results of PEF performed twice daily with a Personal Best Peak Flowmeter (Health Scan, Cedar Grove, NJ) was completed by each subject. Eight symptom scores were recorded daily on the diary card: nocturnal wheezing, nocturnal cough,

TABLE I. Subject characteristics

	AA-2414	Placebo
No. of subjects (M:F)	16 (10:6)	15 (10:5)
Age (y)	31 (16-54)	29 (16-50)
FEV ₁ (L)	2.65 (1.54-3.34)	2.44 (1.68-3.26)
FEV ₁ (% predicted)	72.9 (57.9-85.3)	71.7 (55.6-85.3)
AHR (units)	1.18 (0.12-3.32)	1.08 (0.30-3.23)
IgE (IU/mL)	753.0 (28.6-2618.4)	756.4 (33.0-1150.0)

Data are expressed as means (ranges).

AHR, Airway hyperresponsiveness measured as the minimum cumulative value; units, 1 minute of 1.0 mg/mL aerosol inhalation of methacholine.

morning chest tightness, wheezing during the day, limitation of activity, and the effects of exercise, cold air, and dust on asthma symptoms. A scoring system ranging from 0 to 3 was used as follows: 0, none; 1, mild; 2, moderate; and 3, severe. Seven days after the initial visit, patients returned having omitted inhaled β_2 -agonist for at least 12 hours. Spirometry was performed, and airway methacholine responsiveness was measured by using an "Astograph" (TCK-6000CV; Chest MI Co, Tokyo, Japan), according to the method of Takishima et al.²² Briefly, respiratory resistance (Rrs) was measured by the forced oscillation method (3 Hz) during inhalation of methacholine continuously with stepwise incremental concentrations, until Rrs reached twice the baseline value. Methacholine chloride was diluted in physiologic saline solution with 2-fold increasing concentrations from 49 to 25,000 μ g/mL. Each concentration of methacholine solution was inhaled for a period of 1 minute. All subjects were examined during quiet breathing in a sitting position. Bronchial hyperresponsiveness was evaluated as the minimum cumulative dose (Dmin) of methacholine for which the reciprocal of Rrs decreased linearly. Dmin was calculated so that 1 unit of Dmin equaled 1 minute of inhalation of aerosol solution at 1.0 mg/mL. All patients with bronchial asthma had Dmin less than 50 units. Seven days later, patients returned, and fiberoptic bronchoscopy was performed. Four months later, patients returned to the hospital for repeat measurement of spirometry, airway responsiveness, blood examination, and fiberoptic bronchoscopy.

Fiberoptic bronchoscopy

Premedication was provided by intramuscular injection of 0.5 mg of atropine sulphate and 15 mg of pentazocine. After the throat had been anaesthetized with 4% lidocaine spray, the subjects inhaled 200 μ g of salbutamol to prevent bronchoconstriction. A bronchoscope (BF type 1T-30; Olympus Co, Tokyo, Japan) was inserted through the mouth. The pharynx, trachea, and bronchi were anaesthetized with 2% lidocaine. Oxygen was administered at 4 L/min through the nasal cannulae throughout the procedure. Biopsy forceps (FB-15C, Olympus) were used to collect 2 to 3 biopsy specimens from the spur of a basal segment bronchus of the right lower lobe and from the subcarina of the right upper lobe. Inhalation of 200 μ g of salbutamol was performed after the procedure.

