Paradoxical CysLT$_2$R Antagonism by Leukotriene D$_4$ Limits Platelet-Dependent LTC$_4$-driven lung immunopathology

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Introduction

The three cysteinyl leukotrienes (cysLTs), (LT)C$_4$, LTD$_4$, and LTE$_4$, have different biological half-lives, cellular targets, and receptor specificities. LTC$_4$, but not LTD$_4$, activates platelets and elicits their CysLT$_2$R-dependent release of IL-33 ex vivo. LTC$_4$ strongly potentiates allergen-induced pulmonary eosinophilia through a CysLT$_2$R-mediated, platelet- and interleukin-33 dependent pathway in vivo that is not recapitulated by LTD$_4$. We now report that LTD$_4$ unexpectedly behaves as a functional antagonist for LTC$_4$ signaling at CysLT$_2$R in vitro and in vivo.

Methods

Mouse platelets were stimulated for surface CD62P and HMGB1 by FACS analysis. Ovalbumin(OVA)-sensitized wild-type mice were challenged with aerosolized LTs for lung inflammation determination. Airway resistance (R$_L$) in response to lysine aspirin (Lys-ASA) challenge was assessed in *Ptges*$^{-/-}$ mice with an Invasive Pulmonary Function Device, and R$_L$ was recorded for 45 min.

Results

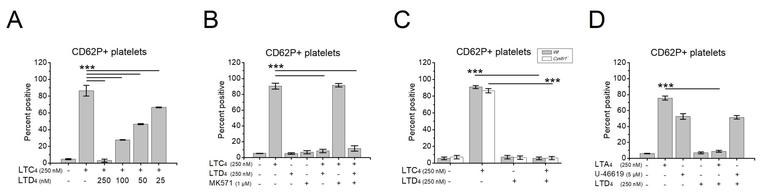

Figure 1. LTD$_4$ blocks LTC$_4$/CysLT$_2$R-dependent platelet activation. Platelet rich plasma from the indicated mouse strains was stimulated with LTC$_4$ (250 nM) for 10 min with or without the simultaneous addition of the indicated agonists and antagonists. Surface expression of CD62P was monitored by flow cytometry. **A.** Dose-dependent effect of LTD$_4$ on LTC$_4$-elicited expression of CD62P. **B.** Lack of effect of the CysLT$_1$R-selective antagonist MK571 on LTC$_4$-elciited CD62P expression and its suppression by LTD$_4$. **C.** Lack of effect of *Cysltr1* deletion on LTC$_4$-elicited CD62P expression. **D.** Effect of LTD$_4$ on CD62P expression elicited by the indicated agonists. ***p<0.001.

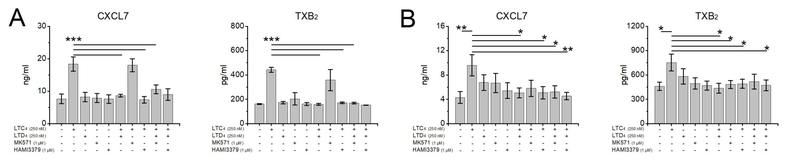

Figure 2. LTD$_4$ blocks the release of soluble mediators from mouse and human platelets activated by LTC$_4$. PRP from WT mice (**A**) or healthy human volunteers (**B**) were stimulated for 30 min with LTC$_4$ in the absence or presence of the indicated agonists or antagonists. CXCL7 and TXB$_2$ (as a surrogate for TXA$_2$ production) were measured in the supernatants by ELISA. * p<0.05, **p<0.01, ***p<0.001.

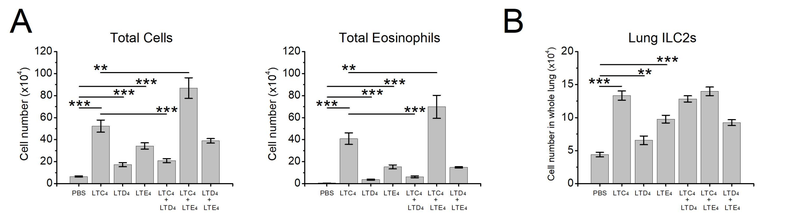

Figure 3. LTD$_4$ blocks amplification of OVA-induced pulmonary inflammation induced by LTC$_4$ but not by LTE$_4$. WT mice were sensitized on days 0 and 7 with OVA/Alum IP. On day 14-16, mice received single intranasal doses (2.2 nmol) of the indicated cysLTs, followed 12 h later by inhaled OVA (0.1% for 30 min). BAL fluid and lung tissue were collected 24 h after the last dose of OVA. **A.** Total BAL fluid cell counts (left) and eosinophil counts (right). **B.** Numbers of ILC2s in dispersed lung tissue from the indicated groups. **p<0.01, ***p<0.001.

