

Atopic children with cystic fibrosis have increased urinary leukotriene E₄ concentrations and more severe pulmonary disease

Peter Greally, MRCPI,^a Arlene J. Cook, BSc,^b Anthony P. Sampson, PhD,^b
Rosemary Coleman, MRCPI,^c Susan Chambers, MSc,^d
Priscilla J. Piper, PhD,^b and John F. Price, MD^a London, England

Background: We investigated the hypothesis that cysteinyl leukotriene (LT) production is altered in atopic patients with cystic fibrosis (CF).

Methods: Urinary LTE₄ was measured in two groups of children with CF: atopic (ACF group, n = 22) and nonatopic (NACF group, n = 13); and in two groups of unaffected children, those with atopic asthma (AA group, n = 11) and nonatopic normal control subjects (NN group, n = 12).

Results: Atopic groups excreted significantly more urinary LTE₄ (geometric means [95% confidence intervals] in picomoles per millimole creatinine), ACF group: 104 (73-147) and AA group: 195 (136-282) compared with NACF group: 19 (9-39) and NN group: 27 (15-48). The ACF group had significantly more airflow obstruction than the NACF group, with forced expiratory volume in 1 second (percent predicted, mean $\pm$ SD) in ACF: 58 $\pm$ 21 versus NACF: 81 $\pm$ 23, and forced vital capacity (percent predicted, mean $\pm$ SD) 72 $\pm$ 17 versus 87 $\pm$ 23, respectively. There were significant correlations between the degree of airflow obstruction, bronchodilator responsiveness, and urinary LTE₄ concentration within the entire CF group. We used multiple regression analysis to assess the respective influence of age, atopy, sensitization to *Aspergillus fumigatus*, and colonization with *Pseudomonas aeruginosa* on urinary LTE₄ concentration. The atopic state was the only significant variable associated with urinary LTE₄ production in subjects with CF.

Conclusions: The similarities in urinary LTE₄ between ACF and AA groups suggest that the atopic state is the prime determinant of urinary LTE₄ excretion. Enhanced cysteinyl LT production associated with atopy in CF may increase the severity of pulmonary disease. (*J ALLERGY CLIN IMMUNOL* 1994;93:100-7.)

Key words: Cystic fibrosis, atopy, leukotrienes, pulmonary disease, airway reactivity

Cysteinyl leukotrienes (LTs), arachidonic acid products of the 5-lipoxygenase pathway, have been implicated in the pathophysiology of airway inflammation observed in patients with cystic fibrosis (CF).^{1, 2} They are present in CF sputum at

From ^athe Departments of Child Health and Thoracic Medicine, King's College Hospital; ^bthe Department of Pharmacology, Hunterian Institute, Royal College of Surgeons of England; ^cthe Department of Dermatology, Institute of Child Health; and ^dthe Department of Clinical Biochemistry, King's College Hospital.

Supported in part by the Cystic Fibrosis Trust (United Kingdom).

Received for publication Oct. 30, 1992; revised May 24, 1993; accepted for publication May 24, 1993.

Reprint requests: Peter Greally, MRCPI, Division of Pediatric Pulmonology, Children's Hospital of Pittsburgh, 3705 Fifth Ave., Pittsburgh, PA 15213.

Copyright © 1994 by Mosby-Year Book, Inc.
0091-6749/94 \$1.00 + .10 1/1/48997

Abbreviations used

AA:	Atopic asthmatic
ACF:	Atopic CF
Af:	<i>Aspergillus fumigatus</i>
CF:	Cystic fibrosis
FEV ₁ :	Forced expiratory volume in 1 second
FVC:	Forced vital capacity
LT:	Leukotriene
NACF:	Nonatopic CF
NN:	Nonatopic normal
PA:	<i>Pseudomonas aeruginosa</i>
TNF- α :	Tumor necrosis factor- α

concentrations sufficient to cause bronchial lability, mucous hypersecretion, and mucosal inflammation.³ Sputum cysteinyl LT concentration correlates significantly with the Chrispin-Norman

score of radiologic abnormality, which suggests that they are biologically active.⁴

In patients with CF a higher prevalence of positive cutaneous reactions to inhalant allergens is reported. Patients with positive skin test results tend to have more advanced pulmonary disease.^{5, 6} However, the pattern of response in CF is different than that observed in the general population. Patients with CF have a marked predisposition to develop immediate hypersensitivity reactions to *Aspergillus fumigatus* (Af).^{5, 6} The prevalence of positive reactions to inhalant allergens (other than Af) in subjects with CF is no greater than that expected in the general population.^{7, 8} This suggests that the development of an IgE response to Af is an acquired characteristic, related to disease severity, rather than indicative of an innate atopic tendency.

It has been established that peripheral blood leukocytes from healthy individuals who are atopic exhibit enhanced production of leukotrienes in vitro compared with cells from nonatopic individuals.⁹ We postulated that patients with CF who are atopic have increased LT production and that this contributes to the severity of their lung disease.