Sample processing and immunohistochemistry

Biopsy specimens were mounted in an OCT compound (Miles, Scientific, Naperville, Ill), frozen rapidly in dry ice/acetone, and stored at -70°C until use. Four-micrometer cryostat serial sections were cut, fixed in acetone, and stained with hematoxylin and eosin for conventional histologic examination. The sections were also immunostained by using the alkaline phosphatase antialkaline phosphatase system (Dako, Glostrup, Denmark),²³ according to the

TABLE II. Clinical data before and after treatment with AA-2414 or placebo

	AA-2414		Placebo	
	Before	After	Before	After
Symptom score	6.4 ± 2.5	4.8 ± 2.0**	6.3 ± 3.0	6.8 ± 3.1
PEF (L/min)	364.2 ± 108.6	405.1 ± 108.0**‡	332.6 ± 62.9	325.4 ± 62.4
PEF variation (%)	24.0 ± 10.3	17.4 ± 7.8*‡	22.8 ± 9.8	24.1 ± 8.8
FEV ₁ (L)	2.65 ± 0.61	2.88 ± 0.60	2.44 ± 0.45	2.42 ± 0.49
FEV ₁ (% predicted)	72.9 ± 9.6	75.3 ± 10.0	71.7 ± 9.3	69.7 ± 8.3
AHR (units)	1.18 ± 0.78	2.96 ± 2.00*‡	1.08 ± 0.75	0.99 ± 0.75

Data are expressed as means ± SD.

PEF variation, Diurnal variation of PEF; AHR, airway hyperresponsiveness measured as the minimum cumulative value; units, 1 minute of 1.0 mg/mL aerosol inhalation of methacholine.

* $P < .01$ for comparison between values before and after treatment (within group).

‡ $P < .05$ or $P < .01$ for comparison of treatment effects on values (before minus after treatment) for AA-2414 versus placebo.

manufacturer's instructions, and developed with fast red substrate. Finally, sections were rinsed with distilled water, counterstained with Mayer's hematoxylin, and dried by brief consecutive dips in ethyl alcohol followed by xylene; coverslips were mounted. Control slides were performed by substitution of PBS and incubation with an irrelevant mAb of the same isotype and concentration.

Detection of inflammatory cells. Anti-major basic protein antibody (BMK-13; Harlan Sera-Lab, Hophurst Lanc, UK) was used to stain total eosinophils. The degree of "activation" of infiltrating eosinophils was assessed by immunostaining with EG2 mAb (Kabi Pharmacia, Uppsala, Sweden), which stains only activated eosinophil cationic protein-secreting eosinophils. AA1 (Dako), developed against human mast cell tryptase, was applied for detection of mast cells. T lymphocytes were stained with anti-CD3 (Becton Dickinson, Cowley, UK), helper T cells with anti-CD4 (Becton Dickinson), cytotoxic T cells with anti-CD8 (Becton Dickinson), and macrophages with anti-CD68 (Dako).

Detection of chemokines. Immunolocalization of chemokines in bronchial tissue was examined with mAbs against monocyte chemotactic protein (MCP)-3 (R&D Systems, Abington, UK), against RANTES (R&D), against macrophage inflammatory protein (MIP)-1 α (R&D), and against eotaxin (R&D).

Quantitation. To avoid observer bias, all microscopic slides were coded before analysis by one observer and read blind. All measurements were performed in at least 2 nonserial sections per biopsy specimen. Subepithelial chemokine⁺ cell counts were made per millimeter of basement membrane length. The numbers of inflammatory and chemokine⁺ cells were counted for the entire submucosal area beneath the epithelial basement membrane, excluding mucosal glands and vessels, at a magnification of $\times 400$. The total area examined was calculated by delineating the area of the section on a video micrometer system (VM-50, Olympus), using software for measurements (Analytical Measuring System, Olympus). Results were expressed as the number of positive cells per square millimeter of submucosa.

