Suppression of Leukotriene B₄ Biosynthesis by Endogenous Adenosine in Ligand-activated Human Neutrophils

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Summary

Adenosine (Ado) has been shown to suppress several functional responses of human polymorphonuclear leukocytes (PMNs). The current study investigated whether endogenous Ado regulates the biosynthesis of leukotriene (LT)B₄ in ligand-stimulated PMNs. Measurements of Ado in PMNs resuspended in Hank's buffered salt solution (HBSS) or plasma showed a cell concentration- and time-dependent accumulation of the nucleoside. The removal of endogenous Ado with either Ado deaminase or the blockade of its action by the Ado A₂ receptor antagonist, 8-(3-chlorostyryl) caffeine, markedly increased LTB₄ biosynthesis upon ligand stimulation in HBSS. Similarly, LTB₄ synthesis by ligand-stimulated PMNs in plasma (containing recombinant LTA₄ hydrolase to allow the conversion of protein-bound LTA₄) was strongly enhanced by addition of Ado deaminase. Addition of red blood cells to suspensions of PMNs in plasma mimicked the effect of adding Ado deaminase and LTA₄ hydrolase in enhancing LTB₄ biosynthesis upon ligand stimulation. This effect of red blood cells on LTB₄ biosynthesis was blocked by dipyridamole, an inhibitor of Ado transport, or captopril, an inhibitor of LTA₄ hydrolase. These results demonstrate that endogenous Ado efficiently downregulates ligand-stimulated LTB₄ biosynthesis in PMN suspensions, pointing out a potentially important regulatory function of Ado in inflammatory exudates. These results also unveil a dual role for red blood cells in upregulating LTB₄ biosynthesis, namely, the removal of endogenous Ado and the conversion of LTA₄ released by activated PMNs.

Materials and Methods

Materials. Ado deaminase (EC 3.5.4.4., calf intestinal type VIII), captopril, dipyridamole, N-formyl-Met-Leu-Phe (fMLP),...
and LPS (Escherichia coli 0111B4) were from Sigma Chemical Co. (St. Louis, MO). Ado deaminase was diazylated against NaCl 0.9% before use. 2-p-(carboxyethyl)phenethylamino-5’S-N-ethyl-carboxamido-adenosine HCl (CGS 21680) and 8-(3-chlorostyryl) caffeine (CSC) were from Research Biochemicals International (Natick, MA). 5’S(N-ethy)carboxamidoadenosine (NECA) was from ICN Biomedicals Canada Ltd. (Mississauga, Canada). rLTA<sub>4</sub> hydrolase was obtained from Sf9 cells infected with LTA<sub>4</sub> hydrolase using the baculovirus system (13). The 100,000 g supernatant of infected Sf9 cells was used directly as the source of LTA<sub>4</sub> hydrolase and contained a specific activity of 60 nmol LTB<sub>4</sub>/mg of protein (12 mg of protein/ml). The 100,000 g supernatant of the wild-type Sf9 cells (uninfected cells) was used as control. Recombinant GM-CSF and TNF-α were provided by the Genetics Institute (Cambridge, MA) and Knoll Pharmaceuticals (Wilmington, DE), respectively.

Cells. Human PMNs were isolated as previously described (4). In brief, human venous peripheral blood was collected into heparinized tubes. RBCs were allowed to sediment at 1 g after mixing 4 volumes of blood and 1 volume of dextran 2% in HBS; M mononuclear cells were removed by centrifugation on Ficoll-Paque cushions. Contaminating RBCs in the PMN pellet were eliminated by a 20-h hypotonic lysis in PBS. PMNs were resuspended in 10 mM Hepes-buffered HBS (pH 7.4) containing 1.6 mM CaCl<sub>2</sub>. RBCs obtained from dextran sedimentation were freed of contaminating leukocytes by repeated removal of the buffy coat after four successive centrifugations (200 g, 15 min at 20°C) and resuspensions in four volumes of 10 mM Hepes-buffered HBS (pH 7.4) containing 1.6 mM CaCl<sub>2</sub>. In some experiments, RBCs were treated with 1 mM captopril or its diluent (NaCl 0.9%) for 30 min at room temperature before the fourth washing.

