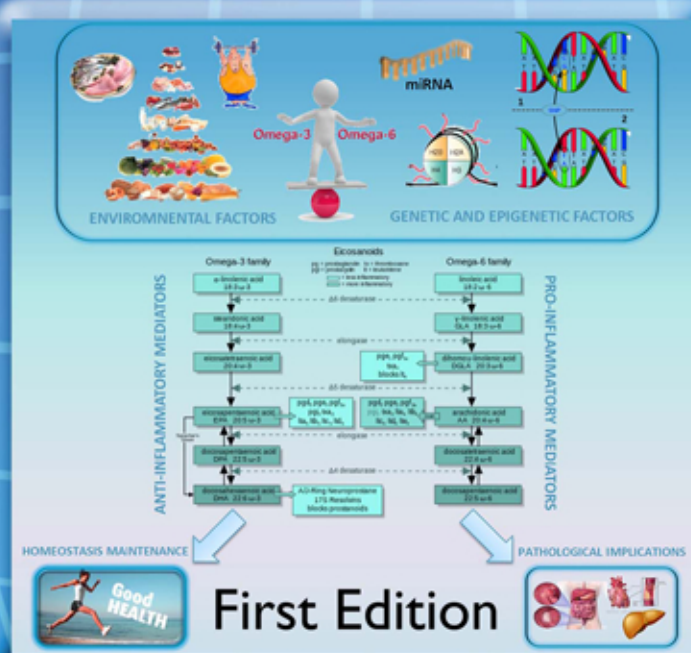


# Eicosanoids, Inflammation and Chronic Inflammatory Diseases

*Pathophysiology, Health Effects  
and Targets for Therapies*

Nova Biomedical



CARMELA RITA BALISTRERI, PH.D.  
EDITOR

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**BIOCHEMISTRY AND MOLECULAR BIOLOGY IN THE POST GENOMIC ERA**

**EICOSANOIDS, INFLAMMATION  
AND CHRONIC INFLAMMATORY  
DISEASES**

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*Dedicated to all components of my family: my brothers, Francesco and Vincenzo,  
and my sister, Luisa, and my mom, Lina*

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## Preface

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The extraordinary increase of the elderly in developed countries underlines the importance of studies on aging and longevity. The need for the spread of knowledge about aging to decrease the medical, economic and social problems associated with advancing years is important because of the increased number of individuals affected by invalidating pathologies, such as age-related chronic inflammatory diseases. Human aging is, indeed, characterized by a chronic, low-grade inflammation, and this phenomenon has been termed as "inflammaging." Inflammaging is a highly significant risk factor for both morbidity and mortality in elderly people; most age-related diseases share an inflammatory pathogenesis. Nevertheless, the precise etiology of inflammaging and its potential causal role in contributing to adverse health outcomes remain largely unknown. The identification of pathways that control age-related inflammation across multiple systems is therefore important in order to understand whether treatments that modulate inflammaging may be beneficial in old people. Thus, the scientific community is currently researching pathways to co-temporally use as appropriate biomarkers and targets for new and more efficient therapeutic treatments, i.e., personalized therapies, for age-related diseases. Among the inflammatory pathways, eicosanoids are of crucial importance. They constitute a large and expanding family of bioactive lipids synthesized from polyunsaturated fatty acids (PUFA) to either pro-inflammatory omega-6 arachidonic acid (AA) or anti-inflammatory omega-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In these last cases, two essential fatty acids (FAs),  $\omega$ -6 linoleic acid (C18:2n6) and  $\omega$ -3 linolenic acid (LA) (C18:3n3) utilized as substrates and a series of desaturase and elongase enzymes are essential for their production.

Among these different members, the AA-derived eicosanoids operate as potent signaling mediators able in providing an efficient way to cells to respond to various stimuli. As result, they act as part of a complex regulatory network and control a number of important physiological processes. These bodily functions include smooth muscle tone, vascular permeability, platelet aggregation, broncho-constriction/dilation, intestine motility, inhibition of gastric acid secretion, uterus contraction, kidney filtration and renal blood flow, increase in hypothalamic and pituitary hormone secretion. Their action is mediated through the binding to specific G-protein-coupled membrane receptors which can trigger an increase or decrease in the rate of cytosolic second messenger generation (cAMP or  $\text{Ca}^{2+}$ ), activation of specific protein kinases or changes in membrane potential. Different cellular types are involved in their production from classical inflammatory cells, including polymorphonuclear leukocytes,

macrophages (important producers) and mast cells to dendritic cells, which represent both a source and target of AA-derived eicosanoids. In addition, human activated T and B cells produce a significant amount of eicosanoids, particularly prostaglandins, such as PGD<sub>2</sub> and PGE<sub>2</sub>. This propriety might be principally central to several functions of B cells. However, AA-derived eicosanoids also constitute the optimal modulators of the immune/inflammatory responses by mediating their effects on macrophages, mast cells, dendritic cells, lymphocytes and natural killer cells. Thus, they are also able to exert both the evocation of immune responses and immune-modulation.

In addition to their capacity to elicit biological responses, eicosanoids, and particularly AA-derived eicosanoids, are now understood to regulate immunopathological processes ranging from inflammatory responses to chronic tissue remodeling, obesity, insulin resistance, diabetes, atherosclerosis, allergic diseases, cardiovascular complications (i.e., coronaropathies, aneurysm, etc.), cancer, rheumatoid and autoimmune disorders. A genetic basis has been postulated for susceptibility to each of these diseases. Each of these medical conditions is syndromic; that is, it is caused by more than 1 molecular defect. On the other hand, they are multifactorial diseases. Recently, it has been suggested the role of genetic variants of eicosanoid pathways in the risk of these diseases. Their combinations have been observed in patients affected by these diseases. Thus, they might be used as promising biomarkers in a pre- and post-treatment clinical setting of these diseases. Indeed, their identification may hold promise for the realization of a personalized medicine.

The evidence is growing in terms of the eicisaoid role in tissue regeneration and wound healing. It is also interesting how a Mediterranean diet might be suggested as an advantageous and new form of anti-inflammatory therapy. This implies the possibility to use eicosanoids as both health and disease biomarkers, and to consider them as potential therapeutic targets.

Many of these aspects are summarized in this book describing the data based on an expert opinion derived on the findings from author's studies on ageing, age-related diseases and inflammation.

This book is focused on addressing a wide range of audiences: health care professionals, nutritionists, food scientists, biologists, physicians and diverse scientific community. It will be a valuable resource for clinical scientists and researchers, university professors, nutritionists, health practitioners, nursing and dieticians, food and nutraceutical researchers, gerontologists and geriatricians, students, and for all those who wish to broaden their knowledge in the allied field. Policy makers and agencies involved in implementing food and dietary supplement policies may also use this book as an updated integral resource. All government and private organizations, including libraries at the college level, academic universities, and research institutions will benefited as resource complied in this text book for their reference.

Overall, we are very enthusiastic in saying this book is a unique and versatile piece which covers and interlinks several processes: inflammation, aging and pathophysiology of age-related diseases, genetic and epigenetic factors in the modulation of physiological and pathological eicosanoid related effects, intricate mechanistic aspects and how nutrition, supplements and exercise. These factors can prevent a broad spectrum of diseases that occur due to aging and promote healthy aging.

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# **Eicosanoids: Structure, Function and Role in Local and Systemic Inflammation**

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## **Abstract**

Lipid mediators derived from polyunsaturated fatty acids, (such as arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid) are recognized as potent signalling molecules that regulate a multitude of cellular responses via receptor-mediated pathways. In particular, metabolism of arachidonic acid leads to several families of lipid mediators, including prostanoids, leukotrienes, 5-oxo-6,8,11,14-eicosatetraenoic acid, lipoxins and epoxyeicosatrienoic acids along two major metabolic pathways: the cyclooxygenase and lipoxygenase pathways. These compounds are collectively known as eicosanoids and possess potent biological activities; they are involved in maintenance of normal hemostasis, regulation of blood pressure, renal function, and reproduction as well as host defense. Recently, new families of local acting mediators were discovered and biosynthesized from EPA (E-series resolvins) and DHA (D-series resolvins, protectins and maresins). These mediators are endogenously generated in the termination program of acute inflammation. In this chapter structure, pathways involved in biosynthesis and their role in inflammation and its resolution will be treated.

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## Introduction

Phospholipids, originally regarded as just membrane constituents and energy storing molecules, are now recognized as potent signaling molecules. In fact, they regulate a multitude of cellular responses via receptor-mediated pathways, including cell growth, death and inflammation. They are of critical importance in mammalian cell biology, acting as substrates for generation of important lipid mediators. One of the most studied families of bioactive lipids are the eicosanoids. The importance of these hormones was recognized when the 1982 Nobel Prize in Physiology and Medicine was awarded for the initial discoveries. In particular it was found how they control virtually every function in the human body, and how aspirin works by altering eicosanoid levels. The term “eicosanoids” is used to describe the bioactive derivatives of three fatty acids with 20-carbonacyl-chains, namely: arachidonic acid (AA), eicosapentaenoic acid (EPA), and dihomo-gammalinolenic (DGLA;20:3n-6). In particular, the principal eicosanoids of biological significance to humans are derived from AA. Additional biologically significant eicosanoids are derived from DGLA that is produced in the reaction pathway leading to AA from linoleic acid. Minor eicosanoids are derived from EPA, which is itself derived from  $\alpha$ -linolenic acid or obtained in the diet. The major source of AA is through its release from membrane phospholipid stores. Within the cell, it resides predominantly at the C-2 position of membrane phospholipids and is released from there upon the activation of several phospholipase (PL) enzymes, mainly PLA<sub>2</sub>. The family of PLA<sub>2</sub> comprises a large number of enzymes with distinct characteristics in terms of their activation, cellular localization, and substrate specificity. These enzymes are activated by various cellular agonists, such as IL-8, platelet activating factor, microorganisms or even non specific stimuli such as damage or injury. The immediate dietary precursor of arachidonate is linoleic acid. The activity of the  $\Delta^6$ -desaturase is slow and can be further compromised; this is due to nutritional deficiencies as well as during inflammatory conditions. Therefore, maximal capacity for synthesis of AA occurs with ingested  $\gamma$ -linolenic acid (GLA), the product of the  $\Delta^6$ -desaturase. GLA is converted to DGLA and then to arachidonic acid. Like the  $\Delta^6$ -desaturase, the activity of the  $\Delta^5$ -desaturase is limiting in arachidonic acid synthesis and its activity is also influenced by diet and environmental factors. Due to the limited activity of the  $\Delta^5$ -desaturase most of the DGLA formed from GLA is inserted into membrane phospholipids at the same C-2 position as for arachidonic acid. The major dietary sources of GLA are borage oil, evening primrose seed oil, hemp seed oil, and black currant seed oil. Diets containing sources of GLA have been shown have distinct cardiovascular benefits similar to diets rich in omega-3 polyunsaturated fatty acids (PUFA), as found in cold water fishes. The eicosanoids derived from AA are generally powerful pro-inflammatory eicosanoids, whereas those derived from DGLA are powerful anti-inflammatory eicosanoids. Although those derived from EPA are virtually neutral in their inflammatory actions, EPA can play an important role in modulating the balance of DGLA and AA, and thus the balance of pro- and anti-inflammatory eicosanoids derived from them. Derived oxygenated PUFAs originate from three main pathways: the cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP) reactions. Eicosanoids include a broad number of mediators: *Prostaglandins*, *Thromboxanes*, *Leukotrienes*, *Hydroxylated essential fatty acids*, *Endocannabinoids*, *Lipoxins*, *Protectins* and *Resolvins*. Understanding the relationship of eicosanoids with

inflammation, development of chronic disease and their effects on gene expression is one of the prime research areas in today's biotechnology industry [1-3].

## Cyclooxygenase Pathway

The eicosanoid cascade starts with the activation of phospholipases, mainly PLA<sub>2</sub>. They release AA and other PUFA from the cellular membrane. Free AA is then metabolized via the constitutive and inducible COX isoforms (COX-1 and -2, respectively) to the unstable endoperoxide PGH<sub>2</sub>. While COX-1 is constitutively expressed in most cells and tissues, COX-2 is usually undetectable, but is rapidly induced when cells are challenged with inflammatory stimuli. Although not exclusive, it is generally accepted that COX-1 is involved in cellular housekeeping functions necessary for normal physiological activity, whereas COX-2 acts primarily at sites of inflammation. PGH<sub>2</sub> is then transformed to prostaglandins (PG), thromboxanes (TX) and prostacyclin (PGI<sub>2</sub>) via tissue specific synthases; these COX-derived mediators belong to the family of eicosanoids and are collectively known as prostanoids. Apart from AA, prostanoids are formed from DGLA, and EPA, with the result in gmetabolites having different activities and being considered less-inflammatory than the AA-derived ones. PGE<sub>2</sub> is produced by prostaglandin E synthase that is found as membrane bound or cytosolic. PGD<sub>2</sub> is produced by the hematopoietic-type or the lipocalin-type synthases, while PGF<sub>2</sub> $\alpha$  is produced either directly from PGH<sub>2</sub> via the prostaglandin F synthase or through further metabolism of PGE<sub>2</sub> and PGD<sub>2</sub> by PGE 9-ketoreductase and PGD 11-ketoreductase, respectively. Prostacyclin (PGI<sub>2</sub>) is produced via the prostacyclin synthase (PGIS) and is usually detected as its stable but inactive metabolite 6-keto-PGF<sub>1</sub> $\alpha$ . Finally, thromboxane synthase (TXS) converts PGH<sub>2</sub> to TXA<sub>2</sub>, an unstable prostanoid that is quickly hydrolyzed to the stable but inert metabolite TXB<sub>2</sub>. The bioactivity of prostanoids is mediated through G protein-coupled receptors for PGE<sub>2</sub>, PGD<sub>2</sub>, PGF<sub>2</sub> $\alpha$ , PGI<sub>2</sub>, and TXA<sub>2</sub>, designated EP, DP, FP, IP, and TP, respectively [4, 5]

## Prostanoids

Overall, prostanoids are potent autacoids, generated in most tissues and cells. Their biosynthesis is the target of non-steroidal anti-inflammatory drugs (NSAIDs), one of the most widely used classes of pharmaco-therapeutic agents for the treatment of chronic inflammatory diseases. Prostanoids modulate biological processes such as smooth muscle tone, vascular permeability, hyperalgesia, fever and platelet aggregation (References in [5-10])

### PGE<sub>2</sub>

PGE<sub>2</sub> is one of the most abundant PGs produced in the body. PGE<sub>2</sub> is an important mediator of many biological functions, such as regulation of immune responses, blood pressure, gastrointestinal integrity. Although considered to be a, primarily, pro-inflammatory eicosanoid, PGE<sub>2</sub> can also mediate anti-inflammatory signals. After PGE<sub>2</sub> is formed, it is

transported or diffuses across the plasma membrane to act at or near its site of secretion. PGE2 acts locally through binding to its receptors, termed EP1, EP2, EP3 and EP4. Each EP shows a distinct cellular localization within tissues. PGE2 can regulate the function of many cell types including macrophages, dendritic cells, T and B lymphocytes. In particular PGE2 stimulates the differentiation of CD4+CD8+ thymocytes, while in later stages it regulates the development and balance of Th1, Th2, and Th17 subsets and, overall, influences proliferation, differentiation, cytokine production, and apoptosis of mature T cells. Interestingly, the activity of PGE2 on T cells appears to be concentration dependent: while at low concentrations, it is involved in homeostatic events and inhibits the activation and differentiation of T lymphocytes, at high concentrations, PGE2 has the opposite effect, increasing T cell proliferation and suppressing immune functions. PGE2 can also affect the maturation of DC and alter DC-produced cytokines, thus influencing the differentiation of T cell subtypes. PGE2 can also enhance the proliferation of T cells through the induction of costimulatory molecules on DC. PGE2 has also been demonstrated to suppress Th1 differentiation and B cell functions. Moreover PGE2 can exert anti-inflammatory actions on innate immune cells, such as neutrophils, monocytes and NK.

## PGD2

PGD2 is considered an immunomodulatory prostaglandin. PGD2 is a major eicosanoid that is synthesized in both the central nervous system and peripheral tissues and appears to function in both an inflammatory and homeostatic capacity. In the brain PGD2 is involved in the regulation of sleep and pain perception. PGD2 is the predominant prostanoid produced by activated mast cells, which initiate IgE mediated type I responses. However production of PGD2 has been detected in Th2 cells. The downstream product of PGD2 dehydration, 15d-PGJ2, has also been detected in human T cell cultures. PGD2 mediates its effects through two receptors DP1 and DP2. DP1 belongs to the prostanoid family of receptors, signals through cAMP and has been detected in Th1, Th2, and CD8+ cells. DP1 receptor is expressed on bronchial epithelium and mediate production of chemokines and cytokines that recruit inflammatory lymphocytes and eosinophils leading to airway inflammation and hyperreactivity in asthma. DP2 belongs to the cytokine receptor family; it signals through increased calcium and inhibition of cAMP and has been found to be preferentially expressed by activated Th2 cells mediating their recruitment and motility. Furthermore, both receptors have been reported involved in T cell proliferation, and DP1 has been suggested to promote T cell apoptosis and down regulate immune responses, while DP2 has been reported to delay Th2 apoptosis. PGD2 and its degradation product 15-deoxy  $\Delta^{12,14}$ -PGJ2 (15d-PGJ2), are involved in the resolution of inflammation. A potentially anti-inflammatory protective effect of 15dPGJ2 in pregnancy has been attributed to its suppression of Th1 response and promotion of Th2 immunity through DP2. Finally, PGD2 has been shown to affect the maturation of monocyte derived DC impacting on their ability to stimulate naive T cells and favoring their differentiation toward Th2 cells.



## PGF2 $\alpha$

PGF2 $\alpha$ , synthesized in the female reproductive system, acts via the FP. It plays an important role in ovulation, luteolysis, contraction of uterine smooth muscle and initiation of parturition. Also plays a significant role in renal function, contraction of arteries, myocardial dysfunction, brain injury and pain. To date, there is very limited information on the contribution of PGF2 $\alpha$  on T cell function. There are no reports on the production of PGF2 $\alpha$  or expression of the relevant synthases on T lymphocytes. However, a recent report on allergic lung inflammation presents evidence for the contribution of PGF2 $\alpha$  in Th17 cell differentiation, an autocrine effect mediated through cell surface FP receptors.

## PGI2

PGI2 is one of the most important prostanoid that regulates cardiovascular homeostasis. In addition to its cardiovascular effects, PGI2 is an important mediator of the edema and pain that accompany acute inflammation. The major source of PGI2 are vascular cells, including endothelial cells and vascular smooth muscle cells (VSMC). PGI2 is best known as a potent vasodilator, an inhibitor of platelet aggregation, leukocyte adhesion and VSMC proliferation and DNA synthesis, anti-mitogenic. Recent finding has shown its involvement in immune regulation with particular importance in airway inflammation. These actions are mediated through specific IP receptors, expressed in kidney, liver, lung, platelets, heart, aorta and in a number of immune cells, including Th1 and Th2 lymphocytes. However, there is very little information on the actual production of PGI2 by T cells with only some indirect evidence for possible transcellular biosynthesis operating between platelets and lymphocytes. Studies in various models suggest that PGI2 is involved in regulating the balance of Th1 and Th2 responses, as well as promoting Th17 cell differentiation. Finally, the anti-inflammatory effect of PGI2 has been explored through analogs that reduced the production of pro-inflammatory cytokines and chemokines by DC, increased the production of anti-inflammatory IL-10, and inhibited their ability to stimulate CD4<sup>+</sup> T cell proliferation.

## TXA2

TXA2 is predominantly derived from platelet COX-1, but it can also be produced by other cell types including macrophage COX-2. Although production of TXA2 by T cells has been reported, albeit at very low levels, the expression of the relevant synthase has not yet been shown. Its activity is principally mediated through the TP receptor and include platelet adhesion and aggregation, smooth muscle contraction and proliferation, and activation of endothelial inflammatory responses. Also in human lymphocytes it has been suggested that TXA2 is involved in the inhibition of T cell proliferation and related cytokine production. Following production of TXA2 by DC, stimulation in TP expression was observed and this appeared to be involved in the random movement of naive but not memory T cells, suggesting that TXA2 can mediate DC–T cell interactions.

## Lipoxygenase Pathway

Lipoxygenase reactions mediate the oxygenation of free fatty acids including AA and other PUFA and are catalyzed by non heme iron-containing dioxygenases. They are found widely in plants, fungi and animals and are classified according to the regioselectivity, i.e., the number of the carbon subjected to dioxygenation as well as their stereoselectivity that can be either “S” or “R”. There are no fewer than 6 lipoxygenases, but the main mammalian LOX enzymes are defined as 5-, 12-, and 15-LOX. The products of LOX reactions are unstable hydroperoxides that are then reduced to hydroxy acids. Cellular 5-LOX activity is dependent on a small membrane protein, 5-lipoxygenase activating protein (FLAP), devoid of enzyme activity; This complex metabolizes AA to 5S-hydroperoxyeicosatetraenoic acid (HPETE) that is further reduced to 5S-HETE or dehydrated to leukotriene (LT) A<sub>4</sub>, an unstable epoxide containing a conjugated triene system characteristic of all LT. LTA<sub>4</sub> can be metabolized to LTB<sub>4</sub> or form the cysteinyl LT, LTC<sub>4</sub>, LTD<sub>4</sub> and LTE<sub>4</sub> following conjugation with reduced glutathione. 5S-HETE can be also enzymatically reduced to the 5-oxo-eicosatetraenoic acid (5-oxo-EETE), a chemoattractant mediator. Mammalian 12- and 15-LOX isozymes oxygenate a range of PUFA, both free and esterified in membrane phospholipids and lipoproteins, forming a multitude of mono and poly-hydroxy fatty acids: e.g., AA produces hydroxyeicosatetraenoic acids (HETE), EPA generates hydroxyeicosapentaenoic acids (HEPE), DHA produces docosanoids including hydroxydocosahexaenoic acids (HDHA), linoleic acid (LA; 18:2n-6) forms octadecanoids such as hydroxyoctadecadienoic acids (HODE), DGLA forms hydroxyeicosatrienoic acids (HETrE), etc. [10, 11]

## Leukotrienes

As indicated by their name, *leuko*-trienes are primarily formed in various types of *leuko*-cytes in particular granulocytes, monocytes/macrophages, mastcells and dendritic cells. Like other eicosanoids, leukotrienes are paracrine mediators exerting their actions in the local cellular milieu. Just like other eicosanoids such as PGs and TX, leukotrienes (LT) may be formed via transcellular biosynthesis. This concept involves a donor cell capable of generating the intermediate LTA<sub>4</sub> which is exported to a recipient cell equipped with enzyme for further metabolism into LTB<sub>4</sub> or cys-LTs. LTs act through specific heptahelical receptors of the rodopsin class that are located on the outer leaflet of the plasma membrane of structural and inflammatory cells (References in 5, 8, 10-13).

## LTB<sub>4</sub>

The main activity attributed to LTB<sub>4</sub> is chemotaxis, in fact attracts and activates neutrophils, monocytes and lymphocytes. This property is mediated through two types of receptors BLT1 and BLT2 with different affinities and cellular expression profiles. BLT1 shows a high degree of specificity and is expressed in a variety of inflammatory cells including mastcells, CD4<sup>+</sup> and CD8<sup>+</sup> T cell subtypes. BLT1 is also important for homing events, as it enables the adhesion of T cells to epithelial cells, and appears of particular

importance for the recruitment and direction of T cells to the airways in asthma. Blockade of LTB<sub>4</sub>/BLT1 pathway has also been shown to improve CD8<sup>+</sup> T cell mediated colitis and beneficial for the treatment of various diseases such as bronchial asthma, multiple sclerosis, contact dermatitis and postmenopausal osteoporosis. The second receptor, BLT2, has a broader ligand specificity; in contrast to the BLT1 receptor, which is predominantly found in leukocytes, BLT2 is ubiquitously expressed in various tissues. BLT2 plays different roles from BLT1 and one of important role of BLT2 is the maintenance of mucosal integrity in the colon. Finally, LTB<sub>4</sub> appears involved in Th17 cell differentiation, Th1 and Th2 proliferation, and cytokine production.

### LTC<sub>4</sub>, LTD<sub>4</sub>, LTE<sub>4</sub>

The cysteinyl LTs are recognized by two specific receptors CysLT1 and CysLT2. The preferred ligands for the CysLT1 receptor are LTD<sub>4</sub>, followed by LTC<sub>4</sub> and LTE<sub>4</sub> in decreasing order of potency. It is generally believed that the classical bioactions elicited by CysLTs, such as smooth muscle contraction, increased vascular permeability and plasma leakage, emanate from CysLT1 signaling, and this receptor is targeted by antiasthma drugs such as montelukast. The CysLT2 receptor binds LTC<sub>4</sub> and LTD<sub>4</sub> equally well, whereas LTE<sub>4</sub> shows low affinity to the receptor. This receptor contributes to inflammation, vascular permeability and tissue fibrosis in lungs. A potent and selective antagonist of CysLT2 has recently been described. Furthermore both receptors have been found to be expressed by peripheral blood T cells. Interestingly, it has been reported that resting Th2 cells display higher expression of the CysLT1 receptor compared to Th1 or activated Th2 cells, suggesting its involvement in Th2 cell differentiation. The family of receptors for Cys-LTs has expanded and now includes gpr17, P2Y12 and CysLTE which are functionally interconnected allowing complex signaling patterns.

### 5-HETE, 5-oxo-EETE, 12-, 15-HETE

Oxidative stress appears to stimulate the metabolism of 5-HETE to 5-oxo-EETE in peripheral blood lymphocytes, although the role of this lipid mediator in T cell function is not clear. Furthermore 5-oxo-EETE is produced by eosinophils, neutrophils, basophils and monocytes. Although its most potent bioaction is as chemoattractant for eosinophils, it also induces calcium mobilization, actin polymerization, CD11b expression and L-selectin shedding. Furthermore it induces degranulation and superoxide production in leukocytes primed with cytokines.

Finally another important effect is its ability to stimulate human monocytes to secrete GM-CSF, a potent survival factor for eosinophils. 5-Oxo-EETE acts via a distinct orphan receptor recently termed the OXE receptor, highly expressed in human peripheral leukocytes, lungs, kidney, liver and spleen. 12-HETE is a neutrophil chemoattractant and has been involved in T cell function, with particular relevance to allergic disease. Increased levels of 12-HETE were also associated with metabolic changes in T cells leading to development of autoimmune disease. It has been reported that 15-HETE regulates T cell division and displays anti-proliferative effects on a leukemia T cell line. 15-LOX metabolites have also been

involved in Th1 responses in a mouse model of Th1 allergic inflammation induced by double-stranded RNA.

## **Anti-Inflammatory and Proresolving Lipid Mediators**

There are some lipid mediators derived from essential omega-6 and omega-3 PUFA that have been shown to control and resolve inflammation in a variety of experimental models of inflammatory disorders [14-16].

### **Lipoxins**

Lipoxins (LXs) are a series of trihydroxytetraene-containing bioactive eicosanoids first isolated from human leucocytes. LXs, such as LXA4 and B4, were the first proresolving mediators to be recognized; they act as anti-inflammatory and proresolution lipid mediators and are generated through cell-cell interaction by a process known as transcellular biosynthesis. In humans there are two major routes of LXs biosynthesis: the first pathway involves the oxygenation of AA at C-15 by 15-LOX in eosinophils, monocytes or epithelial cells, yielding 15S-HPETE. Following secretion, 15S-HPETE is taken up by either PMNs or monocytes and rapidly converted into 5,6-epoxytetraene by 5-LOX, which is hydrolysed within these recipient cells by either LXA4 or LXB4 hydrolase to bioactive LXA4 or LXB4. The second major route of LX biosynthesis occurs in a LTA4-dependent manner, involving peripheral blood platelet-leukocyte interactions. Leukocyte 5-LOX converts AA into LTA4, which is released, taken up by adherent platelets, and subsequently transformed to LXA4 and LXB4 via the LX synthase activity of human 12-LOX. A third route of LX generation occurs after the exogenous administration of aspirin, which irreversibly acetylates COX-2 in endothelial cells and other cell types. Rather than COX-2 converting AA into PGG2, acetylation causes the transformation of AA into 15R-HETE (C-15 alcohol carried in the R-configuration). This is then rapidly metabolised in a transcellular manner by adherent leukocyte, vascular endothelial or epithelial 5-LOX to form 15 epimeric-LX (15-epi-LXs) or aspirin-triggered LXs (ATL) that carry their C-15 alcohol in the R-configuration rather than 15S native LX. These mediators are anti-inflammatory because they regulate both granulocyte and monocyte entry to sites of inflammation; in fact they inhibit the transmigration of neutrophils and promote non inflammatory infiltration of monocytes required for resolution. LXs stimulate macrophages to ingest and clear apoptotic neutrophils. Furthermore they can elevate the levels of TGF- $\beta$ 1, an anti-inflammatory cytokine, which down regulates a number of pro-inflammatory pathways. Finally LXs may also counteract the fibrotic response and thus improve tissue remodeling by reducing the proliferation of fibroblasts.

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## Resolvins, Protectins and Maresins

These products of EPA and DHA are formed through transcellular metabolism and contribute functionally to the resolution of inflammatory exudates and neuroprotection. The term resolvins (Rsv) is derived from “resolution phase interaction products”. Rsv derived from EPA are members of the E-series (RvE1 and RvE2) whereas those derived from DHA are members of the D-series (RvD1, D2, D3, D4). DHA also serves as a precursor for the biosynthesis of protectins (PDs) enzymatically converted by 15-LOX to a 17S-hydroperoxide-containing intermediate which is rapidly converted by human leukocytes into a 16 (17)-epoxide that is enzymatically converted in these cells to a 10,17-dihydroxy-containing compound. PDs are distinguished by the presence of a conjugated triene double bond and by their potent bioactivity. One specific DHA-derived lipid mediator, 10,17S-docosatriene was termed protectin D1 (PD1). When generated in neural tissue however, this compound is called neuroprotectin D1 (NPD1). Similarly to RsvPD1 exerts potent immunoregulatory effects: inhibits neutrophil migration, suppresses Th2 inflammatory cytokines and pro-inflammatory lipid mediators, blocks T-cell migration and promotes T-cell apoptosis. Maresins (MaR) were identified in 2008. Although the exact biosynthetic pathway is yet to be elucidated, it is thought that DHA is converted to 14S-hydroperoxydocosahexaenoic acid (14S-HPDHA; maresin, MaR1) via 12- or 15-LOX. As maresins have recently been identified, little is known about how these novel lipid mediators function. However, it has been reported that, as with Rvs and PD1, MaR1 blocks the infiltration of PMNs, while stimulating macrophage phagocytosis of apoptotic PMNs/zymosan.