Statistical analysis

Clinical data are expressed as means ± SD. A mean symptom score was calculated from the daily mean of the sum of the 8 symptoms for both the 2 weeks of the baseline period and the last 2 weeks of the treatment period. The mean daily PEF was calculated from the mean of the morning and evening readings, and means were calculated for the 2 weeks of the baseline period and the last 2 weeks of treatment. Diurnal variation of PEF was calculated from the difference between the morning and evening readings and expressed as a percentage of the daily mean. The results for clinical data were normally distributed and were analyzed by using the paired Student *t* test for within-group comparisons and the unpaired

Student *t* test for between-group comparisons. The number of positive cells exhibited a positive skewed distribution. Nonparametric data were expressed as median values and ranges. Paired data for cell counts were analyzed by using the Wilcoxon matched-pairs signed-rank test. For between-group comparisons, the Mann-Whitney *U* test was performed on the Δ values (pretreatment minus post-treatment) for the 2 treatment groups. Changes in numbers of different cell types were also compared by using a *t* test, with Bonferroni correction to allow for multiple comparisons. Intraobserver reproducibility was assessed with the coefficient of variation, and at least 3 replicate measurements of morphometric parameters were performed by the same observer. The mean coefficient of variation for repeated measurements ranged between 5% and 14% for each cell and between 9% and 15% for each chemokine. Correlations between individual data were tested for significance by using Spearman's rank correlation test and corrected for multiple correlations. Probability (*P*) values of less than .05 were considered significant.

RESULTS

Sixteen subjects in the AA-2414 group and 15 subjects in the placebo group completed the study. There was no significant difference between the 2 groups in sex, age, FEV₁, Dmin, or IgE (Table I). Two patients from the AA-2414 group were discharged for poor compliance with therapy (serum concentration of AA-2414 < 10 ng/mL). The mean concentration of AA-2414 was 6394.4 ng/mL and ranged from 3929 to 10,036 ng/mL in the actively treated group. There were no adverse effects of treatment, including bleeding tendency and blood chemistry values. Hematologic examination revealed that platelet count, bleeding time, and whole blood clotting time were normal. Liver and kidney function tests were also within normal limits. Three patients in the placebo group did not complete the study: 2 experienced deterioration of asthma that required steroids or theophylline, and 1 refused to submit to a second bronchoscopy.

Clinical data

After treatment, symptom score ($P < .01$), PEF ($P < .01$), diurnal variation of PEF ($P < .01$), and Dmin ($P < .01$) were significantly improved from baseline in the AA-2414-treated, but not in the placebo-treated, subjects. There was no change in FEV₁ before to after treatment with AA-2414. When the AA-2414- and placebo-treated groups were compared, significant improvements