5-LO Product Analysis. Incubations were stopped by adding cold (0°C) methanol/acetonitrile (50/50; vol/vol) containing 12.5 ng each of 19-OH prostaglandin B<sub>2</sub> and prostaglandin B<sub>2</sub> as internal standards to aliquots of cell suspensions and stored at −20°C until reverse phase HPLC (R-P-HPLC) analysis. Denatured samples were centrifuged at 2,000 g for 10 min, and the supernatants were subjected to R-P-HPLC using on-line extraction procedures as previously described for PMN suspensions in HBS (14) or samples containing plasma (15). LT<sub>B</sub> bioactivity was measured by photometry at 280 and 229 nm, using fixed wavelength UV detectors. The lower limits of detection were 0.5 ng at 280 nm and 1 ng at 229 nm.

Analysis of Ado. PMN incubations (1 ml) were stopped by adding 100 μl of 22% TCA. N ECA was added (10 ng/sample) as an internal standard and the denatured cell suspensions were placed at −20°C for at least 30 min. The samples were then centrifuged at 2,000 g for 10 min and the supernatants were extracted on Sep Pak C<sub>18</sub> cartridges (3 cc, C-18 sorbent) as follows. The samples were loaded on the cartridges which were washed with water; Ado was then eluted with 3.5 ml of methanol/water (50:50, containing 0.1% acetic acid). The eluates were evaporated to dryness using a Speed Vac evaporator. The residues were dissolved in 200 μl of methanol/water (25:75, containing 0.05% acetic acid). The samples were analyzed by liquid chromatography-mass spectrometry using nebulizer-assisted electrospray ionization in the positive mode and by monitoring the transitions m/z 309 and m/z 268 (protonated parent ions) to m/z 136 (protonated adenine), corresponding to the loss of the carbohydrate moieties from NECA and Ado. The samples (1–2 μl) were injected onto a C-18 column (Ultrosphere, 2 × 150 mm, 5-μm particles; Beckman, Fullerton, CA) and eluted at a flow rate of 200 μl/min using methanol/water (40:60, containing 0.1% acetic acid) as the mobile phase. Ado was quantitated by extrapolating the measured Ado/NECA ratio on a calibration curve generated from standard solutions containing 1 ng NECA and 0–4 ng Ado in 5 μl. The limit of detection for Ado was 5 pg injected (signal to noise ratio <5).

Results
Ado concentrations in PMN suspensions. Isolated PMNs in suspension in salt buffers have been reported to release Ado at levels that influence their functions (12). We thus performed measurements of endogenous Ado concentrations in PMN suspensions under conditions used for the assessment of leukotriene biosynthesis. After the centrifugation and resuspension of PMNs in fresh buffers, aliquots were removed for up to 60 min and the concentration of Ado was measured. PMNs resuspended in HBS or autologous plasma released Ado in a time- and cell concentration-dependent manner (Fig. 1). Cell-depleted plasma was found to contain 30 ± 3 nM (mean ± SE, n = 6) of Ado. The addition of 0.1 U Ado deaminase after the incubation of 2.0 × 10<sup>7</sup> PMN/ml for 15 min in HBS reduced the concentration of Ado within seconds to <4 nM, and remained below this level for up to 30 min. Stimulation of PMNs with 0.6 μM platelet-activating factor (PAF) did not have any effect on the levels of endogenous Ado (not shown).

