

LEUKOTRIENE-RECEPTOR EXPRESSION ON NASAL MUCOSAL INFLAMMATORY CELLS IN ASPIRIN-SENSITIVE RHINOSINUSITIS

ANA R. SOUSA, PH.D., ABHI PARIKH, M.S., GLENIS SCADDING, M.D., CHRISTOPHER J. CORRIGAN, M.D., PH.D.,
AND TAK H. LEE, M.D., SC.D.

ABSTRACT

Background Patients with asthma who have aspirin sensitivity have greater cysteinyl leukotriene production and greater airway hyperresponsiveness to the effects of inhaled cysteinyl leukotrienes than their aspirin-tolerant counterparts. We hypothesized that the latter effect reflects elevated expression of the cysteinyl leukotriene receptor CysLT₁ on inflammatory cells in the target organ and that its expression is down-regulated by aspirin desensitization.

Methods We obtained nasal-biopsy specimens from 22 aspirin-sensitive and 12 non-aspirin-sensitive patients with chronic rhinosinusitis and nasal polyps. Additional specimens were then obtained from subgroups of the aspirin-sensitive patients after intranasal application of lysine aspirin or placebo for two weeks (five and four patients, respectively) or for six months (five and four patients, respectively). The numbers of leukocytes expressing the CysLT₁ and leukotriene B₄ (LTB₄) receptors per unit area of sections of the nasal submucosa were determined by immunohistochemistry.

Results The absolute number of cells expressing the CysLT₁ receptor was significantly higher in the aspirin-sensitive patients than in the non-aspirin-sensitive patients (median, 542 cells per square millimeter [range, 148 to 1390] vs. 116 cells per square millimeter [range, 40 to 259]; $P < 0.001$). The percentage of CD45+ leukocytes expressing the CysLT₁ receptor was also higher in the aspirin-sensitive subjects (25 percent of CD45+ leukocytes [range, 4 to 50] vs. 5 percent of CD45+ leukocytes [range, 2 to 11]; $P < 0.001$); the percentage of CD45+ leukocytes expressing the LTB₄ receptor did not differ significantly between these two groups. Desensitization was associated with a decrease in the numbers of inflammatory cells expressing CysLT₁.

Conclusions The elevated numbers of nasal inflammatory leukocytes expressing the CysLT₁ receptor in aspirin-sensitive patients with chronic rhinosinusitis as compared with their non-aspirin-sensitive counterparts and the down-regulation of receptor expression after desensitization to aspirin are probably fundamental in the pathogenesis of aspirin sensitivity and in the mechanism of aspirin desensitization. (N Engl J Med 2002;347:1493-9.)

Copyright © 2002 Massachusetts Medical Society.

PATIENTS with idiosyncratic reactions to aspirin ingestion characteristically have what has become known as Samter's triad of aspirin sensitivity, asthma, and nasal polyposis.¹ A fundamental defect in aspirin sensitivity is excessive production of cysteinyl leukotrienes, both at base line^{2,3} and after aspirin challenge.²⁻⁷ These mediators are derived from arachidonic acid by the actions of 5-lipoxygenase and leukotriene C₄ synthase.⁸ Leukotriene B₄ (LTB₄) is a dihydroxy leukotriene that is produced in similar amounts in patients with aspirin sensitivity and those without it.

Enhanced activity of key synthetic enzymes,⁹ perhaps genetically determined,¹⁰ has been implicated as the cause of the elevated production of cysteinyl leukotrienes in patients with aspirin-sensitive asthma. The cellular sources of cysteinyl leukotrienes may include mast cells and eosinophils,^{6,11,12} which are increased in number in bronchial-biopsy specimens from aspirin-sensitive persons with asthma.^{9,13} The cysteinyl leukotrienes and LTB₄ exert their effects through distinct, G protein-coupled, high-affinity receptors termed CysLT₁ and CysLT₂,^{14,15} and through the LTB₄ receptor. Most of the proinflammatory actions of the cysteinyl leukotrienes are mediated by their binding to the CysLT₁ receptor.^{8,16-18} CysLT₁-receptor antagonists attenuate the asthma elicited by aspirin challenge in aspirin-sensitive subjects.¹⁹

In addition to enhanced production of the leukotrienes, aspirin-sensitive patients with asthma have greater airway hyperresponsiveness on inhalation challenge with leukotriene E₄ than non-aspirin-sensitive patients.²⁰ These data suggest the presence of augmented target-organ responsiveness to the cysteinyl leukotrienes.

Aspirin-sensitive patients can be desensitized by oral administration of incremental doses of aspirin or by topical administration of soluble lysine aspirin.^{21,22} Desensitization of aspirin-sensitive patients to orally administered aspirin is associated with reduced responsiveness to inhaled cysteinyl leukotrienes and only partial attenuation of endogenous production of these leukotrienes.^{20,23} We therefore hypothesized that the CysLT₁ receptor is overexpressed in the mucosal in-

From Guy's, King's and St. Thomas' School of Medicine (A.R.S., C.J.C., T.H.L.) and the Royal National Throat, Nose and Ear Hospital (A.P., G.S.) — both in London. Address reprint requests to Dr. Lee at the Department of Asthma, Allergy and Respiratory Science, Guy's Hospital, London SE1 9RT, United Kingdom, or at tak.lee@kcl.ac.uk.

flammatory infiltrate in these patients and that desensitization is accompanied by down-regulation of its expression.

