0031-3998/94/3605-0628$03.00/0
PEDIATRIC RESEARCH

Vol. 36, No. 5, 1994
Printed in U.S.A.

Altered Leukotriene Generation in Leukocytes from Cystinotic Children

GUILLEM PINTOS-MORELL, PATRICK SALEM, GENEVIÈVE JEAN, PATRICK NIAUDET, AND JEAN MICHEL MENCIA-HUERTA

University Hospital "Germans Trias i Pujol," Badalona, Spain [G.P.-M.]; INSERM U.200, Université Paris-Sud, Clamart, France [P.S.]; INSERM U.192, Hôpital Necker-Enfants Malades, Paris, France [G.J., P.N.]; and Institut Henri Beaufour, Les Ulis, France [J.M.M.-H.]

ABSTRACT

Upon *in vitro* stimulation with 10 μM ionophore A 23187 for 5 min at 37°C, the generation of leukotriene (LT) C_4 in polymorphonuclear leukocytes (PMNL) from nine untreated cystinotic children was significantly increased compared with that in eight control children ($p < 0.01$) and 25 normal adults ($p < 0.001$) (417.4 ± 70.0 *versus* 177.0 ± 30.9 and 164.9 ± 19.5 pmol/1 × 10^7 cells, respectively). Concomitantly with the increased generation of LTC_4, LTB_4 production in PMNL from untreated cystinotic children was decreased compared with controls, whereas the total amount of LTA_4 derivatives was similar in the three groups. The increase in LTC_4 production was not related to the number of eosinophils present in the PMNL preparations from cystinotic children, which was similar to that of control subjects. PMNL from cystinotic children treated with cysteamine, an aminothiol compound that decreases the intracellular cystine content, generated smaller amounts of LTC_4 upon ionophore A 23187 stimulation than PMNL from untreated cystinotic children. In addition, abrogation of the cysteamine treatment for 3 or 4 d led to an increase in LTC_4 production. These findings suggest that the metabolic abnormalities taking place in infantile cystinosis may favor the biosynthesis of LTC_4 from PMNL. (***Pediatr Res* 36: 628–634, 1994**)

Abbreviations
CDE, cystine dimethylester
GSH, glutathione
HBSS, Hanks' balanced salt solution
LT, leukotriene
PMNL, polymorphonuclear leukocyte
RP-HPLC, reverse-phase HPLC

LT are lipid mediators derived from arachidonic acid through the 5-lypoxygenase pathway (1). The C-6-sulfidopeptide LT, 5(S),6(R)5-hydroxy-6-S-glutathionyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid (LTC_4) and its derivatives LTD_4 and LTE_4, are the constituents of the slow-reacting substance of anaphylaxis (2). C-6-sulfidopeptide LT are potent inducers of bronchial and ileal smooth muscle contraction (3). They also increase vascular permeability (4) and induce synthesis of prostacyclin by endothelial cells (5) and synthesis of prostaglandin E_2 by macrophages (6). Another LT, 5(S),12(R)-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid (LTB_4), is a potent chemotactic factor for human neutrophils and monocytes *in vitro* (7). LTB_4 also increases the expression of C_3b receptors and induces lysosomal enzyme release from and aggregation of neutrophils (8).

The biosynthetic pathway of LT has been elucidated (9). Once released from membrane lipids, arachidonic acid is converted by the 5-lipoxygenase enzyme into 5-hydroperoxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid, which is transformed into 5,6-oxido-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid (LTA_4) by the same enzyme. LTC_4 is formed upon the adduction of GSH onto LTA_4 by a GSH-S-transferase. LTA_4 is also converted into LTB_4 by a specific hydrolase or nonenzymatically hydrolyzed primarily into 5(S),12(R)- and 5(S),12(S)-6-*trans*-LTB_4-diastereoisomers. The latter compounds have also been shown to originate from a myeloperoxidase-dependent metabolism of LTC_4 by activated human PMNL (10).

Infantile cystinosis is a recessively inherited metabolic disease characterized by a high intracellular cystine content (11) and the development of renal insufficiency during the first decade of life (12). The primary metabolic defect in cystinosis is a defect of the lysosomal cystine transport (13). Although GSH content is comparable in cystinotic and healthy subjects (14), the presence of several metabolic disturbances in cystinotic cells, such as an accelerated GSH turnover and an increased γ-glutamyl

Received April 5, 1993; accepted February 21, 1994.
Correspondence and reprint requests: Dr. G. Pintos Morell, University Hospital "Germans Trias i Pujol," Department of Pediatrics, 08916 Badalona, Spain.
G.P.M. was supported by grants from the "Comissió Interdepartamental per la Recerca i Innovació Tecnològica" (CIRIT), Generalitat de Catalunya (Spain), and from the PIRMED-Centre National de la Recherche Scientifique (CNRS), France.

transpeptidase activity (15, 16), are considered to be secondary to the increased cystine content.

Cysteamine (β-mercaptoethylamine), which decreases the intracellular cystine content *in vitro* and *in vivo* (17), has proven its efficiency in the treatment of cystinosis by improving growth and maintaining glomerular function in cystinotic children (18).