## Cytochrome P450 Pathway

There is a third less well-characterized pathway of AA metabolism: cytochrome P450 (CYP). Cytochrome P450 mono-oxygenases relevant to PUFA metabolism catalyze the conversion of fatty acids including AA into products which have been denoted epoxyeicosatrienoic acids (EETs), hydroxyeicosatetraenoic acids (HETEs), and dihydroxyeicosatrienoic acids (DHETs). All these mediators are involved in inflammation and immunity exhibiting a range of protective roles. They block the adhesion of PMNs to the vascular wall by suppressing the expression of cell-adhesion molecules, have the propensity to down-regulate various cytokine-induced pro-inflammatory signalling pathways downstream of NF- $\kappa$ B activation. It was also shown that EETs can directly activate peroxisome-proliferator-activator receptor-gamma (PPAR- $\gamma$ ) in endothelial cells. The EETs-mediated anti-inflammatory effects were later demonstrated to be blocked by the PPAR- $\gamma$  antagonist. EETs released from platelets have been shown to exert anti-thrombotic properties by inhibiting platelet aggregation induced by AA and vascular injury. It was also demonstrated that EETs could act in a pro-fibrinolytic manner by increasing the expression of tissue plasminogen activator in a cAMP-dependent mechanism, thus suggesting that they could play an important role in controlling the fibrinolytic balance in the vessel wall. It was very recently suggested that the anti-inflammatory properties of EETs occurred through its binding to a cell surface receptor [10].

## Endocannabinoids

The endocannabinoids anandamide (arachidonylethanolamide, AEA) and 2-arachidonoyl glycerol (2AG) are derivatives of AA and act as endogenous ligands to the cannabinoid receptors CB1 and CB2. Although endocannabinoids can be metabolized by COX and LOX, their precursor phospholipids and metabolism are different to eicosanoids. The biochemical precursors of AEA and its congeners are various N-acylated ethanolamine phospholipids (NAPE) that are found in very low concentrations in the biological membranes and are hydrolyzed by NAPE-specific PLD or PLC-type lipases. 2AG production is mediated by PLC-diacylglycerol lipase. The biological actions of these endocannabinoids are terminated by their hydrolysis by specific lipases, fatty acid amide hydrolase (FAAH) and N-acylethanolamine hydrolyzing acid amidase (NAAA) for AEA and monoacylglycerol lipase (MAGL), and a/b hydrolase domain 6 (ABHD6) for 2-AG. These enzymes hydrolyze AEA and 2-AG into AA and ethanolamine or glycerol, respectively. In addition to COX-2 and the lipases mentioned here, endocannabinoids can also be metabolized by other enzymes such as lipoxygenases and cytochrome P450. The endocannabinoids AEA and 2-AG exert numerous biological effects. They modulate food intake and energy balance, and exert anxiolytic effects as well as analgesic and anti-inflammatory effects. Classically cannabinoids exert their effects by binding to and activating two receptors CB1 and CB2. AEA and 2-AG can also activate the PPAR receptors, and AEA is a ligand for the transient receptor potential cation channel subfamily V member 1. Furthermore, the endocannabinoid system is considered an important regulator of the immune response with AEA, 2AG, and related enzymes and receptors being involved in T cell function. Production of AEA and 2AG have been shown in human T lymphocytes, while the receptors CB1 and CB2 have been identified in primary T cells and T cell lines where their expression is stimulated upon activation. Some studies suggested that AEA inhibits T cell proliferation and induces apoptosis. The immunosuppressive effect of AEA extends to Th17 cell and this is of particular interest for the development of immunotherapeutic approaches. Endogenous AEA or inhibition of FAAH leading to increased AEA levels, were effective in reducing cytokine levels, decreased liver injury, and increased numbers of Treg cells in a murine model of immune-mediated liver inflammation. AEA inhibited the migration of CD8+ T through the CB2 receptor. Other studies have reported proinflammatory effects by AEA. In a mouse model of atherosclerosis, reduced levels of FAAH that resulted in increased AEA and its congeners, palmitoyl- and oleoyl-ethanolamide, were accompanied by reduced CD4+FoxP3+ regulatory T cells, suggesting a pro-inflammatory effect on the overall immune response. As regards 2AG, its chemotactic properties are also mediated through the CB2 receptor and this has been shown in various immune cells including migration of splenocytes, homing of B cells, and motility of human natural killer cells. Furthermore, 2AG can act as DC chemoattractant and indirectly shift the memory response toward a Th1 phenotype in a CB2mediated fashion (References in [17-18]).

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## **Eicosanoids and Immune/Inflammatory Cells**

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### **Abstract**

Eicosanoids are an important class of lipid signaling mediators generated by hydrolysis of membrane phospholipids by phospholipase A2 to omega-3 and omega-6 C20 fatty acids, and successively converted to leukotrienes (LTs), prostaglandins (PGs), prostacyclins (PCs), and thromboxanes (TXAs). They have long been extensively studied for their proinflammatory functions. In recent years, however, evidence sustains not only their involvement in inflammation promotion, but also their anti-inflammatory actions. In addition, they show more complex and nuanced roles in the regulation of immune and inflammatory responses. Prolonged inflammation involves the production and release of numerous mediators, such as cytokines inducing the expression of COX-2, the enzyme responsible for prostanoids production. Anyway, the excessive production of inflammatory mediators, such as prostaglandins and leukotrienes with an exacerbated sensing response to inflammatory triggers, correlates with progression from acute inflammation to chronic inflammation in many diseases. Some inflammatory processes are, however, self-limiting, and mediated by endogenous anti-inflammation and pro-resolution pathways. They seem induced by eicosanoids, which given rise to classical signs of inflammation such as redness, swelling, pain, and heat, but some eicosanoids also regulate immune cells, and particularly T cell functions.

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## Introduction

Inflammation is a response that protects tissues affected by various types of tissue insults, foreign pathogens or physical trauma. However, inflammation often persists and becomes chronic. Growing evidence now suggests involvement of chronic inflammatory processes in pathogenesis of a variety of diseases, including cancer, metabolic syndrome, and vascular diseases. Inflammation is evoked by inflammatory cells (neutrophils, monocytes/macrophages, and lymphocytes) after the initial insult. The trafficking of these cells into the inflammatory sites is regulated by numerous cell surface receptors and ligands, such as chemokines, cytokines, vasoactive amines, and bioactive lipid mediators. Lipid mediators essentially represented by sphingolipids and eicosanoid derivatives, are known to play a decisive role in inflammatory response, but also paradoxically in its resolution. Their biosynthesis from polyunsaturated fatty acids can be catalyzed by cyclooxygenase (COX-2), lipoxygenases (LOX), and cytochrome P450 enzymes. Depending on the mechanism/pathway of biosynthesis and parent molecules, different classes of eicosanoids are defined. Eicosanoid lipids are derived from the activity of phospholipase A2 on the 20-carbon membrane phospholipid arachidonic acid. Multiple divergent metabolic pathways use arachidonic acid as their substrate. One pathway metabolizes arachidonic acid via the lipoxygenase pathway to leukotrienes (LTs) and lipoxins. A second major pathway forms the Prostaglandins (PGs) and thromboxanes (TX) from arachidonic acid via COX pathway. PGs including PGD<sub>2</sub>, PGE<sub>2</sub>, PGF<sub>2α</sub>, PGI<sub>2</sub> and thromboxane (TX) A<sub>2</sub> are a group of lipid mediators produced and released in response to various stimuli. They are synthesized from arachidonic acid by sequential actions of COX and respective synthases, and exert their actions through a family of G-protein-coupled receptors (GPCRs), prostaglandin D receptor (DP), EP1, EP2, EP3 and EP4 subtypes of prostaglandin E receptor, prostaglandin F receptor (FP), prostaglandin I receptor (IP) and thromboxane A receptor (TP). Because COX is the target of aspirin-like nonsteroidal anti-inflammatory drugs (NSAIDs) that effectively suppress various symptoms of acute inflammation, many symptoms of acute inflammation were presumed to be mediated by PGs. Moreover PG signaling is involved in transition to and maintenance of chronic inflammation.

The biosynthesis of LTs start from the release of arachidonic acid by phospholipases, primarily cytosolic phospholipase A<sub>2</sub> (cPLA<sub>2</sub>) acting at perinuclear membranes. Arachidonic acid is oxygenated by the enzyme 5-lipoxygenase (5-LO) in cooperation with the 5-LO activating protein (FLAP), leading to the production of the intermediate, LTA<sub>4</sub>. LT synthesis is completed by the downstream enzymes LTA<sub>4</sub> hydrolase (LTA<sub>4</sub>H) and LTC<sub>4</sub> synthase (LTC<sub>4</sub>S), which produce LTB<sub>4</sub> and LTC<sub>4</sub>, respectively. LTC<sub>4</sub> is produced by the attachment of glutathione to LTA<sub>4</sub>, by a sulfide linkage involving the central cysteine residue of glutathione. Both LTs appear to be actively exported from cells through ATP-binding cassette (ABC) transporters. Following the export of LTC<sub>4</sub> from the cell, glutamate may be removed by  $\gamma$ -glutamyl transferase ( $\gamma$ -GT) to produce LTD<sub>4</sub>, which in turn may lose glycine, through dipeptidase (DiP) action, to yield LTE<sub>4</sub>. LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub> are known collectively as 'cysteinyl LTs', as they, but not LTB<sub>4</sub>, have cysteine linked to arachidonic acid.

As pro-inflammatory mediators, LTs at concentrations in the low nanomolar range stimulate cellular responses that are quick in onset but do not last long, such as smooth muscle contraction, phagocyte chemotaxis, and increased vascular permeability, all of which are mediated via specific G-protein coupled receptors.

## Regulation of Immune Response by Prostaglandin and Thromboxanes

### *PGE<sub>2</sub>*

PGE<sub>2</sub> is an essential homeostatic factor and a key mediator of immune-pathology in chronic infections and cancer. The impact of PGE<sub>2</sub> reflects the balance between its COX-2-regulated synthesis and 15-hydroxyprostaglandin dehydrogenase-driven degradation and the pattern of expression of PGE<sub>2</sub> receptors.

### *Regulation of PGE<sub>2</sub>*

The receptors for PGE<sub>2</sub> (EP1–EP4) are present on multiple cell types, reflecting the ubiquitous functions of PGE<sub>2</sub>, which extent nociception and other aspects of neuronal signaling, hematopoiesis, regulation of blood flow, renal filtration and blood pressure, regulation of mucosal integrity, vascular permeability, and smooth muscle function. The PGE<sub>2</sub> and its receptors are also involved in the regulation of different phases of immune responses and different effector mechanisms of immunity. PGE<sub>2</sub> can be produced by all cell types of the body, i.e., epithelia, fibroblasts and infiltrating inflammatory cells, representing the major sources of PGE<sub>2</sub> in the course of an immune response. PGE<sub>2</sub> has a very rapid turnover rate in vivo and is rapidly eliminated from tissues and circulation. The rate of PGE<sub>2</sub> degradation is controlled by 15-PGDH and the suppression of 15-PGDH activity is observed in many forms of cancer suggesting that, in addition to the rate of PGE<sub>2</sub> synthesis, the rate of PGE<sub>2</sub> decompose may contribute to immune pathology and constitute a potential target for immunomodulation. The heterogeneous effects of PGE<sub>2</sub> are reflected by the existence of four different PGE<sub>2</sub> receptors, designated EP1, EP2, EP3 and EP4, with an additional level of functional diversity resulting from multiple splice variants of EP3 that exists in at least eight forms in humans. EP3 and EP4 are high-affinity receptors, whereas EP1 and EP2 require significantly higher concentrations of PGE<sub>2</sub> for effective signaling. The signaling through the two Gs-coupled receptors, EP2 and EP4, is mediated by the adenylate cyclase-triggered cAMP/PKA/CREB pathway, and is responsible of the anti-inflammatory and suppressive activity of PGE<sub>2</sub>. Despite their similar nominal functions, the signaling by EP2 and EP4 is triggered by different concentrations of PGE<sub>2</sub> and differs in duration. Indeed, EP4 signaling is rapidly desensitized following its PGE<sub>2</sub> interaction, while EP2 is resistant to ligand-induced desensitization, implicating its ability to mediate PGE<sub>2</sub> functions over prolonged periods of time and at later time points of inflammation. In contrast to EP2 and EP4, low-affinity EP1 and high-affinity EP3 are not coupled to Gs and lack cAMP-activating functions. Most of the splice variants of EP3 represent Gi-coupled PGE<sub>2</sub> receptors that inhibit adenylate cyclase. Signaling via EP1 involves calcium release. The differences in sensitivity, susceptibility to desensitization and ability to activate different signaling pathways between the different PGE<sub>2</sub> receptor systems allow for adaptable patterns of responses of different cell types at different stages of immune responses. Additional flexibility of the PGE<sub>2</sub> receptor system results from different sensitivity of the individual receptors to regulation by additional factors. The expression of EP2 and the resulting responsiveness to PGE<sub>2</sub> can be suppressed by hypermethylation, as observed in patients with idiopathic lung fibrosis. These observations raise the possibility that, in addition to the regulation of PGE<sub>2</sub> production and its degradation, the regulation of PGE<sub>2</sub> responsiveness at the level of expression of individual PGE<sub>2</sub> receptors can also contribute to the pathogenesis of human disease and be exploited in their therapy.

### *PGE<sub>2</sub> and Activity of Innate Immune Cells*

PGE<sub>2</sub> promotes the tissue influx of granulocytes, macrophages, mast cells and NK cells, but it differentially affects the function of different innate effector cells. PGE<sub>2</sub> has been shown to inhibit granulocyte functions, contributing to the defective innate host defense in patients after bone marrow transplantation or with cancer, as well as other conditions associated with overproduction of PGE<sub>2</sub>. This limits the phagocytosis by alveolar macrophages and their pathogen-killing function acting in an EP2-dependent manner or via the induction of IL-1R-associated kinase-M, which blocks the scavenger receptor-mediated phagocytosis and the TLR-dependent activation of TNF- $\alpha$ . Concerning mast cell, PGE<sub>2</sub> promotes both their induction, local attraction and degranulation by a mechanism involving EP1 and EP3. It also promotes the degranulation-independent production of the proangiogenic and immunosuppressive vascular endothelial growth factor and MCP-1 by mast cells, which contributes to the overall disease-promoting activity of PGE<sub>2</sub> in cancer. PGE<sub>2</sub> also suppresses the cytolytic effector functions of NK cells, in a mechanism involving suppression of IL-12 and IL-15 responsiveness, and most likely IL-2. It also inhibits NK cell production of IFN- $\gamma$ , abrogating NK cell “helper” function in the DC-mediated induction of Th1 and CTL responses.

### *PGE<sub>2</sub> and Specific Immune Response*

PGE<sub>2</sub> affects several key phenomena relevant to the induction of immune responses. In addition to its multifaceted regulation of Dendritic Cells (DC) functions during the priming of naïve T cells, it also directly inhibits T cell production of IL-2 and IL-2 responsiveness, suppressing the activation and expansion of Ag-specific T cells. PGE<sub>2</sub> has been shown to disrupt early stages of DC differentiation, contributing to local and systemic DC dysfunction in cancer.

Although the ability of PGE<sub>2</sub> to suppress the differentiation of functionally competent Th1-inducing DCs has been long recognized, it has been recently shown that these kind of myeloid-derived suppressor cells induce the suppression of CTL responses. In contrast, PGE<sub>2</sub> has been shown to support the induction of fully mature DCs capable of homing to lymph nodes and to be highly effective in priming naïve T cells. The addition of PGE<sub>2</sub> to the mixture of pro-inflammatory cytokines involving IL-1 $\beta$  and TNF- $\alpha$  accelerates DC maturation and elevates their expression of costimulatory molecules. It has been shown that PGE<sub>2</sub> promote high-level expression of CCR7 and responsiveness to these lymph node-type chemokines in maturing monocyte-derived DCs. This activity and its roles in podosome dissolution and induction of matrix metalloproteinase-9 suggested the role for PGE<sub>2</sub> in DC migration to the lymph nodes. DCs matured in the presence of PGE<sub>2</sub> develop a distinct “exhausted” phenotype, manifested by their impaired ability (compared with alternatively matured DCs) to induce the CTL-, Th1-, and NK cell-mediated type 1 immunity, while promoting Th2 responses.

CCR7 ligands (CCL19 and CCL21) and CXCR4 ligand (CXCL12) represent two groups of chemokines needed for effective T cell entry into lymph nodes. Although the role of PGE<sub>2</sub> in the local regulation of these two chemokines within the lymph nodes remains unclear, PGE<sub>2</sub> has been recently shown to suppress the ability of DCs to produce CCL19 (the only CCR7 ligand produced by human monocyte-derived DCs) and to block the ability of DCs to attract naïve T cells. In contrast, PGE<sub>2</sub> was shown to enhance the production of CXCL12 by vascular endothelium, raising the possibility that a similar effect may also operate in the

lymph nodes, resulting in an opposite impact of PGE<sub>2</sub> on the CCR7- versus CXCR4-driven events governing T cell accumulation in the lymph nodes and their interaction with different types of APCs.

The suppressive effects of PGE<sub>2</sub> on the activation and expansion of naïve T cells also include the direct inhibitory effects of PGE<sub>2</sub> on IL-2 production and the expression of IL-2 receptor and JAK3, which mediate the responsiveness of T cells to IL-2. In particular, PGE<sub>2</sub> suppresses IL-2 production and IL-2 responsiveness in T cells, at high doses, while much lower concentrations of PGE<sub>2</sub> show modulatory effects shifting the pattern of CD4<sup>+</sup> helper T cell responses from the aggressive Th1 cells (promoting the inflammatory/cytotoxic form of immunity) toward Th2 and Th17 cells that mediate less tissue destructive forms of immunity. In addition to its direct impact on CD4<sup>+</sup> T cells, the Th1-suppressive impact of PGE<sub>2</sub> also relies on its ability to suppress the production of IL-12 in monocytes and DCs. Additional mechanisms of the IL-12 antagonistic activity of PGE<sub>2</sub> include its ability to suppress the expression of IL-12 receptor and the resulting responsiveness to IL-12, and may include the induction of IL-12p40 homodimer, a competitive inhibitor of the IL-12 receptor in mice. Thus, PGE<sub>2</sub> shifts the balance away from Th1 responses toward other forms of immunity, such as Th2 responses. In support of its involvement in Th2-mediated human pathology, overproduction of PGE<sub>2</sub> is observed in multiple Th2-associated diseases, among which atopic dermatitis and asthma.

EP2- and EP4-dependent signals from PGE<sub>2</sub> have also been shown to promote the development of IL-17-producing T cells (Th17) in multiple models of infection and autoimmunity. The Th17-promoting activity of PGE<sub>2</sub> is related to its ability to suppress the production of the Th17-inhibitory cytokine IL-12p70, while enhancing the Th17-inducer cytokine IL-23.

It has been demonstrated that the induction of CTL activity against viral Ags and alloantigens is highly sensitive to PGE<sub>2</sub> and cAMP elevation. Apart from the interference with the de novo development of CTL activity, PGE<sub>2</sub> can also suppress the ability of fully developed CTLs to interact with their targets and kill tumor cells. In addition to its direct effects on CD8<sup>+</sup> T cells, PGE<sub>2</sub> has also been shown to suppress the ability of maturing DCs to develop CTL-inducing function by suppressing their ability to secrete IL-12 during the subsequent interaction with naïve CD8<sup>+</sup> T cells. Interestingly, CTLs can produce PGE<sub>2</sub> by themselves, resulting in the acquisition of their suppressive function, although the implications of this phenomenon to the overall regulation of CTL cell function remain unclear.

PGE<sub>2</sub> has been shown to interfere with early stages of B cell activation and show profound cAMP-mediated regulation of the process of Ig class switch in activated B cells. Perhaps the most conspicuous of these effects is the ability of PGE<sub>2</sub> to promote IgE production, the phenomenon contributing to atopic diseases, together with the ability of PGE<sub>2</sub> to support the induction, attraction, and degranulation of mast cells.

PGE<sub>2</sub> has been shown to promote the development of regulatory T cells (Tregs) in humans and in mice. COX2 and PGE<sub>2</sub> have been shown to be essential for the EP2- and EP4-dependent induction of Tregs in human tumor tissues. In addition to promoting de novo Treg differentiation from naïve precursors, PGE<sub>2</sub> also promotes the interaction of DCs with Tregs, suggesting that it may also promote the expansion of pre-existing Tregs, as observed in cancer patients vaccinated with PGE<sub>2</sub>-matured DCs.

### *Trafficking of Innate and Specific Immune Cells to Target Tissues*

In addition to its opposite impact on the development and function of the effector versus suppressive cells, PGE<sub>2</sub> also differentially regulates their influx to affected tissues. PGE<sub>2</sub> enhances the production of CXCL8/IL-8, the attractant for neutrophils and macrophage-recruiting CCL2/MCP-1. It is also a chemo-attractant for mast cells, helping to recruit the three members of innate immune system specialized in fighting extracellular pathogens at early stages of immune responses. However, the macrophage-attracting properties of PGE<sub>2</sub> are limited by its ability to block the expression of CCR5 and Mac-1 on monocytes and macrophages, leading to interference with their extravasation and functions. The PGE<sub>2</sub>-driven suppression of CCL5, as well as all three CXCR3 ligands, CXCL9/MIG, CXCL10/IP10, and CXCL11/ITAC, results in its powerful inhibition of the attraction of not only the pro-inflammatory-type macrophages but also the CCR5<sup>+</sup> and CXCR3<sup>+</sup> type 1 effector cells as CTLs, NK cells and Th1 cells. At the same time, PGE<sub>2</sub> enhances the production of Th2-attracting and promotes the production of CCL22/MDC and the resulting attraction of Tregs. In addition, PGE<sub>2</sub> also interferes with the expression of chemokine receptors. It blocks the induction of CCR5 on monocytes and suppresses the DC and IL-12-driven induction of CCR5 and CXCR3 on CD8<sup>+</sup> T cells, whereas it induces and stabilizes the expression of CXCR4 on cancer-associated myeloid-derived suppressor cells. Additionally, PGE<sub>2</sub> has also been shown to block the trans-endothelial migration of human and murine T lymphocytes, interfering with the expression and functions of relevant integrins and directly suppressing CTL motility.

### *PGD<sub>2</sub>*

PGD<sub>2</sub> is considered an immune-modulatory prostaglandin with anti-inflammatory activities. Production of PGD<sub>2</sub> has been detected in Th2 cells and this was linked to expression of the hematopoietic-type PGD synthases (H-PGDS), while the lipocalin-type (L-PGDS) has not been identified in any T cell subtype. PGD<sub>2</sub> mediates its effects through two receptors, DP1 and DP2, the latter better known as chemo-attractant receptor-homologous molecule expressed on Th2 cells. DP1 belongs to the prostanoid family of receptors, signals through cAMP and has been detected in Th1, Th2 and CD8<sup>+</sup> T cells. DP2 has similarity to prostanoid receptors and belongs to the cytokine receptor family; it signals through increased calcium and inhibition of cAMP and has been found to be preferentially expressed by activated Th2 cells mediating their recruitment and motility. PGD<sub>2</sub> can mediate different effects depending on the target receptor and related signaling events. DP1 can induce differentiation of Th2, while DP2 is mostly involved in their recruitment, although the two receptors may exert opposing effects, as examined in an animal model of contact hypersensitivity, where DP2 appeared to mediate inflammatory events while DP1 was inhibitory. Furthermore, both receptors have been reported involved in T cell proliferation, and DP1 has been suggested to promote T cell apoptosis and downregulate immune responses, while DP2 has been reported to delay Th2 apoptosis. Activation of Th2 cells by PGD<sub>2</sub> is thought to occur predominantly through DP2 with concomitant increase in the production of cytokines and pro-inflammatory proteins. PGD<sub>2</sub> binding to this receptor is also very important for CD4<sup>+</sup> T cell trafficking and motility. When produced at high concentrations by mast cells, as seen in allergic inflammation, there is a consequent activation and recruitment of Th2 cells toward the PGD<sub>2</sub> producing sites. Activated T cells can also produce PGD<sub>2</sub> and this may promote further accumulation of Th2 in the inflamed tissue.

Finally,  $\text{PGD}_2$  has been shown to affect the maturation of monocyte-derived DC impacting on their ability to stimulate naïve T cells and favoring their differentiation toward Th2 cells.

### *PGI<sub>2</sub>*

$\text{PGI}_2$  is best known as an inhibitor of platelet aggregation and potent vasodilator, while recent finding has shown its involvement in immune-regulation with particular importance in airway inflammation. The IP receptor is expressed in a number of immune cells in the lung, including T lymphocytes of the Th1 and Th2 lineage. However, there is very little information on the actual production of  $\text{PGI}_2$  by T cells with only some indirect evidence for possible transcellular biosynthesis operating between platelets and lymphocytes, and some recent work showing PGI synthases (PGIS) mRNA in an animal model of contact hypersensitivity. Studies in various models suggest that  $\text{PGI}_2$  is involved in regulating the balance of Th1 and Th2 responses, as well as promoting Th17 cell differentiation. In a mouse model of asthma has been shown that  $\text{PGI}_2$  produced by endothelial cells and signaling through the G protein-coupled IP receptor prevents the recruitment of Th2 in the airways. Furthermore,  $\text{PGI}_2$  increased the ratio of IL-23/IL-12 leading to differentiation of Th17 cells and exacerbation of EAE in mice. Finally, it has been also demonstrated that  $\text{PGI}_2$  shows anti-inflammatory effect. Indeed it induces a reduced production of pro-inflammatory cytokines and chemokines by DC, while induce an increased production of anti-inflammatory IL-10.

### *TXA<sub>2</sub>*

Although production of  $\text{TXA}_2$  by T cells has been reported, albeit at very low levels, the expression of the relevant synthase has not yet been shown. However, the G protein-coupled TP receptor has been found in a range of T cell populations and a polymorphism identified in Th2 cells has been linked to aspirin-exacerbated respiratory disease. Recent findings indicate that  $\text{TXA}_2$  is involved in the inhibition of T cell proliferation and related cytokine production. Following production of  $\text{TXA}_2$  by DC, stimulation in TP expression was observed and this appeared to be involved in the random movement of naïve but not memory T cells, suggesting that  $\text{TXA}_2$  can mediate DC-T cell interactions.

## Regulation of Immune Response by Leukotrienes

The leukotrienes are important lipid mediators with immune modulatory and pro-inflammatory properties. Classical bio-actions of leukotrienes include chemotaxis, endothelial adherence, and activation of leukocytes, chemokine production, as well as contraction of smooth muscles in the microcirculation and respiratory tract. When formed in excess, these compounds play a pathogenic role in several acute and chronic inflammatory diseases, such as asthma, rheumatoid arthritis, and inflammatory bowel disease. An increasing number of diseases have been linked to inflammation implicating the leukotrienes as potential mediators. Indeed, it has been demonstrated the involvement of leukotrienes in cardiovascular diseases including atherosclerosis, myocardial infarction, stroke, and abdominal aortic aneurysm. Moreover, new insights have changed the previous notion of leukotrienes as mediators of inflammatory reactions to molecules that can modify the innate and adaptive immune response.

LOX enzymes mediate the oxygenation of free fatty acids including arachidonic acid. Their activities are commonly defined by their positional selectivity when they oxygenate arachidonic acid and, following this system, the main mammalian LOX enzymes are defined as 5-, 12-, and 15-LOX. The products of LOX reactions are unstable hydroperoxides that are then reduced to hydroxy acids. 5-LOX acts in concert with FLAP to metabolize arachidonic acid to 5S-hydroperoxy eicosatetraenoic acid (HPETE) that is further reduced to 5S-HETE or dehydrated to LTA<sub>4</sub>, an unstable epoxide containing a conjugated triene system characteristic of all LTs. LTA<sub>4</sub> can be metabolized to LTB<sub>4</sub> or form the cysteinyl LT (cysLT), LTC<sub>4</sub>, LTD<sub>4</sub>, LTE<sub>4</sub> following conjugation with reduced glutathione.

### *LTB<sub>4</sub>*

LTB<sub>4</sub> is a powerful chemotactic agent that, in contrast to its cysLT counterparts, has no direct role in broncho-constriction or pulmonary vasoconstriction. LTB<sub>4</sub> has two extracellular receptors, BLT1 and BLT2. BLT1 has been characterized as a 43-kDa G-protein-coupled receptor (GPCR) expressed only in inflammatory cells (neutrophils predominantly) and with a high affinity for only LTB<sub>4</sub>. The second LTB<sub>4</sub> receptor, BLT2, has only recently been described and, although a GPCR similar to its BLT1 homolog, the BLT2 is ubiquitously expressed in all mammalian tissues. Activation of both BLTRs serves as a powerful stimulus for *in vitro* leukocyte chemotaxis, especially of neutrophils, while *in vivo* experiments have shown LTB<sub>4</sub> to increase neutrophil rolling, adhesion and its release from blood vessels through increased expression of adhesion proteins as integrins and selectins. Additional actions of LTB<sub>4</sub> include stimulation of IL-5 in T lymphocytes, chemotactic effects on IL-5-activated eosinophils, neutrophil secretion of superoxide anion radicals, and antiapoptotic effects on neutrophils. LTB<sub>4</sub>, along with LTC<sub>4</sub> and LTD<sub>4</sub>, has been shown to promote eosinophil survival by inhibiting apoptosis.