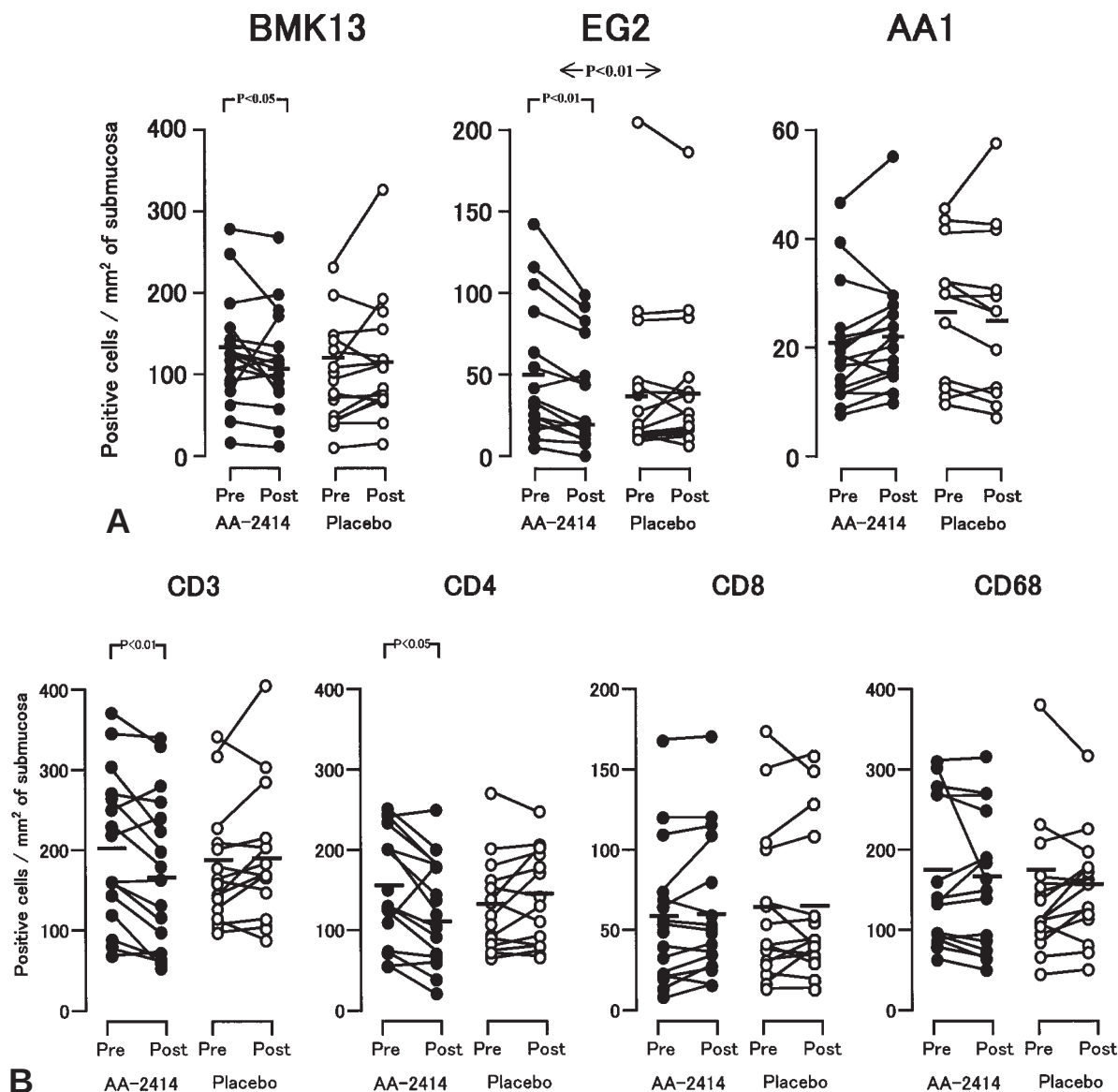


FIG 1. A, Cellular infiltration with total eosinophils (BMK13⁺), activated eosinophils (EG2⁺), and mast cells (AA1⁺) in bronchial mucosa before and after treatment with AA-2414 and placebo. *Horizontal bars represent median values. B,* Cellular infiltration with T lymphocytes (CD3⁺, CD4⁺, and CD8⁺) and macrophages (CD68⁺) in bronchial mucosa before and after treatment with AA-2414 and placebo. *Horizontal bars represent median values.*

TABLE III. Numbers of immunoreactive cells in bronchial specimens from AA-2414- and placebo-treated subjects

	BMK13	EG2	AA1	CD3	CD4	CD8	CD68
AA-2414							
Before treatment	134 (17-280)	50 (7-143)	20.5 (8-47)	202.5 (70-370)	154.5 (56-250)	58 (8-168)	176 (63-310)
After treatment	113* (15-270)	21.5†‡ (2-100)	22 (12-55)	175† (55-340)	126* (22-250)	63 (16-170)	168 (50-315)
Placebo							
Before treatment	121 (13-235)	43 (10-205)	26.5 (10-46)	187 (100-342)	131 (68-275)	64.5 (15-175)	174 (48-385)
After treatment	116.5 (16-330)	44 (7-187)	25 (8-58)	192 (90-405)	146 (70-250)	65 (14-160)	156 (55-320)

Data are expressed as medians (ranges) and represented number of cells per square millimeter of mucosa.

BMK13, Identified total eosinophils; EG2, "activated" eosinophils; AA1, mast cells; CD3, mature T cells; CD4, helper/inducer T cells; CD8, suppressor/cytotoxic T cells; CD68, macrophages.