To test these hypotheses, we compared the expression of the CysLT₁ receptor with that of the LTB₄ receptor on inflammatory leukocytes in nasal-biopsy specimens from aspirin-sensitive and non-aspirin-sensitive patients with rhinosinusitis. In several aspirin-sensitive subjects, additional biopsy specimens were obtained after two weeks or six months of desensitization with topical lysine aspirin or placebo.

METHODS

Base-Line Measurements

Nasal-biopsy specimens were obtained from 22 aspirin-sensitive patients (median age, 38 years [range, 20 to 68]), 7 of whom were men, and from 12 non-aspirin-sensitive patients (median age, 50 years [range, 21 to 69]), 7 of whom were men. Twenty of the 22 aspirin-sensitive patients and 8 of the 12 non-aspirin-sensitive patients had asthma, which was defined as a clinical history of episodic wheezing and reversibility of the forced expiratory volume in one second or peak expiratory flow rate by at least 15 percent, either spontaneously or after inhalation of 400 μ g of albuterol. Eighteen of the 22 aspirin-sensitive patients and 6 of the 12 non-aspirin-sensitive patients also had atopy, as determined by a positive skin-prick test to 1 or more of a panel of 10 standard aeroallergens in the presence of a positive histamine test and a negative test with diluent (as a control).

Trial of Aspirin Desensitization

Eighteen of the 22 aspirin-sensitive patients took part in a randomized, double-blind, placebo-controlled trial of topical desensitization with intranasal lysine aspirin. To be included in this trial, patients had to be older than 18 years of age and had to have proven sensitivity to aspirin and recurrent nasal polyposis. Those with asthma used inhaled corticosteroids to control their asthma and continued to take their medication at the usual dosages; those requiring oral corticosteroids to control their asthma was excluded from the trial. Use of intranasal corticosteroids or any other medication to control polyp growth was not permitted during the study period. The trial was explained in detail to all the patients, and they were advised that they might receive either active drug or diluent (placebo) for therapy.

The participating patients took part in either a two-week trial or a six-month trial. In the six-month study, each participant was seen in the research clinic once every six weeks. Participants who had a mild reaction during treatment were advised to take an antihistamine and to document both the reaction and the use of the antihistamine in a study diary. If the reaction was severe, they were advised to contact one of the investigators, who would then offer suggestions about treatment options and make a decision about whether to withdraw the patient from the study. Ethical approval for the study was obtained from the ethics committee at the Royal National Throat, Nose and Ear Hospital in London. Written informed consent was obtained from all the patients.

Diagnosis of Aspirin Sensitivity

If a patient had taken aspirin or a nonsteroidal antiinflammatory drug at clinical doses within the previous six months without any untoward reaction, he or she was considered to be non-aspirin-sensitive. All other patients underwent bilateral intranasal challenge with incremental doses of lysine aspirin in a single-blind, placebo-controlled fashion.²⁴ Reaction to the instillation was assessed subjectively with the use of symptom scores and objectively by means of

acoustic rhinometry. A response was defined as a reduction in nasal patency (in terms of minimal cross-sectional area and volume, as determined by rhinometry) of at least 25 percent. Such a response was usually accompanied by symptoms of rhinorrhea and sneezing within 45 to 60 minutes after instillation. The mean (\pm SD) threshold for these changes was 4.6 ± 4.0 mg (range, 2.0 to 16.0). None of the patients had any concurrent bronchoconstriction during the challenge. Absence of aspirin sensitivity was defined as recent aspirin ingestion without resultant symptoms or absence of the above changes in response to 16 mg of intranasal aspirin. All the patients who reported a history of clinical reactions to aspirin had a positive response to the lysine aspirin challenge.

Topical Nasal Desensitization with Lysine Aspirin

After a 30-minute acclimatization period, the patients received lysine aspirin by intranasal application in incremental doses 40 minutes apart, beginning with 2 mg and progressing to 4 mg and finally 8 mg, with the head in a downward forward position.²⁴ Nasal and chest symptoms, nasal inspiratory peak flow rate, peak expiratory flow rate, and results of acoustic rhinometry testing were recorded at base line (immediately before the first dose) and 20 minutes after each dose of lysine aspirin. When a reaction occurred, application of the next dose of lysine aspirin was delayed for one to two hours, until acoustic rhinometry values had returned to within 5 percent of base-line values. If a patient had a reaction to 8 mg of lysine aspirin, this dose was repeatedly administered at intervals of one to two hours until it was tolerated. Two subjects had a reaction to 16 mg of lysine aspirin at diagnosis, and they decided not to participate in the desensitization trial.

When the aspirin-sensitive patients had been rendered tolerant to 8 mg of lysine aspirin, nine of them took part in a two-week trial and nine of them in a six-month trial in which we studied the effects of topical desensitization. Patients were randomly assigned in a double-blind fashion to receive 8 mg of lysine aspirin or matching placebo, applied topically to both nostrils every other day. Assignment to active therapy or placebo was determined with a list of random numbers in a protocol administered by members of the hospital pharmacy staff. In the two-week trial, five patients received lysine aspirin and four received placebo. In the six-month trial, five patients received lysine aspirin and four received placebo. Compliance was verified by examining, at random intervals, the volume of solution remaining in the supplied bottles containing lysine aspirin or placebo.