The high affinity receptor, BLT1, is also expressed in many CD4<sup>+</sup> and CD8<sup>+</sup> T cell subtypes and it is important for homing events, as it enables the adhesion of T cells to epithelial cells, and appears of particular importance for the recruitment and direction of T cells to the airways in asthma. Blockade of LTB<sub>4</sub>/BLT1 pathway has also been shown to improve CD8<sup>+</sup> T cell-mediated colitis. Finally, LTB<sub>4</sub> appears involved in Th17 cell differentiation, Th1 and Th2 proliferation, and cytokine production.

### *LTC<sub>4</sub>, LTD<sub>4</sub>, LTE<sub>4</sub>*

LTC<sub>4</sub> is formed by conjugation of LTA<sub>4</sub> with the tripeptide glutathione through the catalysis of LTC<sub>4</sub> synthase. Stimulated by divalent cations and phosphatidylcholine, LTC<sub>4</sub> synthase is an 18-kDa protein with a wide tissue distribution. After LTC<sub>4</sub> synthesis, the multidrug transporter ATP-binding cassette (Abc)c1 actively transports LTC<sub>4</sub> out of the cell, where LTD<sub>4</sub> and LTE<sub>4</sub> are formed through the elimination of glutamine and glycine, respectively, by  $\gamma$ -glutamyl transpeptidase and dipeptidase. Failure to express Abcc1 has been shown to increase intracellular accumulation of LTC<sub>4</sub>. This leukotrien has been shown in animal models to be relatively short-lived with rapid conversion to LTD<sub>4</sub> and LTE<sub>4</sub>. The transport of LTC<sub>4</sub> by Abcc1 has also been shown to regulate dendritic and T cell migration to peripheral lymph nodes. Antagonism of Abcc1 or 5-LOX activity inhibits dendritic and T cell migration, which is restored after the addition of exogenous cysLTs. The cysteinylLT specific receptors CysLT1 and CysLT2 have been found to be expressed by peripheral blood T cells. Interestingly, it has been reported that resting Th2 cells display higher expression of the



CysLT1 receptor compared to Th1 or activated Th2 cells, suggesting its involvement in Th2 cell differentiation. Accordingly, in the presence of PGD<sub>2</sub>, LTD<sub>4</sub> and LTE<sub>4</sub> have been shown to enhance Th2 cell activation and cytokine production, in a more than additive effect. Furthermore, LTC<sub>4</sub> appears to induce T cell proliferation, while LTC<sub>4</sub>-maturated DC appear to stimulate CD4<sup>+</sup> responses and induce cytotoxic T cells *in vitro* without concomitant recruitment of Treg cells.

## Conclusion

Eicosanoids are an important class of lipid signaling mediators and have long been studied for their proinflammatory functions. Recently it has become evident that these molecules not only promote inflammation, but can also act as anti-inflammatory mediators and have more complex roles in the regulation of immune-inflammatory responses. There are evidences for the expression of and signaling by some important eicosanoids, the arachidonic acid-derived prostanoids and the leukotrienes, in innate and specific cells of immune system. These lipid mediators regulate a number of functions in immune cells, including proliferation, apoptosis, cytokine secretion, differentiation and chemotaxis. Eicosanoids regulate a wide array of physiological processes, ranging from inflammatory processes such as asthma and allergies, to immune regulation and involvement in graft rejection, as well as diseases such as cancer and AIDS. There is significant interest in targeting some of these pathways for therapeutic gain and it is therefore crucial to develop a complete understanding of all the different physiological functions of these important signaling mediators. Other important issues that remain to be resolved include the roles of these lipid mediators in adaptive and/or innate immunity and whether they play a role as autocrine, paracrine, or endocrine ligands.

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## Paraoxonases, Eicosanoids and Their Role in Age Related Diseases

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### Abstract

The Paraoxonase family represents one of the most important classes of enzymes responsible for the protection against oxidative damage and lipid peroxidation. It is composed by three members: PON1, PON2 and PON3 that show high similarity in their structural characteristics. PON1 and PON3 are active in the serum where they are associated with HDL. On the other hand, PON2 is not detectable in serum but expressed in several tissues. These enzymes have been extensively studied especially for their ability to counteract oxidative stress and ameliorate atherosclerosis. Together with paraoxonases, eicosanoids are involved in the pathway underlying the reactive oxygen species (ROS) production and are considered key players in the atherosclerosis process. The topic of this chapter is to focus on the relationship between eicosanoids and paraoxonases and their involvement in the onset of age related diseases, especially cardiovascular diseases.

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## Introduction

The large family of eicosanoids is composed of the following: prostaglandins, prostacyclins, thromboxanes, leukotrienes, lipoxins, and epoxyeicosatrienoic acids. The networks of controls depending on eicosanoids' family are among the most complex in the human body. Eicosanoids are not stored within cells, but are *synthesized* as required. All mammalian cells are designated to synthesize eicosanoids except erythrocytes. All eicosanoids function locally at the site of synthesis, through receptor-mediated G-protein linked signaling pathways. Arachidonic acid (AA), the precursor in the production of eicosanoids, is derived from storage within the cells. Specifically, it resides predominantly at the C-2 position of membrane phospholipids and is released upon the activation of phospholipase A<sub>2</sub> (PLA<sub>2</sub>). Eicosanoids are derived from either *omega-6* ( $\omega$ -6) or *omega-3* ( $\omega$ -3) fatty acids. In general, the first type are considered pro-inflammatory while the latter are much less. The biosynthesis of eicosanoids consists of two distinct pathways: cyclic and linear pathway. The synthesis of prostaglandins and thromboxanes is performed by the cyclic pathway, otherwise the leukotrienes by the linear pathway. Two families of enzymes catalyze fatty acid oxygenation to produce the eicosanoids: i) *Cyclooxygenase*, or COX, generates the prostanoids; ii) *Lipoxygenase*, or LOX, generates the leukotrienes and via transcellular biosynthesis is also involved in *lipoxin* generation. The eicosanoid biosynthetic pathways are intimately related with the production of reactive oxygen species (ROS). In this chapter we will show how one of the most important classes of detoxifying enzymes involved in the removal of oxygen peroxides is involved in the biosynthesis and in the functions of eicosanoids pathways, and together with eicosanoids can modulate atherosclerosis and inflammation and thus affect the development of cardiovascular disease. Oxidation by either COX or lipoxygenase releases ROS and the initial products in eicosanoid generation are themselves highly reactive *peroxides*. Other reactions of lipoxygenases generate cellular damage, i.e., in *murine* models it has been demonstrated that 15-lipoxygenase contributes to the *pathogenesis* of *atherosclerosis* [1]. The oxidation in eicosanoid generation is compartmentalized; this limits the peroxides' damage. Some of the enzymes which are involved in eicosanoid biosynthetic pathway, such as microsomal glutathione-S-transferase 2 (GST2) and epoxide hydrolases, belong to families that play a role in detoxification [2-5]. The fact raises the intriguing possibility that leukotrienes (LT) signaling may have evolved as an adaptive response to oxidative stress [2]. In this context, it is not surprising to find that Paraoxonase, one of the enzyme protein families most important in detoxifying oxidate biomolecules in blood and other tissues, is involved in eicosanoid biosynthesis and function. The paraoxonase gene family in humans is composed of three members, PON1, PON2, and PON3, placed adjacently on the long arm of chromosome 7q21.3-22.1. They show high similarity in their structural characteristics and have ~ 65% identity at the amino acid level [6]. Phylogenetic analysis has determined that the three genes are well conserved in mammals, and that the oldest member of this family is PON2 [7]; this enzyme is not detectable in serum and it is expressed in many tissues: lung, liver, heart and intestine, with a primary localization in the plasma membrane [8]. Human PON1 is synthesized in the liver and secreted into the blood, where it is associated exclusively with HDLs. Similar to PON1, PON3 is expressed mostly in the liver and associated with HDL, but at a less order of magnitude than PON1 [8].

## Paraoxonases and Eicosanoids in Atherosclerosis

Atherosclerosis and associated cardiovascular diseases (CVDs) still remain today, despite of the remarkable scientific innovations that have occurred, one of the hot topics of the modern medicine because CVDs are still the leading cause of death in developed countries. Atherosclerosis is the primary mechanism underlying the development of coronary artery disease, and is characterized by lipoprotein accumulation and oxidation, aberrant lipoprotein metabolism, and systemic inflammation. In recent years, HDL function has emerged as an effective biomarker for atherosclerosis risk. Together with HDL, paraoxonase has been considered as an antioxidant enzyme with protective functions. HDL's ability to prevent oxidative modifications of LDL has been attributed to PON1, and serum PON1 levels are inversely proportional to the risk of coronary heart disease. In the last fifteen years, also two of us have extensively analysed the relationship between PON1 and its possible role in the achievement of a "successful ageing". To explore this hypothesis we have adopted a model of "successful ageing", represented by centenarian people. Centenarians are exceptional individuals who have reached extreme ages escaping or delaying the onset of the major age-related diseases, including CVD. Since it is well known that ~ 25% of the "extreme longevity" character depends on the own genetic background, we have tried to explore if the genetic of paraoxonase was important in achieving longevity, by the analysis of two polymorphisms of the PON1 gene (PON1 Q192R and L55M polymorphisms). We first reported that PON1 R allele (R+) carriers are significantly more represented in Italian centenarians; subsequently this topic has been addressed by many other groups [9-13]. After these innovative studies we have published other papers that have considered the role of PON genetics in the onset of CVD [14-16]. The antiatherogenic role of PON1 is supported by studies in transgenic mice lacking or overexpressing the enzyme. PON1-knockout mice are more susceptible to developing atherosclerosis than are wild-type mice, and their HDLs, in contrast to wild-type HDL, fail to prevent LDL oxidation in cultured artery wall cells. Like PON1, both human PON2 and PON3 have been shown to prevent cell-mediated oxidative modification of LDL. However, the exact endogenous substrates and mechanism of the PONs' protective activities remain to be elucidated [8]. The complex role of eicosanoids in atherosclerosis, and at the same time a possible functional connection with paraoxonases, is shown by a series of investigations on cyclooxygenase 2 (COX-2). The COX-2 is an enzyme that during acute and/or chronic inflammation is up-regulated, and since its discovery in 1991 its activity inhibition has been considered for the treatment of inflammatory diseases. However, it has been demonstrated by several clinical trials that inhibition by the COX-2-selective inhibitors drugs, such as celecoxib and rofecoxib, caused an increase in the incidence of cardiovascular events, such as heart attacks and strokes [17-19]. While the literature suggests that COX-2 inhibition may have pro-inflammatory effects in cardiovascular physiology, the underlying mechanisms are poorly understood. By using a COX-2<sup>-/-</sup> mouse, Narasimha et al. [20] observed: (i) lipid accumulation in the circulation and liver, (ii) accumulation of dysfunctional, pro-inflammatory HDL, and (iii) increase in thromboxane B<sub>2</sub> (TXB<sub>2</sub>) levels. During inflammation, HDL is characterized by (i) increased ROS accumulation, (ii) decreased levels and activity of anti-inflammatory, anti-oxidant factors including apolipoprotein A-1 (apoA-1) and paraoxonase 1 (PON1), (iii) reduced potential to efflux cholesterol and (iv) diminished ability to prevent LDL oxidation. Moreover, when mice are feeded with an atherogenic diet the situation worsened; so, the

authors concluded that the lack of COX-2 may be responsible for a worsening of atherosclerosis and thus a greater risk of cardiovascular diseases. Interestingly, although this COX-2<sup>-/-</sup> mouse possesses high levels of HDL, these turn out to be pro-inflammatory causing an excessive ROS content, decreased apoA-1 levels, deficient PON1 activity, decreased cholesterol efflux potential, and inability to prevent LDL-induced monocyte chemotactic activity. The authors also have investigated the influence of an atherogenic diet into systemic inflammation and they have found an increase in TNF and IL-6 cytokines, thus reinforcing the link between atherosclerosis and inflammation. This mouse model also determines an imbalance of prostanoid production which in turn determines a pronounced aggregation in the circulation, a 7 fold decrease in the anti-inflammatory prostanoid prostacyclin coupled with a concomitant increase in the pro-thrombotic and pro-inflammatory eicosanoids thromboxane A<sub>2</sub> (TXA<sub>2</sub>) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>). The authors believe that this aberrant shift in eicosanoid profile may be due to either augmented or unopposed COX-1 activity in the absence of COX-2 [20]. Taken together, these results suggest that COX-2 deficiency may affect the cardiovascular physiology in mice, by an accumulation of dysfunctional/pro-inflammatory HDL.

### Influence of Paraoxonases in the Biosynthesis of Eicosanoids

PON1 is by far the best studied member of the family and at first it has been investigated especially for its capacity to hydrolyze the toxic oxon metabolites of a number of organophosphorous insecticides such as parathion, diazinon, and chlorpyrifos and even nerve agents such as sarin and soman (PON activity) [21]. Also, PON1 hydrolyzes aromatic esters, and phenyl acetate as arylesterase activity. Organophosphatase activity is limited to PON1; PON3 has very low PON activity and does not hydrolyze diazoxon and chlorpyrifos oxon. All three PONs hydrolyze aromatic esters, but with some noticeable differences. Phenyl acetate is one of the best substrates for PON1 but is hydrolyzed at a modest rate by PON3 and very slowly by PON2. Obviously, these substrates could not be considered the physiologic substrates, and so, for many years, several researchers have been involved in the finding of the “real” substrate. Only recently, it has been found that the primary activity of the paraoxonases is that of a lactonase [22]. In fact, PON1 hydrolyzes a variety of aromatic and aliphatic lactones, and it also catalyses the reverse reaction, i.e., the lactonization of  $\gamma$  and  $\delta$ -hydroxycarboxylic acids [23]. Interestingly, Draganov et al. [24] proposed that “the physiological role of the PONs is the metabolism of lipid mediators arising from oxidation of polyunsaturated fatty acids, resulting in modulation of the local anti-inflammatory response”. Indeed, it has been reported that many of the compounds of the eicosanoid pathway are PON substrates. In particular, highly purified serum PON1 is reported to possess phospholipase A<sub>2</sub> activity [24]. In addition, all the three PONs are able to metabolize with very high efficiency 5-hydroxy-6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid 1,5-lactone (5-HETEL) and 4-hydroxy-5*E*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*-docosahexa-enoic acid (4-HDoHE), products of both enzymatic and non-enzymatic oxidation of arachidonic acid and docosahexaenoic acid, respectively. Given that 5-HETEL appears to have an inhibitory effect on the synthesis of various eicosanoid pathways intermediates (by acting on PLA<sub>2</sub>, 5-lipoxygenase, and/or cyclooxygenases) in different cell models, its metabolization by PONs could modulate indirectly this regulatory mechanism in eicosanoid biosynthesis [24]. The lactonase activity of PON1 is also

demonstrated by its capacity to convert 5,6-epoxyeicosatrienoic acid (5,6-EET) into dihydroxyeicosatrienoic acid (5,6-DHET), through the hydrolysis of a lactone intermediate (5,6-DHTL) [24-26]. Interestingly, it has been recently demonstrated a possible feedback mechanism to control paraoxonase activity in response to eicosanoid pathway activation. Indeed, arachidonic acid (AA) has been shown to decrease the macrophage cholesterol biosynthesis rate and increase the PON2 expression, resulting in the protection from cholesterol accumulation, foam cell formation and oxidation, typical hallmarks of atherogenesis [27, 28].

## PON1, Eicosanoids and Hypertension

Hypertension is a multifactorial disease in which dietary salt intake and a tendency toward salt retention are important risk factors for its pathogenesis. The underlying mechanism responsible for the increase in blood pressure is very complex, but it has been recently proposed that the activation of transient receptor potential vanilloid 4 (TRPV4) could sense and convey a compensatory counteracting mechanism to depress the salt-induced increase in blood pressure [29].

Eicosanoid pathway is considered to be involved in the pathogenic mechanism induced by high salt. Indeed, high salt is responsible both for upregulating TRPV4 and cytochrome P450 arachidonic acid epoxygenases, that in turn contribute to enhanced vasorelaxation. The underlying mechanism involves the action of 5,6-EET as a direct endogenous agonist of TRPV4, which activates large-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels in smooth muscle, leading to final vasodilation [30]. PON1 is thought to be involved in blood pressure control, via regulating the levels of 5,6-EET [31]. In fact, in PON1KO mice it has been demonstrated an accumulation of 5,6-EET and an inverse association between blood pressure and 5,6-EET level, while upon addition of PON1 a down regulation of this eicosanoid is observed [31]. Together with the above suggested role of paraoxonase in mediating blood pressure regulation via 5,6-EET, some authors suggested an additional, inverse, effect on blood pressure regulation mediated by preserving endothelial function in condition of excessive oxidative stress [31]. This hypothesis is based on the results obtained in apolipoprotein E-deficient mice, expressing high oxidative stress, where gene transfer of human PON1 was shown to improve the impaired endothelial-dependent relaxations [32]. The importance of paraoxonase enzymes in the development of hypertension is also supported by the results of genetic studies showing that paraoxonase's polymorphisms are associated with the risk of developing this multifactorial disease. One of us performed a study that considered PON1 192 polymorphism in two groups of carefully selected subjects: the first group was composed of 219 healthy controls and the second of 119 hypertensive patients with untreated essential arterial hypertension. In hypertensive patients, a significant increase of the frequency of PON1 192 RR genotype with respect to healthy controls was found and the PON1 192RR genotype was independently associated with a four-fold increase in susceptibility to arterial hypertension [33].

## Conclusion

Besides its importance in explaining the pathogenetic mechanisms of inflammation and different cardiovascular diseases, the complex relationship between eicosanoids and paraoxonase enzymes should be taken into consideration also for a better understanding of the effects of many drugs and nutrients. For example, it has been demonstrated that aspirin, which irreversibly inhibits platelet cyclooxygenase-1 (COX-1), also increases PON1 concentration and activity in patients with coronary artery disease, thus reinforcing the antioxidant effects of HDLs [34]. Although the modulation of paraoxonase activity by diet is well known [reviewed in 35], only recently has it been found that diet supplementation with different eicosanoids in mice models can result into a modulation of paraoxonase activity [36]. Given the recent emphasis on studies concerning the importance of nutrients for health and wellness, these preliminary results deserve to be translated in humans.

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# Eicosanoids: Functions in Physiological Processes

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## Abstract

Eicosanoids are an extremely complex group of lipidic mediators involved in controlling a plethora of functions in different pathways of many key biological processes, including their well known pivotal contribution to inflammation. In general, the activation of eicosanoids' receptors on plasmatic membranes of target cells in various tissues, stimulates or inhibits the synthesis of second messengers, such as cyclic AMP. The deepening of both systemic and organ-specific physiological molecular dynamics, represents the base for the development of new personalized therapies for the treatment of various pathological conditions. This objective is also reached considering the individual differences in terms of genetically determined quali/quantitative eicosanoids profile. Certainly, new findings provided by intense researchers' work on eicosanoid physiology, are necessary to allow the setup of new drugs.. This might lead to an increased/reduced production of specific eicosanoids to re-establish the delicate equilibrium, broken in response to altered environmental conditions and responsible for the maintenance of whole organism homeostasis.

## Introduction

Eicosanoids are biological compounds derived from the metabolism of twenty carbon atoms fatty acids, such as arachidonic acid. Their action mechanism both like-hormone

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paracrine and autocrine, even if there is a substantial difference, consisting in the delivery not mediated by the blood circulation. They act on their target cells by binding to seven-trans-membrane receptors and inducing changes in levels of intracellular second messengers, such as cyclic AMP (cAMP). The synthesis of eicosanoids consists in the hydrolysis of fatty acids present in cell membrane, mediated by fosfolipases, and a subsequent targeted oxygenation catalyzed mainly by Ciclo-OXigenases (COXs) and LipOXygenases (LOXs). The COX pathway of the arachidonic acid cascade leads to the biosynthesis of prostanoids encompassing prostaglandins, tromboxanes and prostacyclins. The LOX pathway leads to the production of leukotrienes, lipoxins and resolvins. The main physiological activity of eicosanoid compounds can be summarized in: vaso-constriction/dilatation, platelets aggregation, broncho-constriction/dilation, intestine motility, inhibition of gastric acid secretion, uterus contraction, kidney filtration and renal blood flow, modulation of hypothalamic and pituitary hormone secretion. The major number of these physiological activities are described in this chapter, by subdividing in class-eicosanoid mediated activities and organ specific activities.

### Prostaglandins: Their Physiological Activities

Prostaglandins (classified in those of D, E, F, G, H, I and J classes) have been exhaustively investigated in various tissue types, showing a crucial contribution in different biological processes [1]. PGD<sub>2</sub>, mainly produced by eosinophils and mast cells, is responsible for the relaxation of vascular and lung bronchioli smooth muscles. Moreover, it recruits particular white blood cells such as Th<sub>2</sub> cells, eosinophils and basophils. PGEs exert different physiological effect. In fact, they regulate the blood pressure by dilating arterioles and capillaries, relax vascular smooth muscle, cause contraction of gut musculature, open lung bronchioli, enhance renal blood flow and increase urinary volume as well as the excretion of sodium ions [2]. Lejeune's group demonstrated also the involvement of PGE<sub>2</sub> in the regulation of gut mucosa permeability. In particular, they evidenced as the administration of EP2 receptor antanogist determines a significant decrease in the Trans-Epithelial Electrical Resistance (TEER), through the down-regulation of the tight junction protein Claudin-4 [3].

Prostanoids have been demonstrated to be involved in metabolism regulation through interfering with hormonal signals. In fact, PGE<sub>1</sub> inhibits glycerol release in adipose tissue after stimulation with epinephrine, glucagon, ACTH and TSH [4]. The latter, augments thyroid levels of PGE<sub>2</sub>, PGF<sub>2α</sub> and 6-oxo-PGF<sub>1α</sub> with a consequent increase of cAMP in thyrocytes [5].

Human erythrocytes are cells subjected to mechanical stress due to their role in body circulation. Experiments employing the sollicitation of red blood cells by a fine needle in presence of extracellular Ca<sup>2+</sup>, demonstrated their ability in producing PGE<sub>1</sub> and PGE<sub>2</sub> within 30 min. These evidences have been confirmed by the reveal of COX2 bands by Western-blot assay, suggesting an active role of red blood cells in microcirculation through the production of specific prostanoids [6]. On the other hand, experiments conducted on pig erythrocytes in the 1985 by Ledwozyw A and colleagues, suggested a role of PGE<sub>1</sub> and PGE<sub>2</sub> in increasing intracellular sodium levels to counteract the hemolysis in hypotonic environment [7].

PGE<sub>1</sub> intervenes also in the regulation of coagulation process, by inhibiting platelet aggregation induced by P2Y1 receptor activation. On the contrary, PGE<sub>2</sub> shows prothrombotic effects. An impaired inhibitory response to PGE<sub>1</sub> may contribute to the high platelet reactivity in subjects affected by diabetes mellitus treated with clopidogrel [8].

PGE<sub>3</sub> shows anti-inflammatory effects compared to PGE<sub>2</sub>. In fact, it evokes a reduction of COX-2 expression and IL-6 production. The evidence of a lower affinity of PGE<sub>3</sub> binding to EP receptors than PGE<sub>2</sub>, gives a possible explanation [9].

PGFs are responsible of opposite biological effects in blood pressure homeostasis, respect to those mediated by PGEs. In particular, they increase blood pressure by causing venule vasoconstriction. This effect is evoked by the interaction of PGFs with their specific FP receptors [10]. This determines a signal transduction responsible of subsequent musculature contraction in different tissue districts, such as blood vessels, but also in lung bronchioli and uterus myometrium (inducing also in some cases abortion). PGF<sub>2 $\alpha$</sub>  is involved in the smooth muscle contraction of bronchioli and uterus. In uterus, PGF<sub>2 $\alpha$</sub>  is produced in response to oxytocin. In addition, it is involved in inducing luteolysis with the subsequent formation of the corpus albicans, reciprocally in stopping progesterone production. These physiological functions of PGF<sub>2 $\alpha$</sub>  are used in inducing both labor and abortifacient [11].

PGJ<sub>2</sub>, also called cyclopentenone prostaglandin (cyPG), has anti-inflammatory properties by evocating in target cells the inhibition of NF- $\kappa$ B (Nuclear Factor Kappa-light-chain-enhancer of activated B cells), through a covalent modification of p50 subunit or via PPAR $\gamma$  (Peroxisome Proliferator Activated Receptor) activation [13, 14].

Moreover, PGJ<sub>2</sub> counteracts tissue destruction associated with different inflammatory and autoimmune diseases, probably by inhibiting TRAIL (Tumor necrosis factor-Related Apoptosis-Inducing Ligand) in T lymphocytes [15]. Systemic effects of PGs are summarized in Table 1 and those organ specific in Table 4.

## Tromboxanes and Prostacyclins

Thromboxanes occur in two known forms, the TXA<sub>2</sub> active and TXB<sub>2</sub> inactive form. Among these, TXA<sub>2</sub> mediates blood vessel contraction through the reduction of intracellular cyclic AMP. Moreover, TXA<sub>2</sub> regulates the coagulation process through the induction of platelet aggregation [16, 17]. Prostacyclin (PGI<sub>2</sub>) is released by healthy endothelial cells and has several functions, acting in via paracrine form. In particular, it exerts opposite effects compared to leukotrienes, inducing lung bronchiole dilation. Moreover, PGI<sub>2</sub> induces an inhibition of platelet activation (anti-thrombotic effect), by counteracting TXA<sub>2</sub> effects and acting as a physiological antagonist [18]. Effects of tromboxanes are described in Table 2.

## Leukotrienes

Leukotrienes are pleiotropic lipid mediators with multiple roles ranging from inflammation, immunological responses and maintenance of biological homeostasis in several tissue cells. Regarding their biosynthesis, it is very interesting that LTA<sub>4</sub>, synthesized from myeloid cells, represents the precursor for the production of the other leukotrienes in a

process called “transcellular biosynthesis”. In fact, once LTA<sub>4</sub> is transferred to red blood cells, it gives rise to LTB<sub>4</sub>, as well as LTC<sub>4</sub> in endothelial cells and LXA<sub>4</sub> in platelets [19, 20].

**Table 1. Prostaglandin components, cells involved in their release and their physiological functions**

PROSTAGLANDINS		
Eicosanoids	Main Secreting cells	Physiological functions
PGD <sub>2</sub>	Mast-cells, eosinophils	Th2 cells, eosinophils and basophils recruitment. Sleep induction. Relaxation of blood vessels and lung bronchioli smooth muscles. Increase of the urinary volume as well as the excretion of sodium ions
PGE <sub>2</sub>	Macrophages, astrocytes	Direct vasodilation. Inhibition of noradrenaline release from sympathetic nerve terminals. Inhibition of platelet aggregation
PGF <sub>2α</sub>	Granulosa cells	Smooth muscle contraction in uterus
PGJ <sub>2</sub>	Macrophages, astrocytes	Anti-inflammatory effects
PGI <sub>2</sub>	Endothelial cells	Antithrombotic action

**Table 2. Thromboxan components, cells associated with their production and their actions**

THROMBOXANES		
Eicosanoid	Main Secreting cells	Physiological functions
TXA <sub>2</sub>	Endothelial cells	Contraction of blood vessels
TXB <sub>2</sub>	Endothelial cells	Antagonist of TXA <sub>2</sub>

Leukotrienes modulate the functionality of different organs, such as liver, adrenal gland, uterus and pancreas. This evidence originates from different experimental studies. For example, it has been demonstrated that incubation of rat adrenal gland with A<sub>4</sub>, B<sub>4</sub>, C<sub>4</sub>, D<sub>4</sub> and E<sub>4</sub> leukotrienes determines significant inhibitions of corticosterone production in response to ACTH [21]. About functions exerted by leukotrienes in pancreas and uterus, they will be deepened in the following dedicated sections.






Since the main source of leukotrienes is represented by leukocytes, the latter represent in the same time the sources and the targets of these eicosanoid mediators. In particular, LTA<sub>4</sub> triggers signal transduction cascades in neutrophils by modulating Ca<sup>2+</sup> mobilization [22], whereas LTB<sub>4</sub> and LTC<sub>4</sub> induce the adhesion of leukocytes on the endothelium, inducing them to cross in the inflamed tissue.

**Table 3. Leukotrienes and the cells releasing these molecules and their functions**


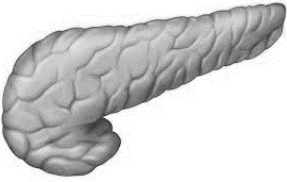
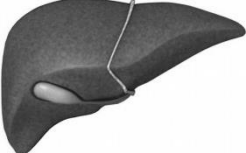

LEUKOTRIENES		
Eicosanoid	Main Secreting cells	Physiological functions
LTA <sub>4</sub>	Myeloid cells	Ca <sup>2+</sup> mobilization in Neutrophils
LTB <sub>4</sub>	Endothelial cells	Polymorphonuclear leucocytes chemotaxis.
LTC <sub>4</sub>	Macrophages, Neutrophils, Mast cells	Vasodilation, bronchocostriction
LTD <sub>4</sub>	Macrophages, Neutrophils	Effects comparable of those evoked by LTC <sub>4</sub>
LTE <sub>4</sub>	Macrophages, Neutrophils	Type of effects equal to those of LTC <sub>4</sub> and LTD <sub>4</sub> but with lower intensity



**Table 4. Physiological effects mediated by eicosanoids in specific tissue and organ targets**

PHYSIOLOGICAL FUNCTION OF EICOSANOIDS IN SPECIFIC TARGETS	
Organ	Physiological functions
	PGs induce gut contraction; in stomach inhibit acid secretion, promotion of mucus and bicarbonate secretion.
	PGEs increase urinary volume and ions excretion. TXA2 evokes opposite effects.
	Bronchiole contraction by Leukotrienes and dilation by PGE2 and PGI2
	EETs induce vasodilation and prevent amylin accumulation.
	12(S)-HETE supports reparative processes.