Because of the direct implication of cyst(e)ine and GSH in LTC_4 synthesis, we investigated the possible relationship between the intracellular cystine content and LTC_4 generation in PMNL from cystinosis patients. This was performed by comparing ionophore A 23187-induced generation of LT from PMNL of cystinotic children with that of control subjects and heterozygotes.

METHODS

Patients and controls. Eighteen cystinotic children, aged 2 to 10 y, before end-stage renal failure and presenting a glomerular filtration rate greater than 30 mL/min/1.73 m^2 were selected. Kidney-transplanted cystinotic children were not included because they were receiving immunosuppressive therapy. All the patients, except two, were receiving cysteamine treatment at the beginning of the study. Three other groups were also studied: the first consisted of eight children aged 3 to 11 y [three presenting chronic obstructive uropathy with mild renal insufficiency (glomerular filtration rate> 30 mL/min/1.73 m^2) and five normal children]. Because in these subjects no difference in the ionophore A 23187-induced generation of the various LTA_4 metabolites was observed, the results were analyzed as those of an homogeneous group. The second group included 10 adult cystinotic heterozygotes, and the third consisted of 25 healthy adult blood donors. All patients and controls were free of any sign of infection at the time of the experiments. In the case of cysteamine-treated children, blood was collected 6 h after the last ingestion of the drug. In seven cystinosis patients, experiments were performed under cysteamine, and 3 to 4 d after interruption of treatment. Informed consent was obtained from the parents of the cystinotic and control children and from the adults before collection of blood samples.

Cell separation procedures. Ten-mL blood samples were collected using heparin (10 U/mL) (Liquemine, Roche, Basel, Switzerland) as anticoagulant. PMNL were isolated upon dextran sedimentation, Ficoll separation, and erythrocyte lysis with ammonium chloride as previously described (19). The cells were finally resuspended in Ca^{2+}- and Mg^{2+}-containing HBSS without phenol red (Eurobio, Paris, France) at a final concentration of 1.1×10^7 cells/mL. PMNL purity averaged 97–98% and viability was greater than 99% as assessed by the trypan blue exclusion method. These cell populations contained mostly neutrophils and less than 10% eosinophils, as determined on smears obtained with a cytocentrifuge (Cytospin 2, Shandon, Runcorn, England) and stained with May-Grunwald-Giemsa.

Cell stimulation. Replicate tubes containing 5×10^6 PMNL in 450 μL of HBSS were preincubated at 37°C for 5 min. A sufficient amount of calcium ionophore A 23187 (Calbiochem-Boehring Corp., La Jolla, CA) in 50 μL of HBSS was then added to reach a final concentration of 10 μM. After 5 min of incubation at 37°C, the reaction was stopped upon addition of 500 μL methanol. This incubation period with this concentration of Ionophore were determined to be optimal for LT generation. In preliminary experiments, the dose-response to ionophore A 23187 and time course for LT generation were shown to be similar for PMNL from all groups of subjects (data not shown). The samples were centrifuged at $500 \times g$ for 10 min, and the supernatants collected and stored at −80°C under nitrogen.

In nine different experiments, PMNL from normal adults (1.1×10^7 cells/mL) were suspended in Ca^{2+}- and Mg^{2+}-free HBSS, pH 7.0, and aliquots (500 μL) were transferred into tubes containing a sufficient amount of CDE to reach a final concentration of 0.25 mM (20). Experiments were performed in parallel with tubes containing CDE and without CDE. CDE was introduced into the tubes as a methanol solution and dried under nitrogen before the cells were added. Then, the cells were incubated for 1 h at 37°C with gentle shaking, washed twice by centrifugation in Ca^{2+}- and Mg^{2+}-free HBSS for 5 min at $500 \times g$, counted, and finally resuspended at a concentration of 5×10^6 PMNL in 450 μL of Ca^{2+}- and Mg^{2+}-containing HBSS before ionophore A 23187 stimulation as described above. Cell viability was not affected by the CDE treatment as assessed by the trypan blue exclusion method.

LT analysis. The samples from stimulated and unstimulated PMNL were analyzed by RP-HPLC as previously described (21). RP-HPLC was carried out with a Waters apparatus (Waters Associates, Millipore Corp., Milford, MA) using a μBondapak C18 column (4.6 × 250 mm) (Waters) eluted at a flow rate of 1 mL/min with methanol:water:acetic acid (60:40:0.1, vol/vol; pH 5.6) as solvent. Five-hundred-μL samples were injected, and the elution was monitored at 280 nm using a 440 Waters spectrophotometer. LTC_4, 6-*trans*-LTB_4 diastereoisomers, and LTB_4 were quantified by integration of the UV absorbance peak according to a standard curve established with known amounts of authentic standards. In selected experiments, 1-min fractions were collected and dried under vacuum. The dry residues were resuspended in 10 mM Tris, 0.15 M NaCl, 0.1% gelatin, pH 7.4, and the samples were assayed for C-6-sulfidopeptide LT content using an RIA. Synthetic LTC_4 and rabbit antibodies against LTC_4 were a gift from Dr. Rokach (Merck Frosst, Montreal, Canada). [^{3}H]LTC_4 was purchased from New England Nuclear (Paris, France).