LTC₄ is rapidly degraded by enzymes present in tissues, blood, and urine to LTD₄ and then to LTE₄, which is their stable and active metabolite.¹⁰ LTE₄ is excreted in urine and its concentration is widely accepted as a reliable indicator of systemic cysteinyl LT release.¹¹ Therefore we investigated whether the atopic state in children with CF influenced urinary LTE₄ levels and what effect this had on the severity of airflow obstruction.

METHODS

Informed written consent was obtained in each case, and the study was approved by the hospital's ethics committee. All patients with CF were recruited from the CF clinic at King's College Hospital and had confirmed CF (sweat Na⁺ > 70 mmol/L). Children were studied and grouped on the basis of their atopic status. Power calculations indicated that approximately 30 patients (15 atopic and 15 nonatopic) were required to demonstrate a significant difference in urinary LTE₄ concentration between the groups at a 5% probability level. Thirty-five subjects aged 5 to 19 years (mean age, 11.1 years) were studied; 22 were atopic and 13 were nonatopic. Patients with CF were selected on the basis of clinical stability (no change in pulmonary symptoms over the previous month, stable weight, no new physical signs on respiratory examination, and less than 10% variability from recent pulmonary function tests) with the criteria for pulmonary exacerbation as adapted

from Smith et al.¹² Twelve normal nonatopic children aged 4 to 14 years (mean age, 9.2 years) and 11 children with atopic asthma aged 5 to 17 years (mean age, 10.2 years) acted as control subjects. The children with asthma were recruited from the pediatric asthma clinic at King's College Hospital or an allergy clinic at the Institute of Child Health. These children also had been clinically stable for at least 1 month before evaluation and had peak flow rates in excess of 90% of predicted values. No patient or control subject was receiving oral or inhaled antiinflammatory medications or theophylline. Patients refrained from use of β_2 -agonists for at least 4 hours before evaluation.

A personal history of recurrent wheeze, allergic rhinitis, or atopic dermatitis was sought in each patient. Atopic status was determined by performing skin prick tests on the flexor surface of each subject's right forearm with the following allergens: house dust, *Der-matophagoides pteronyssinus*, cat fur, dog hair, mixed feathers, mixed grass pollens, mixed tree pollens, *Alternaria alternata*, *Cladosporium herbarium*, and *Aspergillus fumigatus* (Bencard, Middlesex, U.K.) Tests also included phenol (0.5%)/saline solution, and histamine controls. A wheal diameter 3 mm greater than the negative control, 15 minutes after testing, in response to at least one of the allergens was considered to be indicative of atopy. However, in the CF group isolated positive responses to Af were not considered to be evidence of atopy and such patients were classified as being nonatopic. Total serum IgE was measured in the CF group ($n = 35$) by radioimmunoassay.

Sputum or cough swabs from the children with CF were sent for routine bacterial culture to determine any relationship between *Pseudomonas aeruginosa* (PA) colonization and urinary LTE₄ concentration. Patients were regarded as chronically colonized with PA or *Staphylococcus aureus* if the organisms had been cultured on at least three occasions over the previous 6 months. Forced expiratory volume in 1 second (FEV₁) and forced vital capacity (FVC) were measured by spirometry and expressed as a percent of the predicted value for age, height, and sex.¹³ Bronchodilator responsiveness in the CF group (34 of 35) was assessed 15 minutes after the administration of nebulized albuterol (2.5 mg) and expressed as a percentage change from baseline. Clinical National Institutes of Health scores (best possible score = 100) were calculated for each patient with CF.

DNA, extracted from buccal epithelial cells from 31 of the 35 patients with CF, was analyzed for the four most common mutations (Δ F508, G551D, G542X, and 621 + 1 G > T) with a nonisotopic, polymerase chain reaction-based amplification refractory mutation system (Cellmark Diagnostics, ICI, Abingdon, U.K.).

Urine sampling and spirometry were performed during the same visit. Specimens were coded and stored at -70° C. Samples were spiked with tritiated LTE₄ (3 nCi) and treated with ice-cold methanol to precipi-