*P < .05 and †P < .01 for comparison of values before and after treatment (within group).

‡P < .01 for comparison of treatment effects on values (before minus after treatment) for AA-2414 versus placebo.

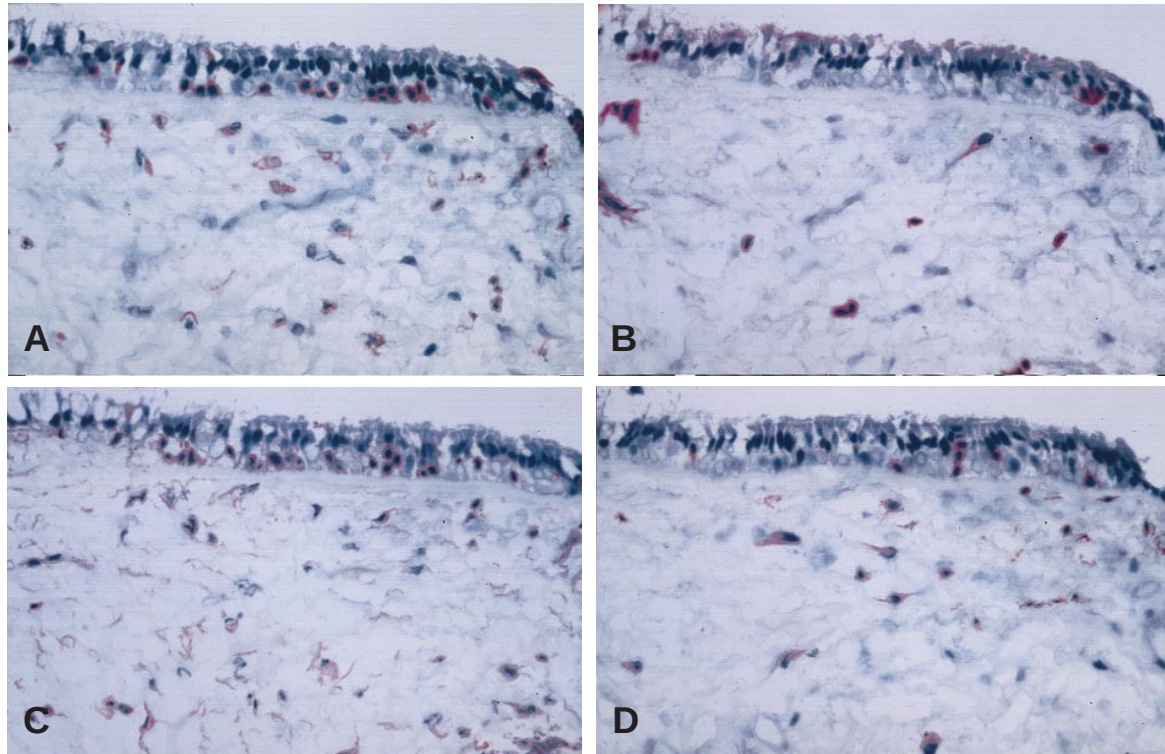


FIG 2. Positive staining for MCP-3+ (**A**), RANTES+ (**B**), MIP-1 α + (**C**), and eotaxin+ (**D**) cells of both the epithelium and submucosa in frozen sections of biopsy specimens before treatment.

TABLE IV. Counts of cells staining with anti-MCP-3, anti-RANTES, anti-MIP-1 α , and anti-eotaxin in bronchial biopsy specimens

