Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 11598–11602, December 1992
Medical Sciences

Leukotriene C_4 uses a probenecid-sensitive export carrier that does not recognize leukotriene B_4

(organic anion transporters/eosinophils/KG-1 cells/HL-60 cells)

BING K. LAM*†, KONGYI XU*, MICHAEL B. ATKINS‡, AND K. FRANK AUSTEN*

*Department of Medicine, Harvard Medical School, and Department of Rheumatology and Immunology, Brigham and Women's Hospital, Boston, MA 02115; and ‡Division of Hematology-Oncology, Tufts-New England Medical Center Hospital, Boston, MA 02111

Contributed by K. Frank Austen, August 17, 1992

ABSTRACT **The export of leukotriene (LT) C_4 from human eosinophils, a carrier-mediated process that is temperature-dependent and saturable, was characterized further in eosinophils and in two human leukemia cell lines that do not present an intact 5-lipoxygenase pathway. In eosinophils, KG-1 cells, and dimethyl sulfoxide (DMSO)-differentiated HL-60 cells, the respective Q_{10} values for temperature-dependent LTC_4 export were 3.7, 3.3, and 3.4 and for energy of activation were 28.2 kcal/mol, 23.0 kcal/mol, and 27.8 kcal/mol (1 kcal = 4.18 kJ). When human eosinophils, KG-1 cells, and DMSO-differentiated HL-60 cells were preloaded with defined amounts of intracellular LTC_4 by incubation with LTA_4 and with incremental amounts of a glutathione conjugate, *S*-dinitrophenyl glutathione (GS-DNP) by sequential incubation with 1-chloro-2,4-dinitrobenzene, GS-DNP inhibited the export of LTC_4 in a dose-dependent manner. By plotting the ratio of total GS-DNP (cell retained plus released) to the sum of total GS-DNP plus total LTC_4 against the percentage inhibition of LTC_4 release, IC_{40} values of 0.839, 0.803, and 0.841 were obtained for eosinophils, KG-1 cells, and DMSO-differentiated HL-60 cells, respectively. When cells preloaded with LTC_4 were resuspended in incremental concentrations of the organic acid transport inhibitor, probenecid, there was a dose-dependent decrease in LTC_4 release; GS-DNP and probenecid inhibited LTC_4 release in a cumulative fashion, whereas neither inhibited the release of LTB_4 from preloaded nondifferentiated HL-60 cells. Therefore, LTC_4 export from cells of bone marrow origin occurs through a probenecid-sensitive membrane carrier shared by other glutathione conjugates and distinct from the LTB_4 carrier export system.**

Leukotrienes (LTs) are potent lipid mediators that are preferentially synthesized and released by bone marrow-derived granulocytes (1–3). LTB_4 elicits polymorphonuclear leukocyte (PMN) functions such as chemotaxis, chemokinesis, and degranulation (4–6), and the cysteinyl leukotrienes, LTC_4 and its metabolites LTD_4 and LTE_4, contract vascular and nonvascular smooth muscles (7–11). The biosynthesis of LTs is initiated by 5-lipoxygenase, which inserts molecular oxygen at the carbon-5 position of arachidonic acid to form 5-hydroperoxyeicosatetraenoic acid and then catalyzes a dehydration to form the epoxide intermediate LTA_4 (12–15). In PMNs and macrophages, LTA_4 is converted to LTB_4 by cytosolic LTA_4 hydrolase (16, 17). In eosinophils, mast cells, basophils, and monocytes, a microsomal LTC_4 synthase conjugates LTA_4 to reduced glutathione to form LTC_4 (18, 19). Once LTB_4 and LTC_4 are formed, they are released by specific membrane transporters (20, 21).