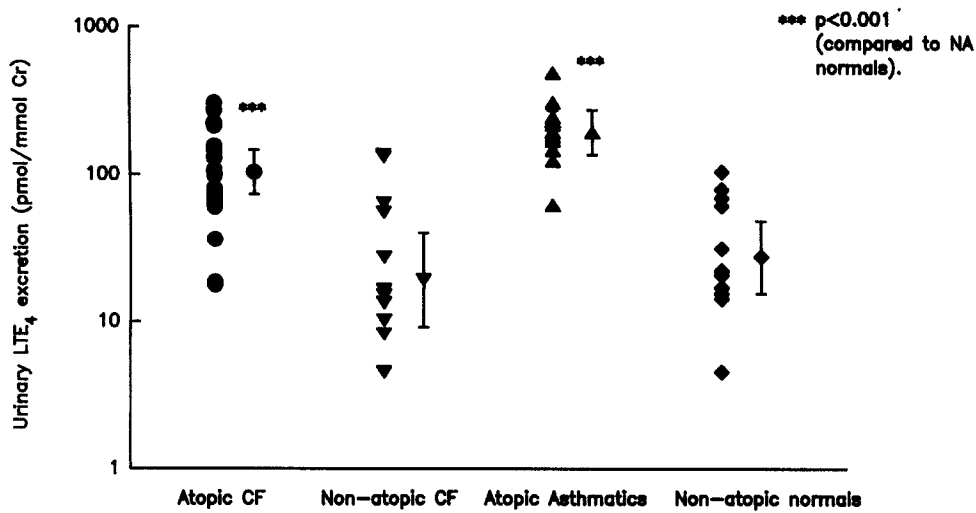


FIG. 1. Urinary LTE_4 excretion in patients with atopic CF, nonatopic CF, and atopic asthma and nonatopic normal control subjects.

TABLE I. Details of atopic patients with CF (ACF) and nonatopic patients with CF (NACF)

	ACF (n = 22)	NACF (n = 13)	p Value
Mean age (yr) (range)	11.9 (5-19)	10.2 (5-17)	NS*
PA	13 (59%)	5 (38%)	NS†
Af	10 (45%)	3 (23%)	NS†
IgE median kU/L (range)	272 (2-1000)	17 (1.8-78)	<0.001‡
$\Delta\text{F508}/\Delta\text{F508}$	10/20	10/11	<0.05†
Clinical NIH score	71 $\pm$ 17	84 $\pm$ 15	<0.05*
FEV ₁ (% pred) mean ($\pm$ SD)	58 ($\pm$ 21)	81 ($\pm$ 23)	<0.01*
FVC (% pred) mean ($\pm$ SD)	72($\pm$ 17)	87 ($\pm$ 23)	=0.05*
Bronchodilator response (% baseline)	7 (0-50%)	1.3 (0-40%)	=0.09‡
Median (range)			

NIH, National Institutes of Health.
*Unpaired Student's *t* test.
†Chi square test with Yates' correction.
‡Mann-Whitney U test.

tate proteins. Dried supernatants were reconstituted in water and loaded onto C18 Sep-Pak cartridges; after washing with 5 ml of distilled water, LT-containing material was eluted in 5 ml of methanol and stored at -20°C . Reverse-phase high-performance liquid chromatography was performed with a 5 μm ODS Techsphere analytical column and ODS guard column (HPLC Technology, Macclesfield, U.K.) with a mobile phase of methanol:water:acetic acid (75/25/0.01 vol/vol/vol, pH 5.6) at a flow rate of 1 ml/min. Fractions (1 ml) were collected for 40 minutes; 0.8 ml aliquots were added to OptiPhase "Hisafe" II (LKB scintillation products, Loughborough, U.K.) scintillant (5 ml) and counted for 2 minutes on a scintillation counter. LTE_4 elution time was identified by the tritiated internal standard. The remaining 200 μl aliquots were evaporated to dryness under vacuum (Gyro-Vap, V.A. Howe, Cambridge, U.K.) and stored at -20°C before radioimmunoassay. Each sample was

reconstituted in 300 μl Tris-HCl and gelatin buffer (pH 7.4), and aliquots (100 μl) were assayed in duplicate. These were incubated overnight at 4°C with assay buffer (600 μl), containing LTE_4 antiserum and tritiated LTE_4 (2.5 nCi). The next day, dextran-coated activated charcoal was added for 10 minutes, and each sample was centrifuged at 2500 *g* for 15 minutes to remove unbound tritiated LTE_4 . A standard curve with known concentrations of unlabeled LTE_4 (8 to 4096 pg/tube) was constructed, and results were obtained by interpolation on the straight-line portion of the curve. LTE_4 antiserum recognized LTE_4 (100%), LTC_4 (90%), LTD_4 (280%), N-acetyl- LTE_4 , and 11-trans LTE_4 but not LTB_4 (0.005%), various prostanoids, arachidonic acid, or glutathione. Immunoreactivity was corrected for the percentage of total tritiated LTE_4 recovered. Urinary LTE_4 levels were corrected for volume and creatinine concentration of each specimen and expressed in picomoles per millimole of creatinine.