Nasal Biopsies, Processing of Samples, and Immunohistochemical Analysis

Nasal biopsies and the processing of coded specimens were performed as previously described.^{9,13,25} Polyclonal rabbit antibodies selective and specific for the CysLT₁ receptor or the LTB₄ receptor (Cayman Chemicals) and extensively validated by others²⁶ were used for immunohistochemical analysis. Immunostaining with the avidin-biotin complex was performed as described previously.^{9,13} In brief, primary antibodies specific for the CysLT₁ receptor and the LTB₄ receptor were used at dilutions of 1:50 and 1:600, respectively. The primary antibodies were detected with the use of a swine antirabbit biotinylated secondary antibody (Dako) (used at a dilution of 1:200) and subsequent application of avidin-biotin solution (Dako). The brown immunoperoxidase reaction was developed with diaminobenzidine (Sigma).

Avidin-biotin complex immunostaining was used to detect cells expressing CD45.^{9,13} In brief, sections were stained with murine monoclonal antihuman CD45 primary antibody (Dako) at a dilution of 1:100. The primary antibodies were then detected with biotin-conjugated, second-layer rabbit antimouse antibody (Dako) at a 1:400 dilution and subsequent application of avidin-biotin complex (Dako). The brown reaction was developed with diaminobenzidine.

Double immunostaining for cells expressing the CysLT₁ receptor and the LTB₄ receptor and leukocyte-phenotype markers was performed as previously described.^{9,13} In brief, the murine monoclonal antibodies UCHT1 (anti-CD3, Dako; 1:50 dilution), PGM1 (anti-CD68, Dako; 1:100 dilution), EG2 (antieosinophil cationic protein, Pharmacia; 1:100 dilution), AA1 (anti-mast cell tryptase, Dako; 1:50 dilution), and NP57 (antineutrophil elastase, Dako; 1:100 dilution) were used to identify T cells, macrophages, activated eosinophils, mast cells, and neutrophils, respectively. Alkaline phosphatase-conjugated goat antimouse secondary antibody (Seralab, 1:100 dilution) was used to detect these antibodies. A blue reaction was developed with 5-bromo-4-chloro-3-indolyl phosphate-nitroblue tetrazolium (Sigma). The rabbit polyclonal antibodies were detected as described above. Double-stained cells were identified by expression of both a blue and a brown precipitate.

Cells in the coded biopsy sections were counted by an investigator without previous knowledge of the protocol. Positive cells were counted with a microscope (Olympus) connected to an image analyzer and associated software (KSS 300, Zeiss). Positive cells were counted in the total area of the submucosa, which was measured automatically by the analyzer. Cell counts were expressed per square millimeter of nasal submucosa.

Statistical Analysis

All statistical analyses were nonparametric. Within-group comparisons were performed by the Wilcoxon signed-rank test, and between-group comparisons were performed by the Mann-Whitney U test. Differences (two-sided) were considered significant when the P value was calculated to be less than 0.05.

RESULTS

Immunohistochemical Analysis of the CysLT₁ and LTB₄ Receptors

The total number of CD45+ leukocytes in the nasal submucosa did not differ significantly between the aspirin-sensitive and non-aspirin-sensitive patients (P=0.16) (Fig. 1A). The number of nasal submucosal cells expressing the CysLT₁ receptor was higher in the aspirin-sensitive group than in the non-aspirin-sensitive group (median, 542 cells per square millimeter [range, 148 to 1390] vs. 116 cells per square millimeter [range, 40 to 259]; P<0.001), although there was some overlap (Fig. 1B). In contrast, the number of cells expressing the LTB₄ receptor was not significantly different between the two groups (P=0.76) (Fig. 1C). The percentage of CD45+ leukocytes expressing the CysLT₁ receptor was significantly higher in the aspirin-sensitive patients (25 percent of CD45+ leukocytes [range, 4 to 50] vs. 5 percent of CD45+ leukocytes [range, 2 to 11], P<0.001) (Fig. 2A); the percentage of leukocytes expressing the LTB₄ receptor did not differ significantly between the groups (P=0.19) (Fig. 2B).

Phenotypes of Cells Expressing the CysLT₁ or LTB₄ Receptor

The proportions of cells expressing the CysLT₁ or LTB₄ receptor that were accounted for by macrophages, T cells, eosinophils, neutrophils, and mast cells were similar in the aspirin-sensitive and non-aspirin-sensitive patients (Table 1).