Intracellular cystine assay. Leukocytes were prepared by mixing fresh heparinized blood with an equal volume of a solution containing 3 g of dextran, 2.1 g of dextrose, 0.33 g of sodium citrate, and 0.11 g of anhydrous citric acid in 100 mL of 0.15 N NaCl. After sedimentation for 45

min at room temperature, the supernatant was removed and centrifuged at 450 × *g* for 10 min. The leukocyte pellet was resuspended in 3.2 mL of 0.22% NaCl for 90 s to lyse the remaining erythrocytes, and isotonicity was then restored by the addition of 0.8 mL of 3.6 NaCl. The cells were sedimented by centrifugation at 450 × *g* for 5 min, and the pellet was washed twice with 0.15 N NaCl. Finally, the pellet was sonicated in 0.3 mL of distilled water and mixed with 0.1 mL of 12% sulfosalicylic acid. Cystine determinations were performed on the acid-soluble fraction by means of the cystine binding protein assay (22). The protein content was measured in the acid-insoluble fraction using the Folin-Lowry method.

Statistical analysis. Results are expressed as means ± SEM of the indicated number of experiments. Significance was assessed by the paired and unpaired *t* test, the nonparametric Wilcoxon signed-rank test and the linear regression test.

RESULTS

Ionophore-induced generation of LT from PMNL from healthy and cystinotic children. RP-HPLC analysis of supernatants from ionophore-stimulated PMNL of cystinotic children resulted in elution patterns similar to those from PMNL of control adults and children (Fig. 1). Four peaks of 280-nm absorbing material corresponding to the retention time of authentic LTC_4, 5(S),12(R)- and 5(S),12(S)-6-*trans*-LTB_4 diastereoisomers, and LTB_4 were detected. However, the amount of UV-absorbing material exhibiting the retention time of LTC_4 was markedly higher in the supernatants of ionophore-stimulated PMNL from cystinotic patients (Fig. 1*B*) compared with control adults and control children (Fig. 1*A*). In addition, a slight decrease in the amount of the product eluting with the retention time of LTB_4 was noted. In contrast, no marked variations in the amounts of products eluting with the retention times of the 6-*trans*-LTB_4 diastereoisomers were observed. No UV-absorbing peak was observed when the supernatants of unstimulated PMNL from either cystinotic or control subjects were analyzed by RP-HPLC.

Fractions collected during RP-HPLC of the supernatants from ionophore A 23187–stimulated PMNL of cystinotic children were assessed for C-6-sulfidopeptide LT. As presented in Figure 2, only the UV-absorbing material exhibiting the retention time of authentic LTC_4 was immunoreactive. In three experiments, the amounts of LTC_4 quantitated by integration of UV absorbance were similar to those obtained using RIA: 245.7 and 237.5, 187.6, and 177.5, and 280.0 and 255.9 pmol/1 × 10^7 cells, respectively. The correlation coefficient between the two methods was 0.996; thus, the use of the RIA was discontinued.

The amount of LTA_4 metabolites, namely, LTC_4, 6-*trans*-LTB_4 diasteroisomers, and LTB_4 in the supernatants of ionophore-stimulated PMNL from control adults and control children, cystinotic adult heterozygotes, and cystinotic children with and without cysteamine treatment was measured by integration of the respective UV-absorbing peak during RP-HPLC (Table 1). For seven of the nine untreated cystinotic children, cysteamine admin-

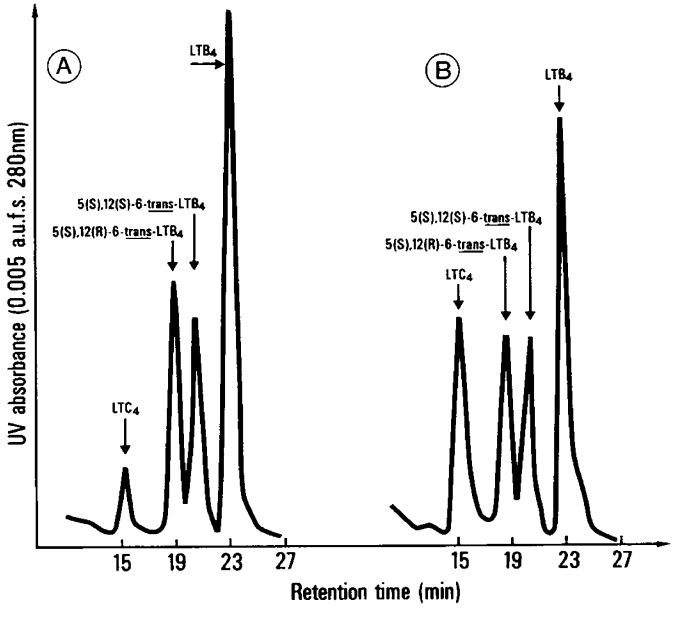

Figure 1. Representative RP-HPLC elution patterns of the products generated from ionophore A 23187–stimulated PMNL from control adults (n = 25) and control children (n = 8) (*A*) and cystinotic children (*B*). The cells were preincubated for 5 min at 37°C and then stimulated with 10 μM ionophore for an additional 5 min. RP-HPLC was carried out using a μBondapak C18 column eluted at 1 mL/min with methanol:water:acetic acid (60:40:0.1, pH 5.6). The elution was monitored at 280 nm, and the *arrows* indicate the retention times of authentic standards. *a.u.f.s.*, absorbance unit full scale.