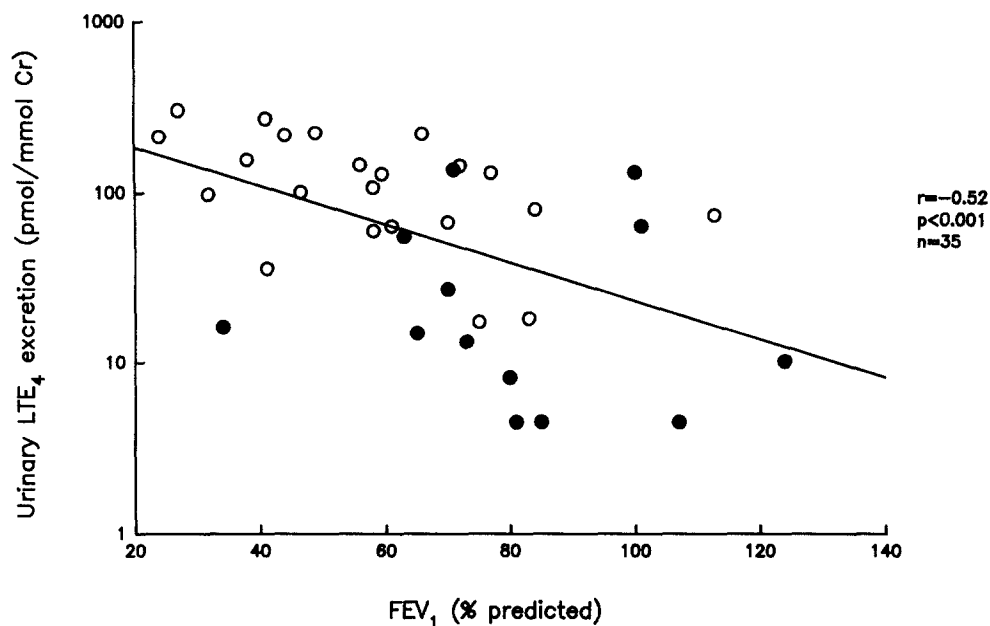


FIG. 2. The relationship between urinary LTE₄ excretion and FEV₁ in children with CF. Filled circles represent the NACF group, and open circles represent the ACF group. Cr, Creatinine.

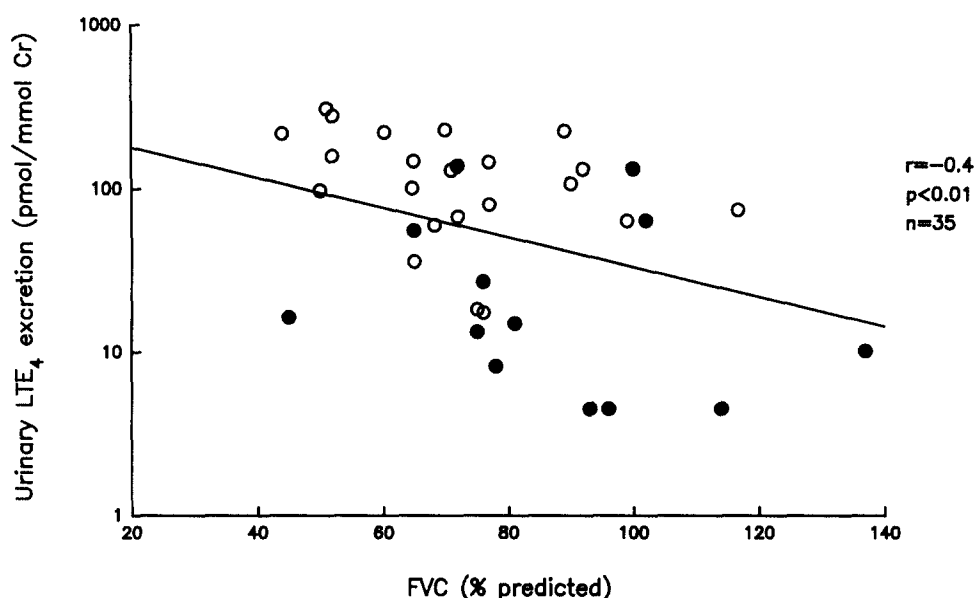


FIG. 3. The relationship between urinary LTE₄ and FVC in children with CF. Filled circles represent the NACF group, and open circles represent the ACF group. Cr, Creatinine.

Because urinary LTE₄ concentrations were log₁₀ normally distributed, they were expressed as geometric means with 95% confidence intervals. Unpaired Student's *t* tests were used to compare age, FEV₁, FVC, and log₁₀ urinary LTE₄ in atopic CF (ACF) and non-atopic CF (NACF) groups. One-way analysis of variance was used to assess differences among ACF, NACF, atopic asthmatic (AA), and normal control (NN) groups. FEV₁ and FVC were plotted against urinary LTE₄ concentration; Pearson's correlation coefficient was calculated, and linear regression analysis was per-

formed with the least squares method. In addition, the respective strengths of the associations among atopy, age, presence of PA, or Af sensitization, and urine LTE₄ concentration were assessed by multiple regression analysis.