**Table 4. Physiological effects mediated by eicosanoids in specific tissue and organ targets (continued)**

PHYSIOLOGICAL FUNCTION OF EICOSANOIDS IN SPECIFIC TARGETS	
	<p>Prostaglandin D2 supports development of male gonads. LTB4 is luteotropic during the estrous cycle by supporting early pregnancy, whereas LTC4 is luteolytic.</p>
	<p>PGEs and Leukotrienes regulate negatively exocrine and endocrine secretion.</p>
	<p>PGs, LTC4 and LTD4 induce glucose and lactate output. PGs reduce portal flow and reduce bile acids secretion.</p>
	<p>PGD2 exerts soporific effects. PGE2 and PGD2 produce increased amounts of neurotrophins. PGs sensitize C fibers. Endocannabinoids induces hyperpolarization in neurons through activation of CB1 receptors.</p>

Moreover, LTB4 and LTB5 act as potent chemoattractant for neutrophils, by potentiating their effector activity through the enhancement of reactive oxygen species formation and the release of lysosome enzymes [23]. The effect evoked by LTB4 and LTB5 are due to their IL-1 like activities, even if LTB5 is less potent compared to LTB4 [24].

LTD4 is involved, like the major part of leukotrienes, in the regulation of respiratory apparatus homeostasis, by controlling contraction of smooth musculature in lung bronchioli, stimulating mucus secretion in airways and enhancing growth of human airway epithelial cells. Furthermore, LTD4 influences the circulatory apparatus by reducing myocardial contractility, coronary blood flow and thus, inducing long lasting hypotension [25]. Systemic biological responses evoked by leukotrienes are summarized in Table 3 and those organ specific in Table 4.

## Lipoxins

The immunomodulation is a very important process for the re-establishment of homeostasis through the participation of cellular mediators endowed with anti-inflammatory effects. Lipoxins are short lived endogenously produced non classic eicosanoids very important to stop inflammatory signals and promote the resolution of inflammation.

Lipoxin A4 counteracts LTB4 through hardly binding to its receptor ALX (shared with resolvins) [26, 27], inhibiting chemotactic responses and degranulation induced by LTB4. Over the effects on leukocyte, LXA4 is also involved in regulation of blood circulation by antagonizing vasoconstriction induced by LTD4 [28]. Similarly to the leukotrienes, LXA4 is the precursor of cysteinyl-lipoxins LXC4, LXD4 and LXE4 [29].

The anti-inflammatory effects of both LXA4 and LXB4 consist in inhibiting leukotriene-stimulated interactions between neutrophils and endothelial cells, through a mechanism involving the rapid remodeling of neutrophil phospholipids finalized to block either aggregation or the formation of lipoxygenase-derived products [30, 31].

## Endocannabinoids

The endocannabinoids (endogenous cannabis-like substances) are small lipidic mediators derived from arachidonic acid conjugated with ethanolamine or glycerol [32]. These pleiotropic compounds exert their biological activities through the binding to G-protein-coupled receptors (GPCRs) distributed in different organs such as adipose tissue, muscle, liver and endocrine pancreas.

Recent studies demonstrated the existence of complex mechanisms involving cannabinoid signaling in negatively modulating insulin sensitivity and substrate oxidation in skeletal muscle [33]. Cannabinoid receptors type 1 (CB1), might become overactive in the skeletal muscle during obesity due to increased levels of endocannabinoids. However, quite surprisingly, one of the most studied endocannabinoids, anandamide, when administered in a sufficient dose shows to improve muscle glucose uptake and to activate some key molecules of insulin signaling and mitochondrial biogenesis. A probable reason is given to fact that anandamide is only a partial agonist for CB1 receptors and interacts with other receptors (PPAR $\gamma$ , TRPV1), which may trigger positive metabolic effects [34].

Furthermore, CB1 is densely distributed in brain areas deputed to emotional responses, motivated behaviour, motor control, cognition and homeostasis control. Outside the brain, the endocannabinoids modulate autonomic nervous system, the immune system and microcirculation. Immune cells express both CB1 and CB2 receptors, secrete

endocannabinoids and have specific transport and breakdown systems [35, 36]. B cells are the most expressing CB receptors followed by NK cells, monocytes, polymorphonuclear neutrophils, CD8<sup>+</sup> lymphocytes and CD4<sup>+</sup> lymphocytes [37]. The expression levels of CB2 receptors in immune cells are 10–100 times greater than CB1 receptors. Moreover, CB2 receptor mRNA is also detectable in the cortex of lymph nodes and the nodular corona of Peyer's patches [38]. In vivo and in vitro studies reported inhibition of antibody formation by natural and synthetic cannabinoids at micromolar concentrations [39, 40].

The CB1 and CB2 receptors negatively regulate adenylyl cyclase activity through pertussis toxin-sensitive GTP-binding protein. This property represents an important mechanism of lymphocyte regulation [41]. As cAMP signaling cascade activates immune cell function, cannabinoid receptor stimulation could antagonize the early events of immune cell activation. Endocannabinoids have also been shown to induce MAPK (Mitogen Activated Protein Kinase) pathway, which is a CB2 receptor-mediated response, playing a key role in immune homeostasis and control [42]. The importance of CB2 receptor activation in the immune-modulatory effects of endocannabinoids has been recognized and is supported by anti-inflammatory effects of CB2 receptor activation in many pathological conditions and disparate diseases. In the brain, endocannabinoids release is triggered by cellular depolarization or receptor stimulation, in a calcium-dependent way.

## Nervous System

The main brain Poly Unsaturated Fatty Acids (PUFA) are Arachidonic acid (20:4 $\omega$ 6) and Docosa-hexaenoic acid (22:6 $\omega$ 3), synthesized from the essential fatty acids linoleic and alpha-linolenic acids, respectively [43]. Depending on the cerebral necessities, PUFA are released from the glycerophospholipids by the action of various phospholipases (cytosolic phospholipase A2, plasmalogen selective phospholipase A2 and secretory phospholipase A2), undergoing subsequently to the classical COX and LOX metabolisms.

Sleep process is necessary for health and survival, and irregular rest periods lead to mental and physical deficits including disorders in concentration and motivation. In this context, PGD<sub>2</sub> exerts soporific effects and its production results increased under sleep deprivation conditions. Studies conducted on mice, rats and monkeys, revealed that intracerebroventricular infusions of PGD<sub>2</sub> promote NREM and REM sleep, counteracted by selenium Tetrachloride (selective inhibitor of PGD<sub>2</sub> synthase) [44].

It has been demonstrated that synaptic plasticity, and consequently, cognitive processes, involve the participation of PGs. Indeed, cultured mouse astrocytes treated with PGE<sub>2</sub> and PGD<sub>2</sub> produce increased amounts of NGF and BDNF [45]. Subsequently, experiments employing broad spectrum COXs inhibitors led to impairment of LTP and spatial learning capacity in rats, condition rescued by the addition of PGE<sub>2</sub> [46]. PGE<sub>2</sub> acts as inhibitor of GABA from release the GABAergic terminals innervating SON neurons by activating presynaptic EP receptors, presumably of the EP3 subclass [47].

In peripheral nerves, PGs sensitize C fibers to painful stimuli. This effect has been demonstrated in animal pain models in which, intravenous administration of anti-PGs neutralizing antibodies, greatly reduces or abolishes pain behavior. On the other hand, in spinal cord, activation of NMDA receptors induces COX-2 gene transcription and PGs

release [48]. Furthermore, PGE<sub>2</sub> counteracts the release of noradrenaline from sympathetic nerve terminals [49].

In nervous system the endocannabinoid system exerts multiple biological actions activating multiple signaling pathways, very often involving preferential activation of Gi (G inhibitor) proteins that block adenylyl cyclase (AC) reducing thus, cAMP accumulation. Moreover, endocannabinoids activate MAPK pathways in most tissues [50]. In nervous system, CB1R activation interferes with voltage-dependent calcium channels, as well as both activation and inhibition of voltage-dependent potassium channels [51]. The two most important endocannabinoids are anandamide (AEA) and 2-arachidonoyl glycerol (2-AG), acting their neurological effects functioning as neurotransmitters in central nervous system and periphery [52, 53]. In particular, AEA binds preferentially to CB1Rs presenting at the same time a lesser affinity extent to CB2Rs whereas 2-AG is a full agonist for both CB1R and CB2R [54].

Endocannabinoids are released as retrograde signalling messengers in GABAergic neurons including amygdalar and hippocampal neurons and substance P-expressing medium spiny neurons of the outflow nuclei of basal ganglia [55]. Since endocannabinoid signaling elements are highly organized and conserved both in GABAergic and glutamatergic synapses, it can be considered play a pivotal role in synaptic transmission. The action mechanisms consist in the activation of K<sup>+</sup> currents and the inhibition of Ca<sup>2+</sup> entry into cells, effects mediated by the CB1 receptor stimulation that substantially leads to local hyperpolarization and general inhibitory effects [56]. The study of the physiological roles played by the endocannabinoids opened up new strategies in the treatment of different disorders ranging from chronic inflammation to psychiatric.

## Skin

A dose dependent relationship has been reported to exist between UV-irradiation and eicosanoid metabolism in the skin [57]. These data prompted Lajos Kemény group to investigate possible changes in skin responses to 12(S)-HETE (HydroxyEicosaTetraEnoic acid). The latter, seems to be involved in cutaneous reparative processes, being not only a potent chemoattractant for leukocytes, but also for fibroblasts and keratinocytes. Effects of single and repeated UV-B irradiations on human epidermal cell line induces a large decrease in 12(S)-HETE binding in a dose-dependent way not affected by changes in receptor affinity [58]. On the contrary, UV-treatment of human keratinocytes up-regulates 15-LOX expression. This cellular response leads to a suppression of Insulin-like growth factor II-induced 12-LOX expression with a concomitant blockade of cell cycle progression. These findings could be useful for the treatment of epidermal disorders such as hyperplasia in psoriasis [59].

## Kidney

Eicosanoids are responsible for the renal hemodynamic changes occurring in disease states. In particular, prostaglandins modulate renal microcirculation and their production

responses to angiotensin II.  $\text{TXA}_2$ ,  $\text{PGE}_2$  and  $\text{PGI}_2$  (prostacyclin) are the primary COX metabolites that contribute to the increase of renal blood flow and glomerular filtration rate.

$\text{TXA}_2$  causes intrarenal vasoconstriction (ADH-like effect), compromising renal function. For this reason, normally kidney synthesizes only small amounts of  $\text{TXA}_2$ . Some animal models showed that hypertension is associated with increased  $\text{TXA}_2$  and decreased  $\text{PGE}_2$  and  $\text{PGI}_2$  synthesis [60].

Conditions of boosted renal blood pressure trigger the synthesis of PGs, leading to an increased sodium excretion, effect annulled only by COX-1 inhibitors. Indeed, intrarenal infusion of  $\text{PGE}_2$ , but not  $\text{PGI}_2$ , counteracts COX blockade, restoring the pressure-natriuretic response [61]. In particular,  $\text{PGE}_2$  synthesized by collecting duct epithelial and interstitial cells, dilates endothelin precontracted isolated outer medullary descending vasa recta vessels [62]. Thus,  $\text{PGE}_2$  seems to assume a key role in kidney's physiological function through the maintenance of water and electrolyte balance.

Experiments conducted on toad bladder focused on the effect of  $\text{PGE}_1$  on osmotic water flow and cyclic AMP content of the mucosal epithelial cells in the presence of antidiuretic hormone (ADH). Resulting data demonstrated that  $\text{PGE}_1$  is able to stimulate a tissue adenylate cyclase to sufficiently high levels that cyclic AMP spills over into the "water flow compartment" and thus stimulates water flow, also in presence of ADH signal [63].

Also another compound, the monohydroxy fatty acid derivative 16:3(8-OH) (metabolite of 12-HETE), is produced by renal epithelial cells. Experiments conducted on MDCK tubular epithelial cell line revealed that 16:3(8-OH) is released from both the apical and basolateral cellular surfaces, suggesting effects within the kidney parenchyma or renal microcirculation [64].

## Gastro-Intestinal Tract

PGs are highly concentrated in gastric mucosa and gastric juice and exert inhibition on acid secretion. Moreover, they stimulate mucus and bicarbonate secretion, modulate mucosal blood flow and provide dramatic protection against a wide variety of detrimental agents. The action mechanism underlying the inhibition of acid secretion is regulated by blockage of histamine-stimulated cAMP increase within the parietal cell. A mucosa prostaglandin-depleted is more susceptible to damage, but does not spontaneously ulcerate [65].

Experiments conducted on dogs demonstrated that exogenous PGs of E and I series intravenous or intra-arterially administration inhibits intestinal motility, whereas  $\text{PGF}_{2\alpha}$  has opposite effects [66]. Of particular note, it is the physiological action of the prostanoid  $\text{PGD}_2$  in the intestinal mucosa. It, produced by mucosal mast cells and human enterocytes, precisely exerts a protective action. This is confirmed by evocation of an inflammatory condition, the colitis, when its levels decrement [67].

## Liver

Eicosanoid compounds, mainly PGs and Leukotrienes, physiologically regulate also the hemodynamic and metabolic equilibrium in liver. In particular, experiments conducted on rat liver employing indomethacin revealed that PGs, through modulating sympathetic hepatic

nerves, induce glucose and lactate output with a concomitant reduction of portal flow and bile acid secretion [68, 69]. Moreover, experimental approaches based on administration of  $\text{PGD}_2$ ,  $\text{PGE}_2$ ,  $\text{PGF}_2$  and thromboxane analog U46619, revealed the ability of these prostanoids in inducing metabolic and hemodynamic effects comparable to those induced by hepatic nerve stimulation [70]. In particular, seems that  $\text{PGF}_2\alpha$  modulates directly hepatic metabolism without affecting hemodynamics.

Thus, since prostanoids are synthesized only in non-parenchymal cells, nervous control of metabolism appears to depend on complex intra-organ cell-cell interactions between the nerve, non-parenchymal and parenchymal cells.

The present results, together with the previously described effects of prostanoid synthesis inhibitors, suggest that prostanoids, probably prostaglandin F<sub>2</sub> alpha and D<sub>2</sub>, could be involved in the actions of sympathetic hepatic nerves on liver carbohydrate metabolism [71].

Moreover,  $\text{PGD}_2$  induces the glycogenolysis not only in the perfused liver but also in isolated parenchymal liver cells [72, 73].

Tromboxanes participate also to the elevation of portal pressure and glycogenolysis, as well as demonstrated by administration of CGS 13080 (thromboxane synthase inhibitor) or BM 13.177 (thromboxane receptor antagonist) [74].

Leukotrienes  $\text{LTC}_4$  and  $\text{LTD}_4$  (with a concentrations between 1 and 20 nM/liter) lead to a decreased perfusion flow or increased portal pressure [70, 75]. Furthermore, metabolic effects of  $\text{LTC}_4$  and  $\text{LTD}_4$  translate themselves in an increased glucose and lactate output, indicating hepatocellular glycogenolysis. On the other hand, both  $\text{LTE}_4$  and  $\text{LTB}_4$  are inactivated in liver, suggesting their foreignness in hemodynamic and metabolic processes [76]. In conclusion, considering that direct leukotriene actions on hepatocyte receptors have not been detected;  $\text{LTD}_4$  and  $\text{LTC}_4$  actions within the liver may be mediated by secondary signals released from non-parenchymal cells. Thus, the above reported findings show the involvement of eicosanoids also in the regulation of liver and, consequently, whole organism metabolic homeostasis.

## Pancreas

Eicosanoids regulate different processes of pancreas physiology ranging from embryonal period (differentiation of stem cells in endoderma) to adulthood (regulation of glucose secretion).

Studies conducted on zebrafish, revealed an important role of  $\text{PGE}_2$  in the inhibition of pancreatic differentiation, leading rather to the formation of hepatocytes [77]. PGs of the E-series are narrowly involved in the pathophysiology of pancreatic exocrine secretion, by switching off AC in acinar and islets cells. In fact,  $\text{PGE}_2$  treatment in presence of various secretagogues, results in a decreased secretion of cholecystokinin-octapeptide (markedly), bombesin, carbachol and vasoactive intestinal peptide [78]. First evidences about regulation of glucostatic function by eicosanoids dated from 1876, when Ebstein reported the capacity of sodium salicylate in decreasing urinary glucose levels of diabetic patients. Subsequently, this drug showed itself able to partially restore insulin secretion in hyperglycemic type II diabetic subjects [79]. Thus, these evidences prompted many investigators to study various nonsteroidal anti-inflammatory drugs for diabetes treatment. In particular, eicosanoids regulate both negatively and positively glucose-induced insulin secretion. For example, 12-

HPETE (HydroPeroxyEicosaTetraEnoic acid) has been reported to augment glucose-induced insulin secretion [80]. On the other hand, PGEs inhibit insulin secretion. These data have been demonstrated through both exogenous PGEs administration [81] and inhibition of PGEs synthesis in pancreatic cells [82]. Perfusion of rat pancreas with different leukotrienes demonstrated that LTB<sub>4</sub>, LTC<sub>4</sub> and, to a lesser extent, LTE<sub>4</sub> and LTD<sub>4</sub>, stimulate insulin release in a dose-related manner, in the concentration range from 10<sup>-11</sup> to 10<sup>-7</sup> M. LTC<sub>4</sub> stimulates glucagon release only when concentrated 10<sup>-7</sup> M [84]. Moreover, studies employing lipoxygenase inhibitors, reported that leukotrienes inhibit glucose-induced insulin release, but at the same time, promote insulin biosynthesis. Then, the above-mentioned findings confirm the role of lipoxygenase pathway as a positive modulator of pancreas secretive function [83, 84].

Finally, pharmacologically increased levels of EETs (Epoxy Eicosa Trienoic acids) improve glucose homeostasis in diabetic rats [85].

## Heart and Circulating Apparatus

The maintenance of physiological conditions in heart tissue are also influenced by eicosanoids. In particular EETs produced from arachidonic acid by cytochrome P450 epoxygenases and by  $\omega$ -hydrolase present several isomers with various functions through the beneficial effects on microcirculation and the inhibition of proteinaceous deposits. Pharmacological inhibition or genetic deletion of sEH (Soluble epoxide hydrolase) increases EETs concentration leading to therapeutic effects in animal models of cardiovascular disease [86].

In particular, EETs induce vasodilation and angiogenesis meanwhile decreasing inflammation and platelet aggregation to maintain vascular homeostasis. The increase in cardiomyocytes contractility and the augment of coronary blood flow are the two primary EETs actions in the heart. The latter seems to be negatively influenced by amylin, a 37-residue peptide hormone co-secreted with insulin from the pancreatic  $\beta$ -cells in the ratio of approximately 100:1.

In fact, hyperamylinemia represents a common condition responsible for the augmentation of cardiovascular risk disorders detectable in subjects with obesity and insulin resistance. It has been reported that EETs interact directly with circulating amylin aggregates increasing their solubility without altering  $\beta$ -cell secretory function [87].

The cardioprotective effects of EETs reflect on a reduced ischaemia–reperfusion damage with an improved rescue of myocardial function. These data derive from studies employing the over-expression of CYP2J2 (involved in EETs biosynthesis) that showed an increased left ventricular developed pressure, effect reverted by treatment with N-(methylsulfonyl)-2-(2-propynyloxy)-benzenehexanamide [88]. EETs exert vasodilator action on endothelium by modulating KCa3.1 channels (intermediate-conductance Ca<sup>2+</sup>/calmodulin-regulated K<sup>+</sup> channels). In particular, 14,15-EET and 20-HETE, revealed efficient KCa3.1 inhibitors on the contrary of 5,6 and 8,9-EET.

These differences in EETs activity have been supposed due to the hydrophobic carbon stretch from C1–10 of the carboxyl head of the molecule as structural requirement for channel inhibition [89].



## Reproductive Apparatus

The development of male germinal cells sees the up-regulation of L and H Prostaglandin D2 synthase (Pgds) activity, leading to an increase in  $\text{PGD}_2$ , responsible of p21 nuclear localization, cell cycle arrest and repression of pluripotency markers. The activation of DP2 receptor leads to the up-regulation of Nanos 2 gene, contributing to the physiological differentiation of male germ cells [90]. The deletion of Pgds in Sertoli cells is responsible for a lack of SOX9 expression with a consequent blockage in the developmental process of male gonads [91]. It has been demonstrated that COX-2 and  $\text{PGE}_2$  modulate the ovulation process, counteracting the effect of luteinizing hormone (LH) on progesterone release. On the other hand, investigation of leukotrienes role in bovine corpus luteum demonstrated that they are physiologically produced in this site and exert their effects modulating the estrous cycle. In particular, the use of specific LTB4 and LTC4 antagonists (respectively dapsone and azelastine) allowed to ascertain that LTB4 is luteotropic during the estrous cycle by supporting early pregnancy, whereas LTC4 is luteolytic, regarded as undesirable in early pregnancy. In particular, LTB4 supports corpus luteum function inducing  $\text{PGE}_2$  and progesterone secretion, whereas LTC4 exerts its luteolytic by stimulating  $\text{PGF}_{2\alpha}$  secretion. The action of LTs on uterine function was studied by measuring the level of PGs after stimulating uterine slices with LTs on days 8-10 of the cycle. Expression of 5-LOX and LTB4R (Leucotriene B4 Receptor) mRNA and protein were highest on Days 2-4 of the cycle, while CysLTR2 and LTC4 Synthase were highest on days 16-18, whereas the greatest LTC4 level was on Days 16-18 increasing the content of  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$  in endometrial slices at a dose of  $10^{-7}\text{M}$  [92, 93].

## Conclusion

Although eicosanoids have been widely studied in processes related to the regulation of inflammatory response, they represent cellular mediators involved in a plethora of processes. In fact, they assume important roles starting to the commitment of stem cells to the regulation of metabolic responses. Thus, deepening of eicosanoids physiological roles could provide the “reading key” for the elaboration of therapeutic procedures finalized to cure pathological conditions caused by disequilibrium in eicosanoids signaling and, consequently, effector activity.

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# **Eicosanoids and Pathophysiology of Inflammatory Diseases: Their Dual Role As *Enhancers* and *Inhibitors***

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## **Abstract**

Chronic inflammatory diseases, characterized by an incessant augment in their prevalence and incidence (particularly in old individuals), require urgent interventions in preventive measures. Accordingly, medical research is pursuing new ways in trying to face this imposing challenge, from identifying all mechanisms involved to detecting targets for developing personalized treatments.

Among these, the inhibition of inflammatory pathways, i.e., eicosanoid pathways, represents a very therapeutic goal. However, this approach is not simple. Eicosanoid pathways mediate patho/physiological effects, which are the consequence of a complex balance between the magnitude of specific eicosanoids released and actions mediated, influenced by tissue and organ specificity.

Thus, they might exhibit pro-inflammatory and anti-inflammatory actions depending on tissues, organs and state of diseases, or they might have essential physiological roles. Thus, it is imperative for developing therapeutic treatments related to suppression of eicosanoid pathways to detect their real effects taking into consideration their capacity to act as *enhancers* or *inhibitors* of a pathological inflammatory disease.

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## Introduction

The two pathways of arachidonic acid (AA)-derived eicosanoids represented by cyclooxygenases COX-1 and COX-2 (and possibly COX-3 in some species), producing the prostaglandins (PGs) and thromboxanes (TXs), and lipoxygenase pathways (5-LOX, 12-LOX, 15-LOX), producing the leukotrienes (LTs), hydroperoxy fatty acids and derivatives, are involved in both induction and progression of chronic inflammatory diseases, and their suppression. On the other hand, chronic persistent inflammation plays a significant role in patho-physiology of cancer, cardiovascular diseases, metabolic syndrome and Alzheimer disease. In these disorders, abundant infiltration of inflammatory cells and expression of various pro-inflammatory molecules, i.e., AA-derived eicosanoids, are found in affected tissues. Granulocytes, macrophages, neutrophils, platelets, mast cells and endothelial cells initially and subsequently lymphocytes orchestrate inflammation conditions, and also are involved in eicosanoid production during inflammation. LTs, known to be potent pro-inflammatory eicosanoids, have vasomotor properties and induce contraction of smooth muscle. During inflammation, these properties are associated with cardiovascular, renal, pulmonary and cutaneous manifestations. Conversely, PGE<sub>2</sub> induces vasodilation and smooth muscle relaxation (decreasing peripheral resistance and blood pressure). Thus, the effects of PGs appear opposed to those of LTs, although the cardiovascular system is considered to be more sensitive to PGs than to LTs. In general, during inflammation eicosanoids are and act as proinflammatory molecules (PGH<sub>2</sub>), chemoattractants (LTB<sub>4</sub>), platelet aggregating factors (TXA<sub>2</sub>), contractors of smooth muscle (Cys-LTs) and modifiers of vascular permeability (LTs). However, PGs might act as both proinflammatory and anti-inflammatory mediators depending on the context, as before described. Lipoxins (LXs) also appear to play an active part in controlling the resolution of inflammation by stimulating endogenous anti-inflammatory pathways. In addition, both PGs and LTs also contribute with different mechanisms in the transition of inflammation from acute to chronic response and its maintenance. This provokes tissue remodeling and onset and progression of a typical chronic inflammatory disease. These mechanisms include: (i) conversion of acute inflammation to long-lasting immune inflammation; (ii) activation of a positive feedback loop by repetitive stimuli; (iii) sustenance of inflammation by changing active cell populations in affected tissues; and finally (iv) tissue remodeling. Thus, the roles of eicosanoids in biology and pathology are diverse and complex. However, their understanding might be fundamental for developing new and more efficient therapeutic treatments for chronic inflammatory diseases.

On the other hand, chronic inflammatory diseases have a great impact on social health due to a large number of affected patients and a significant therapeutic cost. The new strategies, which might be adopted, might be based on blocking eicosanoid actions. Accordingly, initial and prevalent strategy has been to block selectively the enzymatic pathways leading to the production of inflammatory PGs or LTs (COX or LOX, respectively), or to suppress the production or action of a specific PG or LT, believed to be a key player in a specific disease. However, this approach has yielded mixed results, and high-profile drugs (i.e., some COX-2 inhibitors) have been withdrawn from the market because of safety concerns. This is leading to point and detect the possible reasons for the unsatisfactory results and experience with such inhibitors, in order to develop treatments having more efficacy than

those based on inhibition of a single eicosanoid mediator. Four potential causes are emerging as more plausible:

- a. involvement of particular eicosanoids, produced by alternative pathways of AA metabolism, in the patho-physiology of chronic inflammatory diseases
- b. production by the same pathway of eicosanoids having opposing effects on particular
- c. functions
- d. capacity of the same eicosanoids to mediate opposing effects in different organs and tissues and to have different roles, from pro- to anti-inflammatory, during the course of a specific chronic inflammatory disease.
- e. additional ability of some pro-inflammatory eicosanoids and AA-metabolizing enzymes to have crucial physiological functions

The four reasons underline the potentiality of eicosanoids to act as *enhancers* or *inhibitors* of a chronic inflammatory disease. Thus, it is imperative in developing therapeutic treatments based on eicosanoid pathways for a specific chronic disease to identify the real effects mediated by a particular eicosanoid in a specific tissues, organs, and also depending on clinical state of disease. This might consent to develop agonists or antagonists having a more efficient capacity in suppression and/or activation of particular eicosanoid pathways, and consequently to reduce/or retard the progression of pathology, and favour a better long-time prognosis. These causes are summarised in this chapter by describing some example because of the enormous number of studies on this topic.

### Involvement of Particular Eicosanoids, Produced by Alternative Pathways of AA Metabolism, in the Patho-Physiology of Chronic Inflammatory Diseases

Eicosanoid mediators produced by both COX and LOX pathways cotemporally participate in a inflammatory tissue response mediating different effects in several tissues. For example, the COX-derived TXA2 and PGD2, and the LOX produced cysteinyl leukotrienes (cys-LTs) and LTB4 evocate smooth muscle contraction and/or constriction of airways and blood vessels. They are, in fact, involved in the onset of asthma and cardiovascular diseases. However, the same eicosanoids, as well as PGE2, also induce intestinal inflammation and the onset of related inflammatory bowel diseases, as well as other inflammatory pathological conditions, such as sepsis. As consequence, in case of developing an inhibitor treatment, based on blocking only of one of these pathways, it hypothesizes that it might have no favorable therapeutic effects. Indeed, it suppose that it might determine only the switching of AA into another pathway, exacerbating the pathological condition. It should be more appropriate developing treatments able co-temporally to block the different eicosanoid pathways. For example, the treatment with Aspirin1, the most widely used non-steroidal anti-inflammatory drug (NSAID), summarizes this condition. By inhibiting COX, Aspirin1 can shift the AA metabolism to increased production of LTs, which might contribute to the pathology of asthma or gastrointestinal damage. In addition, it is well recognized that low doses of Aspirin1 are useful drugs. However, in case of non-inflammatory cardiovascular

conditions, the side-effects associated with anti-inflammatory doses have been a cause for concern for decades and have been a driving force behind the enormous effort invested in searching for safer alternatives NSAIDs. These limitations have led to use the treatment with selective COX-2 inhibitors. However, such safety concerns have been not fully resolved by this treatment, as suggested by some studies. In particular, they have underlined that cardiovascular side-effects are possibly evoked by reversing the protective constraint of PGI<sub>2</sub> on thrombogenesis, hypertension and atherogenesis *in vivo*. Moreover, this could increase the availability of AA to COX-1-derived TxA<sub>2</sub>, and to 5-LOX-derived LTs, which might further accelerate thrombogenesis and increase blood pressure. As a result of these and other safety concerns, some COX-2 inhibitors, despite having provided pain relief to many millions of patients, have in recent times been withdrawn from the market or had their usage severely curtailed.

### Production by the Same Pathway of Eicosanoids Having Opposing Effects on Particular Functions

Current evidence suggests that AA-derived eicosanoids are not only involved in the induction and maintenance of tissue and systemic inflammation, but also in mediating anti-inflammatory actions and resolution of inflammatory conditions. An example is given by the anti-thrombotic PGI<sub>2</sub>, arising from both COX-2 or COX-1 activity. However, COX-1 also produces the pro-thrombotic TXA<sub>2</sub>. PGD<sub>2</sub> and PGE<sub>2</sub> constitute another example. In case of asthma, PGE<sub>2</sub> acts as bronchodilator, and PGD<sub>2</sub> has the role of bronchoconstrictor. Other examples of known anti-inflammatory prostaglandins include the PGD<sub>2</sub>-derived 'cyclopentenone' prostaglandins (PGA<sub>2</sub>, PGJ<sub>2</sub> and 15-deoxy-delta (12,14) PGJ<sub>2</sub>) which contain reactive carbonyl residues within the cyclopentene structures that can form glutathione adducts under some circumstances. A similar condition exists for LOX pathway. Indeed, 5- LOX-derived cys-LTs, and LTB<sub>4</sub> are a typical instance. They are vaso- and bronchoconstrictors and pro-inflammatory, but their actions can be neutralized by the related 15-LOX-produced LXs, anti-inflammatory lipid mediators.