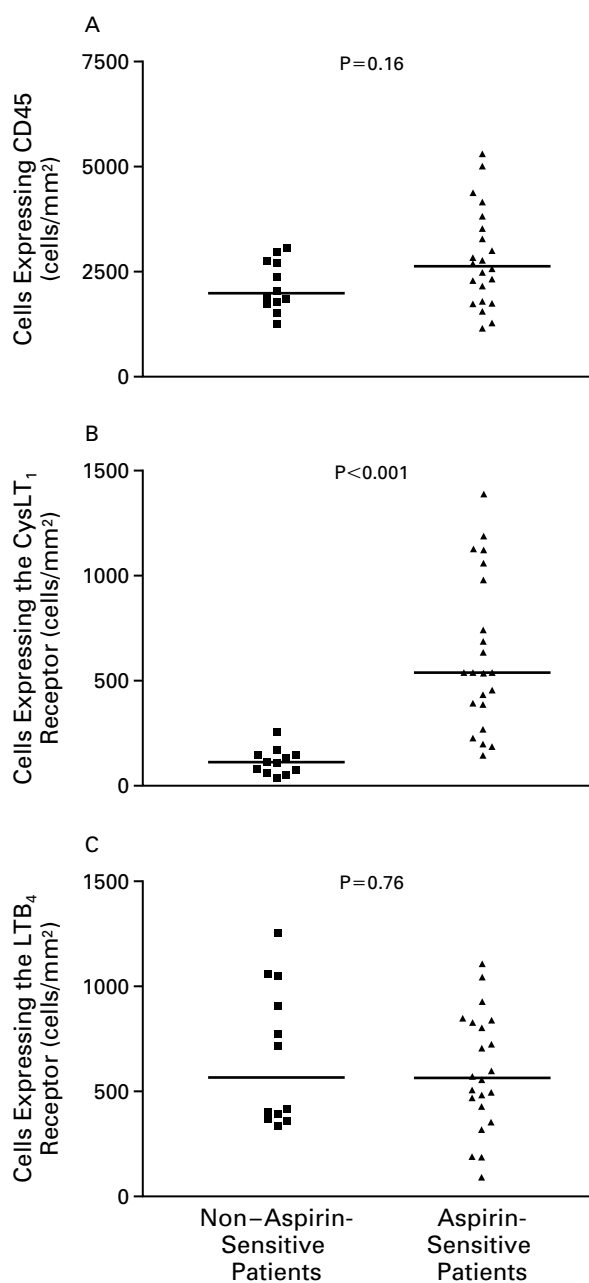


Figure 1. Numbers of Cells Expressing the Leukocyte Marker CD45 (Panel A), the Cysteinyll Leukotriene Receptor CysLT₁ (Panel B), or the Leukotriene B₄ (LTB₄) Receptor (Panel C) in Non-Aspirin-Sensitive and Aspirin-Sensitive Patients with Chronic Rhinosinusitis.

Immunoreactive cells were counted in specimens of nasal submucosa from 12 non-aspirin-sensitive and 22 aspirin-sensitive patients. The bars show median values.

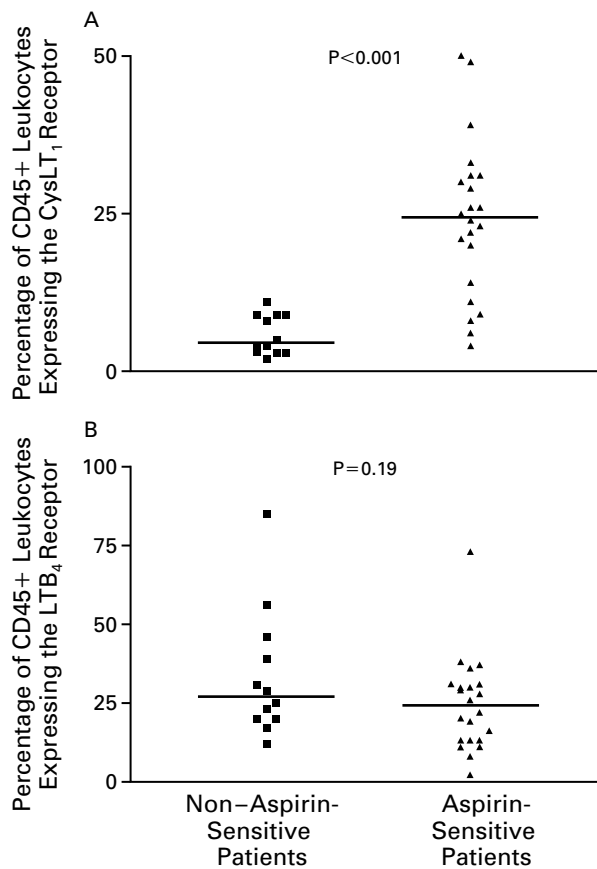


Figure 2. Percentages of CD45+ Inflammatory Leukocytes Expressing the Cysteinyl Leukotriene Receptor CysLT₁ (Panel A) or the Leukotriene B₄ (LTB₄) Receptor (Panel B) in Non-Aspirin-Sensitive and Aspirin-Sensitive Patients with Chronic Rhinosinusitis.

Immunoreactive cells were counted in specimens of nasal submucosa from 12 non-aspirin-sensitive patients and 22 aspirin-sensitive patients. The bars show median values.

Effect of Lysine Aspirin or Placebo on the Numbers of Cells Expressing the CysLT₁ and LTB₄ Receptors

There were very few mild reactions necessitating antihistamine treatment and no severe reactions during either two weeks of treatment or six months of treatment with lysine aspirin or placebo. We observed a significant reduction from base line in the percentages of CD45+ leukocytes expressing the CysLT₁ receptor in the patients given lysine aspirin ($P=0.008$ in those treated for two weeks and $P=0.02$ in those treated for six months) but not in those given placebo ($P=0.68$ and $P=0.89$, respectively). In contrast, regardless of the length of treatment, there was no significant change from base line in the percentage of CD45+ leukocytes expressing the LTB₄ receptor after treatment with either lysine aspirin ($P=0.22$ after two weeks and $P=0.84$ after six months) or placebo ($P=0.34$ and $P=1.0$, respectively).