RESULTS

Urinary LTE₄ levels, expressed as geometric means with 95% confidence intervals in picomoles per millimole creatinine, were higher in the ACF

TABLE II. Results of multiple regression analysis ($n = 35$)

	t Value	r Value	p Value
Age (yr)	0.94	0.28	0.62
Atopy	3.25	0.57	0.002
PA	0.59	0.23	0.56
Af	-0.37	0.36	0.72

and AA groups 104 (73 to 147) and 195 (136 to 282) than in the NACF group: 19 (9-39) in which urinary LTE_4 concentration approximated that of the NN group: 27 (15 to 48) ($p < 0.0001$, analysis of variance, Fig. 1). The ACF group also had more severe airflow obstruction than the NACF group (Table I). A significant inverse correlation existed between urinary LTE_4 concentration of the CF group ($n = 35$) and lung function (Figs. 2 and 3). Bronchodilator responsiveness did not significantly differ between ACF and NACF groups ($p = 0.09$, Table I). However, if a single NACF outlier (with a history of wheezing, 41% reversibility in FEV_1 , and a urinary LTE_4 concentration of 137 pmol/mmol creatinine) was excluded, the differences between the groups became significant ($p < 0.03$, $n = 34$). Moreover, bronchodilator responsiveness correlated with urinary LTE_4 concentration in the ACF group ($r = 0.57$, $p < 0.005$, $n = 22$). When the non-atopic subjects were included, the result remained significant ($r = 0.42$, $p < 0.03$, $n = 34$).

In the ACF group 14 of 22 (64%) had a personal history of atopic disease: recurrent wheezing in six, allergic rhinitis in 10, and atopic dermatitis in two. Only one child in the NACF group repeatedly wheezed. Seventeen of the 22 subjects in the ACF group reacted to two or more common inhalant allergens. Thirteen of the 22 children in the ACF group and 5 of 13 of the children in the NACF group were chronically colonized with PA. Ten subjects in the ACF group, 3 of 13 subjects in the NACF group (Table I), and none of the control subjects exhibited hypersensitivity to Af. Eighteen members of the entire CF group ($n = 35$) were colonized with PA. Thirteen of 35 (37%) had positive skin reactions to Af. Ten were atopic (eight had chronic PA infection) and three were nonatopic (two had chronic PA infection). Thus although atopy, colonization with PA, and Af skin reactivity appeared to be closely associated, the only significant variable associated with urinary LTE_4 concentration was the atopic state ($p < 0.002$ as determined by multiple regression analysis) (Table II).

The results of mutation analysis were as follows. In the ACF group (20 of 22 tested), 10 subjects were homozygous for the ΔF508 deletion, three were $\Delta\text{F508}/\text{G551D}$ heterozygotes, one had $\Delta\text{F508}/621 + 1 \text{ G} > \text{T}$, four had $\Delta\text{F508}/-$, one had $\text{G551D}/-$, and one had unknown mutations ($-/-$). In the NACF group (11 of 13 tested), 10 subjects were homozygous for the ΔF508 mutation and one had $\Delta\text{F508}/-$. Overall, 20 of 31 (64.5%) were ΔF508 homozygotes and 8 of 31 (25%) were ΔF508 heterozygotes. Ten of the 20 ΔF508 homozygotes (6ACF:4NACF) and four of the eight ΔF508 heterozygotes (all in the ACF group) were colonized with PA. More detailed information on individual patients can be found in Table III.

DISCUSSION

We have demonstrated a marked difference in urinary LTE_4 excretion between atopic and non-atopic groups. The ACF group had significantly higher urinary LTE_4 concentrations and greater airflow limitation than the NACF group. They also exhibited a tendency toward more colonization with PA and sensitization with Af (Table I). Even during periods of clinical stability, LTE_4 is present in nanomolar concentrations in CF sputum and correlates with urinary LTE_4 concentrations, suggesting that urinary LTE_4 is partly derived from cysteinyl LTs generated in the lungs.³ LTE_4 , although less potent than its precursors, if inhaled, can produce sustained bronchial hyper-responsiveness in asthmatic airways.¹⁴ In the present study we too have identified a relationship between airway reactivity (as measured by bronchodilator responsiveness) and urinary LTE_4 excretion.

The precise cellular origins of LTE_4 and its precursors in CF have yet to be determined. Cysteinyl LTs are usually produced by mast cells and eosinophils and to a lesser degree by macrophage/monocytes. It is presently not clear whether human airway epithelial cells can express the enzymes of the 5-lipoxygenase pathway, although porcine epithelia express LTA_4 hydrolase in vitro.¹⁵ Histology of the airway in established CF lung disease reveals an infiltrate consisting mainly of neutrophils, which produce LTB_4 but not LTC_4 , and occasional monocytes.^{16, 17} Eosinophils are a rich source of LTC_4 , but they are not commonly seen unless allergic bronchopulmonary aspergillosis has supervened.¹⁸ However, mast cells bearing abundant amounts of tryptase and chymase have been observed in large numbers within CF mucosae^{19, 20} and are a likely source of cysteinyl LTs.