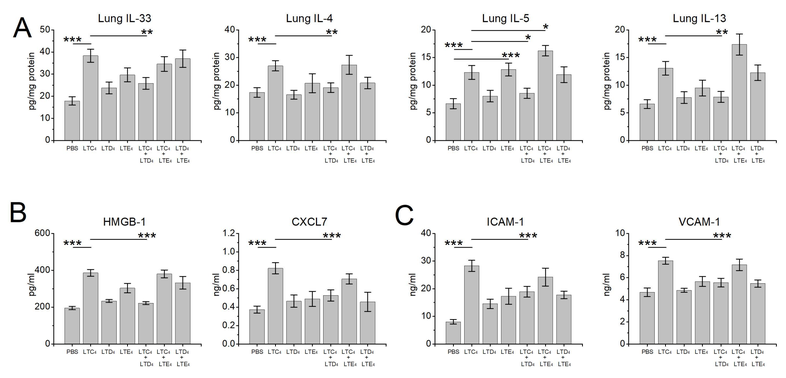

Figure 4. Effect of LTD$_4$ on lung cytokines and BAL fluid mediators induced by LTC$_4$ and LTE$_4$. A. ELISA measurements of IL-33, IL-4, IL-5 and IL-13 in whole lung lysates from WT OVA sensitized and challenged mice receiving the indicated cysLTs. **B.** measurements of HMGB1 and CXCL7, and **C.** soluble adhesion receptors from the same mice as in **A.** * p<0.05, **p<0.01, ***p<0.001.

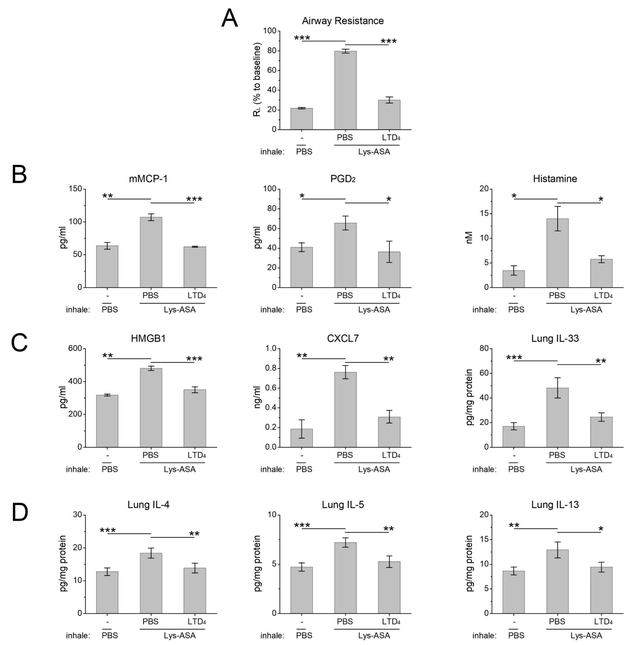

Figure 5. Inhaled LTD$_4$ blocks AERD-like reactions to lysine aspirin challenges of *Ptges*$^{-/-}$ mice. *Df*-primed *Ptges*$^{-/-}$ mice were challenged by inhalation of PBS or Lys-ASA. Some mice received a single inhaled dose of LTD$_4$ 30 min before challenge. **A.** Maximum percent change in R$_L$ monitored continuously for 45 min after the administration of Lys-ASA or PBS. **B.** Levels of MC activation markers (mMCP-1, histamine, PGD$_2$), platelet activation markers (CXCL7 and HMGB1) in BAL fluids from the indicated groups of mice. Whole lung levels of IL-33 (**C**) are shown from the same mice. **D.** Whole lung levels of IL-4, IL-5, and IL-13 from the indicated groups of mice. * p<0.05, **p<0.01, ***p<0.001.

Conclusions

- LTD$_4$ behaves as a functional antagonist of LTC$_4$ signaling at CysLT$_2$R.
- The conversion of LTC$_4$ to LTD$_4$ may limit the duration and extent of potentially pathological signaling through CysLT$_2$R and may contribute to the therapeutic properties of therapeutic desensitization to aspirin.

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