Department of Medical Biochemistry and Biophysics

Studies on the Regulation of Leukotriene Biosynthesis in Antigen Presenting Cells

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Summary

Leukotrienes (LTs) are pro-inflammatory lipid mediators with important roles in host defense and inflammatory disease. Leukotriene B_4 (LTB_4) is a potent chemoattractant for neutrophils and contributes to bacterial killing, while the cysteinyl leukotrienes (LTC_4, LTD_4 and LTE_4) elicit increased vascular permeability, bronchoconstriction, eosinophil trafficking and mucus secretion. Due to their potency to trigger inflammatory responses, the biosynthesis of LTs is highly regulated. This regulation aids to proper pathogen clearance and simultaneously prevents the development of chronic inflammation. It was the aim of this thesis to contribute to a better understanding of the regulatory mechanisms that control LT biosynthesis.

5-lipoxygenase (5-LO), the first enzyme of the LT biosynthetic pathway, catalyzes the conversion of arachidonic acid (AA) to the intermediate leukotriene A_4 (LTA_4). Here, we describe a function of coactosin like protein (CLP) as a stabilizing chaperone for 5-LO. We found that the interaction of 5-LO with CLP, which protects the enzyme from inactivation, depends on lysine 131 in CLP and tryptophan 102 in the regulatory domain of 5-LO. Furthermore, we demonstrate co-localization of 5-LO and CLP in the human monocytic cell line Mono Mac 6 (MM6), implying a regulatory role for CLP in cellular LT formation.

The downstream enzymes of LT biosynthesis (LTA_4 hydrolase (LTA_4H) and LTC_4 synthase (LTC_4S)) further metabolize LTA_4 to the LTs B_4 and C_4, respectively. We studied the regulation of these enzymes during the differentiation of MM6 cells, human monocyte derived macrophages (MDMs) and dendritic cells (MDDCs). Interestingly, a prolonged exposure to the fungal cell wall preparation zymosan (48-96 h) potently downregulated the LTC_4S activity in MM6 cells, MDMs and MDDCs. Acetylsalicylic acid (ASA) and protein kinase inhibitors counteracted this downregulation. Further elucidation of the underlying signaling events indicated that the suppressive effect of zymosan involved toll like receptor 2 ligation, induction of PGE_2 synthesis and protein kinase A and C dependent inhibitory phosphorylation of LTC_4S. These mechanisms for control of cysLT biosynthesis may contribute the resolution of an acute inflammatory response. The counteracting effect of ASA on the suppression of cysLT formation might have implications for aspirin induced asthma.

Antigen presenting cells (APCs), such as macrophages and dendritic cells secrete nano-sized vesicles (exosomes), which serve as messengers in immunity. Here, we investigated if exosomes from MDMs and MDDCs might contain enzymes for LT biosynthesis. We demonstrate that exosomes from MDMs and MDDCs as well as from human plasma and bronchoalveolar lavage fluid (BALF) contain active LTA_4H and LTC_4S. In addition, we show that exosomes from APCs have the capacity to synthesize a variety of eicosanoids and to induce granulocyte migration. Furthermore, BALF exosomes from asthmatics could increase the release of pro-inflammatory mediators (interleukin-8 and cysLTs) from bronchial epithelial cells. Hence, our data implicate exosomes as novel players in LT biosynthesis with potential roles in inflammatory disorders, such as asthma.

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