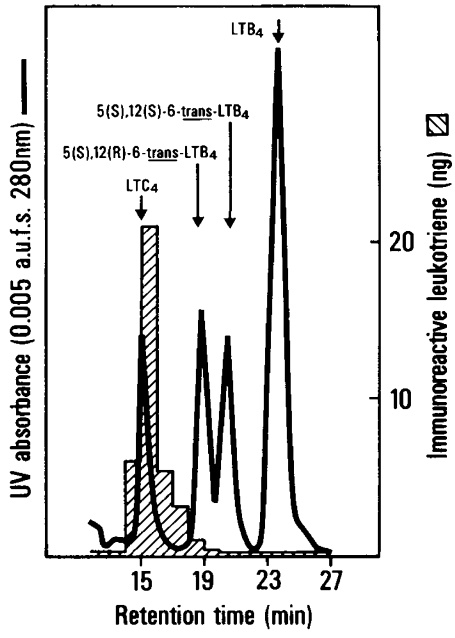

Figure 2. RP-HPLC elution pattern of the products generated from PMNL of a cystinotic child. Same experimental protocol as in Figure 1, except that 1-min fractions were collected and assayed for cross-reactivity in an RIA for C-6-sulfidopeptide LT. One experiment representative of three.

Table 1. *LT production in ionophore A 23187–stimulated PMNL**

	Controls		Adult cystinotic heterozygotes	Cysteamine-treated cystinotic children	Untreated cystinotic children
	Adults	Children			
Number of experiments	25	8	10	18	9
LTC_4	164.9 ± 19.5 (9.8)	177.0 ± 30.9 (9.7)	137.6 ± 32.1 (9.9)	242.3 ± 16.2† (15.4)	417.4 ± 70.0‡ (25.4)
5(S),12(R)-6-*trans*-LTB_4	275.5 ± 14.2 (16.3)	260.0 ± 23.2 (14.2)	240.0 ± 27.8 (17.2)	248.2 ± 25.5 (15.8)	248.9 ± 29.8 (15.1)
5(S),12(S)-6-*trans*-LTB_4	261.0 ± 13.7 (15.5)	242.3 ± 27.1 (13.3)	234.9 ± 22.2 (16.9)	243.2 ± 28.7 (15.4)	210.1 ± 28.9 (12.8)
LTB_4	985.7 ± 56.4 (58.4)	1174.4 ± 120.6 (62.8)	779.2 ± 61.1 (56.0)	839.6 ± 69.2 (63.4)	767.6 ± 72.1† (46.7)
Total LTA_4 metabolites	1687.1 ± 80.3 (100.0)	1826.7 ± 153.1 (100.0)	1391.7 ± 84.0† (100.0)	1573.3 ± 98.3 (100.0)	1644.0 ± 82.7 (100.0)

* A total of 5 × 10^6 PMNL from control adults and control children, adult cystinotic heterozygotes, and cysteamine-treated and untreated cystinotic children were stimulated with 10 μM ionophore A 23187 for 5 min at 37°C. LT were quantitated by integration of the UV absorbance peak during RP-HPLC. Values are expressed as mean ± SEM pmol/1 × 10^7 cells, and the numbers of parentheses indicate the percentage of each product with respect to the total amount of LTA_4 metabolites. Significance of absolute values was assessed by the unpaired *t* test with respect to control children.

† $p < 0.05$.

‡ $p < 0.01$.

istration was interrupted for 3 to 4 d before the experiments. Upon ionophore stimulation, the total amounts of LTA_4 metabolites generated were lower in PMNL from adult cystinotic heterozygotes compared with the other four cell populations. The amount of LTC_4 with respect to the total amount of LTA_4 derivatives generated from PMNL was higher in cysteamine-untreated cystinotic children (25.4%) than in cysteamine-treated cystinotic children (15.4%), cystinotic heterozygotes (9.9%), control children (9.7%), and normal adults (9.8%). The increased LTC_4 production in PMNL from untreated cystinotic children was associated with a decrease in the generation of LTB_4, which was significantly ($p < 0.05$) lower than that in children and adult controls.

Role of eosinophils in generation of LTC_4 from PMNL. Human eosinophils release large amounts of LTC_4 (23). However, the possibility that the different release of LTC_4 in the various PMNL populations demonstrated in Table 1 was merely due to the variation in the number of eosinophils is excluded, because the percentages of this cell type were similar in the PMNL populations from control adults (5.5 ± 4.5%) and control children (5.5 ± 2.0%), adult cystinotic heterozygotes (4.5 ± 2.9%), and cysteamine-treated (6.8 ± 3.5%) and untreated cystinotic children (6.9 ± 4.1%). In addition, statistical analysis showed no correlation between the amounts of LTC_4 generated upon ionophore A 23187 stimulation of PMNL from cystinotic children and the number of eosinophils present in the cell preparation ($r = 0.335$; $p > 0.10$, $n = 27$).