We have demonstrated that LTC_4 is released from human eosinophils by a temperature-sensitive, saturable transport system (21). In the present study, this transport system is characterized further in three different human cell types, eosinophils, myelocytic KG-1 cells, and promyelocytic HL-60 cells, each with a different profile of 5-lipoxygenase pathway enzymes. We show that the release of LTC_4 from these cells is inhibited by probenecid, a classic inhibitor of organic acid transport, and that this LTC_4 transport system is shared by xenobiotic glutathione conjugates. Therefore, organic anion transporters and LTC_4/glutathione conjugate carriers may recognize common structural domains on their substrates. In addition, the carrier-mediated export process for LTC_4 is identified even in the absence of a 5-lipoxygenase and is distinct from that for LTB_4.

METHODS

Materials purchased from suppliers are as follows: Ficoll–Paque (Pharmacia LKB Biotechnology); RPMI 1640, penicillin, L-glutamine, streptomycin (GIBCO); fatty acid-free bovine serum albumin, fetal calf serum, dextran (molecular weight, 153,000), 1-chloro-2,4-dinitrobenzene (CDNB), probenecid (Sigma); prostaglandin B_2 (PGB_2), LTB_4 (Cayman Chemicals, Ann Arbor, MI); HPLC-grade methanol and acetonitrile (Burdick and Jackson). Biosynthetic recombinant human granulocyte/macrophage colony-stimulating factor (GM-CSF) was provided by Genetics Institute (Cambridge, MA). LTC_4 and LTA_4 methyl ester were synthesized and provided by E. J. Corey and B. Spur (Harvard University). The methyl ester of LTA_4 was hydrolyzed as described (22).

Cell Culture. Eosinophils were isolated from peripheral venous blood of patients with solid tumors at the end of their therapy with interleukin 2 as described (21) and were cultured in enriched medium (RPMI 1640 containing 100 units of penicillin per ml, 100 g of streptomycin per ml, 2 mM glutamine, and 10% fetal calf serum) supplemented with 5 pM recombinant GM-CSF under a humidified atmosphere of 95% O_2/5% CO_2 for 3–4 days (21). Human myelocytic KG-1 cells and promyelocytic HL-60 cells (American Type Culture Collection) were cultured under identical conditions except that no GM-CSF was added to the culture medium.

Assay of LTC_4 Export. Cultured eosinophils, KG-1 cells, and HL-60 cells were harvested, were washed twice with Hanks' balanced salt solution containing 2 mg of bovine serum albumin per ml (HBSA), and were resuspended in HBSA at a concentration of 2×10^7 cells per ml. The cell suspensions were incubated with 25 μM LTA_4 for 3 min at 37°C and then for 57 min on ice as described for preloading

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: LT, leukotriene; CDNB, 1-chloro-2,4-dinitrobenzene; RP-HPLC, reverse-phase high-performance liquid chromatography; GM-CSF, granulocyte/macrophage colony-stimulating factor; HBSA, Hanks' balanced salt solution containing 2 mg of bovine serum albumin per ml; GS-DNP, *S*-dinitrophenyl glutathione; DMSO, dimethyl sulfoxide; PGB_2, prostaglandin B_2; PMN, polymorphonuclear leukocyte.

†To whom reprint requests should be addressed.

of LTC_4 (21). The reactions were stopped by centrifugation at 3000 × g for 2 min at 4°C, and the cells were washed with ice-cold HBSA. The preloaded cells were resuspended in HBSA at 37°C and incubated at the same temperature for 3 min to release LTC_4. The reactions were terminated by the addition of 3 vol of ice-cold HBSA and immediate centrifugation at 3000 × g for 20 sec at 4°C in a prechilled Eppendorf centrifuge. Supernatants were separated from pellets and were mixed with 3 vol of methanol containing 200 ng of PGB_2. The cell pellets were resuspended in 200 μl of HBSA and were mixed with 3 vol of methanol with 200 ng of PGB_2. Samples were agitated and sonicated in an ultrasonic water bath. Proteins and cell debris were sedimented at 14,000 × g for 4 min, and the clear methanolic solutions were analyzed for LTC_4 content by reverse-phase high-performance liquid chromatography (RP-HPLC) as described (21).

