Introduction

The three cysteinyl leukotrienes (cysLTs), (LT)C$_4$, LTD$_4$, and LTE$_4$, have different biological half-lives, cellular targets, and receptor specificities. LTD$_4$, but not LTE$_4$, activates platelets and elicits their CysLT$_R$-dependent release of IL-33 ex vivo. LTD$_4$ strongly potentiates allergen-induced pulmonary eosinophilia through a CysLT$_R$-mediated, platelet- and interleukin-33 dependent pathway in vivo that is not recapitulated by LTE$_4$. We now report that LTD$_4$ unexpectedly behaves as a functional antagonist for LTC$_4$ signaling at CysLT$_R$ 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 LTD$_4$/CysLT$_R$-dependent platelet activation. Platelet-rich plasma from the indicated mouse strains was stimulated with LTD$_4$ (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 LTD$_4$-elicited expression of CD62P. B. Lack of effect of the CysLT,R-selective antagonist MK571 on LTD$_4$-elicited CD62P expression and its suppression by LTD$_4$. C. Lack of effect of Cystitr1 deletion on LTD$_4$-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. DP-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$_R$.
- The conversion of LTC$_4$ to LTD$_4$ may limit the duration and extent of potentially pathological signaling through CysLT$_R$ and may contribute to the therapeutic properties of therapeutic desensitization to aspirin.

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