This evidence suggests as the inhibition of a eicosanoid production pathway might disturb the delicate balance between the pro-and anti-inflammatory actions of its metabolites.

### Capacity of the Same Eicosanoids to Mediate Opposing Effects in Different Organs and Tissues and to Have Different Roles, from Pro- to Anti-Inflammatory, during the Course of a Specific Chronic Inflammatory Disease

As mentioned above, eicosanoids, produced by the same or different enzymatic pathways, may have opposing actions. However, it has been also demonstrated that a same lipid mediator might show different actions in diverse tissues. This feature of tissue-specificity represents a very concern in developing its inhibitors. Among eicosanoids, PGE<sub>2</sub> is a remarkable example of prostanoids able to have advantageous or damaging actions depending on tissue, organ and timing of its expression and the phase of an inflammatory

disease. In particular, its inflammatory role has been evidenced in different pathologies, from arthritis to cancer, by inducing cell migration and proliferation, and tumor-associated angiogenesis and neovascularization. In the same time, inflammatory resolving roles have been also observed. This seems linked to the capacity of PGE2 to interact with four different receptors, E-prostanoid (EP) receptors, EP1 to EP4. In addition, all EPs are not expressed in the different tissues. Some tissues show all four EPs, while others express only some or not EP receptors. Interaction between PGE2 and each EP receptors also evokes different signaling and different actions.

For example, interaction between PGE2 with EP1 and EP4 determines pro-inflammatory actions. Thus, it supposes that antagonists of these receptors might be useful for inhibiting the unnecessary actions of COX-2. However, it has been also demonstrated that PGE2-based EP agonists by interacting with EP1 and EP3 receptors modulate histamine release from cultured mast cells and ameliorating asthma in mice. Furthermore, beneficial effects of PGE2 have been found in respiratory disorders, by using inhalators of a sPLA2 inhibitor, which suppress cys-LTs production and increased the production of the bronchodilating PGE2.

Recently, tissue-specific actions have been also observed for other prostanoids. PGD2 has been shown to reduce inflammatory bowel diseases in rats, and to exacerbate inflammation in the lungs. PGI2, a known vasodilator, mediates aortic contraction in acetylcholine-stimulated hypertensive rats, and might decrease blood pressure in the kidney medulla, but increase it in the cortex.

This evidence suggests that dual effects might be identified for other inflammatory eicosanoids by performing further and future studies.

As mentioned above, some pro-inflammatory eicosanoids may exhibit anti-inflammatory activity. Among these, PGE2 is a prominent instance, which switches at some point during the course of a disease ('class-switching'). This topic has been described in several recent studies. In particular, it has been found that PGE2 propagates the onset of inflammatory conditions in the first phase, but later facilitates the production of the pro-resolving eicosanoids and related compounds derived from AA, including lipoxins, resolvins, docosatrienes and neuroprotectins. The initial release of PGs and LTs triggers the inflammatory process, for example by inducing leukocyte extravasation (PGE2) and amplifying the recruitment of polymorphonuclear (PMN) cells through the synthesis of LTB4 produced by 5-LOX. During the course of the inflammatory process, the interaction between leukocytes and platelets initiates LX production, which serves as a stop signal for PMN cell recruitment.

This step is further augmented by the inhibitory action of PGE2 on 5-LOX (and the subsequent reduction of the pro-inflammatory LTB4) in PMN cells and the concomitant enhancement of 15-LOX expression which metabolizes AA into the proresolving and anti-inflammatory LXs. This class-switching of AA metabolism, from pro-inflammatory PGs and LTs to anti-inflammatory LXs, also features the production of resolvins of the E and D series as well as the protectins. In an interesting recent development, the generation by the aspirin-inhibited COX-2 isoform in vascular tissues of a 15-epi product of eicosapentaenoic acid (EPA; one of the principal fatty acids of marine animals) has been reported. This product is subsequently transformed (by leukocytes) to resolvin E1 (RvE1), a compound also found during the resolution phase of murine inflammation.

It seems that in addition to producing an anti-inflammatory effect through inhibition of COX, aspirin (and possibly other NSAIDs) might promote the generation of anti-

inflammatory eicosanoids. Although less well studied, similar changes from pro- to anti-inflammatory activity are also exhibited by PGD<sub>2</sub>, which is produced by both COX-1 and COX-2. In addition to facilitating the further metabolism of LTs to LXs, PGD<sub>2</sub> itself is metabolized to 15dPGJ<sub>2</sub>, which inhibits COX-2 and is a potent anti-inflammatory and protective lipid mediator.

### Additional Ability of Some Pro-Inflammatory Eicosanoids and AA-Metabolizing Enzymes to Have Crucial Physiological Functions

Research efforts to control lipid mediators as a strategy for the treatment of inflammatory processes have been outshined by the fact that eicosanoids also show an essential homeostatic role. The most obvious example is that of the upper gastrointestinal tract where PGE<sub>2</sub> (and PGI<sub>2</sub>) have strong 'cytoprotective effects'. It is the removal of these protective local hormones that is responsible for the principal unwanted effect of the NSAIDs, commonly used in the treatment of arthritis and associated diseases.

Many therapeutic strategies have been devised to mitigate these negative effects. Some take the form of more selective enzyme inhibitors (e.g., COX-2 or m-PGES inhibitors) to accomplish this, whereas other strategies are dependent upon some form of replacement therapy -in effect, giving the 'poison' and supplying a (partial) antidote at the same time. Thus, combination therapies of NSAIDs with cytoprotective eicosanoids such as misoprostil, or other substances are used. Other examples include human labour in which PGE<sub>2</sub> reduces the release of factors associated with cervical ripening in myometrial smooth muscle, delaying the onset of early labour and maintaining pregnancy towards term.

There is also evidence that PGE<sub>2</sub> (again via EP<sub>2</sub> and EP<sub>4</sub>) is neuroprotective and might even be involved in sleep and memory functions. Taken together, the above examples demonstrate that blanket eradication of an AA-metabolizing enzyme, and the subsequent production of pro-inflammatory eicosanoids, might also eliminate their positive/essential functions.

## Conclusion

The biological effects of eicosanoids are particularly important in inflammation conditions. The roles of eicosanoids in biology and pathology are diverse and complex. This diversity is due to their variety in composition, targets and GPCR signaling.

Further understanding of eicosanoid biology will be important for understanding the organ-specific effects of these unique compounds in health and disease.

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## Eicosanoids in Adipocyte Metabolism and Obesity

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### Abstract

The aim of this chapter is to shed light on the controversial issue of eicosanoids and their relationship with obesity and adipocyte metabolism. Eicosanoids are a big family of bioactive lipids. Prostaglandins, tromboxanes and leucotrienes are the main members, but endocannabinoids and isoprostanes are also to be considered. In each of these subgroups there are a lot of different compounds.

Eicosanoids play an important role as modulators of adipocyte metabolism. Each member of that big family has a specific and even controversial role. Frequently, the effect of a specific substance can vary in the different phases of adipocyte differentiation. They can promote the differentiation of adipocyte or they can act as adipogenesis inhibitors. Another important effect is the control of the energy balance through the regulation of the UCP 1 gene expression and the transformation of white adipose tissue

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(WAT) in brown adipose tissue (BAT). In this chapter, we reviewed the role of every eicosanoid's subgroup in adipocyte metabolism.

Further studies are needed to clarify the role of all eicosanoids members in different tissues and to find new pharmacological targets to treat obesity and metabolic syndrome (MetS).

## Introduction

Eicosanoids are a big family of modulators. They play important roles in different tissues. They regulate inflammatory response. One of the most useful and every day- use group of drugs, COX inhibitors exert their effect through the modulation of eicosanoids (prostaglandins) production. Eicosanoids influence the function of the smooth muscle of bowels and uterus. They can regulate blood pressure, bronchial constriction and platelet aggregation. Endocannabinoids have a role in regulating appetite and pain perception.

In the last few years, the scientific community has been trying to delineate the relationship between eicosanoids and obesity through the study of their effects on adipocyte differentiation. Before describing every step forward in this interesting world, it is important to clarify the production cascade of every subgroup of eicosanoids. In the scheme below (Figure 1) the synthesis of leukotrienes, prostacyclines, thromboxanes and prostaglandins from arachidonic acid is described. Isoprostanes are synthesized from radicalic non enzymatic reactions and endocannabinoids from the reaction between arachidonic acid and etanolamine.

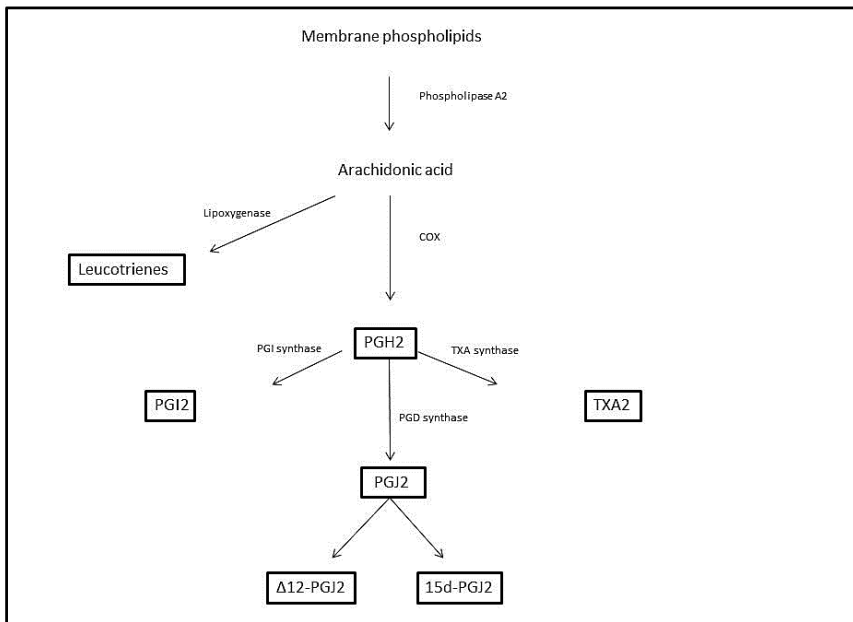


Figure 1. The contribution of eicosanoids in adipocyte metabolism is controversial. Every subgroup acts in a different way and can exert different actions in each phase of the differentiation. It is impossible to summarize the role of eicosanoids in adipocyte differentiation, without considering separately each member of the family. However, we can assume that eicosanoids play the role of modulators MODULATORS in adipocyte metabolism.

In biological research on adipose tissue, the use of 3T3-L1 cell is very frequent. This is a cell line with fibroblast-like morphology able to differentiate under appropriate conditions into adipocyte-like phenotype. Before starting consider the different function of each eicosanoid, it is important to focus the attention on the role of PPAR $\gamma$  in adipogenesis (adipocyte differentiation).

Peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) regulates adipocyte genes involved in adipogenesis and lipid metabolism. Also, they regulate fatty acid storage and glucose metabolism. PPAR $\gamma$  knock out mice are not able to synthesize adipose tissue when fed with a high fat diet. It seems that PPAR $\gamma$  can modulate the expression of adipose triglyceride lipase (ATGL) in adipocytes [1]. ATGL is induced during adipogenesis and remains expressed in mature cells. PPAR $\gamma$  acts as gene modulator when binded with its ligands.

Thiazolidinediones oxidate fatty acids, and some prostaglandins are PPAR $\gamma$  ligands. As we will describe, the relationship between eicosanoids and adipose tissue is strictly related to the interaction with PPAR $\gamma$ .

## **PGE2**

PGE2 suppresses adipogenesis through the suppression of PPAR $\gamma$  function [2]. Activated PPAR $\gamma$  promotes adipocyte differentiation by modulating the expression of genes involved in the differentiation of these cells [3]. PGE2 is synthesized in adipocytes by mPGES-1 (microsomal PGE2 producing enzyme) and a peak of concentration is detected after 3 hours from the start of adipogenesis [4]. PGE2 exerts its effect through its receptor EP4, probably acting as antagonist [5]. It is proved that the use of AE1-329 (an EP4 antagonist) reduces the expression of adipogenetic genes as PPAR $\gamma$  in 3T3-L1 cells. In conclusion, PGE(2), by acting on EP4 receptor, plays an important role in adipocyte differentiation, blocking one crucial step of the process [6]. Another interesting aspect in the study of eicosanoids is their ability of influencing leptin production. Leptin, also known as the “satiety hormone”, has an important anorexigenic effect and is synthesized in adipose tissue.

PGE2 seems able to stimulate leptin release in mice. Using a selective COX2 inhibitor, i.e., “NS-398”, Jhon N. Fain et al. found a reduction in leptin levels and an improved lypolysis associated to reduction PGE2 levels [7, 8] Anyway there are controversial data regarding the relationship between PGE2 and the satiety hormone and further studies are needed.

## **PGF2 $\alpha$**

Similarly to PGE2, PGF2 $\alpha$  is a suppressor of the adipocyte differentiation. It exerts its function through the FP receptor [9]. PGF2 $\alpha$  is able to block adipogenesis by acting on PPAR $\gamma$  activation, similarly to PGE2 [10]. The production peak has been detected three hours after the differentiation start. [11]. This molecule is an early phase suppressor of adipocyte differentiation. An interesting experiment, conducted by Volat FE et al., clarified the key function of PGF2 $\alpha$  in adipocyte metabolism. They focused the attention on an aldo-keto reductase (Akr1b7) which probably has the ability to synthesize PGF2 $\alpha$ . This enzyme is well expressed in adipose stromal vascular fraction but is absent in mature adipocytes.

In Akr1b7 knock out mice they found a lower level of PGF2 $\alpha$ . In those mice, there were an improvement of basal adiposity (hyperplasia and hypertrophy). The mice have an increased predisposition to diet induced obesity. An impressive result was achieved supplementing with PGF2 $\alpha$  the knock out mice. They found the normalization of white adipose tissue through the block of adipocyte differentiation [12]. The conclusion of different authors is that PGF2 $\alpha$  is a potent antiadipogenic factor in in vitro and animal models, but further studies are needed to clarify its role in humans.

## **PGD2**

Only few data have been published about PGD2, but there are many studies regarding PGD metabolites ( $\Delta$ 12-PGJ2, 15d-PGJ2) and the synthesizing enzyme (L-PGDS). Fujitani Y. et al. demonstrated that an overproduction of PGD2 in transgenic mice improves adipogenesis, insulin sensitivity and reduces triglyceride level. They also found a weight increase in those mice compared to another non transgenic group fed with the same diet. [13]. The role of PGD2 in adiponectin and leptin metabolism is controversial. James P. Hardwick and colleagues in their paper "Eicosanoids in metabolic syndrome" demonstrated that PGD2 can inhibit the production of both leptin and adiponectin in adipocyte [8]. However, some other authors support the idea that PGD2 should improve leptin and adiponectin synthesis [13]. 15d-PGJ2, a PGD2 metabolite, seems to promote at high concentrations, adipocyte differentiation in vitro but not in vivo where the levels are usually lower than those used in vitro. 15d-PGJ2 stimulates adipogenesis by acting as a PPAR $\gamma$  ligand [14].

Sarbani Ghoshal et al. notice in mice, in which the production of 15d-PGJ2 is blocked, an important weight loss. In their study, mice with a COX2 deficiency were used and there was a control group fed with the same diet. COX-2-deficient mice showed increased metabolic activity and the wild type group showed a greater level of 15d-PGJ2 in epididymal adipose tissue [3].  $\Delta$ 12-PGJ2 is another PGD2 metabolite and, similarly to 15d-PGJ2, it promotes adipogenesis. It is produced in adipocytes and activates the expression of adipogenic genes in 3T3-L1 cells [15]. Other authors have studied the role in adipocyte metabolism of Lipocalin-type prostaglandin D2 synthase, which produces PGD2 in adipocytes [16]. L-PGDS promotes the use of triglycerides in brown adipose tissue, as energy substrate. Mice lacking this enzyme use carbohydrate to provide fuel for thermogenesis and have an increased lipid content in brown adipose tissue. L-PDGS knock out presents a better glucose tolerance compared to the lean, probably because they have an increased glucose utilization in B.A.T. [17]. However, Ragolia and colleagues found a decreased insulin sensitivity and glucose tolerance in L-PGDS knock-out mice. Also they noticed an increased adipocyte size in this group of mice, fed with normal or high fat diet [18].

## **PGI 2 (Prostacyclin)**

Prostacyclin is well known as a potent vasodilator and inhibitor of platelet aggregation but only few studies focused their attention on its role in adipocyte metabolism. The PGI2 receptor is called IP and is a G-protein coupled receptor.

When binding PGI<sub>2</sub>, the receptor changes conformation and the cAMP level increases with consequent activation of protein kinase A (PKA). The final effect is exerted through the phosphorylation of different substrates. In adipocyte metabolism the result of PGI<sub>2</sub> binding with IP receptor is the promotion of the differentiation of adipose precursor cells [19, 20].

## Leukotrienes and Lipoxygenases

Leukotrienes are produced from arachidonic acid by different types of lipoxygenases. They were first discovered in leukocytes but they are synthesized also in other types of cells. Their most important function is to determine bronchoconstriction and increase vascular permeability. They are involved in asthma and allergic rhinitis. They also act as proinflammatory compounds. It is recently becoming clear a relationship between leukotrienes and metabolic syndrome. Martinez-Clemente et al. in 2010, studied 12 and 15 LOX and described their role in promoting insulin resistance and chronic inflammation in adipose tissue of high fat fed mice. They demonstrated an upregulation of 12/15 pathway in those mice. Supporting this hypothesis, they found a decrease cytokine production in adipose tissue and a reduced insulin resistance in 12/15-LOX-null mice. They described also a lower resistin production and an improved expression of GLUT 4, strictly related with insulin resistance [21]. Similar data have been published by Isabelle Mothe-Satney and her colleagues. They studied directly the role of leukotriene in obese and high fat diet fed mice. An overexpression of LTs has been detected during obesity. They observed that both human and mouse adipocytes can secrete large amounts of LTs.

The production increases during a high-fat diet (HFD), also the bigger the adipocyte, the greater the LTs concentration is. It is well demonstrated the macrophages and T cells chemotaxis effects of leukotrienes in vitro. Similar in vivo data are becoming clear. In 5-lipoxygenase null mice with lower LTs levels, Mothe-Satney et al. found reduced macrophages and T cells infiltration in adipose tissue, during a high fat diet. Those mice also present a higher insulin sensitivity. Similar results have been achieved using, in normal mice, a 5-Lo inhibitor *Zileuton* [22].

## Endocannabinoids

Humans and animals are able to synthesize cannabinoid compounds that are very similar to exogenous cannabinoids, like  $\Delta$ -9-tetrahydrocannabinol (THC) presents in *cannabis sativa*. Endocannabinoids are anandamide (N-arachidonylethanolamide, AEA) and 2-arachidonoylglycerol (2-AG), both produced from arachidonic acid. Their role in controlling appetite, pain-sensation, mood and memory is well known. Novel studies are focusing on endocannabinoid effects in adipogenesis and energy expenditure. Anandamide can stimulate adipogenesis via CB1 receptor and PPAR $\gamma$  [23]. CB1 is a G protein coupled receptor. When the ligand binds its receptor, the adenylate cyclase is blocked and cAMP concentration decreases. Stimulation of CB1 receptor in animals by anandamide provokes an increase in food intake and body weight gain. This is a central effect of endocannabinoids. An interesting fact is the presence of CB1 receptors also in peripheral tissues like adipose tissue and liver.

Preclinical studies, conducted by blocking CB1 receptor, had shown an impressive weight reduction that goes beyond the simple food intake reduction. Endocannabinoids can modulate body weight by acting not only in appetite system but also controlling adipogenesis and energy expenditure [24].

Wagner et al. published in 2011 interesting results by studying CB1 receptor knock out mice. They found a reduced differentiation rate in visceral adipocytes whereas, in subcutaneous ones, differentiation was promoted and apoptosis rate was decreased (25). Considering IL-6 and TNF $\alpha$ , they also noticed an augmented inflammatory level in visceral fat and a reduction in subcutaneous. Subcutaneous CB 1- receptor knock out cells express higher Uncoupling protein-1 (UCP 1) levels compared with control cells and are more sensitive toward a conversion into brown fat phenotype. Wagner and colleagues also found an increased oxygen consumption in these subcutaneous CB-1 receptor knock-out cells.

They concluded that the block of CB 1 receptor could lead to a reduced cardiometabolic risk in mice through a double effect: the enhancement of thermogenesis in subcutaneous adipocytes and the reduction of visceral fat [25].

Rimonabant (withdrawn from the market for its side effects) is a selective CB 1 receptor antagonist and a potent anti obesity drug. It probably exerts its effects not only by mediating an appetite reduction but also acting in peripheral tissue as described above.

In conclusion, endocannabinoids have an important role in metabolic disorders like obesity and metabolic syndrome. They act centrally, in SNC, by modulating the appetite-satiety system but they also play an important role in regulating energy homeostasis through the presence of CB 1 receptors in peripheral tissues.

## **Tromboxanes**

Tromboxane A<sub>2</sub> (TXA<sub>2</sub>) is produced in activated platelet, from prostaglandin H<sub>2</sub> (Figure 1) through the action of Tromboxane-A synthase. Tromboxane B<sub>2</sub> is a stable degradation product of TXA<sub>2</sub>. TXA<sub>2</sub> is a strong vasoconstrictor, has prothrombotic properties and plays an important role in platelet aggregation and activation. No data were found about the relationship between tromboxanes and adipocyte metabolism.

## **Conclusion**

In conclusion, a new important role of eicosanoid is emerging. Every eicosanoid's subgroup can act differently and can exert its effect in different phases of adipogenesis.

A relationship between eicosanoids and energy expenditure regulation is also described. They are able to regulate the transdifferentiation of white adipose tissue in brown adipose tissue. Ultimately, we can assume that eicosanoids play a key role as adipocyte metabolism modulators. In the scheme below (Figure 2), we resumed the influence of each eicosanoid's subgroup in the different phases of adipogenesis and their effects in energy expenditure and insulin resistance.

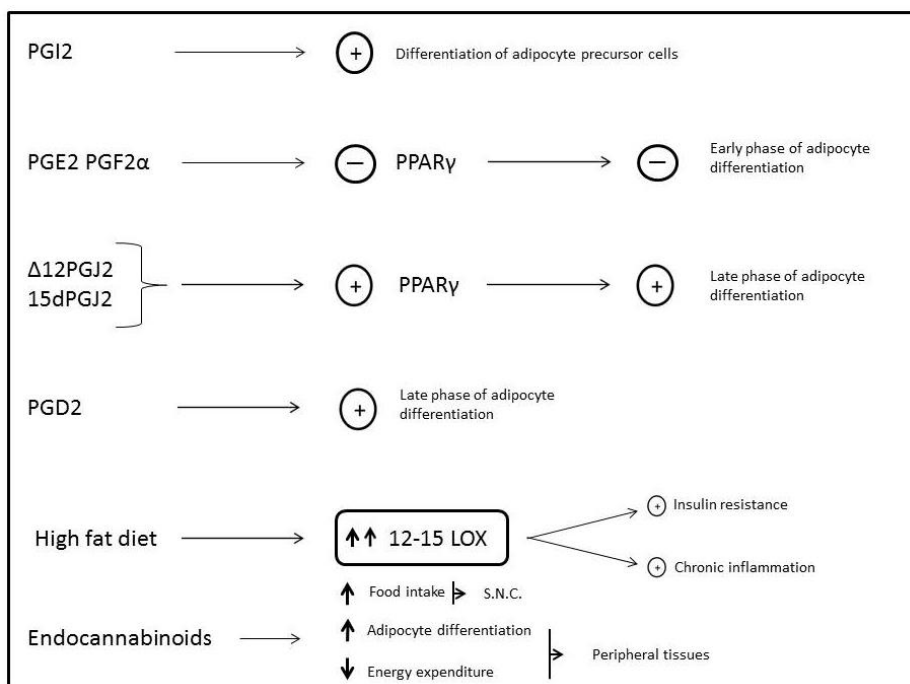


Figure 2. The influence of each eicosanoid's subgroup in the different phases of adipogenesis and their effects in energy expenditure and insulin resistance.

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# **Eicosanids in Atherosclerosis, Coronaropathies and Complications**

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## **Abstract**

Cardiovascular diseases represent the leading cause of morbidity and mortality in the western world and are mainly caused by atherosclerosis and its complications (such as myocardial infarction, stroke and peripheral vascular events). Atherosclerosis is a systemic disease with an immune-inflammatory pathogenesis. In this context, the intricate eicosanoids pathway is strictly involved in almost all the steps that lead from endothelial dysfunction to atherothrombosis and plaque instability.

In this chapter, the cardiovascular effects of the principal eicosanoids family, prostanoids, leukotrienes, and cytochrome P450-derived eicosanoids will be discussed according to the latest acquisitions available in the literature. Current therapeutics perspectives of eicosanoids pathway regulation will also be analyzed.

## **Introduction**

Cardiovascular diseases (CVDs) represent the leading cause of morbidity and mortality in the western world and recognize their principal pathophysiological basis in the atherosclerotic process.

Atherosclerosis is a systemic disease, involving the intima and media tunicae of large and medium caliber arteries with an immune and inflammatory pathogenesis. It is responsible, when complicated by plaque instability, for myocardial, cerebral and peripheral vascular

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events. Eicosanoids are oxidative metabolites of arachidonic acid (AA), originated through three primary pathways via cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450; they are prostanoids, leukotrienes and epoxyeicosanoids. Eicosanoids are involved in almost all the steps that lead to endothelial dysfunction and atherothrombosis. In fact eicosanoids initiate and propagate diverse signaling cascades, primarily through their interaction with cellular receptors and ion channels.

The resulting intricate pathway influences the immune and inflammatory system by modulating cytokine signaling, cell differentiation, survival, migration, antigen presentation, and cell death. Thus, eicosanoids influence the balance between vasoconstriction and vasodilation, modulate platelet aggregation, and govern inflammation by inducing chemotaxis, enzyme release, superoxide generation, matrix deposition and fibrosis [1].

In this chapter, the pathophysiological involvement and the clinical impact on CVDs of the principal eicosanoids family, prostanoids, leukotrienes, and cytochrome P450-derived eicosanoids will be discussed according to the latest acquisitions available in the literature. Therapeutics perspectives of eicosanoids pathway regulation will be also analyzed.

## Role of Prostanoids

According to a cardiovascular (CV) point of view, the main effects of prostaglandins (PG) and their oxidized derivatives (Isoprostanes) include effects on vasculature, platelets and leukocytes. In this context, the most important products of COX activity are thromboxane A<sub>2</sub> (TxA<sub>2</sub>) and prostacyclin (PGI<sub>2</sub>). TxA<sub>2</sub> has a relevant role in platelet activation and vasoconstriction [2], on the contrary PGI<sub>2</sub> is a potent vasodilator and inhibits platelet aggregation, leukocyte adhesion and vascular smooth muscle cells (SMCs) proliferation [3, 4]. Although a recent interest in the modulation of platelet reactivity through prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and its receptors (EP), research in the field of CVDs has principally focused on PGI<sub>2</sub>/TxA<sub>2</sub> balance, due to their preponderant involvement in CV physiology [5] and because their biosynthesis is altered in patients with atherosclerosis [6]. Below are listed in details the specific roles of each prostanoids, while a schematic view is provided in table 1. TxA<sub>2</sub>, mainly produced in platelet by COX-1 or synthesized by monocytes and vascular SMCs [7], causes an increase in peripherals resistances by promoting SMCs depolarization and intracellular calcium release [8]. Even platelet activation and aggregation is induced by stimulation of intracellular calcium release, through a self-perpetuating positive feedback loop, via G<sub>q</sub> protein [8]. Anyway, the atherogenic influence of TxA<sub>2</sub> is not limited to the vasoconstrictive and pro-aggregating mechanism. In fact, it exerts also proliferative and migratory effects on SMCs, enhanced by platelet-released serotonin at sub-threshold levels [9], accounting for an important role of platelet TxA<sub>2</sub> release in restenosis. Further supporting this evidence, mesenchymal stem cells, derived from human adipose tissue, become migratory and proliferative in response to thromboxane receptor (TP) agonism (with U46619) and show markers of differentiation into SMCs, through ERK and p38 MAPK [10]. A similar pro-migratory effect of TP activity has been demonstrated on endothelial cells, where either COX-2 inhibition or TxA<sub>2</sub> antagonism, attenuated fibroblast growth factor-induced corneal angiogenesis, while TxA<sub>2</sub> stimulation amplifies the angiogenic response [11].

Stimulation of TP receptors on endothelial cells increases the expression of the intercellular adhesion molecule 1 [12], known to promote monocyte adhesion and to be associated with atherosclerosis progression [13].