To examine the effect of glutathione conjugates on LTC_4 release, cells were preloaded with LTC_4 as described above and were incubated with various concentrations of CDNB for 10 min on ice. The doubly preloaded cells were pelleted and resuspended in HBSA at 37°C for 3 min. The release reactions were stopped by the addition of 3 vol of ice-cold HBSA. Supernatants and pellets were separated and processed as described above. LTC_4 and *S*-dinitrophenyl glutathione (GS-DNP) were resolved by RP-HPLC (21).

To study the effect of probenecid on LTC_4 export, cells preloaded with LTC_4 were resuspended at 37°C in HBSA containing incremental concentrations of probenecid and incubated at 37°C for 3 min. The reactions were terminated by the addition of 3 vol of ice-cold HBSA, the supernatants and pellets were separated and processed as described above, and LTC_4 was quantitated by RP-HPLC. The data are expressed as pmol per 10^6 cells unless specified otherwise.

RESULTS

Effect of Temperature on LTC_4 Release. Human myelocytic KG-1 cells, unlike human eosinophils (23), do not generate LTC_4 in response to stimulation with calcium ionophore. However, they are able to produce LTC_4 from the intermediate substrate LTA_4 (data not shown), thereby indicating that KG-1 cells possess LTC_4 synthase but not 5-lipoxygenase. Human HL-60 cells also lack 5-lipoxygenase activity, but expression of the enzyme can be induced by dimethyl sulfoxide (DMSO) differentiation of the cells, as measured by the ability of these cells to generate LTC_4 and LTB_4 after stimulation with calcium ionophore (24). Similarly, as measured in the presence of the substrate LTA_4, HL-60 cells did not show detectable LTC_4 synthase activity until it was induced with 1.3% DMSO (Fig. 1). The enzyme activity plateaued after 4–5 days of culture with DMSO, at which time 115.3 pmol of LTC_4 was generated per 10^6 cells from 25 μM LTA_4 at 37°C for 10 min (Fig. 1, a representative of two experiments). These same HL-60 cells generated 4.5 pmol of LTB_4 per 10^6 cells from LTA_4 before culture with DMSO. However, no LTB_4 was detected after 3 days of culture with DMSO (data not shown), and therefore 4-day DMSO-differentiated HL-60 cells were routinely used for the experiments.

Similar to human eosinophils, at 0°C KG-1 cells and DMSO-differentiated HL-60 cells synthesized LTC_4 from LTA_4 but did not export it (data not shown). Because of this property, we were able to preload the cells with LTC_4. Replicate samples of eosinophils, preloaded with LTC_4 and held at 0°C, were resuspended at various temperatures for 3 min and sedimented to separate supernatants and pellets for analysis for LTC_4 by RP-HPLC. The release of LTC_4 was temperature-dependent, with a Q_{10} of 3.7 and an energy of activation of 28.2 kcal/mol (mean, $n = 2$; 1 kcal = 4.18 kJ) (Fig. 2). For KG-1 cells and DMSO-differentiated HL-60

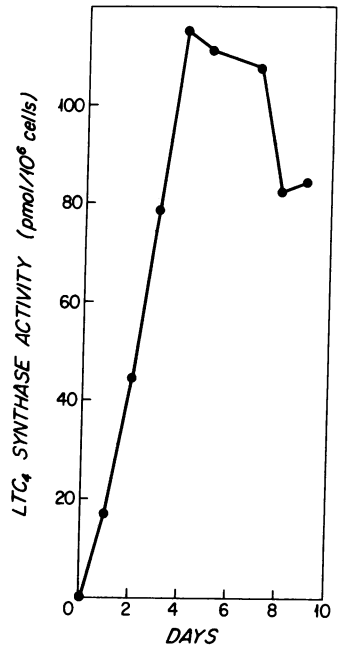

FIG. 1. Time-dependent induction of LTC_4 synthase activity in HL-60 cells cultured with 1.3% DMSO. Cells (10^7) were harvested and incubated with 25 μM LTA_4 at 37°C for 10 min, and the reactions were stopped by the addition of 2 vol of methanol. Total LTC_4 content was analyzed by RP-HPLC.

cells, the Q_{10} values were 3.3 and 3.4, respectively, and the values for energy of activation were 23.0 and 27.8 kcal/mol, respectively. The similarity of these values to those for the eosinophils suggests that LTC_4 export systems are similar in these cells.