	MCP-3	RANTES	MIP-1 α	Eotaxin
Epithelium				
AA-2414				
Before treatment	15 (2-28)	4 (0-10)	15 (7-40)	8 (2-33)
After treatment	11 [†] (3-20)	2* [‡] (0-5)	8 ^{†‡} (1-33)	5.5 [†] (0-15)
Placebo				
Before treatment	14 (2-27)	3 (0-8)	14 (2-30)	7.5 (1-21)
After treatment	14 (2-30)	3.5 (1-10)	16.5 (3-30)	8 (0-18)
Submucosa				
AA-2414				
Before treatment	16 (6-24)	4.5 (1-10)	22.5 (5-40)	17.5 (7-37)
After treatment	11 ^{†§} (2-31)	3* [‡] (0-9)	14.5 ^{†§} (6-33)	12.5 ^{†§} (2-28)
Placebo				
Before treatment	14 (3-32)	4 (1-8)	23 (4-44)	18 (6-43)
After treatment	13.5 (3-32)	5 (1-10)	25 (6-48)	19 (8-40)

Data are expressed as medians (ranges) per millimeter of basement membrane length of epithelium and per square millimeter of submucosa.

* $P < .05$ or $^{\dagger}P < .01$ for comparison of values before and after treatment (within group).

$^{\ddagger}P < .05$ or $^{\S}P < .01$ for comparison of treatment effects on values (before minus after treatment) for AA-2414 versus placebo.

in symptom score ($P < .05$), PEF ($P < .01$), PEF variation ($P < .01$), and Dmin ($P < .01$) were found in the former (Table II).

Bronchial biopsies

At baseline, there were no significant differences between the subjects receiving AA-2414 and those undergoing placebo treatment in bronchial cell pheno-

type immunohistochemistry. Within the AA-2414-treated group, there were significant reductions in median counts of submucosal BMK⁺ eosinophils ($P < .05$), EG2⁺ eosinophils ($P < .01$), CD3⁺ T lymphocytes ($P < .01$), and CD4⁺ T lymphocytes ($P < .05$) before to after treatment. Within the placebo-treated group, there was no significant change in any cell count before to after treatment. Comparison between the treatment groups