TABLE III. Details of individual patients with CF (*n* = 35)

Age (yr)	Sex	Sputum	Atopy	FEV ₁	NIH score	CF genotype	Urinary LTE ₄
16.4	F	PA	+	44	63	621 + 1 > G/ΔF508	221.0
9.2	M	SA	+ *	24	40	ΔF508/ΔF508	215.0
9.5	F	PA	+	66	81	ΔF508/ΔF508	225.0
12.4	M	O	+	84	84	ΔF508/-	80.4
10.5	M	PA	+	59	58	ΔF508/G551D	129.6
13.7	M	SA	+ *	58	79	ND	108.0
16.0	M	PA	+ *	49	66	ΔF508/ΔF508	228.0
15.7	M	PA	+ *	46	57	ΔF508/ΔF508	101.3†
13.6	M	PA	+ *	58	68	ΔF508/ΔF508	60.1†
8.5	F	O	+	70	89	ΔF508/ΔF508	67.8†
16.5	F	SA	+	38	61	ΔF508/ΔF508	157.2
19.5	M	PA	+ *	27	37	ΔF508/ΔF508	307.8
10.1	M	PA	+	56	70	ND	147.0†
14.8	F	PA	+ *	77	88	ΔF508/ΔF508	132.0
5.4	F	PA	+	41	60	ΔF508/-	277.0
16.5	F	PA	+ *	32	49	ΔF508/-	98.0†
11.8	F	PA	+ *	41	77	G551D/-	36.0
10.3	M	O	+	72	84	ΔF508/ΔF508	145.0
11.0	M	O	+	75	92	-/-	17.6†
7.0	F	O	+	75	94	ΔF508/G551D	74.5
6.0	M	PA	+ *	61	71	ΔF508/G551D	64.0
9.4	M	O	+	83	96	ΔF508/-	18.4
7.0	F	O	o	80	93	ΔF508/ΔF508	8.2
15.5	F	O	o	124	90	ND	10.2
7.5	F	O	o	107	97	ΔF508/ΔF508	4.5
6.5	F	O	o	101	97	ΔF508/ΔF508	64.0
7.5	M	PA	o	65	72	ΔF508/ΔF508	15.1
11.0	F	PA	o *	34	38	ΔF508/ΔF508	16.4
16.5	F	PA	o *	85	84	ΔF508/ΔF508	4.5
11.4	M	PA	o	81	81	ND	4.5
10.5	F	SA + HI	o *	63	81	ΔF508/ΔF508	55.0
6.0	M	O	o	71	93	ΔF508/ΔF508	137.5†
15.5	M	PA	o	73	86	ΔF508/ΔF508	13.4
9.0	F	O	o	100	91	ΔF508/-	132.6
8.8	M	O	o	70	92	ΔF508/ΔF508	27.2

Dashes represent unknown mutations.
SA, *Staphylococcus aureus*; O, sterile culture; ND, not done; HI, *Haemophilus influenzae*.
*Positive skin reaction to Af.
†Personal history of recurrent wheeze.

In subjects with CF the reported prevalence of positive skin reactions to Af ranges between 35% and 88%.^{6, 21} Most studies suggest that these patients are more likely to be colonized with PA and have poorer lung function.^{5, 6} In contrast, the prevalence of positive reactions to Af in an unselected normal population ranges from 4% to 10%.²² Many theories have been proposed for this hypersensitivity to Af in CF including: selective retention of spores caused by gas trapping, increased permeability of inflamed bronchial mucosae, or an adjuvant effect of PA on the IgE response.^{5, 23, 24} It was for these reasons that we regarded isolated positive skin responses to Af as

an indication of the degree of bronchial injury rather than as a reflection of an individual's underlying atopic predisposition.

Although not a primary aim of the study, we have identified a tendency toward ΔF508 homozygosity with the nonatopic group and more ΔF508 heterozygosity in the atopic group. However, the distribution of ΔF508 within the group as a whole is similar to that observed within the Caucasian population in the United Kingdom.²⁵ Homozygotes for the ΔF508 mutation have been shown to present at an earlier age and have higher annual rates of PA acquisition.²⁶ Those homozygotes who were colonized with PA were equally distributed

between the two groups (6ACF:4NACF). In a more detailed recent evaluation of CF mutations and their clinical correlates, it was demonstrated $\Delta F508$ heterozygotes with the second CF allele in the nucleotide binding fold encoding exons had an increased risk, even higher than $\Delta F508$ homozygotes, for earlier acquisition of PA.²⁷ One might speculate that the unknown CF alleles could be mutations in the exon coding for the nucleotide binding fold, which might explain the poorer pulmonary function and tendency to more PA colonization in the ACF group. Further studies are necessary to determine any relationship among CF genotype, atopy, and pulmonary disease in larger groups of patients.