Effect of GS-DNP on LTC_4 Release. GS-DNP competitively inhibits LTC_4 transport in eosinophils and in liver canaliculi membrane vesicles (21, 25). When KG-1 cells, eosinophils, and DMSO-differentiated HL-60 cells were preloaded with LTC_4 and then were loaded with incremental amounts of GS-DNP, the release of LTC_4 from KG-1 cells, eosinophils, and HL-60 cells decreased in a dose-dependent fashion from 39.3 pmol to 7.4 pmol per 10^6 cells, 47.5 to 18.5 pmol per 10^6 cells, and 22.3 to 3.7 pmol per 10^6 cells, respectively (Fig. 3).

Effect of Probenecid on LTC_4 Release. Because LTC_4 is an organic anion, we examined the dose–response effects of probenecid, an inhibitor of anion transport. Replicates of eosinophils, KG-1 cells, and HL-60 cells were preloaded with LTC_4 by incubation with 25 μM LTA_4, washed, and resuspended in 37°C HBSA for 3 min in the presence of incremental concentrations of probenecid. As shown in a repre-

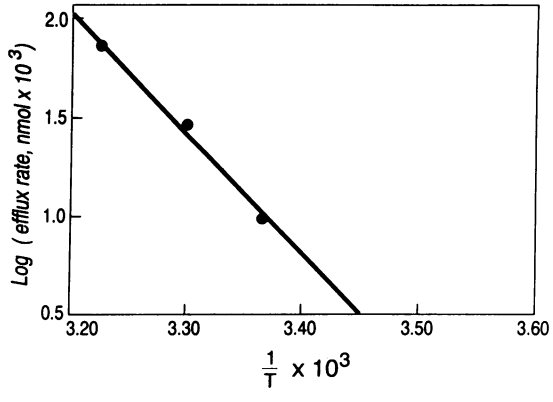

FIG. 2. Arrhenius plot of the effect of temperature on LTC_4 release from human eosinophils.

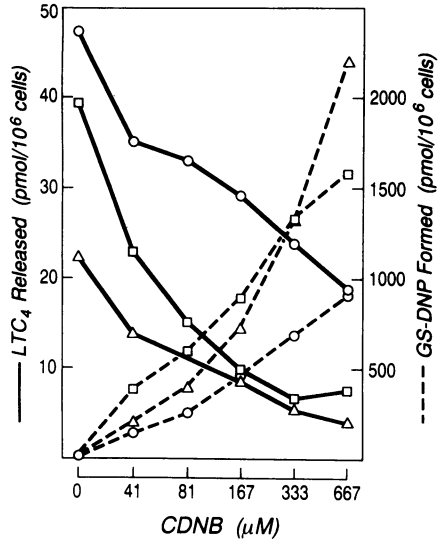

FIG. 3. Effect of GS-DNP on LTC_4 release from human eosinophils (○) (n = 4), KG-1 cells (□) (n = 4), and DMSO-differentiated HL-60 cells (△) (n = 3). LTC_4 released (——) and total GS-DNP (– – –) formed (released plus residual) are plotted.

sentative experiment in Fig. 4, probenecid inhibited LTC_4 release in a dose-dependent manner in all three types of cells. The amount of LTC_4 released decreased from 40.2 to 10.2 pmol per 10^6 cells in eosinophils, from 51.3 to 7.8 pmol per 10^6 cells in KG-1 cells, and from 8.0 to 0.0 pmol per 10^6 cells in DMSO-differentiated HL-60 cells.