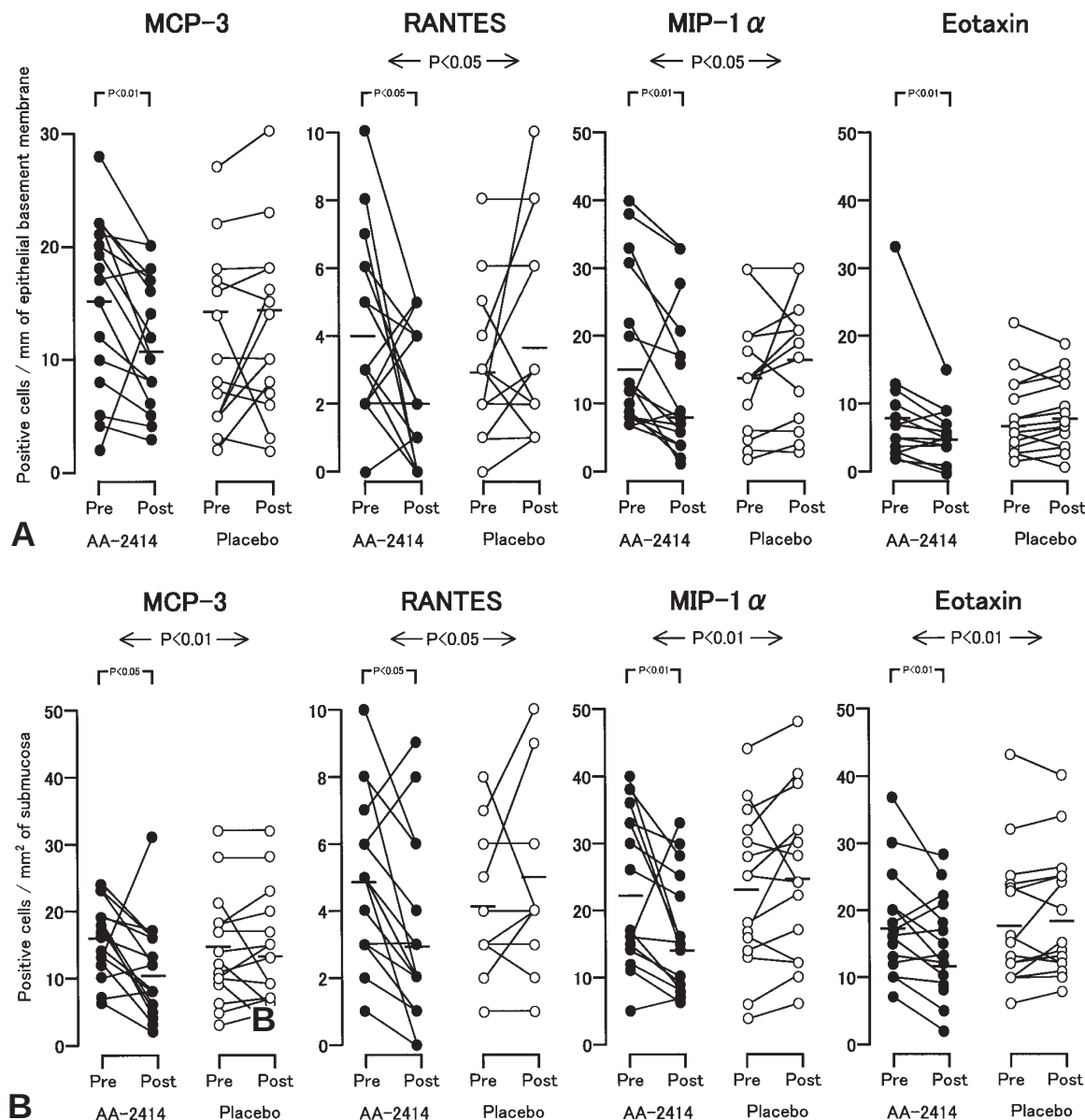


FIG 3. Effect of treatment on epithelial (**A**) and submucosal (**B**) counts of MCP-3⁺, RANTES⁺, MIP-1 α ⁺, and eotaxin⁺ cells. Horizontal bars represent median values.

revealed that the difference in submucosal EG2⁺ eosinophils was significant ($P < .01$) (Table III and Fig 1, A and B).

Immunostaining for MCP-3, RANTES, MIP-1 α , and eotaxin in bronchial biopsy specimens was localized to the epithelium, some inflammatory cells, and endothelial cells (Fig 2). In subjects treated with AA-2414, there were significant reductions in median epithelial counts of MCP-3⁺ cells ($P < .01$), RANTES⁺ cells ($P < .05$), MIP-1 α ⁺ cells ($P < .01$), and eotaxin⁺ cells ($P < .01$) and in submucosal cell counts of MCP-3⁺ cells ($P < .01$), RANTES⁺ cells ($P < .05$), MIP-1 α ⁺ cells ($P < .01$), and eotaxin⁺ cells ($P < .01$). No significant change before to

after treatment was observed in cell numbers in biopsy specimens from the placebo-treated group. When the changes in cell counts for subjects treated with AA-2414 and those treated with placebo were compared, significant reductions in epithelial RANTES⁺ ($P < .05$) and MIP-1 α ⁺ cells ($P < .05$) (Fig 3, A) and in submucosal MCP-3⁺ ($P < .01$), RANTES⁺ ($P < .05$), MIP-1 α ⁺ ($P < .01$), and eotaxin⁺ cells ($P < .01$) were found in the former (Fig 3, B, and Table IV). The numbers of submucosal MCP-3⁺ ($r_s = 0.52$, $P < .005$), MIP-1 α ⁺ ($r_s = 0.34$, $P < .05$), and eotaxin⁺ cells ($r_s = 0.47$, $P < .01$) were significantly correlated with number of EG2⁺ cells. There was a significant negative correlation between the

increase in Dmin and the magnitude of change in the number of submucosal EG2⁺ cells ($r_s = -0.65$, $P < .001$).