Role of intracellular cystine content in generation of LTC_4 from PMNL. In seven patients, the ionophore A 23187–induced generation of LT from PMNL was assessed during cysteamine treatment and 3 to 4 d after withdrawal of the drug (Table 2). In four of the seven patients (patients 1, 2, 4, and 7), a marked increase in the ionophore A 23187–induced LTC_4 production was observed with the interruption of the drug, whereas in the three other patients (patients 3, 5, and 6) no significant difference was observed. Notably, the four patients presenting an increase in ionophore-induced LTC_4 production were also those presenting the highest increment in cystine content after cysteamine withdrawal for 3 to 4 d. In addition, the increment in LTC_4 production and cystine content were highly correlated ($p = 0.004$). In these experiments, the increase in LTC_4 generation upon stimulation of PMNL from untreated patients also appeared to be concomitant with a decrease in LTB_4 production. In contrast, the amounts of 6-*trans*-LTB_4 diastereoisomers were unchanged, and the total amount of LTA_4 metabolites produced was similar before (1701.0 ± 193.0 pmol/1 × 10^7 cells) and after (1629.3 ± 98.9 pmol/1 × 10^7 cells) interruption of the cysteamine treatment.

CDE has been shown to increase several times the intracellular cystine content (20). To investigate the possible relationship between the intracellular cystine content and LTC_4 production, PMNL from normal adults were incubated for 1 h in the presence of 0.25 mM CDE

Table 2. *Cystine content (Cyst) and LTC_4 generation from ionophore-stimulated PMNL from cystinotic children**

	Cysteamine treatment					
	+		−			
	LTC_4	Cyst	LTC_4	Cyst	ΔLTC_4	ΔCyst
Patient						
1	182.4	2.0	435.2	5.1	252.8	3.1
2	336.0	1.6	684.8	7.1	348.8	5.5
3	547.2	2.4	454.4	4.3	−92.8	2.0
4	150.4	1.6	326.4	5.3	176.0	3.7
5	156.8	0.6	166.4	1.3	9.6	0.7
6	211.2	3.4	185.6	4.2	−25.6	0.7
7	265.6	0.9	800.0	6.7	534.4	5.8
Mean	264.7	1.8	436.1	4.9		
SEM	53.3	0.3	90.2	0.7		
r	0.239		0.835		0.910	
p	0.606		0.019		0.004	

* A total of 5 × 10^6 PMNL obtained from seven cystinotic children both during (+) and 3 to 4 d after interruption (−) of treatment with cysteamine were stimulated with 10 μM ionophore A 23187 for 5 min at 37°C. LT were quantitated by integration of the UV absorbance peak during RP-HPLC, and results are expressed as means ± SEM pmol LTC_4/1 × 10^7 cells. Cystine was measured by a cystine binding assay as defined in "Methods," and results are expressed as nmol half cystine/mg protein. Δ represents the difference between LTC_4 generation and cystine content after drug withdrawal and the values obtained during treatment.

before ionophore A 23187 stimulation. As presented in Table 3, incubation of PMNL with CDE led to a significant ($p < 0.05$) increase in LTC_4 generation after ionophore stimulation compared with LTC_4 generation in untreated PMNL. In contrast, the production of the 6-*trans*-LTB_4 diastereoisomers and LTB_4 as well as the total amount of LTA_4 metabolites were not significantly altered in CDE-treated PMNL compared with untreated ones. In these experiments, the cystine content was 0.29 ± 0.07 and 3.47 ± 0.67 nmol half cystine/mg protein in control and CDE-treated PMNL, respectively.

Finally, the intracellular cystine content and the amounts of LTC_4 generated upon ionophore stimulation were measured in PMNL preparations from 10 cysteamine-treated cystinotic children (Table 4). In these patients, the intracellular cystine content was 3 to 17 times greater than normal values, which were less than 0.2 nmol half cystine/mg protein. However, no correlation ($r = 0.239$) was observed between the intracellular cystine content and LTC_4 generation in these experiments.

Table 3. *Effect of incubation of PMNL with CDE on ionophore-induced production of LT**

	Control PMNL	CDE-treated PMNL
LTC_4	78.6 ± 15.4 (7.9)	120.2 ± 17.5 (11.5)†
5(S),12(R)-6-*trans*-LTB_4	167.1 ± 10.5 (16.8)	178.5 ± 13.2 (17.0)
5(S),12(S)-6-*trans*-LTB_4	150.4 ± 10.4 (15.1)	163.9 ± 12.8 (15.7)
LTB_4	597.1 ± 37.4 (60.1)	584.2 ± 32.7 (55.8)
Total LTA_4 metabolites	993.2 ± 62.7 (100.0)	1046.8 ± 60.5 (100.0)

* Replicate tubes containing 5×10^6 PMNL from adult controls were incubated for 1 h at 37°C in the presence or absence of 0.25 mM CDE from ionophore stimulation for 5 min. LT were quantitated by integration of the UV absorbance peak during RP-HPLC. Values are expressed as means ± SEM pmol/1 × 10^7 cells of nine experiments and values in parentheses are the percentage of each product with respect to the total amount of LTA_4 metabolites.

† $p < 0.05$. Significance was assessed by the Wilcoxon sign-rank test.