The findings that LTC_4 release occurs through a probenecid-sensitive pathway for which xenobiotic glutathione conjugates can compete prompted a study of the combined

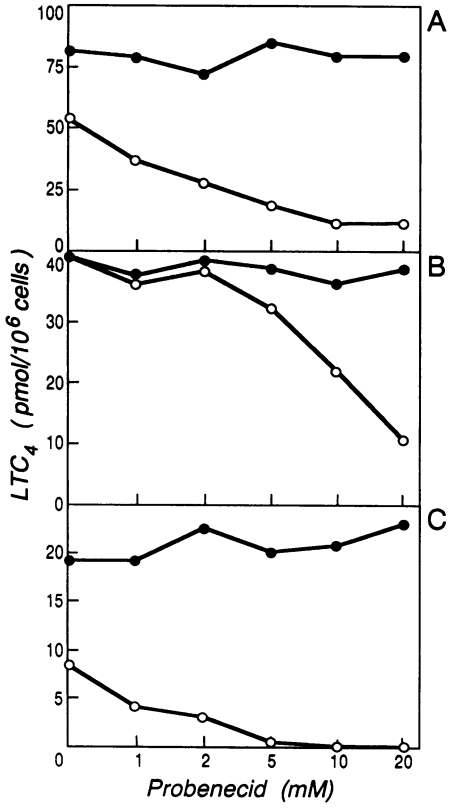

FIG. 4. Effect of incremental concentrations of probenecid on release at 37°C of preloaded LTC_4 from KG-1 cells (A), eosinophils (B), and DMSO-differentiated HL-60 cells (C). LTC_4 released (○) and total LTC_4 (released plus residual) (●) are plotted.

effects of these two interventions on LTC_4 release. Replicate KG-1 cells were preloaded with intracellular LTC_4 and then were incubated without or with CDNB (40 and 667 μM) for 10 min on ice. The preloaded cells were washed and resuspended in 37°C HBSA without and with 2 mM probenecid for 3 min to initiate the release of LTC_4. As shown in Fig. 5, the control cells that did not receive either CDNB or probenecid synthesized 72.1 pmol of LTC_4, of which 49.9 pmol was released into the supernatant. Dose-dependent increases in GS-DNP synthesis did not affect the total LTC_4 synthesis but were associated with a dose-dependent inhibition of LTC_4 release. At 40 μM and 667 μM CDNB, KG-1 cells synthesized 162.6 pmol and 1301.9 pmol of GS-DNP, respectively, and LTC_4 release decreased to 31.3 pmol and 10.4 pmol, respectively. When probenecid was present during the release reactions, LTC_4 release decreased further to 15.8 pmol and 5.8 pmol, respectively. Probenecid alone reduced LTC_4 release to 28.7 pmol. Thus, probenecid alone suppressed LTC_4 release by about 42%. When combined with 40 μM and 667 μM CDNB, it provided an increased inhibition of about the same percentage, 49% and 44%, respectively. In one experiment with eosinophils, 10 mM probenecid suppressed LTC_4 release by about 26%, and 667 μM CDNB alone suppressed LTC_4 export by 41%. A combination of probenecid and CDNB inhibited LTC_4 release by about 84% (data not shown).

Effect of Probenecid and GS-DNP on LTB_4 Release. Although the original undifferentiated HL-60 cells made no LTC_4 when cultured with LTA_4 (Fig. 1), after 6 months of culture under routine conditions, HL-60 cells not exposed to DMSO synthesized both classes of LTs from exogenously added LTA_4. Replicate HL-60 cells were preloaded by incubation with 25 μM LTA_4 for 60 min on ice, incubated with incremental concentrations of CDNB (0 μM, 40 μM, and 667 μM) on ice for 10 min more, washed, and resuspended for 1 min at 37°C. Alternatively, replicate preloaded cells were resuspended in 37°C buffer for 0.5–3 min without and with 10 mM probenecid. Fig. 6 shows a representative experiment from a total of four. In the absence of probenecid there was