Moreover, it is relevant to report that a significant increase in TP receptor expression has been reported in both atherosclerotic aorta and coronary arteries from patients with Coronary Artery Disease (CAD) [14]. Finally, of many downstream effects, TP activation promotes CD40/CD40L stimulation [15] independently of aspirin-mediated COX inhibition [16] suggesting the overlying integration of multiple activating pathways. On the contrary, PGI<sub>2</sub> is synthesized primarily by endothelial cells, via COX-2, possibly induced by physiological rates of shear stress [17], and plays an important inhibitory role in local control of vascular tone and platelet aggregation [18], leukocyte adhesion to endothelium and vascular SMCs proliferation in plaque [19], in a tight and complex interplay with TxA<sub>2</sub> [20, 21]. PGI<sub>2</sub> receptor (IP) in fact, promotes vasodilation and platelets deactivation via G<sub>s</sub> stimulation of adenylyl cyclase, which results in the synthesis of cyclic adenosine monophosphate (cAMP) that blocks calcium influx, and determines membrane hyperpolarization. In a clinical study, a pathological IP receptor variant (R212C), cAMP stimulation deficient, was closely linked to disease severity and adverse CV events in patients with CVDs, suggesting that altered IP receptor functionality may be implicated in hypertension, myocardial infarction (MI) and stroke [22, 23]. Interestingly, the cardiovascular protective role of estrogen in premenopausal females, may arise, at least in part, from the stimulation of prostacyclin synthesis [24], through a well-defined estrogen response element in the promoter of the human IP receptor [25]. In addition to the roles of PGI<sub>2</sub> and TxA<sub>2</sub>, even PGE<sub>2</sub> appears to mediate, in the CV setting, an impressive range of biological processes. However its role is multifaceted largely due to the presence of four cell surface receptors (EP<sub>1-4</sub>) releasing different secondary messengers, often leading to opposing downstream effects [26]. Regarding their signaling cascade, EP<sub>1</sub> is associated with G<sub>q</sub> (the same effector as TP) promoting intracellular calcium release while EP<sub>3</sub> decreases cellular cAMP formation via G<sub>i</sub>. In contrast, EP<sub>2</sub> and EP<sub>4</sub> increase cAMP production through G<sub>s</sub> [1]. Anyway, according to the available literature, the involvement of PGE<sub>2</sub> in the regulation of vascular tone is limited to the pulmonary vessels, causing both constriction of veins (EP<sub>1</sub>) and arteries (EP<sub>3</sub>) [27-29], and veins relaxation (EP<sub>4</sub>) [30]. The effects of PGE<sub>2</sub> on platelets balance between the pro-aggregatory G<sub>q</sub> and calcium signal (EP<sub>1</sub> mediated) and the anti-aggregatory G<sub>s</sub>-cAMP signal (via EP<sub>2</sub>-EP<sub>4</sub>), attenuated by an inhibitory influence on cAMP through G<sub>i</sub> via EP<sub>3</sub> [31-33]. EP<sub>3</sub> and G<sub>i</sub> derived pathway, in fact, doesn't affect aggregation directly, but produces synergistic effects with TxA<sub>2</sub>, making platelets more sensitive to calcium stimulation by reducing cAMP. Regarding the pathophysiological influence of each EP receptor in CVDs, only limited information is available so far on EP<sub>1</sub>, that might play a role in atherosclerosis [34] and be relevant to the pulmonary circulation [27]. Regarding EP<sub>2</sub>, some studies demonstrate that it inhibits platelet aggregation [32, 35, 36]; pharmacological stimulation of EP<sub>2</sub> with the selective agonist ONO-AE1-259 reduces brain damage after ischemic stroke [37], but it has been observed also that shear stress, in endothelial cells from human carotid arteries, induces COX-2 and EP<sub>2</sub> receptor expression, thus triggering the early activation of the inflammatory circuit of NF-κB and CCL-2 [38]. Activation of EP<sub>3</sub> receptor, by low doses of PGE<sub>2</sub> or by selective agonist (sulprostone) has been shown to potentiate platelet aggregation [39], effect that is reverted by specific EP<sub>3</sub> receptor antagonists [35, 40, 41]. More complicated is the involvement of EP<sub>4</sub> receptor in atherothrombosis. Together with the evidence of its implication in the PGE<sub>2</sub>-

mediated anti-inflammatory [42] and anti-aggregatory effect [32, 35, 36], over expression of EP<sub>4</sub> in atherosclerotic vessels, correlated with plaque destabilization and increase in inflammation. Further confirming this evidence, silencing EP<sub>4</sub> receptor inhibited matrix metalloproteinase (MMP-9) expression [43] and reduced aortic atherosclerosis with increased apoptotic cells in the lesions [43, 44]. This discrepancy may be, at least partially, explained assuming distinct roles for PGE<sub>2</sub> in different phases of atherogenesis, such as a protective influence in the early stages and a proinflammatory action in later stages [45].

Isoprostanes (IsoPs), constitute a complex family of PG isomers, first described as products of non-COX oxidative modification of fatty acid precursors, originating from free-radical attack of cell membrane phospholipids [46, 47], or circulating low-density lipoproteins (LDL) [48]. There are three main classes of IsoPs, the F<sub>2</sub>-, E- and D-IsoPs, the latter being isomers to PGE<sub>2</sub> and PGD<sub>2</sub>, respectively [46, 49]. F<sub>2</sub>-IsoPs are considered useful biomarkers of lipid peroxidation and thus of oxidative stress, now considered implicated in the etiology of CV and other human disease. However, they are not only biomarkers, but they also play a direct influence on blood vessels, even if it is still not clear their target receptor [50]. In fact, while some evidence reported the presence of a distinct receptor for IsoPs in aortic SMC and in endothelial cells [51, 52] recent studies suggest that they are effective TP receptor activators [53], thus simulating TxA<sub>2</sub> effects, and probably explaining some cases of observed aspirin resistance [54]. Indeed, in a mice model, TP receptor blockade produced an additional and more potent anti-inflammatory and anti-atherogenic effect compared with TxA<sub>2</sub> synthesis inhibition only [55], and ameliorated endothelial dysfunction in patients with CAD already treated with aspirin [56]. In addition, recent evidence suggests that isoprostanes can also contribute to angiotensin II induced vasoconstriction [57].

Interestingly, while IsoPs are synthesized from oxidized LDL, high-density lipoprotein (HDL) are the main plasmatic carrier of circulating IsoPs, reducing free IsoPs effects, both through sequestration and inactivation [58].

## Role of Leukotrienes

The literature on leukotrienes (LTs) and the biomedical research in this field have been traditionally focused on asthma and other allergic disorders. However, recently, an increasing body of evidence suggests a key role also in CV homeostasis for LTs, generated by 5-lipoxygenase (5-LO). In particular, LTB<sub>4</sub>, seems to be involved not only in the initiation and progression of atherosclerosis, but also in the extracellular matrix remodeling.

In fact, since LTB<sub>4</sub> production has been associated with increased levels and/or activity of matrix metalloproteinase (MMP) proteins in atherosclerotic lesions [59-61], then LTB<sub>4</sub>-stimulated MMP could potentially link inflammation to plaque rupture or aneurysmatic remodeling of vascular wall. A mouse model for abdominal aortic aneurysm (AAA) formation revealed that B leukotriene receptor 1 (BLT<sub>1</sub>R) deficiency results in a lower incidence of AAA with a reduced tissue inflammation [62]. Similarly, BLT<sub>1</sub>R antagonism improves atherosclerotic burden in mice [63] through reduced monocyte recruitment and foam cells formation, thus supporting the key role of LTB<sub>4</sub> as an *inflammatory recruiter* in atherosclerosis, confirmed in other studies [64-67]. Furthermore, genetic studies in healthy subjects, showed that a promoter variant of 5-LO is associated with an increase in carotid

intima-media thickness [68]. Moreover, 5-LO activating protein (FLAP) gene variants, associated with raised LTB<sub>4</sub> production, determine inflammation in the arterial wall and plaque instability, conferring almost two fold increased risk for either MI and stroke [69, 70].

**Table 1. Prostanoids and their cardiovascular activities**

PROSTANOID	RECEPTOR	CARDIOVASCULAR ACTIVITIES
TxA <sub>2</sub>	TP	- vasoconstriction [8] - platelet aggregation [8] - increased migration and proliferation of VSMCs [9] and ECs [10] - increased leucocytes adhesion to endothelium [12, 13]
PGI <sub>2</sub>	IP	- vasodilation [18] - platelet inhibition [18] - reduced proliferation of VSMCs [19] - reduced leucocytes adhesion to endothelium [19]
PGE <sub>2</sub>	EP <sub>1</sub>	- contraction of pulmonary veins [27-29] - platelet aggregation [31] - atherogenesis [34]
	EP <sub>2</sub>	-platelet inhibition [32, 35, 36]
	EP <sub>3</sub>	- contraction of pulmonary arteries [28, 29] - platelet sensitization [31, 33, 39]
	EP <sub>4</sub>	- relaxation of pulmonary veins [30] - platelet inhibition [32, 35, 36] - plaque destabilization and increased inflammation [43, 44, 45]
IsoPs	TP? [52, 53]	- vasoconstriction [57] - endothelial dysfunction in CAD patients [56] - inflammation and atherogenesis [55]

VSMCs, vascular smooth muscle cells; ECs, endothelial cells.

However, the functional impact of the latter polymorphism is debated and not all subsequent studies confirmed the association between FLAP gene polymorphism and the risk of MI [71-73]. In addition, very recent evidence in human studies, found that LTB<sub>4</sub> levels within the advanced human carotid atherosclerotic plaque were not associated with plaque vulnerability or adverse clinical outcome [74]. Nevertheless, specific targeting of LTB<sub>4</sub> pathway, such as knockout or antagonism of the BLT<sub>1</sub>R seems to be strongly associated with positive effects on experimental atherosclerosis aneurysm formation, restenosis and plaque size [63, 64, 75-77]. Finally LTB<sub>4</sub> also activates BLT<sub>1</sub>R expressed on the endothelium and vascular SMCs, inducing migration and proliferation associated with intimal hyperplasia [78] and thickening of atherosclerotic vascular wall [77].

Addition of cysteine-based glutathione to LTB<sub>4</sub>, produces leukotriene LTC<sub>4</sub>, that can be further metabolized to cysteine-containing LTD<sub>4</sub> and LTE<sub>4</sub>, collectively referred as Cysteinyl-Leutrienes (Cys-LTs). These are synthesized by perivascular mast cells, platelet and even by endothelial cells and vascular SMC from neutrophil-derived LTA<sub>4</sub>, via trans-cellular metabolism [45]. They are responsible for several activities of CV relevance, such as

regulating blood pressure, enhancing vascular permeability, reducing coronary blood flow and reducing cardiac inotropism and flow without affecting the heart rate [79-83], through the interaction with specific receptors, CysLT1R and CysLT2R.

CysLT1R mediates a powerful SMC contractile response [84], via promiscuous interactions with Gq and Gi [85]. Moreover, the CysLT1R antagonist montelukast, demonstrated to reduce vascular ROS production, significantly improving endothelial function and ameliorating atherosclerotic plaque generation in a mouse model in vivo [86]. CysLT2R increases endothelial permeability and aggravates ischemia-reperfusion (I/R) injury from MI [87], acting exclusively via Gq calcium release in endothelial cells [88]. Furthermore, CysLT2R overexpression induced intensification of inflammatory gene expression, leading to faster left ventricular remodeling, induction of apoptosis in the peri-infarct zone, and impairment in the cardiac performance [87]. LTE4 instead, demonstrated to be involved in platelet aggregation, directly interacting with P2Y12 receptor, which is also the target of thienopyridines, like clopidogrel [89].

Accordingly, increased urinary excretion of LTE4 have been described after episodes of acute MI, being considered as a predisposing factor [90, 91]. Finally, Cys-LTs can be produced directly by coronary arteries [92] and levels of Cys-LTs have been found raised in CAD patients, either before and after coronary artery bypass surgery [93], as well as after episodes of unstable angina [94]. In accordance with these evidence, neutrophil exposure to FLAP inhibitors such as MK 886 [95] or BAY-X1005 [96], by preventing LTC<sub>4</sub>-LTD<sub>4</sub> production and coronary spasm in isolated rabbit heart preparations, leads to a significant cardioprotection and reduced mortality. In table 2 are summarized the CV effects of each LTs, according to interaction with the specific receptor.

## Role of Cytochrome P450 Derived Eicosanoids

Epoxyeicosatrienoic acids (EETs), produced by *cytochrome P450 epoxigenases* (mainly CYP2C), utilize alternative mechanism to influence vascular tone, and provide a major anti-inflammatory action, that has been recently reviewed [97, 98]. EETs promotes vasodilation through hyperpolarization of vascular SMC, via activation of large conductance calcium-activated potassium channels [99], competitively antagonize the TR receptor [100].

**Table 2. Leukotrienes and their cardiovascular activities**

LEUCOTRIENE	RECEPTOR	CARDIOVASCULAR ACTIVITIES
LTB <sub>4</sub>	BLT <sub>1</sub> R	- development and progression of atherosclerosis. [63, 64, 75-77] - increased migration and proliferation of VSMCs. and ECs [77, 78] - plaque instability? [60, 61, 74]
Cys-LTs (LTC <sub>4</sub> , LTD <sub>4</sub> , LTE <sub>4</sub> )	CysLT1R	- vasoconstriction [84] - atherogenesis [86]
	CysLT2R	- increased endothelial permeability [87] - aggravated I/R injury from MI [87] - left ventricular remodeling [87]
	P2Y12*	- platelet aggregation [89]

\*only for PGE<sub>2</sub>.

VSMCs, vascular smooth muscle cells; ECs, endothelial cells.



Their anti-atherogenic benefits range also from reducing platelet activation and adhesion [101] to reducing endothelial chemiotaxis, preventing leukocyte adhesion to the vascular wall [102]. Angiotensin II reduces Cyp2C synthesis of EETs [103] and promotes its catabolism up-regulating soluble *epoxide hydrolase* (sEH) to lower activity dihydroyeicosanoic acids (DHETs) [104]. A recent study, reported that plasma EET/DHET ratios were significantly higher in patients with established CAD compared to healthy individuals, suggesting a compensatory suppression of sEH metabolic function and higher EETs levels in patients with cardiovascular disease [105].

Another recent study reported that lower EET/DHET ratios, that are marker of enhanced sEH function, were associated with higher plasma monocyte chemo-attractant protein (MCP) and cellular adhesion molecules (CAM) levels, in humans with established CAD, meaning that in this subset of patients, despite a potential overall compensatory increase in EETs levels, those with the highest sEH metabolic function may be predisposed to more advanced vascular inflammation [106]. It is also relevant to report that reactive oxygen species (ROS) or H<sub>2</sub>O<sub>2</sub> production, under oxidative stress conditions, directly inhibits Cyp2C9 and Cyp2J2, impairing EETs synthesis [107], and a similar effect is produced also by nitric oxide (NO) [108].

However, while *cytochrome P450 epoxigenases* synthesizes EETs acids in endothelium, thus promoting vasodilation, *cytochrome P450 ω-hydroxylase* produces hydroxyeicosatrienoic acids (HETEs) promoting vasoconstriction [109]. In particular, 20-HETE plays an important role in the regulation of vascular tone in the brain, kidney, heart and splanchnic circulation [110, 111] by activation of L-type calcium channels and inhibition of calcium-sensitive potassium channels, thus promoting depolarization and constriction of vascular SMC [112, 113].

Anyway, their action is not confined to the physiological modulation of blood supply to those organs, but it also extends to the development of ischemia/reperfusion (I/R) injury. In fact, it has been reported that the content of 20-HETE was enhanced in coronary vessels after I/R injury in dog and rabbit hearts, in association with increased activity of CYP ω-hydroxylase enzymes [114, 115], while administration of inhibitors of CYP ω-hydroxylase enzymes ameliorates cardiac dysfunction, induced by I/R, thus reducing the infarct size [116, 117].

Recent data indicate that the effect of 20-HETE on exacerbation of myocardial I/R injury is attributable to stimulated, NADPH oxidase-derived, generation of ROS, leading to contractile-related protein oxidation and damage [118]. For a synoptic view, see table 3.

**Table 3. Cytochrome P450 derived eicosanoids and their cardiovascular activities**

CYTOCHROME P450 DERIVED EICOSANOID	CARDIOVASCULAR ACTIVITIES
EETs	- vasodilation [99] - platelet inhibition [101] - reduced leucocytes adhesion [102]
DHET <sub>s</sub> (less active metabolite of EETs)	- same activities of EETs, lower intensity [104]
HETEs	- vasoconstriction [111] - aggravate I/R injury from MI [118]

## Therapeutic Implications

Since long time, the intricate eicosanoid network has been a strong and fascinating research area in medical pharmacology. Given the wide physiological and pathological impact of the intricate eicosanoids pathway in several organs and systems, it is easy to understand that molecules able to interfere with the enzymes or receptors involved in this pathway, would have a great therapeutic effect. Just to make an example, acetyl-salicylic acid (ASA) is a cornerstone in the treatment of atherothrombosis, having demonstrated to significantly reduce vascular mortality, MI and stroke either in the acute setting or in primary and in secondary prevention [119].

In fact, with an optimal risk/benefit ratio, achieved at the dose of 75-100mg, it selectively inhibits COX-1, and irreversibly block the formation of thromboxane  $A_2$  in platelets, thus preventing aggregation and thrombosis [120].

Moreover, ASA mediate NO formation, which have been shown, in mice to have anti-inflammatory effects by inhibiting leukocyte-endothelium interactions [121]. However, higher doses of ASA or other non-steroidal anti-inflammatory drugs (NSAIDs) produce a “non-specific” inhibition of eicosanoids synthesis, disrupting their “physiological balance”, and causing, in addition to the well-known gastrointestinal (GI) side effects [122], even renal failure, fluid retention, and an increase in blood pressure in the elderly [123].

Hence, with the intention to find more specific, pain-killer drugs, COX-2 selective inhibitors (COXIBs) were formulated. In 1999, Hawkey proudly demonstrated that COXIBs were associated with lesser rates of GI adverse effects and peptic ulcers [124].

Notwithstanding, even COX-2 selective inhibition showed adverse effects, creating an unbalance between prostaglandins (specifically  $PGI_2$ ) and  $TxA_2$  levels, thus decreasing the protective anti-coagulative effect of  $PGI_2$  and increasing the risk of thrombosis [125].

Despite is undoubted the CV significance of COX products and NSAIDs inhibition, only recently attention has been paid to the use of anti-leukotriene drugs (AD) in this pathological conditions, with heterogeneous and controversial results. Anyway, both synthase inhibitors and BLT and CysLTs receptor antagonists, have been tested in animal models of experimental atherosclerosis, MI, cerebral ischemia and other vascular injury, obtaining promising results. In fact, significant reduction of inflammation, intima-media thickness, plaque size and infarct size has been observed with inhibition of LTs pathway by either selective 5-LO [126, 127] and FLAP [67, 96, 128] inhibitors or by  $BLT_1$  [61, 63, 75, 76] and CysLT<sub>1</sub>R antagonist [129-131]. Moreover, in patients with critical limb ischemia unsuitable for revascularization, prostanoids showed beneficial effects, although evidence is not unique and thus the class of recommendation in guidelines is IIb [132-134].

Finally, eicosanoid synthesis modulation may, at least partially, explain some of the beneficial CV effects of n-3 poly-unsaturated fatty acids (n-3PUFAs) [135]. N-3 PUFAs supplementation, in fact, improves flow-mediated vasodilation [136-140] and lowers circulating markers of endothelial dysfunction and inflammation, such as E-selectin and others cellular adhesion molecules [141-143]. One of the possible biological mechanism underlying the beneficial effects of long-chain n-3 PUFAs is that they may compete with n-6 fatty acids at the COX and LOX levels, modulating prostaglandin metabolism. Particularly they increase the levels of less atherogenic isoforms, such as the weak platelet aggregator and vasoconstrictor  $TxA_3$ , the weak inducer of inflammation  $LTB_5$  and the active vasodilator and

inhibitor of platelet aggregation PGE<sub>3</sub>, and favour the potent and pro-atherosclerotic TxA<sub>2</sub> and LTB<sub>4</sub> [144]. Some recent data reports that circulating levels of total PUFA are reduced in patients with acute MI [145].

Many studies reports that n-3 PUFAs consumption, mainly from oily fish, is potentially associated with beneficial effects on cardiac risk factors, notably reduction in triglycerides and may prevent CVD. In one study the consumption of n-3 PUFAs reduced mortality, in survivors of MI, however in further randomized, controlled trials the reduction of CV events was not confirmed [146–150].

Thus current recommendations are to increase n-3 PUFAs intake through fish consumption, rather than from capsules, giving that, when following the rules of a healthy diet, no dietary supplements are needed [120, 151].

## Conclusion

The complex and fascinating eicosanoids cascade is crucially involved in atherogenesis and its chronic complications. Prostanoids primarily regulate the complex balance between vasodilation/vasoconstriction and induction/inhibition of platelet activation. This plays a key role in atherothrombosis. Leukotrienes are involved in the inflammatory process and consequently in atherosclerosis regulating chemotaxis, enzyme release and superoxide generation. Although less known, Cytochrome P450 derived eicosanoids also interfere with vasodilation, platelet activation and inflammation.

Pharmacological modulation of the eicosanoids network demonstrated to affect cardiovascular outcome. Selective COX-1 inhibition with low dose aspirin reduces cardiovascular mortality. Specific COX-2 inhibition may result in detrimental effects due to thromboembolic events. Targeting the synthesis of a particular mediator or specifically antagonize an eicosanoids receptor will certainly provide further favorable cardiovascular effects. However the structure, function and expression of each receptor are very complex and still poorly understood.

Certainly, the complex eicosanoids signaling is a pivotal and promising research area to better understand the atherosclerotic process and prevent its cardiovascular formidable complications.

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## Chapter 8

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# Eicosanoids and Aneurysms

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## Abstract

In the inflammatory process underlying aneurysm onset and progression, eicosanoids seem to have an important role. Different hypotheses have been tested to describe the course of action.

Interestingly, the imbalanced synthesis of cyclooxygenase-derived thromboxane A<sub>2</sub> and prostacyclins seems to be involved in Marfan syndrome. Neutrophil derived leukotriene B<sub>4</sub> and prostaglandin E<sub>2</sub> simultaneously have an important role in smooth muscle cell viability, inflammatory processes and expansion of aneurysm.

The involvement of eicosanoids in aneurysm pathophysiology suggested the effect of nonsteroidal antiinflammatory drugs by reducing both inflammation and proteolysis in aneurysm wall.

## Introduction

Abdominal aortic aneurysm (AAA) is an inflammatory disorder characterized by localized connective tissue degradation and smooth muscle cell apoptosis, leading aortic dilatation and rupture. A large number of studies have been performed for investigating the different mechanisms related to eicosanoids in aneurysm progression and complications.

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## **Imbalanced Synthesis of Cyclooxygenase-Derived Thromboxane A<sub>2</sub> and Prostacyclin Involved in Marfan Syndrome**

Marfan syndrome is a genetic disorder of connective tissue caused by mutations in the gene encoding fibrillin -1 [1]. The most life-threatening complication is the progressive aortic aneurysm, leading aortic dissection and rupture [2]. The nitric oxide-mediated endothelial-dependent relaxation is impaired in the thoracic aorta in Marfan syndrome [3]. Recent studies showed that the compromised vasomotor function in Marfan thoracic aorta is associated with an imbalanced synthesis of thromboxane A<sub>2</sub> (TXA<sub>2</sub>) and prostacyclin (PGI<sub>2</sub>) resulting from the differential protein expression of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) [4]. TXA<sub>2</sub> and PGI<sub>2</sub> functionally are antagonistic prostanoids, which are generated from the metabolism of arachidonic acid through cyclooxygenases. Under normal physiological conditions, TXA<sub>2</sub> induces vasoconstriction, smooth muscle proliferation and migration, leukocyte activation, as well as platelet aggregation, while PGI<sub>2</sub> opposes these biological actions. COX-1 is constitutively expressed and activated and it is the main source of basal TXA<sub>2</sub>. COX-2 is subject to rapid induction by a number of cardiovascular risk factors, such as cytokines, cholesterol, hypoxia or after vascular injury, and COX-2 derived PGI<sub>2</sub> is the most abundant prostanoid in the vasculature.

Vascular pathologies such as hypertension, vascular ageing, endothelial dysfunction, are recognized and characterized by a reduced release of endothelium-derived relaxing factors and concomitant increase in release of endothelium-derived contracting factor. During the progression of the Marfan syndrome endogenous endothelial NO production and eNOS downstream signaling are significantly impaired in the thoracic aorta; nevertheless, vasoconstriction is greatly suppressed regardless of the means of stimulation.

In a recent study, performed by AWY Chung and colleagues [4], they concluded that the diminished contraction in the Marfan thoracic aorta might be attributed to both reduced basal production of COX-1 derived TXA<sub>2</sub> and enhanced synthesis of COX-2 derived PGI<sub>2</sub>. They observed, in fact, that the contractility of Marfan aorta at 6 and 9 months of age was significantly improved by pretreatment with non specific COX inhibitor indomethacin, but not the specific COX-1 inhibitor.

This might be due to an augmented synthesis of COX-2 derived relaxant PGI<sub>2</sub> in the Marfan aorta. An imbalance in the basal production of COX-derived vasoconstrictors and vasodilators in Marfan thoracic aorta was further revealed by blocking the TXA<sub>2</sub>/PGH<sub>2</sub> receptors. On the other hand, in the Marfan aorta the expression of COX-1 was down-regulated [5]. In the vasculature, COX-1 is constitutively expressed and is the main source of the basal constricting prostanoid TXA<sub>2</sub>. The decreased TXA<sub>2</sub> secretion in the Marfan aorta might be due to a significant reduction of COX-1 expression. The reason for this reduction of COX-1 and TXA<sub>2</sub> in the Marfan aorta is recognized.

Although COX-2 is much less abundant than COX-1, its expression is up-regulated in the Marfan aorta. It is possible that the loss of vessel elasticity and increase in pulse wave velocity in the Marfan aorta might induce COX-2 expression.



On the other hand, COX-2 expression induces release of metalloproteinases, the activation of which and resultant extracellular matrix degradation are essential for the formation of aortic aneurysm and vascular remodeling [6].

## **Role of Neutrophil Derived Leukotriene B<sub>4</sub> As a Major Chemotactic Factor Released from Intraluminal Thrombus in Abdominal Aortic Aneurysm**

Recent study supports a role of a nonocclusive intraluminal thrombus (ILT) in AAA [7]. The ILT is a laminated neotissue comprising a red blood cell-rich luminal layer at the interface with the flowing blood, an intermediate zone, and a brown fibrinolyzed abluminal layer, which lines the aneurismal wall. Neutrophil infiltration within the ILT during AAA progression will hence accumulate neutrophil derived proteases and lead to a more severe degradation of the aneurismal wall lined by a thrombus compared with the adjacent wall in contact with the flowing blood [8]. An increased production of chemotactic factors by neutrophils could potentially lead to further recruitment through autocrine and paracrine signaling and an amplification of the inflammatory and proteolytic responses. The identification of neutrophil chemoattractants within the aneurismal lesions may hence be key target in the search for medical treatment to slow down aneurysm progression [9]. Leukotriene B<sub>4</sub> (LTB<sub>4</sub>) is a potent leukocyte chemoattractant and mediator of inflammation derived from arachidonic acid by the enzymes 5 lipoxygenase and LTA<sub>4</sub> hydrolase [10]. The chemotactic activity of LTB<sub>4</sub> is transduced through high and low affinity membrane receptors denoted BLT<sub>1</sub> and BLT<sub>2</sub> respectively [11].

These receptors are expressed on human leukocytes, including neutrophils, eosinophils, monocytes and T lymphocytes, as well as on structural cell within the vascular wall. In a study performed by Houard and colleagues [12], differential inflammatory activity across human abdominal aortic aneurysms reveals neutrophil-derived leukotriene B<sub>4</sub> as a major chemotactic factor released from the intraluminal thrombus. Histological examination revealed major expression of the leukotriene-producing enzymes 5-lipoxygenase and LTA<sub>4</sub> hydrolase, as well as the two receptors for leukotriene B<sub>4</sub> (BLT1R and BLT2R), corresponding to neutrophils in the luminal part of the thrombus. In contrast, in the vascular wall, the leukotriene pathway mainly localized in macrophage-rich adventitial areas. Furthermore, conditioned media of the intraluminal thrombus contained significantly higher concentration of leukotriene B<sub>4</sub> than that derived from the vascular wall, which were significantly correlated to other neutrophil-derived mediators. Finally, the neutrophil-chemotactic activity of the conditioned media from the intraluminal thrombus exhibited major inhibition by antagonists of leukotriene B<sub>4</sub> receptors. On the other hand, Ahluwalia and colleagues [13], in their study have shown that diminished AAA formation in BLT<sub>1</sub>-deficient mice was associated with significant reductions in mononuclear cell chemoattractants and leukocyte accumulation in the vessel wall, as well as striking reductions in the production of matrix metalloproteinases-2 and 9 (MMP-2 and -9). Thus, BLT<sub>1</sub> contributes to the frequency and size of abdominal aortic aneurysms in mice and that BLT<sub>1</sub> deletion in turn inhibits

proinflammatory circuits and enzymes that modulate vessel wall integrity. Interestingly,  $LTB_4$  has been correlated to MMP-9 activity in the oral cavity smokers and  $BLT_1$  receptor knockout mice display lower MMP-2 and MMP-9 activities in aneurismal lesions. Furthermore, 5 lipoxygenase co-localizes with MMP-9 in unstable human atherosclerotic lesions [14]. Taken together, those studies suggest a link between the  $LTB_4$  pathway and proteolysis, which was further supported by the significant correlations between  $LTB_4$  and MMP-9.

Taken together these data suggest that  $LTB_4$  formation and antagonism of BLT receptors may represent a therapeutic strategy to control aneurysm progression.

## **Implications of Prostaglandin E2 in Smooth Muscle Cell Viability, Inflammatory Processes and Expansion of Aneurysm**

Prostaglandin  $E_2$  ( $PGE_2$ ), which is synthesized at high concentration in the walls of AAAs, appears to have adverse effects *in vitro* on both proliferation of aortic smooth muscle cells and cytokine secretion by the aneurysm wall.  $PGE_2$  has a pivotal role in the dilatation of AAAs. Walton and colleagues [15] have shown that outgrowth of smooth muscle cells from explants of AAA biopsies appears to be limited by an arachidonate metabolite, probably macrophage-derived  $PGE_2$ . It also inhibited DNA synthesis and proliferation of smooth muscle cells cultured from normal and aneurysmal aorta.

Specific receptors for  $PGE_2$  in aortic smooth muscle cells may mediate these effects, with  $EP_1$  receptors being described as having higher affinity for  $PGE_2$  than  $PGE_1$  [16]. The differential expression of EP receptors in smooth muscle cells from young healthy aorta and cells from AAAs could be sufficient to explain why  $PGE_2$  caused cell death, probably by apoptosis, only in cells derived from aneurysmal aorta.