LTB<sub>4</sub> Biosynthesis in HBS. We first examined the effect of endogenous Ado present in PMN suspensions in HBS on the synthesis of LTB<sub>4</sub>. Endogenous Ado was neutralized using two different approaches; the addition of a selective A<sub>2a</sub> receptor antagonist, CSC (16; Fig. 2 A), or Ado deaminase (Fig. 2 B) to the incubation media. The pretreatment of TNF-α/GM-CSF-primed neutrophils with increasing concentrations of either CSC or Ado deaminase before stimulation with 0.6 μM PAF resulted in a progressive enhancement of 5-LO product biosynthesis, as compared to cells stimulated in the absence of CSC or Ado deaminase.

Figure 1. Time course of Ado accumulation in PMN suspensions. Freshly isolated PMNs were resuspended in HBS (A) or autologous plasma (B) at concentrations of 5 × 10<sup>6</sup>/ml (squares) or 20 × 10<sup>6</sup>/ml (circles). At various time points, 1-ml aliquots of the cell suspensions were denatured with TCA and the Ado content was measured by liquid chromatography-mass spectrometry. Results shown are the means ± SD of triplicate incubations from one experiment representative of three. Error bars are not shown when smaller than symbols.

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Figure 2. Effects of CSC and Ado deaminase treatment of PMN suspensions on LTB₄ biosynthesis. PMN suspensions in HBSS (10⁷/ml) were preincubated with 700 pM GM-CSF + 1.2 nM TNF-α for 30 min, and then treated with various concentrations of CSC (A) or Ado deaminase (B) for 5 min and stimulated with 0.6 μM PAF. After 10 min of incubation in the presence (or absence) of PAF, incubations were stopped and 5-LO products were measured by RP-HPLC. In B, CGS 21680 was added at the concentration of 1 μM. Data are the means (± SD) of triplicate incubations from one experiment representative of three. (C) RP-HPLC profiling of 5-LO products generated by TNF-α/LPS-primed PMN suspensions stimulated (or not, control) with PAF, with or without earlier pretreatment with either 0.1 U deaminase Ado or 1 μM CSC. Conditions were as indicated for A and B; 5(S)-hydroxy-6,8,11,14(E,Z,Z,Z)-eicosatetraenoic acid (5-HETE) was not detectable in all conditions tested. Amounts of LTB₄ indicated represent the sum of 20-OH LTB₄, 20-OH LTB₄, and LTB₄, PGF₂α, prostaglandin B₂; ADA, adenosine deaminase.

The stimulatory effect of Ado deaminase was fully reversed by the addition of 1 μM CGS 21680, a selective A₂ₐ receptor agonist (17). Fig. 2C illustrates typical HPLC profiles of the 5-LO products generated by PMNs activated with PAF in the presence or absence of CSC or Ado deaminase. In the absence of PAF stimulation, the biosynthesis of LTB₄ was not detected.

Figure 3. Effect of exogenous LTA₄ hydrolase, Ado deaminase, and RBCs on the biosynthesis of LTB₄ by PMNs in plasma. (A) PMNs (5 × 10⁶) in suspension in autologous plasma (0.5 ml) were treated with 1.2 nM TNF-α and 1 μg/ml LPS for 30 min at 37°C. 10 μl of the preparation of rLTA₄ hydrolase (see Materials and Methods) and/or 4 U of Ado deaminase (or its diluent, NaCl 0.9%) were added (per milliliter of incubation media) 1 and 5 min before stimulation with 1 μM fMLP. After 15 min of stimulation, the incubations were stopped and LTB₄ production was measured by RP-HPLC. (B) PMNs (5 × 10⁶) in suspension in autologous plasma (0.5 ml) were treated with 1.2 nM TNF-α and 1 μg/ml LPS for 30 min at 37°C. RBCs (0.5 ml of packed cells) treated or not with captopril (see Materials and Methods) were next added to PMNs in suspension in autologous plasma (0.5 ml) and treated with 30 μM dipyridamole for 25 min at 37°C, and then stimulated with 1 μM fMLP for 15 min. LTB₄ biosynthesis was measured by RP-HPLC. Results shown are the means ± SD of triplicate incubations from one experiment representative of three. LTA₄-H, LTA₄-hydrolase; DIPY, dipyridamole; CAPT-RBC, captopril-treated red blood cells; ADA, Ado deaminase.
Discussion