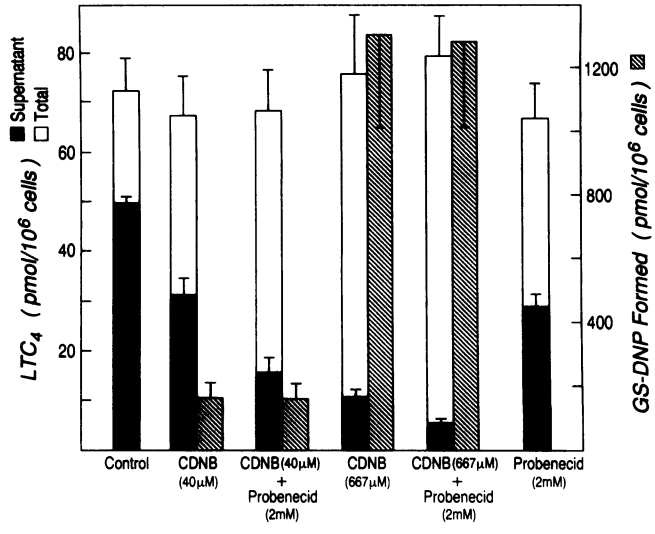

FIG. 5. Effect of GS-DNP and probenecid on LTC_4 release from KG-1 cells. Cells were preloaded with LTC_4 by incubation with LTA_4 at 37°C for 3 min and on ice for 57 min and then were loaded with different amounts of GS-DNP by incubation with 0 (control), 40, and 667 μM CDNB on ice for 10 min more. After the cells were washed, the reaction products were released in the absence or presence of 2 mM probenecid for 3 min at 37°C and then were quantitated as released and retained by RP-HPLC. LTC_4 released into the supernatant (■), total LTC_4 (released plus residual) (□), and GS-DNP (□) are plotted (n = 3, mean ± SEM).

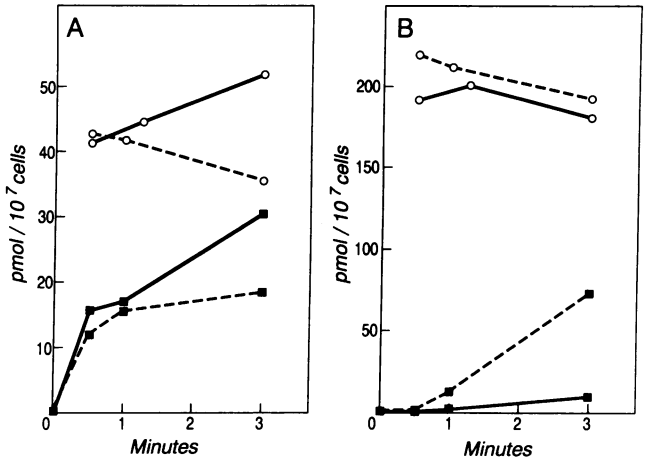

FIG. 6. Effect of probenecid on the LTB_4 (*A*) and LTC_4 (*B*) released from nondifferentiated HL-60 cells. Cells were incubated with 25 μM LTA_4 for 60 min at 4°C, washed, and resuspended in 37°C buffer for 0.5–3 min with (——) or without (– – –) 10 mM probenecid. Total (released plus residual) (open symbols) and released (closed symbols) LTB_4 and LTC_4 are plotted.

a time-dependent increase in LTB_4 release from 12.1 pmol per 10^7 cells at 0.5 min to 18.2 pmol per 10^7 cells at 3 min and in LTC_4 release from 0.0 pmol per 10^7 cells at 0.5 min to 71.6 pmol per 10^7 cells at 3 min. In the presence of 10 mM probenecid the release of LTC_4 was suppressed by 85%, whereas the release of LTB_4 was not inhibited. Similarly, GS-DNP did not affect the release of LTB_4 from these cells (data not shown).

DISCUSSION

The initial study linking the export of cellular LTC_4 and glutathione conjugates (21) was followed by the demonstration that the accumulation of cellular GS-DNP does not affect the carrier-mediated release of LTB_4 in human PMNs (20). These transport systems are now further distinguished by the selective inhibitory effect of the organic acid transport inhibitor probenecid on the export of LTC_4 but not LTB_4. Probenecid inhibited the release of LTC_4 in KG-1 cells, eosinophils, and DMSO-differentiated HL-60 cells in a dose-dependent manner (Fig. 4), indicating that LTC_4 export occurs through a probenecid-sensitive organic acid transport system. In nondifferentiated HL-60 cells that had spontaneously developed the ability to synthesize LTB_4 and LTC_4 from LTA_4, probenecid suppressed the release of LTC_4 by 85% but did not inhibit the release of LTB_4 (Fig. 6). This finding confirmed that LTC_4 and LTB_4 are exported by different transport systems.