Table 4. *Intracellular cystine content and LTC_4 generation from PMNL of cysteamine-treated cystinotic children**

Patient	Intracellular cystine (nmol half cystine/mg protein)	LTC_4 (pmol/1 × 10^7 cells)
1	0.57	169.6 (8.7)
2	0.61	547.2 (28.0)
3	0.91	214.4 (17.0)
4	3.43	211.2 (8.2)
5	1.60	332.8 (20.3)
6	1.60	118.4 (7.6)
7	0.56	230.4 (13.6)
8	2.34	275.2 (15.0)
9	1.30	166.4 (12.3)
10	1.70	342.4 (19.5)

* Intracellular cystine content was measured using a cystine binding assay as described in "Methods" (normal values: 0–0.2 nmol half cystine/mg protein). LTC_4 generated from PMNL upon ionophore stimulation was quantitated by integration of the UV absorbance peak during RP-HPLC. The numbers in parentheses indicate the percentage of LTC_4 with respect to the total amount of LTA_4 metabolites.

DISCUSSION

Ionophore-stimulated PMNL from cystinotic children generated increased amounts of LTC_4 compared with those from control children, control adults, and adult cystinotic heterozygotes. LTC_4 generated from cystinotic PMNL upon ionophore stimulation was identified by its retention time during RP-HPLC and the quantitative correspondence between RIA measurement and integration of the UV absorbance peak.

The total amounts of LTA_4 metabolites, namely LTC_4, 6-*trans*-LTB_4 diastereoisomers, and LTB_4 generated from ionophore-stimulated PMNL from cystinotic children were similar to those generated from children and adult control PMNL. However, upon ionophore stimulation of PMNL from cystinotic children, a significant increase in the amount of LTC_4 and a concomitant decrease in the production of LTB_4 were observed. Thus, increased metabolism of LTA_4 via the GSH-S-transferase pathway appeared to be responsible for the increased LTC_4 generation in PMNL from cystinotic children. In these experiments, the amounts of two of the nonenzymatic degradation products of LTA_4, namely 5,6-dihydroxy-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid diastereoisomers (8, 9), only accounted for less than 2% of the total LTA_4 metabolites. The addition of catalase (500–1000 U) during stimulation did not modify the RP-HPLC elution pattern of the LT generated from PMNL of cystinotic children and controls (data not shown). This excludes the possibility that the increase in LTC_4 production was due to less efficient myeloperoxydase-dependent LT catabolism (10) in PMNL from cystinotic children than in control subjects.

Calcium ionophore stimulation of phospholipase A has no preferential effects *per se* on the different arachidonic metabolites, but it depends on the particular cell type. Ionophore-stimulated human neutrophils preferentially produce LTB_4 (7), and eosinophils have been shown to release significant amounts of LTC_4 (23). However, in the present study, no difference in the percentage of eosinophils was noted between the various PMNL preparations, indicating that the increase in LTC_4 production in PMNL from cystinotic children was not related to a variation in the composition of the cell populations studied. In addition, there was no correlation between the amount of LTC_4 generated and the number of eosinophils present in the PMNL preparations from cystinotic children. Our study, however, does not exclude the possibility that the alteration in LTC_4 generation is related to a modification of the eosinophil response. Unfortunately, purification of eosinophils could not be performed due to the small volume of blood that can be obtained safely from children. Only the comparison of the response of purified cell populations from cystinotic and control subjects will permit us to eventually ascribe the observed alterations with a defined cell type. An alternative possibility, not ruled out in our study, is that cystinotic eosinophils could generate drastically enhanced quantities of LTC_4 and that a second biochemical defect exists in the neutrophils, attenuating LTB_4 production.

The major biochemical alteration of cells from cystinotic children is a defect of the lysosomal cystine transport (13). The association of increased uptake (24) and reduction rates of cystine (25) as well as increased γ-glutamyl transpeptidase activity and accelerated GSH turnover (15, 16) are considered to be secondary findings. Also, an increase in GSH synthetase activity has been shown in lag and early growth phases of cystinotic cells, and it was not reflected in increased GSH content (26). Cysteamine has been shown to deplete transiently the intracellular cystine content in leukocytes from cystinotic children (27). Accordingly, LTC_4 production from PMNL of cysteamine-treated cystinotic children was markedly lower than in untreated cystinotic children and patients for whom the treatment was interrupted. In four of seven cystinotic children studied with and without cysteamine, drug withdrawal led to a further enhancement of LTC_4 generation without alteration of the total production of LTA_4 metabolites. These data suggest that elevated intracellular cystine content is involved in the increase in LTC_4 production. Although most cystine accumulation occurs in the lysosome while the synthesis of LTC_4 takes place in the cytoplasm, the presence of an alternative nonsaturable transmembrane route for cystine transport from the lysosome to the cytoplasm demonstrated in L-929 fibroblasts (28) supports this possibility (Fig. 3). Accordingly, cystinotic cells, mainly granulocytes and monocytes, present multiple alterations of diverse biologic responses (29, 30). This hypothesis was further strengthened by our experiments showing that PMNL previously loaded with CDE generated, when stimulated by ionophore A 23187, higher amounts of LTC_4 (53% increase) than untreated PMNL.