We recently reported that Ado and analogues (particularly A2a agonists) are potent inhibitors of LTB4 biosynthesis in whole blood, as well as in isolated PMNs and monocytes (11). It is also recognized that Ado accumulates in PMN suspensions (12). These observations led us to hypothesize that endogenous Ado present in PMN suspensions might exert a suppressive effect on the biosynthesis of LTB4. Measurements of endogenous Ado in PMN suspension media (HBSS and plasma) clearly indicated that after 15–30 min of incubation in all experimental conditions tested, Ado reaches concentrations (25–400 nM; Fig. 1) likely to severely impact LTB4 synthesis, given the IC50 of 80 and 60 nM measured previously for the inhibition of LTB4 synthesis in blood (11) and HBSS (our unpublished data), respectively. Accordingly, we found that the removal of endogenous Ado using Ado deaminase or the blockade of its effect with the A2a receptor antagonist CSC, strikingly increased the biosynthesis of LTB4. Moreover, we showed that endogenous Ado has a similar inhibitory effect on the biosynthesis of LTB4 when stimulated by fMLP and PAF (21). Since LTB4 biosynthesis is highly dependent on ligand-stimulated Ca2+ influx (22, 23), it is therefore conceivable that the inhibitory effects of Ado might be related to the modulation of arachidonate release and/or 5-LO activation, both of which are Ca2+ dependent (22, 23). Studies are in progress to assess this hypothesis.

One possible site of action of Ado is the transcellular metabolism of LTA4 by RBC LTA4 hydrolase (6). Therefore, inhibition of initial LTA4 biosynthesis by PMNs activated in plasma would be expected to facilitate LTA4 formation, whereas inhibition of extracellular LTA4 uptake would result in the formation of the 6-trans isomers (nonenzymic hydrolysis products) of LTB4, which was not observed in our experiments. Another possible site of action of Ado in the regulation of LTB4 biosynthesis could be at the level of the conversion of LTB4 to its o-oxidation products. However, as seen in Fig. 2C, endogenous Ado suppresses the formation of both LTB4 and 20-OH-LTB4, the sum of LTB4, and its metabolites being two- to threefold greater in incubations performed in presence of Ado deaminase or CSC. Interestingly, previous studies have shown that elevated extracellular concentrations of Ado inhibits external Ca2+ influx in PMN suspensions stimulated by either PAF or fMLP (21). Since LTB4 biosynthesis is highly dependent on ligand-stimulated Ca2+ influx (22), it is therefore conceivable that the inhibitory effects of Ado might be related to the modulation of arachidonate release and/or 5-LO activation, both of which are Ca2+ dependent (22, 23). Studies are in progress to assess this hypothesis.

Another finding of the current study is the involvement of a dual mechanism in the regulation of the biosynthesis of LTB4 by RBCs. Indeed, RBCs have previously been shown to enhance the biosynthesis of LTB4 through the transcellular metabolism of LTA4 by RBC LTA4 hydrolase (6). However, the results of our studies with PMNs in plasma suggested that the presence of RBC LTA4 hydrolase cannot fully account for the increased production of LTB4, and that the reported capacity of RBCs to efficiently take up Ado (18) may contribute to the ability of RBCs to enhance the synthesis of LTB4 by ligand-stimulated PMNs in plasma. In fact, both RBC-mediated events proved to be determinant in the stimulatory effect of RBCs since both captopril, an inhibitor of LTA4 hydrolase, and dipyridamole, an inhibitor of adenosine transport, efficiently reversed the effect of RBCs on LTB4 synthesis, in full agreement with the data obtained by the simultaneous addition of LTA4 hydrolase and Ado deaminase to PMN suspensions in plasma.