On the other hand, Wang et al. [17] showed that deletion of Microsomal Prostaglandin E Synthase-1 (mPGES-1) protects against AAA formation induced by angiotension II in hyperlipidemic mice, coincident with a reduction in oxidative stress. mPGES-1 catalyzed the isomerization of  $PGH_2$  into  $PGE_2$  and is a member of membrane-associated proteins in eicosanoid and glutathione metabolism superfamily [18]. It is often co-regulated with COX-2, but it has been co-localized with both COX isoforms in some setting [19].  $PGE_2$  can regulate MMP-2 expression. Suppression of oxidative stress was associated with attenuated MMP-2 activity in Ang II-infused Apo  $E^{-/-}$  mice. It is presently unclear whether the reduction in activity of MMP-2 and perhaps other proteases derives from suppression of  $PGE_2$  or substrate redirection to other products of COX, such as  $PGI_2$  and  $PGD_2$ .

## **Conclusion**

Eicosanoids have an important role in aneurysm onset and progression through the induction of different mechanisms. In particular, they are involved in the inflammatory process underlying aneurysm onset. Inflammation seems to trigger the proteolysis of aortic wall and evocate changes in normal aortic structure. The involvement of eicosanoids in

aneurysm patho-physiology suggested the effect of non-steroidal anti-inflammatory drugs by reducing aortic complications.

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## Eicosanoids in Alzheimer Disease

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### ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the presence of  $\beta$ -amyloid (A $\beta$ ) plaques and neurofibrillary tangles. This represents the most common cause of dementia in the elderly [1]. An important pathologic hallmark of AD is neuroinflammation, a process characterized by disproportionate activation of cells (microglia and astrocytes, primarily) in the central nervous system.

Neuroinflammation has been proposed as the link between A $\beta$  deposition and formation of neurofibrillary tangles; this suggests the crucial role of persistent neuroinflammation in pathogenesis and progression of age-related neurodegenerative disease. In particular, eicosanoids, metabolized by cyclooxygenases (COX-2), lipoxygenases (LOX) and cytochrome P450 enzymes, are potent lipid mediators of inflammation. It is known that eicosanoids have an important role as critical modulators of neuronal function and regulation of oxidative stress mechanisms in brain health and disease. Epidemiological evidence suggests that the use of non-steroidal anti-inflammatory drugs (NSAIDs) seems to be beneficial in terms of slowing the development and progression of AD. This induces (COX) and (LOX) inhibition and modulation of neuroinflammation. Unfortunately, clinical trials with NSAIDs have thus far yielded disappointing results.

Therefore, it is important to continue to explore the role of neuroinflammatory events in AD in order to provide opportunities toward the development of new therapeutic strategies.

**Keywords:** Alzheimer's disease, neuroinflammation, eicosanoids, cyclooxygenase (COX), lipoxygenase (LOX), non-steroidal anti-inflammatory drugs (NSAIDs)

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## Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder which results in cognitive deficiency, behavioral disturbance and neuropsychiatric symptoms. AD represents the most common cause of dementia in the elderly with ~30 million patients worldwide whose number is expected to triple by 2050 [1].

Although most cases of AD are sporadic, a small fraction of cases have an autosomal dominant inheritance with onset before 65 years of age. Currently, mutations in four genes: amyloid precursor protein (APP), presenilin 1 (PSEN1), presenilin 2 (PSEN2) and the  $\epsilon 4$  allele of the apolipoprotein E (APOE), which are situated respectively on chromosomes 21, 1, 14 and 19, are believed to have a role in this disease [2].

The two major neuropathologic hallmarks of AD are amyloid plaques, which are mainly composed of the peptide amyloid  $\beta$  ( $A\beta$ ) and intracellular neurofibrillary tangles (NFTs) [3].  $A\beta$ , a 40–43 amino acid peptide, is a normal constituent of human brain. It is generated from amyloid precursor protein, also known as amyloid beta ( $A\beta$ ) precursor protein (APP). In the normal state, APP is initially cleaved by  $\alpha$ -secretase to generate sAPP $\alpha$  and a C83 carboxyterminal fragment. The presence of sAPP $\alpha$  is associated with normal synaptic signaling, and results in synaptic plasticity, learning and memory, emotional behaviors, and neuronal survival. In the disease state, APP is cleaved sequentially by  $\beta$ -secretase and  $\gamma$ -secretase, to release an extracellular fragment called  $A\beta_{40/42}$ . This neurotoxic fragment frequently aggregates and results in  $A\beta_{40/42}$  oligomerization and plaque formation. Release of toxic  $A\beta$  fragments lead to the early formation of soluble dimeric and oligomeric aggregates that directly inhibit synaptic function and are highly toxic to neurons [4, 5].

AD is also characterized by the presence of neurofibrillary tangles, which are composed of the tau protein ( $\tau$ ). In healthy neurons,  $\tau$  is an integral component of microtubules, which are the internal support structures that transport nutrients, vesicles, mitochondria, and chromosomes from the cell body to the ends of the axon and backwards.

However in AD,  $\tau$  becomes hyperphosphorylated inducing its oligomerization, its dissociation from the microtubule, and apoptosis of neuronal cells [6, 7]. Another important pathologic hallmark of pathogenesis and disease progression in AD and other age-related neurodegenerative disease is neuroinflammation, which has been proposed to be the link between  $A\beta$  deposition and the formation of neurofibrillary tangles [8]. In particular, it has long been recognized that eicosanoids, which are potent lipid mediators of inflammation. They are known to play an important role as critical modulators of neuronal function and regulation of oxidative stress mechanisms, in brain health and disease.

This chapter describe and provide a general overview on the role of neuroinflammatory process and of eicosanoids in AD, and an update on non-steroidal anti-inflammatory drugs (NSAIDs) treatment in recent epidemiological analyses, and clinical trials.

## Neuroinflammation in Alzheimer's Disease

Although inflammation in the brain is a defense mechanism against neurotoxic stimuli, increasing evidences suggest that chronic inflammation and increased oxidative stress contributes to neurodegeneration in the pathogenesis of AD [9].

A $\beta$  peptides promote pro-inflammatory responses and are activators of neurotoxic pathways which lead to brain cell dysfunction and death [10]. These events include enhanced excitotoxicity via increased calcium flux into neurons, activation of microglia, overproduction of reactive oxygen species and proinflammatory cytokines, and an overall oxidative stress response in the brain [11]. Accordingly, these products of inflammation (such as pro-inflammatory cytokines) might change the substrate specificity of kinases/phosphatases, leading to tau phosphorylation at pathological sites [12].

Another common feature in the brain of AD patients is the presence of dystrophic neurites, activated microglia, and reactive astrocytes [13] surrounding the senile amyloid plaques and tangles, which stimulate a chronic inflammatory reaction.

Activated cells induce the expression of proinflammatory cytokines, chemokines [14], the activation of the complement cascade, prostaglandins, leukotrienes, thromboxanes, coagulation factors, reactive oxygen species (and other radicals), nitric oxide, proteases, pentraxins, and C-reactive protein [15, 16].

This inflammatory mediators reaction, in turn enhance APP production and the amyloidogenic processing of APP to induce A $\beta$ 42 peptide production, providing in this way obvious stimuli for inflammation and a vicious cycle in glia cells [17]. Several lines of evidence suggest that all of these factors can contribute to neuronal dysfunction and cell death, either alone or in concert.

Cytokines play a key role in inflammatory and anti-inflammatory processes in AD. An important factor in the onset of inflammatory process is the overexpression of interleukin (IL)-1, which cause dysfunction and neuronal death. Other important cytokines in neuroinflammation are IL-6 and tumor necrosis factor (TNF)- $\alpha$ . By contrast, other cytokines such as IL-1 receptor antagonist (IL-1ra), IL-4, IL-10, and transforming growth factor (TGF)- $\beta$  can suppress both proinflammatory cytokine production and their action, subsequently protecting the brain [18].

In addition, it was observed that cytokines, such as IL-1, and synthetic amyloid peptides induce cyclooxygenase-2 (COX-2) expression and prostaglandin E2 (PGE2) release in the human neuroblastoma cell line SK-NSH [19]. Considering that it has been long recognized that COX-2 plays a-key role in inflammation and eicosanoids' synthesis, many evidences led to the hypothesis that it may represent a primary target for NSAIDs in AD, consistent with inflammatory processes occurring in AD brain [20].

## The Role of Eicosanoids in the Brain

The brain is the second organ with highest content of lipids in the human body next to adipocytes, at 36-60%. Fatty acids represent integral membrane components, essential for proper neuronal and brain function and serve as both energy substrates [21]. In particular, polyunsaturated fatty acids (PUFAs) are integral membrane lipids, created through hydrolysis mediated crosslinking (double bond formation) along the carbonyl backbone of their saturated fatty acid precursors. The incorporation of PUFAs into neuronal cells increase stability and membrane fluidity, that is essential to maintain synaptic structures, promoting synaptic plasticity and neurotransmission [22].

The main brain PUFAs are arachidonic acid (AA; 20:4 $\omega$ 6) or docosa-hexaenoic acid (DHA; 22:6 $\omega$ 3). In grey matter, the proportion of DHA in glycerophospholipids is higher than AA, whereas in white matter this situation is reversed. Clearly, brain AA is important as a precursor of a wide range of eicosanoids and leukotrienes which play critical roles in many aspects of neural function [23]. Under the control of specific stimuli, PUFAs are released from the glycerophospholipids by the action of various phospholipases (cytosolic phospholipase AII, cPLA2; plasmalogen selective phospholipase AII, PlsEtn- PLA2 and secretory phospholipase AII, sPLA2) and metabolized by cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 enzymes (CYP-450) into a variety of derivatives known as eicosanoids [24, 25].

COX enzyme is present in mammalian in three isoforms (COX-1, COX-2 and COX-3). Both COX-1 and COX-2 enzymes are express in brain tissue [26] and can catalyze the reaction that converts arachidonic acid to a stable hydroxyl endoperoxide (PGH2). PGH2 is then converted into primary prostanoids, which can be classified into prostaglandins (PGE2, PGF2 and PGD2), prostacyclins (PGI2) and thromboxanes (TXA2) [27]. PGD2 is the most abundant prostaglandin synthesized in the central nervous system that protects the brain from excitotoxic injury, PGE2 is involved in brain maturation and in regulation of synaptic activity and plasticity, and instead PGI2 and TXA2 are potent vasodilators and vasoconstrictors respectively. However, a second highly responsive and inducible prostanoid pool of primarily (PGE2) mediates the initiation and propagation of inflammation, and is thought to contribute to the disproportionate inflammatory response in AD, and to many other pathological processes such as carcinogenesis and metastasis [28].

LOXs are a group of enzymes that catalyzes the reaction, which involves the addition of oxygen to AA producing hydroxyperoxyeicosatetraenoic acid (HPETEs) which then reduces to give leukotrienes (LTs) and hydroxyeicosatetraenoic acid (HETEs) [29].

Leukotrienes (LTC4, LTD4 and LTE4), can be involved in inflammatory diseases, attracting leukocytes altering cerebral vessel functions and the blood brain barrier [30]. Moreover, they are also produced in response to numerous acute brain injuries. HETEs are potent vasoactive agents and are altered in cerebrovascular pathologies [31].

(CYP-450), DiHETEs, HETEs and the brain AA undergoes metabolism by CYP-450 to give epoxye-icosatrienoic acid (EETs).

In the brain, EETs are involved in controlling the cerebral blood flow (CBF), representing neuroprotective agents because of their anti-inflammatory and anti-thrombotic effects [32]. Zhang et al. found that deletion of sEH, the enzyme that metabolizes EETs to DiHETEs, is protective against ischemic brain injury [33].

## **Eicosanoids in AD**

In the brain, eicosanoids are important to maintain homeostasis and normal functions (such as synaptic plasticity) and protect cortical neurons against glutamate toxicity, especially in AD. On the other hand, alterations in the levels of these lipids in the brain have been associated with numerous diseases such as Alzheimer's, Parkinson's, Multiple sclerosis, schizophrenia, and epilepsy [21]. (DHA) is a dietary essential omega-3 fatty acid, concentrated in membrane phospholipids, which attenuates increases in the levels of lipid



peroxides and reactive oxygen species in the cerebral cortex and the hippocampus of A $\beta$ -infused rats [34].

It is well documented that, in neural trauma and neurodegenerative diseases, there is a dramatic rise in the levels of AA-derived eicosanoids. In contrast, it was observed that DHA-derived compounds can prevent neuroinflammation.

On the other hand, several clinical trials investigating the effects of omega-3 fatty acid supplementation in AD failed to demonstrate its efficacy in the treatment of AD, suggesting that the beneficial effects of omega-3 fatty acid supplementation may depend on the stage of disease, other dietary mediators, and apolipoprotein E status [35].

DHA has a neuroprotective role by blocking A $\beta$ 40/A $\beta$ 42 neurotoxicity, and it is modified through phospholipase A2 and lipoxygenase to form the docosanoid, neuroprotectin 1 (NPD1). Several studies have demonstrated a reduced activity of phospholipase A2 in brain tissue, and a reduction of DHA and NPD1 in cerebrospinal fluid CSF and from AD subjects. In particular, NPD1 is a potent regulator of an intrinsic neuroprotective, anti-inflammatory, and antiapoptotic gene-expression program that promotes survival in stressed human brain cells, which may play a significant role in the development of AD.

Therefore, decreased levels of these important signaling molecules may contribute to the pathogenesis of AD and other degenerative diseases [36].

In addition, AA and DHA non enzymatic oxidation result in the formation of the biologically active hydroxynonenal F2 isoprostanes (F2IsoPs) and hydroxyhexenal F4 neuroprostanes (F4NPs), respectively [37].

Early studies showed that F2IsoPs and F4NPs were increased in the brains and ventricular fluid of autopsied, late stage, AD patients and in the brains of mild cognitive impairment (MCI) patients compared with normal controls. This study indicates the oxidative damage of AA and DHA is an early event in the pathogenesis of AD and that the F2IsoPs, F4NPs, and other oxidative derivatives of PUFAs could be represent important markers of oxidative stress in neurodegenerative diseases. It was also observed that their biological activities could enhance A $\beta$  oligomerization *in vitro*. The usefulness of assays for F2IsoPs and F4NPs as biomarkers for therapeutic trials investigating PUFA and other antioxidative compounds may prove valuable to assess the biological efficacy of such strategies irrespective of the outcome of primary clinical endpoints [38].

Alteration in COX and LOX pathways in AD has been extensively investigated. It is well documented that COX activity is implicated in neuroinflammation associated with normal aging and in the pathophysiology of experimental AD [39]. While COX-1 (considered a crucial player in the maintenance of cell homeostasis) is expressed in most tissues and cell types and is constitutively active, COX-2 is uniquely inducible and activated by a variety of stimuli (such as cell proliferation, neoplasia, environmental stress, and inflammation), increasing prostanoid levels. It has been documented that COX-2 expression, normally very low in brain, is markedly increased in AD and other neurodegenerative diseases [40].

It was observed in animal models of cerebral ischemic injury, ALS, or PD that COX-2 overexpression in neurons promotes neuroinflammation, toxicity and correlated with cell death. COX-2 expression also occurs in activated microglia, which can contribute to neuronal death [41]. However, the mechanism by which COX-2 contributes to neuronal death is unknown. Some investigators suggest that cyclopentenone PGs, highly reactive dehydration products of PGE2 and PGD2 (mediating the initiation and propagation of inflammation), may be the toxic COX products [42].

In addition to direct neurotoxicity, A $\beta$  mediates increased cellular phospholipase activity, the first step in prostanoid biosynthesis, in addition to stimulation of pro-inflammatory cytokine secretion. PGE<sub>2</sub> is the major effector in the CNS based, and is the most studied with regard to neuroinflammation. In order to understand the biological role of PEG<sub>2</sub>, different groups of research developed genetic mouse models of AD that lacked specific PGE<sub>2</sub> receptor subtypes (EP1, EP2, EP3, and EP4) and then measured outcomes of cognition, amyloid plaque burden, proinflammatory cytokine expression, and oxidative stress. They observed that amyloid plaque burden was significantly reduced in EP1 knockout mice expressing both the Swedish amyloid precursor protein (APP) and PS1 mutations [43]. EP2 receptor knockout AD mice showed reduced oxidative damage and have cognitive deficits. Instead, EP3 receptor deficient AD mice demonstrated reduced proinflammatory gene expression, cytokine production, oxidative stress, and plaque production, as did EP4 receptor knockouts, which additionally demonstrated reduced atherosclerosis [44].

These data suggest that all PGE<sub>2</sub> receptors showed a correlation with A $\beta$  toxicity; in some clinical trials compounds SC-51089 and AE30208, which target EP1 and EP4, respectively, would be a potential therapeutic target for AD, improving cognitive function and reducing A $\beta$  plaques [45].

Different studies also suggested that 5-LOX could participate in AD pathobiology through different mechanisms. It was observed through quantitative Western blot assays that 5-LOX levels were significantly increased in AD brain samples [46].

Ikonomovic et al., investigated the distribution and cellular localization of 5-LOX in the medial temporal lobe, from AD and control subjects founding that in AD subjects LOX immunoreactivity is elevated relative to controls and that its localization is dependent on the antibody-targeted portion of the 5-LOX amino acid sequence.

Thus, carboxy terminus-directed antibodies detected 5-LOX in glial cells and neurons amino terminus-directed antibodies was absent in neurons and abundant in neurofibrillary tangles, neuritic plaques, and glia [47].

Firuzi et al. investigated the effect of 5-lipoxygenase (5-LOX) deficiency on the amyloid-beta pathology of a transgenic mouse model of AD-like amyloidosis, the Tg2576 mice [46]. These authors found that, in the absence of alteration in A $\beta$  precursor protein processing or amyloid-beta catabolism, the genetic disruption of 5-LOX reduced amyloid-beta deposits and A $\beta$  42 levels. Additional in-vitro studies showed that 5-LOX activation and its metabolites increase A $\beta$  deposits, whereas 5-LOX inhibition decreased A $\beta$  formation by modulating the gamma-secretase complex activity [46].

Generally, the conversion of arachidonic acid by 5-LOX leads to production of leukotrienes and, under certain conditions, lipoxins. Various leukotriene receptors are expressed by both neurons and microglia. It has been reported that leukotrienes and their receptors, e.g., the cysteinyl leukotriene receptor 1 (CysLT1) may promote brain injury and that increased 5-LOX expression and activity lead to production of brain-toxic molecules [48]. Growing evidence suggests a role for leukotrienes in brain inflammation associated with age-related dementia, as well as with neurodegenerative diseases.

Recently, Di Francesco et al., studied the epigenetic regulation of (5-LOX). They found a significant increase in 5-LOX and leukotriene B<sub>4</sub> gene expression in peripheral blood mononuclear cells of AD subjects compared to healthy controls, supporting other results present in literature.

In addition, a consistent reduction in DNA methylation at 5-LOX gene promoter was documented in AD versus healthy subjects, supporting the role of 5-LOX in neurodegeneration [49]. The type of the 5-LOX metabolite produced may be influenced also by 5-LOX phosphorylation. For example, 5-LOX phosphorylation at Ser523 determines induce production of LTB<sub>4</sub> or LXA<sub>4</sub> [50]. All these observations suggest that delineate the exact nature of the involvement of the brain COXs and 5-LOX in AD would reinvigorate the search for novel targets for AD therapy.

## **Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) in AD**

Considering that persistent neuroinflammation has a pivotal role in pathogenesis and disease progression in neurodegenerative diseases, epidemiological evidences support a therapeutic benefit of targeted anti-inflammatory pharmacotherapy approaches in AD.

Anti-inflammatory drugs, in particular (NSAIDs), seem to be beneficial in terms of slowing the development and progression of AD, inducing COX inhibition and modulation of neuroinflammation [51].

Early studies regarding the role of cyclooxygenases in AD suggest that COX inhibitors such as (NSAIDs) could be beneficial in AD patients. This line of research led to the development and trials of selective COX-2 inhibitors as a putative therapy for AD. In particular, the development of COX-2 inhibitors (coxibs) and the presence of elevated COX-2 in both post-mortem brain and AD animal models assess the efficacy of COX inhibitors (specific and non-specific) in the treatment or prevention of AD [52]. As summarized in several recent reviews [53, 54], it appears that selective COX-2 inhibitors may not be an effective therapy in AD patients with mild to severe cognitive impairment and it has been suggested that COX-2 inhibition by NSAIDs might be involved in the apparent protection in this setting. Double blind, randomized, placebo-controlled trials using nonselective NSAIDs [55] and COX-2 specific inhibitors have shown no significant effect on cognitive performance in AD patients. Recent studies from larger populations confirm previous studies regarding the use of COX-2 inhibitors in smaller subject groups or for shorter duration, suggesting that NSAID treatment is ineffectual once memory decline [56]. Due to the key role of microglia in neuroinflammation, it has been suggested that selective inhibition of COX-1, rather than COX-2, will be more effective in treating neuroinflammation and neurodegeneration. After observing in some models of neuroinflammation, that no beneficial effects were observed with COX-2 inhibitors, Choi and Bosetti propose the hypothesis that the potential protective effects of NSAIDs in AD may be related to COX-1. Reduction in cognitive decline in AD patients was observed in a 6-month, double-blinded, placebo-controlled study with indomethacin, a non-selective, but a potent COX-1 inhibitor [57, 58]. In addition, a previous study showed that neurons treated with COX-1 selective inhibitors are resistant to A $\beta$ 1-42, induce inhibition of either LPS- or arachidonic acid-induced PGE<sub>2</sub> synthesis in human microglia. However, it remains to be determined how COX-1 inhibition modifies the early beneficial function of activated microglia in A $\beta$  clearance and whether COX-1 inhibition is protective against neuronal loss in other models of AD such as the PS1-APP transgenic mice.

Some NSAIDs, e.g., aspirin, trigger a peculiar interplay between the COXs and 5-LOX pathways, by causing the acetylation of COX-2, that ultimately shifts the 5-LOX end products from leukotrienes to lipoxins [50]. Furthermore, in animal models and human trials, it was observed that many NSAIDs and structurally related enantiomers could influence AD pathobiology independent of their COX inhibitory activity, i.e., by interacting with the gamma-secretase complex [59], and therefore reducing formation and accumulation of A $\beta$ .

Growing evidence suggests a role for 5-LOX as potential target in AD. Compared to the pharmacology of COX inhibition, few clinical studies have yet been reported about the use of specific 5-LOX inhibitors in Alzheimer's patients or in any other neurodegenerative diseases. It was observed that 5-LOX inhibitors (e.g., minocycline) [60], are beneficial in AD animal models.

On the other hand Sobrado et al. found that rosiglitazone, an anti-diabetic drug which induces 5-LOX expression in ischemic rat brain, leading to production of both, putatively neurotoxic mediators such as leukotrienes or neuroprotective mediators such as LXA4 [61].

Furthermore, although leukotriene receptor inhibitors appear to be neuroprotective in animal models of stroke [62] no data are available on their possible effects in AD.

## Conclusion

Increasing evidences underline that inflammation significantly contributes to the pathogenesis and clinical progression of AD. The generation and secretion of pro-inflammatory mediators may interact at multiple levels; they could contribute to neuronal death and also influence classical neurodegenerative pathways such as APP processing and  $\tau$  phosphorylation. Early works regarding the putative role of COXs and 5-LOX in AD indicate that these enzymes and eicosanoids from their activity could influence AD and other neurodegenerative disorders. In particular, 5-LOX could influence AD by producing pro-inflammatory leukotrienes (LTB4 and CysLTs), anti-inflammatory lipoxins and by an interaction with the gamma-secretase complex. Instead, COXs could influence AD by acting on (AA), and producing PGH2 and PGE2. Moreover, the interplay between COXs and 5-LOX pathways, which is exemplified by the generation of anti-inflammatory/ neuroprotective 15-epi-LXA4, may provide novel insights into the role of these pathways in neurodegenerative disorders [50]. In conclusion, elucidation of the role of neuroinflammatory events in AD may provide opportunities toward the development of new therapeutic strategies and reinvigorate the search for novel targets for AD therapy strategies that are targeting inflammatory mechanisms.

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## Eicosanoids and Cancer

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### Abstract

Cancer is a leading cause of death worldwide. Different treatment strategies have been used such as chemotherapy, radiation, surgical resection and immune therapies. However, 5-year survival rate for some forms of cancer still remains too low. Thus, further studies are needed for a deeper understanding of molecular and pathological bases of cancer. This might consent the development of new and more effective therapeutic strategies. Growing findings from clinical, epidemiological and *in vitro* studies highlight a high relationship between inflammation and malignant transformation. Indeed, it has been largely demonstrated that common pro-inflammatory mediators, such as Reactive Oxygen Species (ROS), cytokines, chemokines, and Arachidonic Acid (AA) derived eicosanoids can produce environmental conditions favorable for cancer onset. In particular, COX2-derived eicosanoids are over-expressed during the early steps of malignant transformation, but also during progression. Indeed, they directly contribute to cancer progression and metastasis. The role of eicosanoids in several types of cancer is nowadays well demonstrated by both *in vitro* and *in vivo* experiments.

Thus, a deeper understanding of the complex relationship between these pro-inflammatory molecules and cancer might offer new and more effective rational targets for cancer therapy. Here, we evidenced the role of eicosanoids in different cancer types.

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## Introduction

Cancer is generally recognized as an age-related disease. In fact, incidence and mortality rates of most human cancers increase consistently with age up to 90 years, but they plateau and decline thereafter. In the United States, one in every four deaths is due to cancer. Despite the improvement in different treatment strategies (i.e., chemotherapy, radiation, surgical resection and immune therapies) the 5-year survival rate for some cancers still is too low. Thus, it is imperative a deeper understanding of the molecular and pathological mechanisms of cancer, in order to develop new and more effective therapeutic strategies [1].

A low-grade systemic inflammation characterizes ageing and this pro-inflammatory status underlies biological mechanisms responsible for age-related inflammatory diseases. On the other hand, clinical and epidemiological studies show a strong association between chronic infection, inflammation and cancer and indicate that even in tumours not directly linked to pathogens, the microenvironment is characterized by the presence of a smouldering inflammation, fuelled primarily by stromal leukocytes. This evidence is leading to develop new therapeutic and personalized treatments able in inhibiting all inflammatory pathways associated with cancerogenesis. On the other hand, common pro-inflammatory mediators (i.e., Reactive Oxygen Species (ROS), cytokines, chemokines, and arachidonic Acid (AA)-derived-eicosanoids) and their pathways in chronic conditions are able in determining environmental favorable conditions for malignant transformation [2,3]. In particular, arachidonic Acid (AA)-derived-eicosanoids and their pathways seem to have a crucial role, as evidenced by a large number of literature data, in the onset of tumors, but also in their progression and metastasis [4].

In this chapter, we described the complex interplay between the Acid (AA)-derived-eicosanoids and different tissue types of neoplasia.

## The Acid (AA)-Derived-Eicosanoids and Their Pathways

Cyclooxygenases (COXs) are crucial enzymes involved in eicosanoids (prostaglandins, leukotrienes and thromboxanes) biosynthesis [5]. There are two COX isoforms: COX-1 and COX-2 [6]. COX-1 is constitutively expressed at relatively low levels, whereas COX-2 is the inducible form of the enzyme, and its expression dramatically increases in response to a variety of stimuli. It is well known nowadays that COX-2 is involved in cellular proliferation, angiogenesis, apoptosis and metastasis [7], by determining malignant transformation both in *vitro* and in multiple animal models [9]. Indeed, COX-2 expression has been shown to be increased in various cancer types, such as lung, colon, breast, and pancreatic tumors, as demonstrated in experimental models, i.e., in *vitro* cancer cells [10]. Moreover, COX-2 over-expression also seems to play a leading role in chemotherapy resistance [10], while pharmacologic inhibition of COX-2 activity increases the apoptotic cell death through the nuclear localization of active p53 [11].

Although these findings strongly demonstrate the COX-2 involvement in tumor initiation and maintenance, the precise mechanisms mediated by COX-2 in promoting these events

remain unclear. Recent evidence focused their attention on both the COX-2 derived eicosanoids and signaling pathways leading to COX-2 induction, such as all three members of the Mitogen-Activated protein Kinases (MAPKs) family (ERKs, JNKs and p38 MAPK) and Akt[12].

Of note is the effect of COX-2 derived prostaglandin E2 (PGE2). PGE2 is known to play an important role in inflammatory responses, and it prevents apoptosis, controls cell growth, increases angiogenesis and induces cell motility and adhesion [13]. Indeed, PGE2 inhibits cancer cell apoptosis, increases invasiveness and angiogenesis in the tumor through activation of different molecular pathways, such as NF- $\kappa$ B, MAPkinase/JNK/p38, phosphatidylinositol-3-kinase (PI3k)/Akt [14,9]. Moreover, COX-2 over-expression has been shown to up-regulate B cell lymphoma gene-2 (Bcl-2), with a resulting decrease in apoptosis. Other effects of COX-2 over-expression may contribute to the malignant cell or tissue phenotype, such as the decrease of E-cadherins, by resulting in loss of cell-to-cell adhesion, matrix metalloproteinase (MMP) over-expression and angiogenic factor's release. All these COX-2 mediated effects lead an associated increase in invasiveness of cancer cells [15]. Thus, there is a growing current interest in using selective COX-2 inhibitors, such as celecoxib and rofecoxib both in cancer prevention and therapies. However, the cardiotoxicity of these agents has determined to stop their long-term administration, as evinced in recent guidelines. This is leading in researching biological molecules in aliments having capacity to modulate the COX-2 actions. Interestingly, it has been demonstrated that n-3 fatty acids are natural modulators of COX-2 and are able to alter COX-2 metabolites [16]. These family of fatty acids, especially Eicosapentanoic acid (EPA) and Docosahexaenoic acid (DHA), have anti-inflammatory and immunomodulatory properties and seems to have no deleterious effects in human cardiac, musculoskeletal, gastrointestinal and immune systems [17]. In addition, epidemiological and preclinical evidence propoens EPA and DHA anticancer activities. Several experimental studies in transgenic mice and carcinogen-induced tumors are underlining as n-3 fatty acids can reduce both onset and progression of different tumors [18]. Human studies are also demonstrating a significant association between a higher intake of n-3 fatty acid and the reduced risk of skin, colorectal, lung, prostate and breast cancers [19-23]. Compelling evidence suggests that EPA and other n-3 fatty acids essentially act as competitive inhibitors of AA for COX-2, by resulting in reduction of the 2-series PGs (such as PGE2) and concomitant production of the 3-series PGs, synthesized from n-3 fatty acids [24]. In contrast to PGE2, PGE3 and other 3-series prostaglandins show anti-proliferative and anti-inflammatory activities, and they could potentially antagonize the tumor promoting effect of PGE2 [24]. The synthesis of the 2- and 3- series PG metabolites from AA or EPA, respectively, shares three common steps: a) phospholipase A2 (PLA2) releases AA or EPA from membrane phospholipids; b) AA or EPA are converted to prostaglandin endoperoxide (respectively, PGH2 from AA and PGH3 from EPA) by COX-1 or COX-2; c) specific synthases isomerize PGH to "2-series" or "3-series" mediators (i.e., PGE2 or PGE3, PGD2 or PGD3, PGF2a or PGF3a, PGI2 or PGI3, thromboxane A2 or thromboxane A3) [25].

