Available online at www.sciencedirect.com
SCIENCE DIRECT®
Developmental and Comparative Immunology 29 (2005) 53–59
Developmental & Comparative Immunology
www.elsevier.com/locate/devcompimm

At what stage in metazoan evolution did leukotriene generation first appear?—key insights from cartilaginous fish

Elizabeth L. Morgan, Ben H. Maskrey, Andrew F. Rowley*

School of Biological Sciences, University of Wales Swansea, Singleton Park, Swansea SA2 8PP, Wales, UK

Received 2 March 2004; revised 19 April 2004; accepted 6 May 2004

Available online 15 June 2004

Abstract

Leukotriene (LT) B_4 is a key player in inflammatory responses in mammals. During the generation of this derivative of arachidonic acid, the unstable product of 5-lipoxygenase, termed LTA_4, is converted to LTB_4 by LTA_4 hydrolase. Invertebrates do not generate LTs yet all vertebrates from bony fish onwards synthesize this compound. As cartilaginous fish are the most primitive living jawed vertebrates, we investigated if the leukocytes from such a fish, the Thornback ray (*Raja clavata*) could generate LTB_4. Supernatants from ionophore-challenged leukocytes generated the 5-lipoxygenase products, 6-*trans*-LTB_4 and 6-*trans*-12-*epi*-LTB_4 but were unable to synthesize LTB_4. To determine if these cells contained an active LTA_4 hydrolase, LTA_4 was incubated with lysates from ray leukocytes. Such preparations did not contain any demonstrable LTA_4 hydrolase activity. Our findings imply at the stage of cartilaginous fish evolution over 350 million years ago that the evolution of an active LTA_4 hydrolase had yet to occur.

Keywords: Ray; Leukotriene A hydrolase; Lipid mediators; Inflammation

1. Introduction

Leukotrienes (LT) in particular LTB_4, are key pro-inflammatory molecules that have been shown to be implicated in many disease processes including asthma and rheumatoid arthritis [1]. During the biosynthesis of LTB_4, the C20 polyunsaturated fatty acid, arachidonic acid, is converted by 5-lipoxygenase (5-LO) to the unstable epoxide termed LTA_4, which in turn is subject to enzymatic hydrolysis by LTA_4 hydrolase (EC 3.3.2.6; Fig. 1). In the absence of LTA_4 hydrolase activity, the unstable LTA_4 ($t_{1/2} < 10$ s in physiological conditions) undergoes rapid non-enzymatic hydrolysis to give rise to a number of di-hydroxyeicosatetraenoic acids (di-HETEs) including 6-*trans*-LTB_4, 6-*trans*-12-*epi* LTB_4 and various 5,6 diHETEs (Fig. 1). These non-enzymatic products are largely without the potent biological activity of LTB_4 [2]. LTA_4 hydrolase is a 69 kDa bi-functional, zinc metalloenzyme with both aminopeptidase and LTA_4 hydrolase activities [3–5]. It is widely distributed in mammalian tissues [6] and has been found in all leukocyte types as well as mature erythrocytes [3,7].

Abbreviations: HETE, hydroxyeicosatetraenoic acid; LO, lipoxygenase; LT, leukotriene; PG, prostaglandin; RP-HPLC, reverse phase high performance liquid chromatography.

* Corresponding author. Tel.: +44-1792-295455; fax: +44-1792-295447.

E-mail address: a.f.rowley@swansea.ac.uk (A.F. Rowley).

0145-305X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dci.2004.05.001

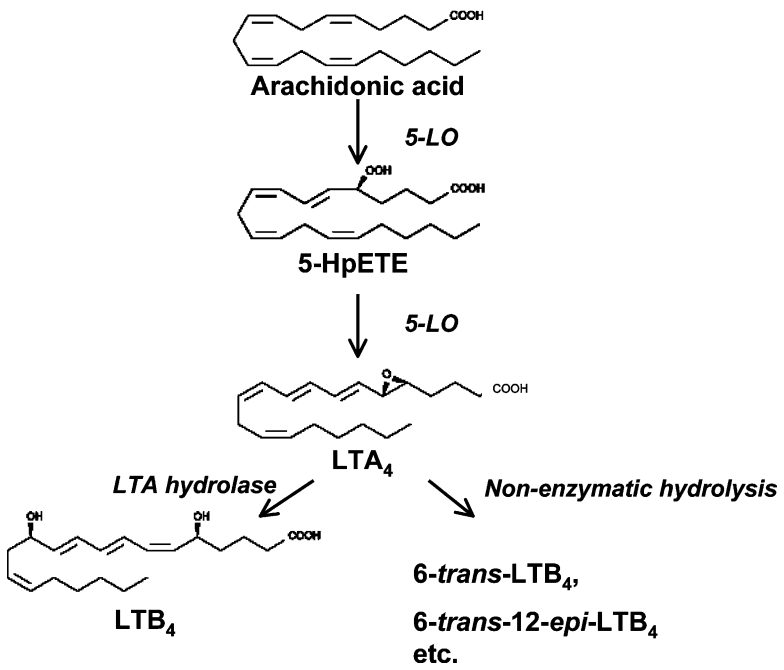

Fig. 1. Schematic diagram showing the major biosynthetic routes of leukotriene (LT) and dihydroxyeicosatetraenoic acid generation from the unstable epoxide, LTA_4. 5-HpETE, 5 hydroperoxyeicosatetraenoic acid; LO, lipoxygenase.

Although invertebrates exhibit potent inflammatory reactions, there is no convincing evidence for the generation of LTs in these animals [8]. This raises the question about the evolution of both 5-LO and LTA_4 hydrolase, and at what stage their products first appeared. Recently, an aminopeptidase has been cloned from the nematode worm, *Caenorhabditis elegans* that although showing strong homology to human LTA_4 hydrolase is unable to convert LTA_4 to LTB_4 [9]. One interpretation of this finding is that in 'lower' animals, such as *C. elegans*, this enzyme originally functioned as an aminopeptidase and, at a later stage in evolution of more complex multicellular animals, the lipid binding site became altered such that the active site could bind LTA_4 and catalyse the biosynthesis of LTB_4 [5]. As leukocytes from bony fish and amphibians generate LTB_4 [10,11] and such animals have been shown to have an active LTA_4 hydrolase [12,13], this implies that the evolution of LT generation probably came about early in vertebrate evolution with the appearance of bony fish. This current study determines whether cartilaginous fish (sharks, rays, etc.) that represent the ancestors of some of the most primitive jawed vertebrates, also generate LTB_4 and express functional LTA_4 hydrolase activity. This was achieved by examining the LT generating capacity of leukocytes from the hemopoietic Leydig organ located in the mucosa of the oesophagus of the Thornback ray, *Raja clavata*.

2. Materials and methods

2.1. Fish

Thornback rays, *Raja clavata*, (25–58 cm length) were collected by trawling from the *R. V. Noctiluca* around the Gower Peninsula, South Wales and either used immediately or maintained in an aquarium overnight. Rays were not fed during this time and were terminally anaesthetised by placing in 3-aminobenzoic acid ethyl ester methanesulfonate salt (MS-222, 0.1 g ml^{-1}, Sigma Aldrich, UK).