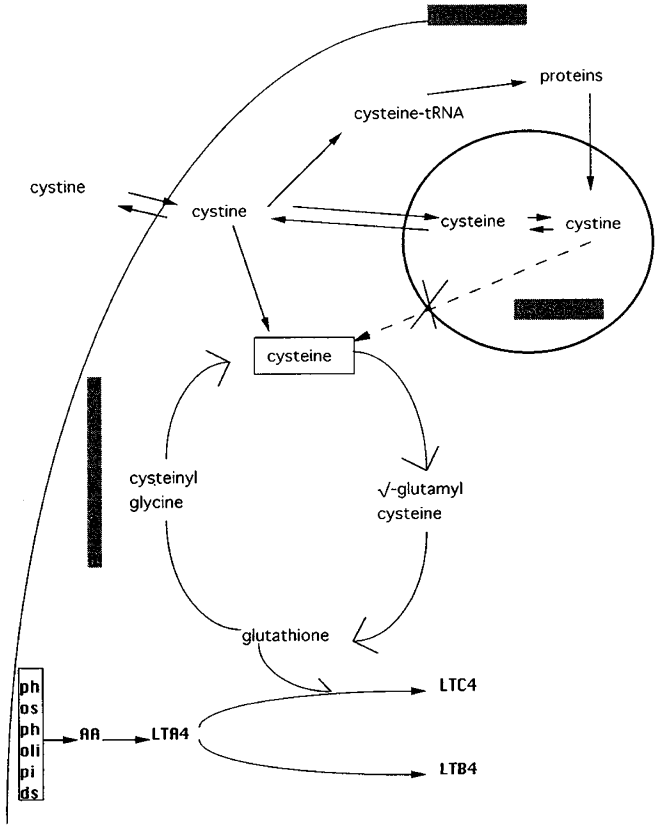

Figure 3. Hypothetical relationship between cystine and LTC_4 synthesis in cystinotic cells. The *broken arrow* indicates that at high intralysosomal cystine concentrations some cystine may exit the lysosome through a nonsaturable mechanism.

The lack of correlation between the intracellular cystine content and the amounts of LTC_4 observed in cysteamine-treated cystinosis patients must be accepted with caution because of the possible drug interactions with the SH-dependent enzymes of the 5-lipoxygenase pathway and with GSH (31).

PMNL from adult cystinotic heterozygotes, which present an intracellular cystine content close to that of cysteamine-treated children (32), generated an amount of LTC_4 similar to that generated by PMNL of control children and adults. These results suggest that the cystine content is not the only reason for the increase in LTC_4 production of PMNL from cystinotic children and that other biochemical alterations present in the complete genetic defect may play a role. These results are in agreement with a previous report showing that PMNL from cystinotic children exhibit an increased oxidative burst upon phorbol-myristate-acetate and formyl-methionyl-leucyl-phenylalanine peptide stimulation that is not correlated with the intracellular cystine content (29).

Additional studies on GSH-S-transferase activity and GSH turnover in purified populations of neutrophils and eosinophils from cystinotic children may provide a better understanding of the alteration of LT production presently demonstrated in mixed granulocyte preparations. LTC_4 produces vasoconstriction, increases vascular permeability, and is a potent agonist for mesangial cell contraction. Excessive LTC_4 production together with increased oxidative burst (29, 30) may contribute to progressive tissue damage in cystinosis. Nevertheless, whether the *in vitro* increased capability of PMNL from cystinotic children to generate LTC_4 participates in the evolution of the disease is presently unknown and thus deserves investigation.

Acknowledgments. The authors thank Dr. J. A. Schneider (Department of Pediatrics, UCSD, La Jolla, CA), who kindly measured intracellular cystine content; Dr. J. Rokach (Merk Frosst, Montreal, Canada), who generously provided the antiserum against LTC_4 and synthetic LTC_4; Dr. M. Broyer and Dr. C. Loirat, who selected the patients; and N. J. Widmer, who provided excellent secretarial assistance.

REFERENCES

1. Parker CW 1987 Lipid mediators produced through the lipoxygenase pathway. Ann Rev Immunol 5:65–84
2. Samuelsson B 1983 Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. Science 220:568–575
3. Hedqvist P, Dahlen SE, Gustafsson L, Hammarstrom S, Samuelsson B 1980 Biological profile of leukotrienes C_4 and D_4. Acta Physiol Scand 110:331–333
4. Dahlen SE, Bjork J, Hedqvist P, Arfors KE, Hammarstrom S, Lindgren JA, Samuelsson B 1981 Leukotrienes promote plasma leakage and leukocyte