The carrier-mediated cellular export of LTC_4 was originally described for cultured human eosinophils isolated from patients treated with interleukin 2. Eosinophils possess 5-lipoxygenase and LTC_4 synthase, and it was not known if this transport system also occurs in other cells of bone marrow origin that do not contain 5-lipoxygenase and would depend entirely upon the transcellular supply of the substrate, LTA_4, to produce LTC_4. Human myelocytic KG-1 cells do not have 5-lipoxygenase but do have LTC_4 synthase, as evidenced by their ability to generate LTC_4 from LTA_4 but not with calcium ionophore stimulation alone. Human promyelocytic HL-60 cells have no 5-lipoxygenase activity, but this enzyme (24) and LTC_4 synthase activity can be induced by culturing the cells with DMSO (Fig. 1). Similar to human eosinophils, KG-1 cells and DMSO-differentiated HL-60 cells synthesized LTC_4 from LTA_4 at 0°C but were not able to export it. The Q_{10} and energy of activation values of the temperature-dependent LTC_4 export for eosinophils (3.7 and 28.2 kcal/mol) (Fig. 2) were similar to those of KG-1 cells (3.3 and 23.0 kcal/mol) and DMSO-differentiated HL-60 cells (3.4 and 27.8 kcal/mol). These data are compatible with a carrier-mediated process for LTC_4 export that is not linked to the presence of 5-lipoxygenase to initiate formation of LTA_4 within the same cell.

This LTC_4 export step in eosinophils is saturable and temperature dependent and is partially inhibited by the intracellular accumulation and release of a glutathione conjugate, GS-DNP. In KG-1 cells and DMSO-differentiated HL-60 cells, GS-DNP inhibited LTC_4 export by >80% (Fig. 3), whereas in eosinophils, GS-DNP inhibition of LTC_4 export plateaued at about 60% in this study and at about 37% in our previous study (21). To determine if the cytokine activation of peripheral blood eosinophils accounted for the failure of these cells to be inhibited in their export of LTC_4 to the same level as the two other cell lines, we analyzed the data again, taking into account the actual intracellular levels of GS-DNP available to compete for the carrier-mediated export of LTC_4. When the data are "normalized" by calculating the ratio of total GS-DNP to total glutathione conjugates (GS-DNP plus LTC_4) required to suppress LTC_4 release by 40% (IC_{40}), the respective results for eosinophils, KG-1 cells, and HL-60 cells, 0.839, 0.803, and 0.841, are virtually the same. Therefore, the difference in total GS-DNP synthesis accounts for the differences among cell types, and LTC_4 shares the same transporter as glutathione conjugates irrespective of the presence of 5-lipoxygenase.

Although glutathione conjugates have been shown to inhibit LTC_4 transport in isolated membrane vesicles (25), this study demonstrates that an organic acid transport inhibitor, probenecid, can inhibit LTC_4 export in intact cells and that glutathione conjugates and probenecid have a cumulative effect in suppressing LTC_4 export in cells of bone marrow origin (Fig. 5). Probenecid was shown to inhibit the transport of LTC_4 across chloroid plexus in rabbits (26). Probenecid has traditionally been used to identify organic anion transporters that mediate transmembrane movement of compounds such as folate and uric acid. Its ability to inhibit the release of LTC_4 suggests that its carrier and the organic acid transporters recognize common structural domains on their substrates.

We thank Dr. R. J. Soberman for valuable discussions and Evelyn Montes and Stephanie Bourdelle for manuscript preparation. This work was supported by Grants AI-22531, AI-22563, HL-36110, AI-31599, AI-07306, RR-05950, and RR-00054 and Contract CM-73702-6 from the National Institutes of Health and by a grant from the Milton Fund of Harvard Medical School.