Since the trends in these changes for both lysine aspirin and placebo were identical after either two weeks or six months of treatment, the two-week and six-month subgroups were combined for the purpose of further statistical analysis. That the trends were identical was confirmed by the absence of a significant difference between the two-week and six-month subgroups in the change from base line in the absolute CD45+ leukocyte count after lysine aspirin treatment ($P=0.29$) or after placebo ($P=1.0$); in the absolute number of cells expressing the CysLT₁ receptor ($P=1.0$ and $P=0.69$, respectively) or the LTB₄ receptor ($P=0.84$ and $P=0.69$, respectively); and in the percentage of CD45+ leukocytes expressing the CysLT₁ receptor ($P=0.42$ and $P=0.11$, respectively) or the LTB₄ receptor ($P=1.0$ and $P=0.89$, respectively).

The data for the combined subgroups are shown in Figure 3, and representative sections are shown in Fig-

TABLE 1. PERCENTAGES OF CysLT₁ RECEPTOR- AND LTB₄ RECEPTOR-EXPRESSING LEUKOCYTES THAT COEXPRESS LEUKOCYTE PHENOTYPIC MARKERS IN THE NASAL MUCOSA OF ASPIRIN-SENSITIVE AND NON-ASPIRIN-SENSITIVE PATIENTS WITH CHRONIC RHINOSINUSITIS.

TYPE OF LEUKOCYTE	PERCENTAGE OF TOTAL CysLT ₁ RECEPTOR-POSITIVE CELLS		EXPRESSION OF THE LEUKOTRIENE B ₄ RECEPTOR	
	ASPIRIN-SENSITIVE PATIENTS (N=3)	NON-ASPIRIN-SENSITIVE PATIENTS (N=3)	ASPIRIN-SENSITIVE PATIENTS (N=3)	NON-ASPIRIN-SENSITIVE PATIENTS (N=3)
	mean percentage of leukocytes (range)			
Macrophages	29.1 (24.8–32.2)	29.8 (28.0–30.8)	31.6 (28.1–35.1)	30.3 (23.6–36.2)
T cells	32.1 (31.8–32.8)	31.6 (29.8–32.7)	25.2 (18.5–33.1)	20.1 (18.6–22.8)
Eosinophils	29.6 (24.5–32.8)	26.6 (23.6–32.3)	20.9 (18.1–22.7)	20.5 (16.8–24.2)
Neutrophils	0.6 (0.5–0.6)	1.6 (1.3–1.9)	19.5 (10.2–32.2)	20.6 (13.2–25.5)
Mast cells	7.2 (5.2–9.2)	7.9 (4.3–11.5)	0.6 (0.4–0.7)	0.7 (0.5–1.0)
Total	98.6	97.5	97.8	92.2

ure 4. In the patients treated with lysine aspirin, there was a small but significant increase from base line in the total number of CD45+ leukocytes in the nasal mucosa ($P=0.03$) (Fig. 3A), a change that was not seen in the patients treated with placebo. The difference between the changes in each group was not, however, significant ($P=0.83$). In all 10 patients treated with lysine aspirin, there was a reduction from base line in the percentage of CD45+ leukocytes expressing the CysLT₁ receptor ($P=0.002$) (Fig. 3B); the response to placebo was heterogeneous but resulted in no overall significant change. The difference between the lysine aspirin and placebo groups in the change in the percentages of CD45+ leukocytes expressing the CysLT₁ receptor was significant ($P=0.02$).

Neither lysine aspirin nor placebo was associated with a significant change in the percentage of CD45+ leukocytes expressing the LTB₄ receptor (Fig. 3C); the difference between the two groups was likewise not significant.

DISCUSSION

Aspirin-sensitive rhinosinusitis is characterized by a selective increase in expression of the CysLT₁ receptor on nasal submucosal inflammatory cells. Because the total number of inflammatory leukocytes and the percentage of these cells expressing the LTB₄ receptor were similar in aspirin-sensitive and non-aspirin-sensitive subjects, this increase in the expression of the CysLT₁ receptor was not due to an increase in the overall inflammatory-cell infiltrate in the nasal mucosa of the aspirin-sensitive patients. There are no previous data on variation over time in the expression of the CysLT₁ receptor or the LTB₄ receptor. It was therefore critical in our study to have longitudinal data after the administration of a placebo control to evaluate receptor expression. All the subjects were offered active therapy when the study concluded.

The increased expression of the CysLT₁ receptor on inflammatory cells probably does not account for the increased production of cysteinyl leukotriene that is characteristic of aspirin sensitivity, unless the receptors change the activation state of the host cell or down-regulate a control element that inhibits cysteinyl leukotriene biosynthesis — changes for which we have no evidence. We therefore propose, as a more likely explanation of aspirin sensitivity, a two-compartment model in which there is both augmentation of cysteinyl leukotriene production and overexpression of the CysLT₁ receptor on inflammatory cells within the respiratory tract. The wide variability of CysLT₁ receptor expression in patients with asthma raises the intriguing possibility that widely different levels of expression of this receptor in a target organ may contribute to the heterogeneity of responses to therapy directed against the CysLT₁ receptor.