adhesion in postcapillary venules: *in vivo* effects with relevance to the acute inflammatory response. Proc Natl Acad Sci USA 78:3887–3891
5. Cramer EB, Pologe L, Pawlowski NA, Cohn ZA, Scott WA 1983 Leukotriene C promotes prostaglandin synthesis by human endothelial cells. Proc Natl Acad Sci USA 80:4109–4113
6. Feuerstein N, Bash JA, Woody JN, Ramwell PW 1981 Leukotriene C stimulates prostaglandin release from rat peritoneal macrophages. Biochem Biophys Res Commun 100:1085–1090
7. Ford-Hutchinson AW, Bray MA, Doig MF, Shipley ME, Smith MJH 1980 Leukotriene B_4, as potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. Nature 286:264–265
8. Lewis RA, Austen KF 1984 The biologically active leukotrienes: biosynthesis, metabolism, receptors, functions, and pharmacology. J Clin Invest 73:889–897
9. Hammarstrom S 1983 Leukotrienes. Ann Rev Biochem 52:355–377
10. Lee CW, Lewis RA, Corey EJ, Barton A, Oh H, Tauber AI, Austen KF 1982 Oxidative inactivation of leukotriene C_4 by stimulated human polymorphonuclear leukocytes. Proc Natl Acad Sci USA 79:4166–4170
11. Schneider JA, Katz B, Melles RB 1990 Update on nephropathic cystinosis. Pediatr Nephrol 4:645–653
12. Gahl WA, Schneider JA, Schulman JD, Thoene JG, Reed GF 1990 Predicted reciprocal serum creatinine at age 10 years as a measure of renal function in children with nephropathic cystinosis treated with oral cysteamine. Pediatr Nephrol 4:129–135
13. Gahl WA, Bashan N, Tietze F, Bernardini I, Schulman JD 1982 Cystine transport is defective in isolated leukocyte lysosomes from patients with cystinosis. Science 217:1263–1265
14. Oshima RG, Rhead WJ, Thoene JG, Schneider JA 1976 Cystine metabolism in human fibroblasts. J Biol Chem 251:4287–4293
15. States B, Segal S 1982 Levels of gamma-glutamyl transpeptidase in cultured skin fibroblasts from cystinotics and normals. Life Sci 27:1985–1990
16. Butler JB, Spielberg SP 1981 Decrease of intracellular cystine content in cystinotic fibroblasts by inhibitors of gamma-glutamyl transpeptidase. J Biol Chem 9:4160–4163
17. Gahl WA, Charnas L, Markello TC, Bernardini I, Ishak KG, Dalakas MC 1992 Parenchymal organ cystine depletion with long-term cysteamine therapy. Biochem Med Metab Biol 48:275–285
18. Gahl WA, Reed GF, Thoene JG, Schulman JD, Rizzo WB, Jonas AJ, Denman DW, Schlesselman JJ, Corden BJ, Schneider JA 1987 Cysteamine therapy for children with nephropathic cystinosis. N Engl J Med 316:971–977
19. Boyum A 1968 Separation of leukocytes from blood and bone marrow. Scand J Clin Lab Invest 21:77–89
20. Steinherz R, Tietze F, Gahl WA, Triche TJ, Chang H, Modesti A, Schulman JD 1982 Cystine accumulation and clearance by normal and cystinotic leukocytes exposed to cystine dimethylester. Proc Natl Acad Sci USA 79:4446–4450
21. Mencia-Huerta JM, Razin E, Ringel EW, Corey EJ, Hoover D, Austen KF, Lewis RA 1983 Immunologic and ionophore-induced generation of leukotriene B_4 from mouse bone marrow derived mast cells. J Immunol 130:1885–1890
22. Oshima RG, Willis RC, Furlong CE, Schneider JA 1974 Binding assays for amino acids: the utilization of a cystine binding protein from *Escherichia coli* for the determination of acid-soluble cystine in small physiological samples. J Biol Chem 249:6033–6039
23. Weller PF, Lee CW, Foster DW, Corey EJ, Austen KF, Lewis RA 1983 Generation and metabolism of 5-lipoxygenase pathway leukotrienes by human eosinophils: predominant production of leukotriene C_4. Proc Natl Acad Sci USA 80:7626–7630
24. States B, Lee J, Segal S 1981 Uptake of cystine by cystine-depleted fibroblasts from patients with cystinosis. Biochem Biophys Res Commun 98:290–296
25. States B, Harris D, Segal S 1977 Patterns of cystine reduction by fibroblasts from normal and cystinotic children. Pediatr Res 11:685–690
26. Butler JB, Key JD, Hughes BF, Tietze F, Raiford DS, Reed GF, Brannon PM, Spielberg SP, Schulman JD 1987 Glutathione metabolism in normal and cystinotic fibroblasts. Exp Cell Res 172:158–167
27. Jonas AJ, Schneider JA 1982 Plasma cysteamine concentrations in children treated for cystinosis. J Pediatr 100:321–323
28. Greene AA, Marcusson EG, Pintos-Morell G, Schneider JA 1990 Characterization of the lysosomal cystine transport system in mouse L-929 fibroblasts. J Biol Chem 265:9888–9895
29. Pintos-Morell G, Niaudet P, Jean G, Descamps-Latscha B 1985 Altered oxidative metabolism, motility and adherence in phagocytic cells from cystinotic children. Pediatr Res 19:1318–1321
30. Pintos-Morell G, Jean G, Dechaux M, Niaudet P 1991 Increased monocyte-dependent suppression of polyclonal activation of B lymphocytes from cystinotic children. Pediatr Nephrol 5:597–602
31. Peters-Golden M, Shelly C 1987 Modulation of alveolar macrophage-derived 5-lipoxygenase products by the sulfhydryl reactant, N-ethylmaleimide. J Biol Chem 262:10594–10600
32. Gahl WA, Tietze F 1987 Lysosomal cystine transport in cystinosis variants and their parents. Pediatr Res 21:193–196