The fact that endogenous Ado exerts a negative regulation of LTB4 biosynthesis by ligand-stimulated PMNs has led to an underestimation of the potential of this cell type to respond to stimulation by physiological agonists such as fMLP and PAF (24) and has also contributed to the generation of controversial data concerning the ability of PMNs to produce LTs in response to such stimuli. Indeed, it is likely that differences in experimental conditions used by different investigators, such as PMN concentration and preincubation temperature and time, directly impact on Ado concentration in the cell suspensions and therefore, on cell responsivity to the stimuli. Moreover, while LTB4 synthesis by PMNs stimulated by soluble agonists such as fMLP and PAF is highly sensitive to inhibition by Ado, the synthesis of LTB4 by PMNs stimulated by the ionophore A23187 is much less sensitive to Ado inhibition (11). It seems important to point out that in an in vivo context, the inhibition of PMN LTA4 biosynthesis by Ado likely has consequences not only on LTB4 formation, but also on the biosynthesis of cysteinyl LTs and lipoxins, since these may be generated, at least in part, from the transcellular metabolism of PMN-derived LTA4 by endothelial cells (25) and platelets (26).
In summary, the current study demonstrates the regulatory role of endogenous Ado on ligand-stimulated LT biosynthesis by PMNs and strongly emphasizes that an elevated level of endogenous Ado in physiological settings can have profound consequences on the ability of PMNs to produce LTA₄, the direct precursor of the lipid mediators LTB₄, LTC₄, and lipoxins, which have been shown to modulate phagocyte functional responses and inflammatory events. Our observations also support the recently proposed concept that Ado is a natural antiinflammatory agent (10). Indeed, it has become increasingly apparent that the antiinflammatory mechanism of methotrexate and sulfasalazine, two potent antiinflammatory drugs, involves an increase of Ado concentration at sites of inflammation (10, 27). Most importantly, these studies showed that leukocyte accumulation at inflammatory sites was diminished and that these effects of the drugs could be antagonized by Ado deaminase or Ado receptor antagonists. In view of the ability of Ado to suppress LTB₄ biosynthesis, it is tempting to speculate that the mechanism by which these antiinflammatory agents act might include the inhibition of LTB₄-dependent extravasation of leukocytes. Further studies are needed to characterize the consequences of increasing Ado levels on LTB₄-mediated inflammatory processes. Finally, taken together, these recent observations and the previously reported inhibitory effects of Ado on PMNs and monocyte functions that support A₂ₐ receptor agonists or agents that can regulate Ado biosynthesis, metabolism, or transport may represent a novel class of potent antiinflammatory agents.

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References

1. Borgeat, P., and P.H. Naccache. 1990. Biosynthesis and biological activity of leukotriene B₄, Clin. Biochem. 23:459–468.
2. Claesson, H.E., B. Odlander, and P.J. Jakobsson. 1992. Leukotriene B₄ in the immune system. Int. J. Immunopharmacol. 14:441–449.
3. Fradin, A., J.A. Zirolii, J. Mclouf, L. Vausbinder, P.M. Henson, and R.C. Murphy. 1989. Platelet-activating factor and leukotriene biosynthesis in whole blood. A model for the study of transcellular arachidonate metabolism. J. Immunol. 143:3680–3685.
4. Surette, M.E., R. Palmantier, J. Gosselin, and P. Borgeat. 1993. Lipopolysaccharides primes whole human blood and isolated neutrophils for the increased synthesis of 5-lipoxygenase products by enhancing arachidonic acid availability: involvement of the CD14 antigen. J. Exp. Med. 178:1347–1355.
5. Palmantier, R., M.E. Surette, A. Sanchez, P. Braquet, and P. Borgeat. 1994. Priming of the synthesis of 5-lipoxygenase products in human blood ex vivo by human granulocyte-macrophage colony-stimulating factor and tumor necrosis factor-α. Lab. Invest. 70:696–704.
6. Sala, A., M. Bolla, S. Zarini, R. M.oller-Peddinghaus, and G. Folco. 1996. Release of leukotriene A₄ versus leukotriene B₄ from human polymorphonuclear leukocytes. J. Biol. Chem. 271:17944–17948.
7. Fitzpatrick, F., W. Liggert, J. Mc Gee, S. Buting, D. Mортin, and B. Samuelsson. 1984. Metabolism of leukotriene A₄ by human erythrocytes. A novel cellular source of leukotriene B₄. J. Biol. Chem. 259:11403–11407.
8. M. Mc Gee, J.E., and F.A. Fitzpatrick. 1986. Erythrocyte-neutrophil interactions formation of leukotriene B₄ by transcellular biosynthesis. Proc. Natl. Acad. Sci. U S A. 83:1349–1353.
9. Lindgren, J.A., and C. Edenius. 1993. Transcellular biosynthesis of leukotrienes and lipoxins via leukotriene A₄ transfer.