Although not proven, it is tempting to ascribe a causal relationship to the association between the atopic state and enhanced LTE_4 excretion observed in CF. However, chronic bacterial exposure could produce a number of immunologic stimuli, which may increase the likelihood of mast cell degranulation and activation of the 5-lipoxygenase pathway. These include increases in total serum IgE concentrations, the development of *Pseudomonas*-specific IgE, and the formation of immune complexes with the subsequent generation of complement degradation products, all of which have been documented in CF.²⁸⁻³¹ In addition, the cytokine tumor necrosis factor- α (TNF- α), which is secreted in response to bacterial lipopolysaccharide³² and other infectious stimuli, may also participate in the inflammatory response seen in CF.³³ TNF- α is also implicated in the pathophysiology of allergic diseases such as asthma and atopic dermatitis,^{34, 35} and it upregulates LT production in human leukocytes in vitro.³⁶ We have previously shown that significant correlations exist between sputum TNF- α and LT concentrations in patients with CF, suggesting that priming of the human 5-lipoxygenase pathway may occur in vivo.³⁷

Using multiple regression analysis, we failed to demonstrate a significant relationship between colonization with PA and urinary LTE_4 concentration. Therefore these data support our original hypothesis that the greater systemic release of cysteinyl LTs in patients with CF is due to the atopic state. The correlations among urinary LTE_4 , severity of airflow obstruction, and airway reactivity suggest that they are biologically active and that they may contribute to airway inflammation. These results suggest a mechanism by which the atopic state places an additional inflammatory burden on the already compromised airways of

patients with CF and may permit their earlier colonization with PA, the major determinant of pulmonary morbidity and death. Further investigation of this association is necessary. If a causal relationship is subsequently proven, novel agents such as 5-lipoxygenase inhibitors and cysteinyl LT receptor antagonists may supplement existing therapeutic strategies for selected patients with well established pulmonary disease, particularly those who are atopic.

We thank Professor B. Peskar (University of Bochum, Germany) for the gift of LTE_4 antiserum. We would especially like to express our gratitude to the children and their families for kindly agreeing to participate in the study.

REFERENCES

1. Cromwell O, Walport MJ, Morris HR, et al. Identification of leukotrienes D and B in sputum from cystic fibrosis patients. *Lancet* 1981;2:164-5.
2. Zakrzewski JT, Barnes NC, Costello JF, Piper PJ. Lipid mediators in cystic fibrosis and chronic obstructive airways disease. *Am Rev Respir Dis* 1987;136:779-82.
3. Sampson AP, Spencer DA, Green CP, Piper PJ, Price JF. Leukotrienes in the sputum and urine of cystic fibrosis children. *Br J Clin Pharmacol* 1990;30:861-9.
4. Spencer DA, Sampson AP, Green CP, Costello JF, Piper PJ, Price JF. Sputum cysteinyl leukotrienes correlate with the severity of pulmonary disease in children with cystic fibrosis. *Pediatr Pulmonol* 1992;12:90-4.
5. Warner JO, Taylor BW, Norman AP, Soothill JF. Association of cystic fibrosis with allergy. *Arch Dis Child* 1976; 51:507-11.
6. Pitcher-Wilmott RW, Levinsky RJ, Gordon I, Turner MW, Matthew DJ. *Pseudomonas* infection, allergy and cystic fibrosis. *Arch Dis Child* 1982;57:582-6.
7. Silverman M, Hobbs FD, Gordon IRS, Carswell F. Cystic fibrosis, atopy and airways lability. *Arch Dis Child* 1978; 53:873-7.
8. Van Asperen P, Mellis CM, South RT. Respiratory allergy in cystic fibrosis. *Aust Paediatr J* 1980;16:53-6.
9. Sampson AP, Thomas RU, Costello JF, Piper PJ. Enhanced leukotriene synthesis in leucocytes of atopic and asthmatic subjects. *Br J Clin Pharmacol* 1992;33:423-30.
10. Drazen JM, Austen KF. Leukotrienes and airway responses. *Am Rev Respir Dis* 1987;136:985-98.
11. Maltby NH, Taylor GW, Ritter JM, Moore K, Fuller RW, Dollery CT. Leukotriene elimination and metabolism in man. *J ALLERGY CLIN IMMUNOL* 1990;85:3-9.
12. Smith AL, Redding G, Doershuk C, et al. Sputum changes associated with endobronchial exacerbation in cystic fibrosis. *J Pediatr* 1986;112:547-54.
13. Cotes JE. Lung function throughout life determinants and reference values. In: Cotes JE, ed. *Lung function: assessment and applications in medicine*. Oxford: Blackwell, 1979:329-87.
14. Arm JP, O'Hickey SP, Hawksworth RJ, et al. Asthmatic airways have a disproportionate hyperresponsiveness to LTE_4 , as compared with normal airways, but not to LTC_4 , LTD_4 , methacholine and histamine. *Am Rev Respir Dis* 1990;142:1112-8.