1. Samuelsson, B. (1983) *Science* **220**, 568–575.
2. Lewis, R. A. & Austen, K. F. (1984) *J. Clin. Invest.* **73**, 889–897.
3. MacGlashan, D. W., Jr., Peters, S. P., Warner, J. & Lichtenstein, L. M. (1986) *J. Immunol.* **136**, 2231–2239.
4. Ford-Hutchinson, A. W., Bray, M. A., Doig, M. V., Shipley, M. E. & Smith, M. J. H. (1980) *Nature (London)* **286**, 264–265.
5. Rollins, T. E., Zanolari, B., Springer, M. S., Guindon, Y., Zamboni, R., Lau, C.-K. & Rokach, J. (1983) *Prostaglandins* **25**, 281–289.
6. Palmblåd, J., Malmsten, C. L., Udén, A. M., Rådmark, O., Engström, L. & Samuelsson, B. (1981) *Blood* **58**, 658–661.
7. Dahlén, S.-E., Hansson, G., Hedqvist, P., Björk, T., Granström, E. & Dahlén, B. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1712–1716.
8. Dahlén, S.-E., Hedqvist, P., Hammarström, S. & Samuelsson, B. (1980) *Nature (London)* **288**, 484–486.
9. Jones, T. R., Davis, C. & Daniel, E. E. (1982) *Can. J. Physiol. Pharmacol.* **60**, 638–643.
10. Chagnon, M., Gentile, J., Gladu, M. & Sirois, P. (1985) *Lung* **163**, 55–62.

11. Lewis, R. A., Austen, K. F. & Soberman, R. J. (1990) *N. Engl. J. Med.* **323,** 645–655.
12. Rouzer, C., Matsumoto, T. & Samuelsson, B. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 857–861.
13. Ueda, N., Kaneko, S., Yoshimoto, T. & Yamamoto, S. (1986) *J. Biol. Chem.* **261,** 7982–7988.
14. Rouzer, C. & Samuelsson, B. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6040–6044.
15. Hogaboom, G. K., Cook, M., Newton, J. F., Varrichio, A., Shorr, R. G. R., Sarall, H. M. & Crooke, S. T. (1986) *Mol. Pharmacol.* **30,** 510–519.
16. Rådmark, O., Shimizu, T., Jörnvall, H. & Samuelsson, B. (1984) *J. Biol. Chem.* **259,** 12339–12345.
17. Evans, J. F., Dupuis, P. & Ford-Hutchinson, A. W. (1985) *Biochim. Biophys. Acta* **840,** 43–50.
18. Yoshimoto, T., Soberman, R. J., Lewis, R. A. & Austen, K. F. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8399–8403.
19. Yoshimoto, T., Soberman, R. J., Spur, B. & Austen, K. F. (1988) *J. Clin. Invest.* **81,** 866–871.
20. Lam, B. K., Gagnon, L., Austen, K. F. & Soberman, R. J. (1990) *J. Biol. Chem.* **265,** 13438–13441.
21. Lam, B. K., Owen, W. F., Jr., Austen, K. F. & Soberman, R. J. (1989) *J. Biol. Chem.* **264,** 12885–12889.
22. Maycock, A. L., Anderson, M. S., DeSousa, D. M. & Kuehl, F. A., Jr. (1982) *J. Biol. Chem.* **257,** 13911–13914.
23. Owen, W. F., Jr., Petersen, J. & Austen, K. F. (1991) *J. Clin. Invest.* **87,** 1958–1963.
24. Reid, G. K., Kargman, S., Vickers, P. J., Mancini, J. A., Léveillé, C., Ethier, D., Miller, D. K., Gillard, J. W., Dixon, R. A. F. & Evans, J. F. (1990) *J. Biol. Chem.* **265,** 19818–19823.
25. Ishikawa, T., Müller, M., Klunemann, C., Schaub, T. & Keppler, D. (1990) *J. Biol. Chem.* **265,** 19279–19286.
26. Spector, R. & Goetzl, E. J. (1985) *Science* **228,** 325–327.