Trends Pharmacol. Sd. 14:351–354.
10. Cronstein, B.N. 1994. Adenosine, an endogenous anti-inflammatory agent. J. Appl. Physiol. 76:5–13.
11. Krump, E., G. Lemay, and P. Borgeat. 1996. Adenosine A₂ receptor-induced inhibition of leukotriene B₄ synthesis in whole blood ex vivo. Br. J. Pharmacol. 117:1639–1644.
12. van Waeg, G., and G. Van den Berghe. 1991. Purine catabolism in polymorphonuclear neutrophils-phorbol myristate acetate-induced accumulation of adenosine owing to inactivation of extracellularly released adenosine deaminase. J. Clin. Invest. 87:305–312.
13. pancini, J.A., and J.F. Evans. 1993. Coupling of recombinant 5-lipoxygenase and leukotriene A₄ hydrolase activities and transcellular metabolism of leukotriene A₄ in Sf9 insect cells. Eur. J. Biochem. 218:477–484.
14. Borgeat, P., S. Picard, P. Vallerand, S. Bourgoin, A. Deimat, P. Sirois, and P.E. Poubelle. 1990. Automated on-line extraction and profiling of lipoxygenase products of arachidonic acid by high-performance liquid chromatography. In Methods in Enzymology. Arachidonate Related Lipid Mediators. R.C. Murphy and F. Fitzpatrick, editors. Academis Press, San Diego. 187:98–116.
15. Surette, M.E., A. Deimat, R. Palmantier, S. Marelle, P.E. Poubelle, and P. Borgeat. 1994. Reverse-phase high-performance liquid chromatography analysis of arachidonic acid metabolites in plasma after stimulation of whole blood ex vivo. Anal. Biochem. 216:392–400.
16. Jacobson, K.A., O. Nikodijevic, W.L. Padgett, C. Gallo-Rodriguez, M. Maillard, and J.W. Daly. 1993. 8-(3-chiorosteryl)caffeine (CSC) is a selective A₂ₐ adenosine antagonist in vitro and in vivo. FEBS Lett. 323:141–144.
17. Gurden, M.F., J. Coates, F. Ellis, B. Evans, M. Foster, E. Hornby, I. Kennedy, D.P. Martini, P. Strong, C.J. Vardey, and A. W. Needon. 1993. Functional characterization of three
The secondary metabolism of arachidonic acid generates a number of compounds, including leukotrienes and prostaglandins. Leukotrienes are potent inflammatory mediators that play a crucial role in the regulation of various physiological processes. The synthesis of leukotrienes is a complex and tightly regulated process that involves the conversion of arachidonic acid into leukotriene A4 (LTA4) by the enzyme 5-lipoxygenase. LTA4 is then converted into leukotriene B4 (LTB4) by the enzyme LTA4 hydrolase.