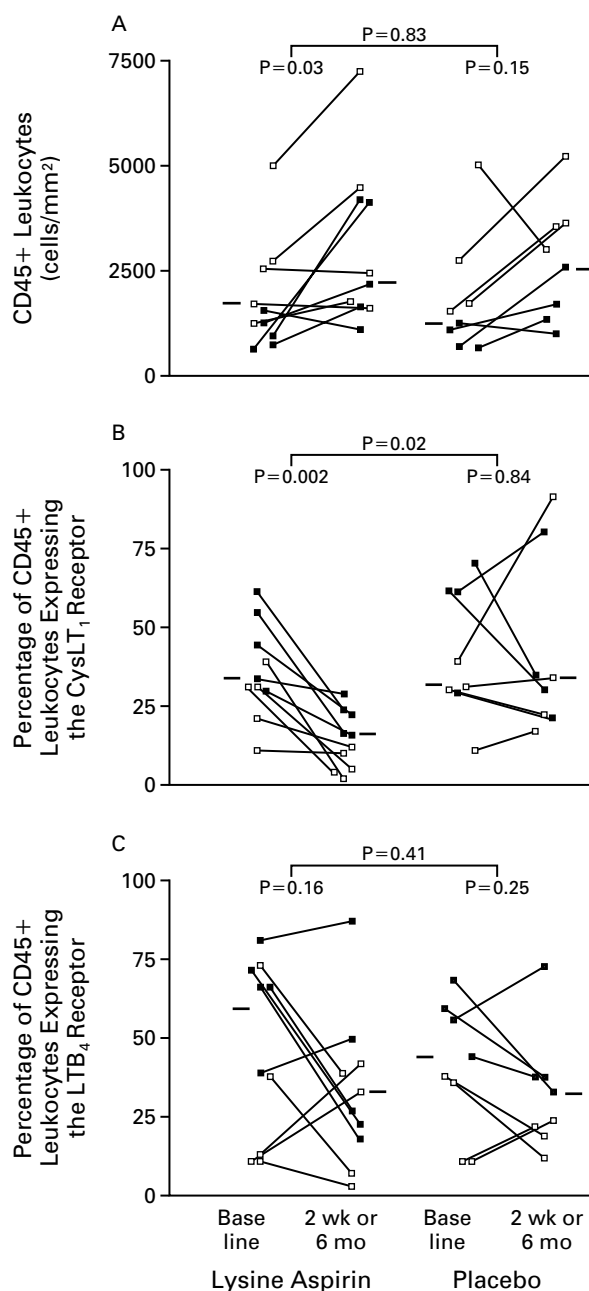


Figure 3. Changes in the Numbers of CD45+ Leukocytes (Panel A) and in the Percentages of These Cells Expressing the Cysteinyl Leukotriene Receptor CysLT₁ (Panel B) or the Leukotriene B₄ (LTB₄) Receptor (Panel C) in 18 Aspirin-Sensitive Subjects with Chronic Rhinosinusitis after Topical Lysine Aspirin or Placebo. Immunoreactive cells were counted in specimens of nasal mucosa from 10 patients given lysine aspirin and 8 given placebo. Solid symbols denote those treated for two weeks, and open symbols those treated for six months. The horizontal bars show median values.