DISCUSSION

In this double-blind, placebo-controlled study, clinical improvement in asthma after treatment with AA-2414 was accompanied by a decrease of EG2⁺ eosinophil infiltration in the bronchial mucosa. This change was associated with reductions in epithelial RANTES⁺ and MIP-1 α ⁺ cells and in submucosal MCP-3⁺, RANTES⁺, MIP-1 α ⁺, and eotaxin⁺ cells, supporting the hypothesis that the clinical effects of AA-2414 in asthma result at least in part from modulation of proinflammatory chemokines and thus reduction of bronchial mucosal inflammation. These findings indicate that AA-2414 reduced eosinophil activation rather than number. The precise mechanisms by which AA-2414 inhibits activated eosinophil recruitment are unclear. However, our findings suggest that the suppression of chemokine expression is involved in this inhibition. The increased expression of cytokines and chemokines relevant to the specific accumulation of eosinophils proved that these mediators play a role in specific eosinophil recruitment in asthma.²⁴ However, our findings suggest the interesting possibility that chemokines may not be required for eosinophil recruitment but are important for eosinophil activity. This hypothesis is supported by the intriguing correlation between the number of EG2⁺ cells and the number of cells staining for chemokines and by the correlation between reduction in EG2⁺ cell numbers and improvement in bronchial sensitivity. Also supporting this hypothesis is the finding that numbers of other types of cells (monocytes, T lymphocytes, and eosinophils) were not significantly reduced by AA-2414 treatment, despite reduction in the number of cells expressing chemokines.

The effects of inhibition of TXA₂ on cytokine production by human alveolar macrophages²⁵ and monocytes²⁶ have been reported. The production of IL-5, IL-2, and IFN- γ by antigen-stimulated splenic mononuclear cells was significantly decreased by treatment with TXA₂ inhibitors.¹⁵ CS-518, a TXA₂ synthetase inhibitor, inhibited not only eosinophil accumulation in BALF, but eosinophil density, which shifted from normodense to hypodense after allergen challenge in guinea pigs, and suppressed eosinophil peroxidase release and chemotaxis by platelet-activating factor in vitro.¹³ Recently, Narita et al²⁷ reported that the eosinophil infiltration into nasal mucosa induced by antigen was inhibited by Bay u 3405, a TXA₂ receptor antagonist, in sensitized guinea pigs. Although the reason for the discrepancy between their findings and those of the present study is unclear, the drugs and species used may account for the difference. In the absence of double-staining procedures, we cannot define the cellular origins of chemokines in this study. Foegh et al²⁸ demonstrated that human peritoneal eosinophils synthesize cyclooxygenase products under basal and stimulated conditions, with TXA₂ predominating. Possible source of TXA₂ include epithelial²⁹⁻³¹ and

endothelial cells,³² and inflammatory cells, such as eosinophils,^{33,34} T lymphocytes, and macrophages,³⁵⁻³⁷ are likely candidates for release of chemokines. It would be interesting to identify the cell populations responsible for the in situ production and release of chemokines with eosinophil chemotactic properties.

Variability in PEF mirrors the clinical severity of asthma and is correlated with bronchial hyperresponsiveness.³⁸ The reduction in asthma symptoms, diurnal variability in PEF, and increase in PEF reflect improvement in asthma control. Because the serum concentrations of AA-2414 obtained in this study were high enough for treatment of asthma, we believe that the improvements in clinical findings we found were the result of AA-2414 administration. Our results support the previous finding that AA-2414 resulted in significant improvement of bronchial responsiveness but did not change baseline value of FEV₁.¹⁹

In conclusion, we have demonstrated that improvement of clinical findings after AA-2414 treatment of asthma is associated with a reduction in the number of activated eosinophils in bronchial mucosa. Although further investigation is needed, our findings suggest that AA-2414 may affect asthma by modulating activated eosinophil infiltration into airways and release of proinflammatory chemokines.

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