Adenosine, a purine nucleoside, has been shown to modulate leukotriene synthesis in various cell types. Adenosine receptor subtypes have been characterized, and they play a role in the regulation of leukotriene synthesis. For example, adenosine receptor subtypes A1 and A2A have been shown to inhibit LTB4 synthesis in neutrophils. This inhibitory effect is likely to be mediated through the activation of phospholipase A2 (PLA2) and the release of arachidonic acid.

Several studies have investigated the interactions between adenosine and leukotriene synthesis. Möser et al. (1989) demonstrated that adenosine turnover in plasma of human and dog blood is influenced by adenosine receptor subtypes. Orning et al. (1991) found that inhibition of LTA4 hydrolase/aminopeptidase by captopril increased LTB4 production.

Inhibition of LTA4 hydrolase by captopril has been shown to increase LTB4 production. This is likely due to the accumulation of LTA4, which can then be converted into LTB4 by the 12-lipoxygenase pathway. Captopril is a diuretic and antihypertensive agent that is known to inhibit the renin-angiotensin system. The inhibition of LTA4 hydrolase by captopril may also be due to the inhibition of other enzymes that are involved in the metabolism of arachidonic acid.

Albumin has been shown to stabilize LTA4 in plasma (Fitzpatrick, 1982). Albumin is a plasma protein that binds a variety of molecules, including LTA4. The stabilization of LTA4 by albumin may be important in the regulation of leukotriene synthesis, as it can prevent the rapid degradation of LTA4 in plasma.

Adenosine has also been shown to inhibit the calcium influx across human neutrophil plasma membrane. This inhibitory effect is likely to be mediated through the activation of adenosine receptors on the cell surface. Tsuruta et al. (1992) demonstrated that adenosine inhibits calcium influx across human neutrophil plasma membrane via surface adenosine A2 receptors.

Calcium-mediated translocation of cytosolic phospholipase A2 (PLA2) to the nuclear envelope and endoplasmic reticulum is a critical step in the regulation of leukotriene synthesis. Schievella et al. (1995) demonstrated that calcium-mediated translocation of cytosolic PLA2 to the nuclear envelope and endoplasmic reticulum is a critical step in the regulation of leukotriene synthesis. Calcium-mediated translocation of cytosolic PLA2 to the nuclear envelope and endoplasmic reticulum is a critical step in the regulation of leukotriene synthesis.

Sulfasalazine is a drug that is used to treat inflammatory bowel disease. Gadangi et al. (1996) demonstrated that the anti-inflammatory mechanism of sulfasalazine is related to adenosine release at inflamed sites. This suggests that adenosine may play a role in the anti-inflammatory effects of sulfasalazine.

24. Haines, K.A., K.N. Giedd, A.M. Rich, H.M. Korchak, and G. Weissmann. 1987. The leukotriene B4 paradox: neutrophils can, but will not, respond to ligand-receptor interactions by forming leukotriene B4 or its ω-metabolites. Biochem. J. 241:55–62.
25. Claesson, H.E., and J. Hagglström. 1988. Human endothelial cells stimulate leukotriene synthesis and convert granulocyte release leukotriene A4 into leukotriene B4, C4, D4, and E4. Eur. J. Biochem. 93:93–100.
26. Serhan, C.N., and K. Sheppard. 1990. Lipoxin formation during neutrophil-platelet interactions: evidence for the transformation of leukotriene A4 by platelet 12-lipoxygenase in vitro. J. Clin. Invest. 85:772–780.
27. Gadangi, P., M. Longaker, D. Naime, R.J. Levin, P.A. Recht, M.C. Montesinos, M.T. Buckley, G. Carlin, and B.N. Cronstein. 1996. The anti-inflammatory mechanism of sulfasalazine is related to adenosine release at inflamed sites. J. Immunol. 156:1937–1941.