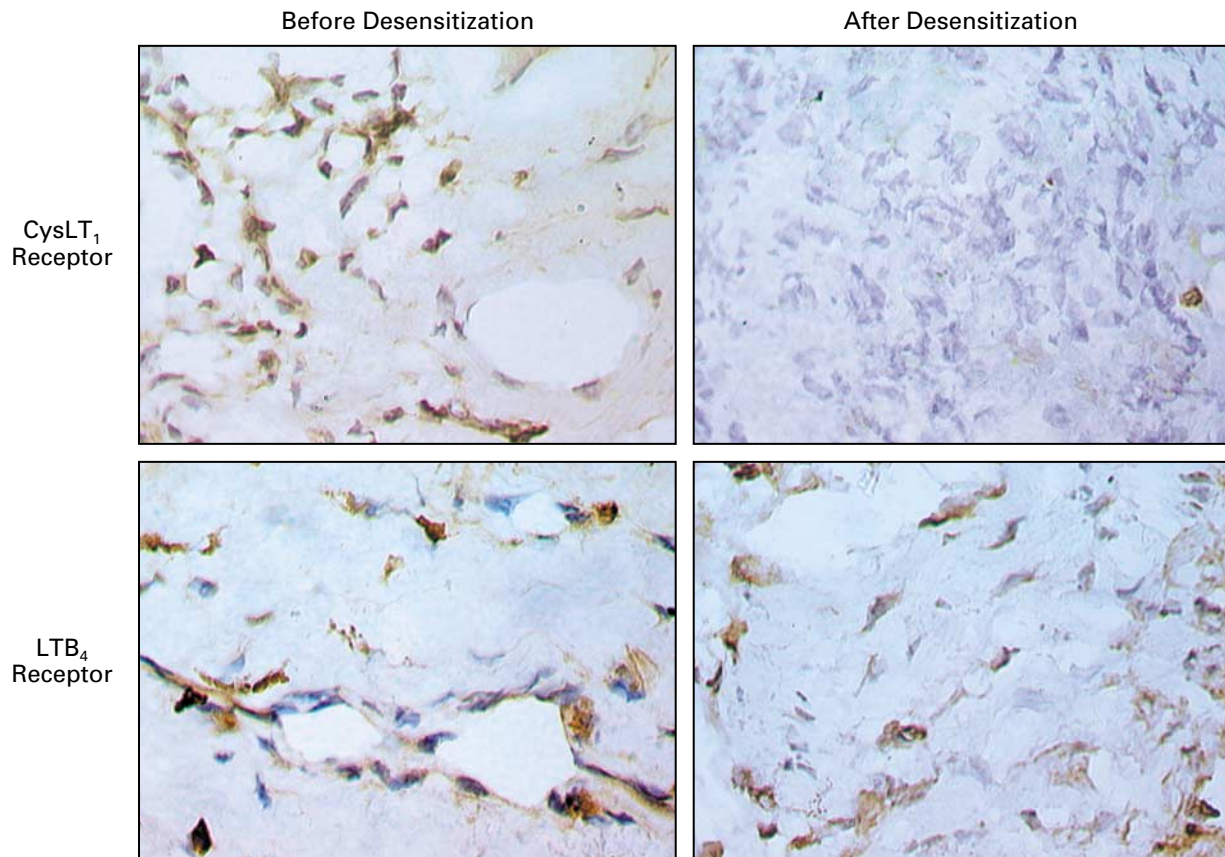


Figure 4. Representative Sections of Nasal-Biopsy Specimens Immunostained for Cysteinyl Leukotriene Receptor CysLT₁ and Leukotriene B₄ (LTB₄) Receptor before and after Desensitization with Topical Lysine Aspirin ($\times 1000$). Brown staining indicates receptor expression.

As expected, macrophages, T cells, eosinophils, mast cells, and neutrophils all expressed both leukotriene receptors.¹⁷ The elevated expression of the CysLT₁ receptor on these cells may augment the effects of cysteinyl leukotrienes on noninflammatory target cells — effects that lead to bronchospasm, plasma exudation, vasoconstriction, and secretion of mucus.¹⁸ For example, inhaled cysteinyl leukotrienes have been shown to recruit eosinophils to the lower airway in patients with asthma,²⁷ or to polyps in the nose in aspirin-sensitive patients as compared with patients with perennial allergic rhinitis.²⁵ Enhanced expression of the CysLT₁ receptor on these cells may lead to the recruitment of additional eosinophils, further enhancing the inflammatory reaction by active secretion of their granule contents.^{27,28}

In aspirin-sensitive patients, desensitization with oral aspirin can improve asthma and rhinitis^{21,22} and topical desensitization with lysine aspirin in the nose reduces the relapse rate of nasal polyps.^{29,30} In aspirin-

sensitive patients with asthma, aspirin desensitization over a period of several months blunts the aspirin-induced elevation of the urinary leukotriene E₄ concentration²³ and reduces airway hyperresponsiveness to inhaled leukotriene E₄.²⁰ In the current study, we show that there is a significant reduction in the percentage of leukocytes expressing the CysLT₁ receptor in the nasal mucosa of patients given intranasal lysine aspirin, beginning as early as two weeks after the commencement of treatment and lasting at least six months. This mechanism may contribute to the therapeutic benefit of aspirin desensitization by reducing the activation of key proinflammatory cells, such as eosinophils and monocytes, by cysteinyl leukotrienes.

Supported in part by the National Asthma Campaign.

We are indebted to the late Dr. Abraham Freedman, of the laboratories of Applied Biology, London, for help and collaboration and for the supplies of lysine aspirin and matching placebo.