15. Komatsy N, Natsui K, Ueda N, Watanabe K, Yamamoto S. Immunohistochemical study on arachidonate 5-lipoxygenase in porcine leukocytes and other tissues. *J Histochem Cytochem* 1991;39:655-62.
16. Bedrossian CWM, Greenberg SD, Singer DB, Hansen JJ, Rosenberg HS. The lung in cystic fibrosis: a qualitative study including the prevalence of pathological findings among different age groups. *Human Pathol* 1976;7:195-204.
17. Dunhill MS. Neonatal and paediatric pulmonary disease. In: Dunhill MS, ed. *Pulmonary pathology*. 2nd ed. London: Churchill-Livingstone, 1987;547-8.
18. Slavin RG, Bedrossian CW, Hutcheson PS, et al. A pathological study of allergic bronchopulmonary aspergillosis. *J ALLERGY CLIN IMMUNOL* 1988;81:718-25.
19. Nadel JA. Roles of mast cell proteases in airways. *Drugs* 1989;37(suppl 1):51-5.
20. Schwartz LB, Irani AA, Mroueh S, Spock A. Reversed ratio of T/TC types of human mast cells in cystic fibrosis. *Clin Res* 1987;35:255-8.
21. Wang JL, Patterson R, Mintzer R. Allergic bronchopulmonary aspergillosis in pediatric practice. *J Pediatr* 1979;94:376-81.
22. Davies RJ, Corrado OJ, Blainey AD. Respiratory allergy. In: Lessof MH, ed. *Allergy*. London: John Wiley & Sons, 1984:256-7.
23. Leskowitz S, Salvaggio JE, Schwartz HE. A hypothesis for the development of atopic allergy in man. *Clin Allergy* 1972;2:237-46.
24. Clarke CW, Hampshire P, Hannant C. Positive skin responses in cystic fibrosis: a possible role for *Pseudomonas* infection. *Br J Dis Chest* 1981;75:15-21.
25. Brock DJH, Shrimpton AE, Jones C, McIntosh I. Cystic fibrosis: the new genetics. *J R Soc Med* 1991;84(suppl 18):2-6.
26. Johansen H, Nir M, Hoiby N, Koch C, Schwartz M. Severity of cystic fibrosis in patients homozygous and heterozygous for the $\Delta F508$ mutation. *Lancet* 1991;337:631-4.
27. Kubesch P, Dork T, Wulbrand U, et al. Genetic determinants of airways colonization with *Pseudomonas aeruginosa* in cystic fibrosis. *Lancet* 1993;341:189-93.
28. Turner MW, Warner JO, Stokes CR, Norman AP. Immunological studies in cystic fibrosis. *Arch Dis Child* 1978;53:631-8.
29. Moss RB, Hsu YP, Lewiston NJ. ^{125}I -C1q binding and specific antibodies as indicators of disease severity in CF. *J Pediatr* 1981;99:215-22.
30. Moss RB, Hsu YP, Lewiston NJ, et al. Association of systemic immune complexes, complement activation, and antibodies to *Pseudomonas aeruginosa* lipopolysaccharide and exotoxin A with mortality in cystic fibrosis. *Am Rev Respir Dis* 1986;133:648-52.
31. Fick RB Jr, Robbins RA, Squier SU, Schoderbek WE, Russ WD. Complement activation in cystic fibrosis respiratory fluids: in vivo and in vitro generation of C5a and chemotactic activity. *Pediatr Res* 1986;20:1258-68.
32. Tabor DR, Burchett SK, Jacobs RF. Enhanced production of monokines by canine alveolar macrophages in response to endotoxin induced shock. *Proc Soc Exp Biol Med* 1988;187:408-15.
33. Norman D, Elborn JS, Cordon SM, et al. Plasma tumour necrosis factor α in cystic fibrosis. *Thorax* 1991;46:91-5.
34. Sumimoto S, Kawai M, Y Kasajima K, Hamamoto T. Increased plasma tumor necrosis factor α concentration in atopic dermatitis. *Arch Dis Child* 1992;67:277-9.
35. Gosset P, Tsicopoulos A, Wallaert B, Joseph M, Capron A, Tonnel AB. Tumor necrosis factor alpha and interleukin 6 production by human mononuclear phagocytes from allergic asthmatic after IgE dependent stimulation. *Am Rev Respir Dis* 1992;146:768-74.
36. Roubin R, Elsas PP, Fiers W, Dessein AJ. Recombinant human TNF α enhances leukotriene synthesis in neutrophils and eosinophils stimulated by Ca^{++} ionophore A23187. *Clin Exp Immunol* 1987;70:484-90.
37. Greally P, Hussain MJ, Sampson AP, Cook AJ, Piper PJ, Price JF. Sputum tumour necrosis factor alpha and leukotriene concentrations in cystic fibrosis. *Arch Dis Child* 1993;68:389-92.