REFERENCES

1. Samter M, Beers RF. Intolerance to aspirin: clinical studies and consideration of its pathogenesis. *Ann Intern Med* 1968;68:975-83.
2. Christie PE, Tagari P, Ford-Hutchinson AW, et al. Urinary leukotriene E₄ concentrations increase after aspirin challenge in aspirin-sensitive asthmatic subjects. *Am Rev Respir Dis* 1991;143:1025-9.
3. Kumlin M, Dahlen B, Björck T, Zetterstrom O, Granstrom E, Dahlen SE. Urinary excretion of leukotriene E₄ and 11-dehydro-thromboxane B₂ in response to bronchial provocations with allergen, aspirin, leukotriene D₄, and histamine in asthmatics. *Am Rev Respir Dis* 1992;146:96-103.
4. Christie PE, Tagari P, Ford-Hutchinson AW, et al. Urinary leukotriene E₄ after lysine-aspirin inhalation in asthmatic subjects. *Am Rev Respir Dis* 1992;146:1531-4.
5. Sladek K, Dworski R, Soja J, et al. Eicosanoids in bronchoalveolar lavage fluid of aspirin-intolerant patients with asthma after aspirin challenge. *Am J Respir Crit Care Med* 1994;149:940-6.
6. Ferreri NR, Howland WC, Stevenson DD, Spiegelberg HL. Release of leukotrienes, prostaglandins, and histamine into nasal secretions of aspirin-sensitive asthmatics during reaction to aspirin. *Am Rev Respir Dis* 1988;137:847-54.
7. Picado C, Ramis I, Rosello J, et al. Release of peptide leukotriene into nasal secretions after local instillation of aspirin in aspirin-sensitive asthmatic patients. *Am Rev Respir Dis* 1992;145:65-9.
8. Drazen JM, Israel E, O'Byrne PM. Treatment of asthma with drugs modifying the leukotriene pathway. *N Engl J Med* 1999;340:197-206. [Errata, *N Engl J Med* 1999;340:663, 341:1632.]
9. Cowburn AS, Sladek K, Soja J, et al. Overexpression of leukotriene C₄ synthase in bronchial biopsies from patients with aspirin-intolerant asthma. *J Clin Invest* 1998;101:834-46.
10. Leff AR. Regulation of leukotrienes in the management of asthma: biology and clinical therapy. *Annu Rev Med* 2001;52:1-14.
11. Sestini P, Armetti L, Gambaro G, et al. Inhaled PGE₂ prevents aspirin-induced bronchoconstriction and urinary LTE₄ excretion in aspirin-sensitive asthma. *Am J Respir Crit Care Med* 1996;153:572-5.
12. Fischer AR, Rosenberg MA, Lilly CM, et al. Direct evidence for a role of the mast cell in the nasal response to aspirin in aspirin-sensitive asthma. *J Allergy Clin Immunol* 1994;94:1046-56.
13. Nasser SM, Pfister R, Christie PE, et al. Inflammatory cell populations in bronchial biopsies from aspirin-sensitive asthmatic subjects. *Am J Respir Crit Care Med* 1996;153:90-6.
14. Lynch KR, O'Neill GP, Liu Q, et al. Characterization of the human cysteinyl leukotriene CysLT₁ receptor. *Nature* 1999;399:789-93.
15. Heise CE, O'Dowd BF, Figueroa DJ, et al. Characterization of the human cysteinyl leukotriene 2 receptor. *J Biol Chem* 2000;275:30531-6.
16. Figueroa DJ, Breyer RM, Defoe SK, et al. Expression of the cysteinyl leukotriene₁ receptor in normal human lung and peripheral blood leukocytes. *Am Rev Respir Crit Care Med* 2001;163:226-33.
17. Panettieri RA, Tan EML, Ciocca V, Luttmann MA, Leonard TB, Hay DW. Effects of LTD₄ on human airway smooth muscle cell proliferation, matrix expression, and contraction in vitro: differential sensitivity to cysteinyl leukotriene receptor antagonists. *Am J Respir Cell Mol Biol* 1998;19:453-61.
18. Nicosia S, Capra V, Rovati GE. Leukotrienes as mediators of asthma. *Pulm Pharmacol Ther* 2001;14:3-19.
19. Christie PE, Smith CM, Lee TH. The potent and selective sulfidopeptide leukotriene antagonist, SK&F 104353, inhibits aspirin-induced asthma. *Am Rev Respir Dis* 1991;144:957-8.
20. Arm JP, O'Hickey SP, Spur BW, Lee TH. Airway responsiveness to histamine and leukotriene E₄ in subjects with aspirin-induced asthma. *Am Rev Respir Dis* 1989;140:148-53.
21. Stevenson DD, Simon RA, Mathison DA. Aspirin-sensitive asthma: tolerance to aspirin after positive oral aspirin challenges. *J Allergy Clin Immunol* 1980;66:82-8.
22. Patriarca G, Nucera E, DiRienzo V, Schiavino D, Pellegrino S, Fais G. Nasal provocation test with lysine acetylsalicylate in aspirin-sensitive patients. *Ann Allergy* 1991;67:60-2.
23. Nasser SM, Patel M, Bell GS, Lee TH. The effect of aspirin desensitization on urinary leukotriene E₄ concentrations in aspirin-sensitive asthma. *Am J Respir Crit Care Med* 1995;151:1326-30.
24. Milewski M, Mastalerz L, Nizankowska E, Szczeklik A. Nasal provocation test with lysine-aspirin for the diagnosis of aspirin-sensitive asthma. *J Allergy Clin Immunol* 1998;101:581-6.
25. Varga EM, Jacobson MR, Masuyama K, et al. Inflammatory cell populations and cytokine mRNA expression in the nasal mucosa in aspirin-sensitive rhinitis. *Eur Respir J* 1999;14:610-5.
26. Thivierge M, Doty M, Johnson J, Stankova J, Rola-Pleszczynski M. IL-5 up-regulates cysteinyl leukotriene 1 receptor expression in HL-60 cells differentiated into eosinophils. *J Immunol* 2000;165:5221-6.
27. Laitinen LA, Laitinen A, Haahela T, Vilkkä V, Spur BW, Lee TH. Leukotriene E₄ and granulocytic infiltration into asthmatic airways. *Lancet* 1993;341:989-90.
28. Diamant Z, Hiltermann JT, Van Rensen EL, et al. The effect of inhaled leukotriene D₄ and methacholine on sputum cell differentials in asthma. *Am J Respir Crit Care Med* 1997;155:1247-53.
29. Patriarca G, Schiavino D, Nucera E, Papa G, Schinco G, Fais G. Prevention of relapse in nasal polyposis. *Lancet* 1991;337:1488.
30. Scadding GK, Hassab M, Darby YC, Lund VJ, Freedman A. Intranasal lysine aspirin in recurrent nasal polyposis. *Clin Otolaryngol* 1995;20:561-3.

Copyright © 2002 Massachusetts Medical Society.

PERSONAL ARCHIVES IN THE JOURNAL ONLINE

Individual subscribers can store articles and searches using a new feature on the *Journal's* Web site (www.nejm.org) called "Personal Archive." Each article and search result links to this feature. Users can create personal folders and move articles into them for convenient retrieval later.