In addition, it has been also found that the expression and activities of the three PLA2, COX-2, and mPGES-1 enzymes, associated with eicosanoids synthesis, markedly differ in cancer than normal tissues. [26]. The anti-proliferative effect of EPA in cancer cells might be mediated not only through reduction of PGE2, but also through concomitant increase of PGE3 produced by COX2. Indeed, the COX-2 selective inhibitor, celecoxib, but not the COX-1 inhibitor SC-560, blockes formation of PGE3 and reduces the anti-proliferative effect

of EPA in non-small cell lung cancer (NSCLC) [24]. In addition, among the multiple signaling pathways implicated in its biological action, PGE3 elicits anticancer activity mainly through AKT, ERK1/2 and PKA activation. PGE3 suppresses tumor growth by inhibiting angiogenesis and cell invasion as well as reducing cell growth and survival. These effects are known to be mediated through activation of both EP2 and EP4 receptors. Furthermore, PGE3 down-regulate PI3-kinase signaling by increasing Phosphatase and tensin homolog (PTEN) expression [26] or suppressing Akt phosphorylation. In sharp contrast, PGE2 increases Akt activation in human lung cancer cells [27]. The anti-tumor activity of PGE3 might be also due to inhibition of Human Epidermal growth factor Receptor 3 (HER3) and cMYC, which are both known to be commonly over-expressed in numerous cancer types.

Several clinical trials are testing the possibility in using plasma n-3 fatty acid levels as beneficial molecules in different pathological conditions, including cancer [28]. However, the biosynthesis of 3-serie PGs in the tumor cells, as well in normal cells, also depends on the expression and activity of enzymes responsible for its synthesis, and consequently by the individual genetic background. Thus, plasma n-3 levels only provide evidence of n-3 fatty acid uptake, not their metabolism or biological effects.

In addition, several soluble forms of PLA2 (sPLA2s) are associated with the development of prostate, colon, gastric, lung and breast cancers. However, their specific role depends on the cancer types as well as the microenvironmental context. The expression of some sPLA2s, most notably the group IIA, III and X enzymes, has been reported to be altered in various malignant tissues, and each particular enzyme may exert pro- or anti-tumourigenic effects depending on cancer types, and more probably microenvironmental context. Thus, there are multiple context-dependent mechanisms of sPLA2 actions in different cancers. In colon, breast, stomach, oesophagus, ovaries and prostate cancer, an aberrant expression of various human sPLA2s has been detected [29]. For example, hGIIA sPLA2 has been found to be increased in the serum or tumor tissue from patients with prostate [30], oesophageal and lung cancer [31]. It strongly correlates with poorer patient survival. In contrast, hGIIA sPLA2 seems to exert anti-tumourigenic effects in gastric cancer cell lines as well as in tumor tissue from patients with gastric cancer, by inducing reduction of cell migration and invasiveness. Thus, hGIIA sPLA2 over-expression correlates with longer survival and favorable outcome for patients with gastric cancer [32]. Despite the role of sPLA2s in cancer, traditionally linked to their enzymatic activity and involvement in the synthesis of potent active lipid mediators, several biological effects of sPLA2s have been found to be independent by its enzymatic activity, suggesting the possibility of an additional receptor-mediated mechanism of action [33]. Importantly, it has been also observed that exogenous addition of AA in the culture media of dendritic cells (DCs) results in generation of DCs having an improved functionality. No differences between the two types of DCs (i.e., DCs generated with and without AA) in terms of morphology, phenotype and antigen uptake have been found, but the AA-treated DCs exhibited an enhanced *in vitro* and *in vivo* homing, T cell stimulatory capacity, cytotoxic T Lymphocytes (CTL) activity and significantly higher transcript levels of COX-2. Furthermore, generated DCs in the presence of AA also show a favorable Th1 cytokine profile than AA-untreated DCs. Thus, the addition of AA in culture media likely induces the DCs to secrete more IL-12 and less of IL-10, and consequently an improved COX-2 mediated release of eicosanoids. These findings lead to propose the possibility to develop DCs based vaccines for cancer immunotherapy [34]. Here, we evidenced the literature data on the role of eicosanoids in different cancer types.

## Breast

Breast cancer represents the most commonly diagnosed cancer and second leading cause of death among women [35]. Obesity is usually linked with a worse breast cancer prognosis[36]. Indeed, increased levels of pro-inflammatory markers, including greater COX-2 expression and enhanced PEG2 release by white adipose tissue-resident macrophages induces aromatase expression and subsequent estrogen synthesis, promoting breast cancer progression. The inhibition of COX-2 activity through non-steroidal anti-inflammatory drug (NSAID) administration seems to reduce estrogen receptor  $\alpha$  (ER $\alpha$ )-positive breast cancer recurrence in obese and overweight women [37,38]. These concepts also emphasizes the role of inflammation in the onset and progression of breast cancer. On the other hand, it has been demonstrated a significant association between chronic inflammatory processes in epithelial breast cells and the onset and progression of breast cancer. It has been observed that epithelial cells switch in mesenchymal, as consequence of the chronic activation of inflammatory pathways. Epithelial mesenchymal transition (EMT) process has been observed to have higher propensity in neoplastic transformation, increased aggressiveness and higher metastatic potentiality. It has been demonstrated in mammary epithelial cells, that EMT is promoted by activation of leukotriene B4 receptor-2 (BLT2), up-regulated by oncogenic Ras in response to transforming growth factor- $\beta$  (TGF- $\beta$ ). Indeed, BLT2 inhibition reduces Ras-induced EMT in presence of TGF- $\beta$ . ROS and nuclear factor  $\kappa$ B (NF- $\kappa$ B) are known to be critical mediators that contribute to EMT [39].

The PI3K/Akt signaling pathway plays a leading role in several cellular processes, including proliferation, growth, survival, angiogenesis and cancer [40]. PI3Ks generates phosphoinositol lipids, which act as second messengers in a number of intracellular signaling pathways and activate PDK and Akt [41]. In particular, the latter is the primary downstream mediator of PI3K and mediates a number of cell processes through the phosphorylation of target substrates. The Akt family of serine–threonine kinases consists of three members: Akt1, Akt2 and Akt3. It was demonstrated that AA induces Akt2 activation and invasion in MDA-MB-231 breast cancer cells. Free AA is metabolized by LOXs and then LOXs metabolites are produced and secreted to the medium. LOXs metabolites bind and activate G-protein coupled receptors (GPCRs) that mediate EGFR transactivation, which promote PI3K/Akt pathway activation. Then, PI3K/Akt pathway activates NF $\kappa$ B and is involved in COX-induced cell migration and invasion [42].

Breast cancer frequently metastasizes to bone. Osteolytic lesions are the most common outcome of breast cancer metastasis to bone and result from increased osteoclast differentiation and activation, which are both mainly mediated by osteoblast production of RANKL (receptor activator for NF $\kappa$ B ligand) a key molecule for osteoclast differentiation. Once breast cancer cells settle in bone, they produce pro-osteolytic factors, which directly induce osteoclast differentiation and activation. On the other hand, cancer cells can induce apoptosis in osteoblasts and thus, metastasis-associated bone loss is due to both increased activation of osteoclasts and suppression of osteoblasts [43]. Bone colonization of breast cancer cells leads to up-regulation of COX-2 and increased PGE2 synthesis in both cancer cells and stromal cells/osteoblasts [44,45]. PGE2 in turn stimulates osteoclasts differentiation by up-regulating RANKL expression mainly via EP4 receptor expressed in osteoblasts and thus, enhances tumor-induced osteolysis [46,47]. The anti-oxidant Trolox, a hydrophilic derivative of  $\alpha$ -tocopherol, has been shown to selectively enhances arsenic-mediated

apoptosis and curcumin-induced cytotoxicity in breast cancer cells, while protect non-malignant cells [48,49]. It has been also demonstrated that Trolox inhibits inflammation induced osteoclast differentiation both by reducing RANKL expression in osteoblasts through inhibition of COX-2 activity and by inhibiting RANKL action on osteoclast precursor cells [50]. Therefore, further studies will be able to explore more in depth the effects of Trolox and its use in osteolytic bone metastasis of breast cancer.

Photodynamic therapy (PDT) is an anti-cancer treatment based on the exposure of a photosensitizing agent to specific wavelength of light, resulting in production of a form of oxygen that kills nearby cancer cells [51]. In effect, PDT destroys tumor tissue through multiple interacting mechanisms that include direct cell killing, microvascular damage and inflammation [52]. However, it has been established that PDT-mediated microvascular damage and the resulting hypoxia can lead to a variety of molecular and physiologic responses, including gene activation, inflammation and angiogenesis, that might facilitate survival of residual tumor cells [53]. For example, PDT induces elevated expression of angiogenic-related factors (VEGF, PGE2) and survival molecules that are associated with tumor regeneration and metastasis [54]. Increased COX-2 and NF- $\kappa$ B expression are just some of the molecular factors that contribute to tumor recurrence [55,56]. Indeed, it was reported that PDT induces the expression of COX-2 that in turn lessens the efficacy of PDT and NF- $\kappa$ B resulting in reduced antitumor effectiveness [57].

## Liver

Liver cancer can directly arise in liver parenchyma or as metastasis of tumors in other parts of the body. The most common form is the human hepatocellular carcinoma (HCC), which affects hepatocytes, the principal type of liver cells. Other cancer types can characterize this organ, as result of malignant transformation in other liver cellular types, even if this circumstance is much less common [58].

It is well known that PGE2 promotes HCC, precisely mediating hepatoma cell growth and migration, as well as invasion. These effects are precisely mediated by PGE2/Akt/NF- $\kappa$ B pathway and evidenced in hepatocellular carcinoma cell lines (Huh-7 and Hep3B cells) after treatment with PGE2, EP4 receptor (EP4R) agonist, Akt inhibitor or NF- $\kappa$ B inhibitor, respectively. PGE2 and EP4R agonist in Huh-7 and Hep3B cells significantly increase the levels of Snail protein, which has been found to be up-regulated in HCC. It has been also demonstrated that PGE2 activates Akt/NF- $\kappa$ B signaling and then up-regulates Snail via the EP4R/EGFR promoting migration and invasion [59].

Recently, it has been reported that Y box-binding protein 1 (YB-1) is closely correlated with malignancy. PGE2 can also increase HCC cell invasion through up-regulation of YB-1 protein, and the EP1 receptor is mainly responsible of this regulation. Furthermore, it has been observed that YB-1 seems to be able to regulate the expression of a series of EMT-associated genes. This seems to indicate that YB-1 could have the potentiality to control the EMT process, known to play a critical role in HCC metastatic disease. Therefore, these findings reveal that PGE2 up-regulates YB-1 expression through EP1/Src/EGFR/p44/42 MAPK/mTOR pathway, which greatly enhances HCC cell invasion and metastasis [60].

Chemotherapy represents until now the most effective treatment for liver cancer, but drug resistance still is the major obstacle. Lipid metabolism plays a critical role in cancer

pathology, particularly due to elevated ether lipid levels. Recently, alkyl-glycerone-phosphate synthase (AGPS), an enzyme which catalyzes the critical step in ether lipid synthesis, has been demonstrated to be up-regulated in multiple types of cancer cells and primary tumors [61]. Silencing of AGPS in chemotherapy resistant hepatic carcinoma HepG2/ADM cell line results in reduced cell proliferation, increased drug sensitivity, cell cycle arrest and cell apoptosis. These effects are all achieved by decreasing intracellular concentration of lysophosphatidic acid (LPA) and PGE2 synthesis. This may determine a reduced activation of LPA receptor- and EP receptor-mediated PI3K/AKT signaling pathway, and an increased expression of several multi-drug resistance genes, such as MDR1, MRP1, ABCG2, B-catenin, caspase 3/8, Bcl-2 and survivin.

Thus, PGE2 in cancer cells seems also to represent an important downstream mediator of AGPS, which plays a pivotal role in cancer chemotherapy resistance [62].

## Lung

Lung cancer is one of the most common and deadliest cancer type in world. . There are diverse lung cancer types, but the forms more common are the following: small cell lung cancer and non-small cell lung cancer (NSCLC). NSCLC accounts for more than 80% of all lung cancers, with a 5-year survival about 15% on average [63]. Significant improvements have been made using conventional therapies. However, the low overall survival and poor prognosis of patients affected by lung cancer suggest that new treatments for this devastating disease are needed [64]

Macrophages in tumor tissues are usually known as tumor-associated macrophages (TAMs) and amount to about 60% of the tumor stroma. Growing evidence shows that cancer cells can recruit and subvert macrophages as active collaborators in their neoplastic program. Persistent activation of macrophages causes local chronic inflammation, production of cytokines and chemokines, promoting tumorigenesis [65]. TREM (Triggering Receptors Expressed on Myeloid cells) proteins are a family of immunoglobulin cell surface receptors expressed on myeloid cells [66], consisting of three members: TREM-1, TREM-2, TREM-3. TREM-1 is highly expressed and may predict cancer aggressiveness as well as disease outcomes in several cancer types, indicating as the expression of TREM-1 in macrophages may also be associated with lung tumor growth and progression [67]. Furthermore, TREM-1 expression in patients with NSCLC has been associated with cancer recurrence and poor survival, suggesting an important role in cancer progression of TREM-1 [68]. Recent studies have shown that lipid mediators, such as prostaglandins, modulate the expression of TREM-1. In particular, it has been observed that expression of TREM-1 is inhibited by PGD2 and PGJ2 in macrophages [69]. It has been also evidenced that lung tumor tissue has increased COX-2 expression as well as PGE2 production. Furthermore, the treatment of macrophages with PGE2 seems to induce an enhanced expression of TREM-1, while the treatment with COX-2 inhibitors attenuate the expression of TREM-1. Expression of TREM-1 in macrophages induced by PGE2 is evoked through the EP2 and EP4 receptor activation. Together, these data suggest that TREM-1 may be a critical link in the tumor microenvironment between tumor-associated macrophage activation, inflammatory response and cancer progression [70]

Recently, thromboxane A2 synthase (TXAS) and receptor (TXA2R) have been also documented to play a pivotal role in lung cancer development [71]. Given the close

relationship between COX-2 and TXAS, it has been firstly hypothesized and demonstrated that thromboxane A2 (TXA2) contributes to the oncogenic activity of COX-2. In particular, it has been found that single TXAS inhibitor/TXA2R antagonist or the dual TXA2 modulators (i.e., the dual blocker of TXAS and TXA2R) offer a similar inhibition of cell proliferation. Moreover, inhibition of TXA2 arrests cell growth in the G2/M phase and induces cancer cell apoptosis. These findings clearly demonstrate that TXA2 functions as a critical mediator for tumor-promoting effects of COX-2 in lung adeno-carcinoma cells. Indeed, dual TXA2 modulators and the single blocker of TXAS or TXA2R offer a similar inhibitory role in lung adenocarcinoma cell. Thus, TXA2 should be regarded as a critical molecule in COX-2-mediated tumor growth and a valuable target for future therapies against lung cancer [72].

Moreover, patients with rheumatoid arthritis (RA) appear to be at a higher risk of lung cancer (LC) [73]. Although the connection between RA and LC has been an active area of research for many years, the molecular pathogenesis of the disease process remains still unclear.

The roles of COX-2-derived TxA2 in LC has been shown to play a potential role in LC development through an auto-regulatory feedback loop. Indeed, it has been reported that an increased expression of COX-2 is present in lung adenocarcinoma specimens, with a concomitant increase in TXAS and PGES, but not PGIS, compared to normal lung tissues [74]. Increased levels of TXA2 have been also found in RA patients [75].

Interestingly, the positive feedback loop for the COX-2/TxA2 pathway has been shown to have a potential function in RA fibroblast-like synoviocytes (RA-FLS), which are known to play a key role in cartilage destruction. TXA2 is able to bind specifically with its receptor TP and activate several intracellular signals, including the ERK and PI3K/Akt pathways [76-78]. The transcription factor CREB can subsequently be activated by both the ERK and PI3K/Akt pathways, whereas NF- $\kappa$ B can only be activated by ERK activation [76]. NF- $\kappa$ B activation in turn determine an increase in the expression of COX-2, TXAS and other oncogenic factors, such as proliferating cell nuclear antigen (PCNA) and vascular endothelial growth factor (VEGF) [77,78]. Thus, there is a positive auto-regulatory feedback loop for the COX-2/TxAS pathway, that contributes to cell proliferation, invasion and angiogenesis in both LC and RA. Therefore, it is possible that COX-2-derived TXA2 could be monitored for the early detection of LC in RA patients, and targeting this molecular pathway may decrease the risk of LC in patients with RA [79]. Lung cancer stem cells (CSCs) are a subpopulation of cells that drive growth, invasiveness, and resistance to chemotherapy [80]. Prostaglandins are known to be implicated in the maintenance of CSCs malignant subpopulation [81]. Specifically, prostaglandin E2 has been demonstrated to be an important driver in proliferation of the CSC subpopulation [82]. Given the role of secretory phospholipase A2 (sPLA2) in prostaglandin production and the importance of sPLA2 in lung tumor growth and invasion in lung cancer, it has been hypothesized that sPLA2 play a significant role in maintaining the CSC phenotype and influence the function of CSC in non-small cell lung cancer. In particular, it has been seen that CSCs contain increased levels of sPLA2 protein and mRNA expression. Furthermore, knockdown and chemical inhibition of sPLA2 effectively decrease the CSC phenotype in non-small cell lung cancer and reduce tumor sphere formation *in vitro*. Thus, sPLA2 plays an important role in CSCs and may be an important therapeutic target for human lung cancer [83].



## Gastro-Intestinal Tract

Colorectal cancer (CRC) is one of the leading causes of cancer-related death, and chronic inflammation has been identified as a major risk factor for CRC onset. It is well known that PGs can protect the gastrointestinal tract by promoting wound healing, limiting the inflammatory response and maintaining of mucosal integrity [84]. However, increased PG production occurs in gastrointestinal mucosa of patients with inflammatory bowel disease (IBD). Furthermore, PGs can also modulate intestinal tumorigenesis. For example, PGE2 is clearly associated with tumor promotion in several experimental models, while PGD2 can have an tumor suppressive effect [85,86]. Specifically, PGE2 enhances cell proliferation, affecting the epidermal growth factor receptor signaling pathway, and inhibits apoptosis through the Bcl-2 and NF- $\kappa$ B activation. In addition, it also promotes angiogenesis through induction of VEGF [87]. However, the exact mechanisms through which PGs contribute to inflammation-associated intestinal tumorigenesis are not well understood. Ishikawa and Herschman reported that neither COX-1 nor COX-2 are critical in the formation of colonic tumors in a mouse model of colitis-associated cancer induced by the azoxymethane (AOM)/dextran sodium sulfate (DSS) [88]. In contrast, other groups have shown that pharmacological inhibition of COX-2 suppresses inflammation-associated colon tumorigenesis, while exogenous administration of PGE2 exerts the opposite effect [89,90]. Furthermore, Montrose and colleagues evaluated the extent of intestinal injury in ApcMin/+ mice with a genetic disruption of PGs synthesis using the DSS injury model. In particular, genetic deletion of cPLA2 (the rate-limiting step in the release of AA from membrane phospholipid stores) and mPGES-1 (the terminal synthase in the formation of PGE2) have been examined. They reported that the ability to maintain sufficient quantities of PGE2 was necessary for dampening the extent of acute DSS-induced inflammation within the intestinal mucosa. In addition, it has been observed that loss of PGE2 was associated with altered levels of other eicosanoids. In addition, cPLA2<sup>-/-</sup> mice and mPGES-1<sup>-/-</sup> mice showed much more extensive mucosal injury compared to WT mice following DSS exposure, that was linked to reduced PGE2 synthesis. The impact of such enhanced intestinal inflammation in DSS-treated mPGES-1<sup>-/-</sup> mice on intestinal tumorigenesis has been also investigated, and the resulting data showed that DSS administration increased the number of colon tumors regardless of mPGES-1 genotype. This effect may be a result of the extensive inflammation occurring in this organ site, masking any beneficial effects of mPGES-1 deletion. In contrast, the effects of PGE2 loss on tumor burden have been observed in the small intestines [91]. Indeed, reduced tumor formation in the small intestines has been promptly observed in ApcMin/+;mPGES-1<sup>-/-</sup> mice following DSS exposure, and it may result from a compensatory increase in PGD2. In fact, the tumor suppressive role of PGD2 has been highlighted by a study carried out by Park and colleagues. In which genetic deletion of hematopoietic prostaglandin D synthase in ApcMin/+ mice resulted in increased intestinal polyps [91,92]. Moreover, genetic deletion of COX-1 or COX-2 have no effect on the development of intestinal tumors induced through AOM/DSS, although COX-2 deletion protect against tumor formation generated by repeated injections with AOM alone [93]. It was also showed that mTORC1 (mechanistic target of rapamycin complex 1) plays an important role in the response of colon cancer cells to PGE2 administration. Indeed, stimulation of human colon cancer LS174T cells with PGE2 increased mTORC1 activity through EP4 receptor activation. Furthermore, PGE2 increased colon cancer cells proliferation as well as the number of colon cancer cells colonies grown in

matrigel and blocking mTORC1 by rapamycin or ATP-competitive inhibitors of mTOR abrogated these effects. In addition, stimulation of LS174T cells with PGE2 increased VEGF production, which was also prevented by mTORC1 inhibition. Taken together, these results show that mTORC1 is an important signaling intermediary in PGE2 mediated colon cancer cells growth and VEGF production, supporting a role for mTORC1 in inflammation-induced tumor growth [94].

Inhibitors of COX and PGE2, are promising molecules in CRC prevention, but they share significant toxicity [95]. Recent findings reveal that Ginger root can also inhibit COX-1 and -2 [96], by decreasing PGE2 concentrations and lowering the incidence of adenomas in subjects at normal risk for CRC [97,98]. Accordingly, it has been observed a significant increase in colonic mucosa concentrations of LTB4 after 28 days-administration of ginger root extracts in participants at elevated risk for CRC. No significant effect in the levels of any other eicosanoids, including PGE2 has been also found. Thus, future and larger studies are needed to clarify the effects of ginger root extracts in intestinal tumor formation, and they might be focused on other biomarker outcomes [99].

As above mentioned, several animal studies plainly indicate that COX-2 and the COX-2-derived PGE2 promote colorectal carcinogenesis and increased COX-2 expression in tumor tissues is associated with reduced survival among the CRC patients [100]. It is recognized that the use of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) generally decreases CRC risk. However, genetic variants in COX-inflammatory pathways may alter their potentiality as preventive agents. Associations between 192 single nucleotide polymorphisms (SNPs) and two variable nucleotide tandem repeats within 17 candidate genes and CRC risk have been evidenced and interactions between these polymorphisms and the effects derived of NSAID use on CRC risk have been also established. In particular, two intronic SNPs (rs11571364 in Arachidonate 12-lipoxygenase and rs45525634 in Prostaglandin E Receptor 2) have been associated with rectal cancer risk. These results may support the development of new genetically targets in cancer prevention strategies using NSAIDs [101].

Eventual associations between PTGS2 (encoding COX-2) gene polymorphisms and aspirin (the most common NSAID) in relation to CRC risk have been also investigated, since it has been demonstrated a significant prolongation of survival related to aspirin administration of patients with COX-2 expressing CRC. Indeed, epidemiological data suggest a significant reduction of the incidence and mortality rate of colorectal cancer administering 75 mg of aspirin every day for several years [102].

Among the genetic variants examined, it has been demonstrated that PTGS2 A-1195G polymorphism, giving rise to a low-activity enzyme, leads to increased risk of ulcerative colitis and CRC. These findings indicate that genetically determined low COX-2 activity is a risk factor for CRC development [103]. Surprisingly, no relations between aspirin use and PTGS2 A-1195G SNP have been detected in relation to CRC risk [104,105]. Aspirin acts as an irreversible inhibitor of COX, and recovery of COX activity, hence, depends on de novo synthesis. It is known that COX-1 is constitutively expressed in the intestine, whereas COX-2 expression is stimulated by cytokines during inflammation. Colon cells are nucleated, and therefore able to generate de novo COX. Aspirin is 170 times more potent in inhibiting COX-1 than COX-2 [106]. Thus, because the evaluated aspirin concentration is sufficient to inhibit COX-1, but not COX-2 in the colon, PTGS2 polymorphisms play a minor role in clinical effects of aspirin in preventing CRC.

The involvement of PGE<sub>2</sub> in cancer development is nowadays well demonstrated. In contrast, the role of PGD<sub>2</sub> in chronic inflammation and tumorigenesis is less understood. Iwanaga and coworkers investigated the action of PGD<sub>2</sub> in colitis and colitis-associated colon cancer (CAC), by using genetically modified mice as an established model of inflammatory colon carcinogenesis. They observed that systemic genetic deficiency in hematopoietic PGD synthase (H-PGDS) aggravates colitis and accelerates tumor formation in a manner associated with increased TNF $\alpha$  expression. Mast cell-specific H-PGDS deficiency also aggravates colitis and accelerates CAC. On the contrary, treatment with a PGD<sub>2</sub> receptor agonist inhibits colitis and CAC. Together, these results suggest mast cell-derived PGD<sub>2</sub> as an inhibitor of colitis and CAC, with implications for its potential use in preventing or treating colon cancer [107]. The effect of aspirin in an experimental model of esophageal adenocarcinoma has been also evaluated. In a rat model of gastroenteroesophageal reflux, it has been detected that aspirin decreases PGE<sub>2</sub> and increases LXA<sub>4</sub> levels in esophageal tissue, but no crucial role in preventing the development of esophageal adenocarcinoma has been found [108].

Furthermore, in gastric cancer cells, COX-2 affects the current of delayed rectifying potassium channel (HERG), that is associated with biological behaviour of gastric cancer cells [109-110]. It has been observed that COX-2 influences the HERG current in gastric cancer cells without affecting the expression of HERG protein in gastric cancer cells. Thus, this finding indicates as regulation of ion current may take place after the translation process. COX-2 regulates human HERG current in gastric cancer cells by altering the levels of cAMP, that interacts with HERG protein and alters HERG current [111].

## Pancreas

Pancreatic cancer is one of the most deadly cancer types in the world, with a 5-year survival rate of about 5%-6%. Its diagnosis usually is performed in the clinical disease process, when curative surgery only represents an option. Thus, the discovery and identification of the early changes in pancreatic tumorigenesis are need for its prevention. Since inflammation represents a key factor in malignant transformation and contributes to genetic changes and DNA damage, its role in pancreatic cancer is of particular interest. Recurring episodes of pancreatitis result in fibrosis, chronic inflammation, and the eventual destruction of the gland [112]. In addition, it has been observed that patients with the longest duration of obesity and diabetes have at the greatest risk for pancreatic cancer [113]. One of the mechanisms proposed for this association is the higher concentration of AA in adipose tissue compared to healthy people. Several epidemiological studies, indicating that the use of NSAIDs reduces the incidence of various solid tumors, also evidence the role of eicosanoids in the carcinogenic pancreas process [4]. However, the exact mechanism linking NSAIDs and pancreatic cancer is still not well understood. Anderson and coworkers conducted a prospective study with 28000 post-menopausal women and demonstrated a decreasing trend in pancreatic cancer incidence in women with more frequent aspirin use [114]. The relationship between COX-2 and pancreatic cancer has been evaluated in multiple studies with the majority of the evidence demonstrating up-regulated COX-2 expression in pancreatic cancer at both the mRNA and protein levels. Indeed, it has been seen that levels of COX-2 mRNA increase 60-fold in pancreatic cancer compared to normal tissue [115]. Furthermore,

increased COX-2 mRNA and protein levels have been also detected in pancreatic carcinoma cell lines BxPC-3, Capan-1, and MDAPanc-3. NSAIDs can inhibit cell proliferation in all the pancreatic cell lines studied in a dose-dependent manner and such inhibition correlate with the expression of COX-2 [116]. Moreover, Maitra and coworkers evaluated COX-2 expression not only in pancreatic adenocarcinoma but also in its precursor, pancreatic intraepithelial neoplasia (PanIN). This study clearly suggests tumorigenic activity of COX-2 in pre-invasive pancreatic lesions and a potential role of COX-2 inhibitors as chemopreventive agents against pancreatic cancer. Over-expression of COX-2 in tumor leads to increased levels of PGE<sub>2</sub>, that shows several tumorigenic effects and is implicated in the inhibition of apoptosis and the induction of proliferation and angiogenesis [117]. Furthermore, Eibl and coworkers [118] demonstrated that PGE<sub>2</sub> in turn subsequently increases VEGF secretion in a subset of pancreatic cancer cell lines. In addition, PGE<sub>2</sub> seems to mediate pancreatic cancer cell invasion through induction of matrix metalloproteinase-2 expression. Extracellular signal-regulated kinase (ERK)/Ets-1 pathway seems to be involved in such induction [119]. In addition, 5-LOX expression is also up-regulated in pancreatic adenocarcinoma [120]. In several pancreatic cancer cell lines, the expression levels of both 5-LOX and its downstream metabolite LTB<sub>4</sub> have been found to be significantly up-regulated in pancreatic tumors compared with normal pancreatic tissue [121]. At the same time, it is well established that 5-LOX plays an important role in pancreatic tumor progression, while few studies have investigated underlying mechanism in this relationship. Notably, Ding and coworkers showed that 5(S)-hydroxyeicosatetraenoic acid, a 5-LOX metabolite, stimulates pancreatic cancer cell proliferation in a time- and concentration-dependent manner and also demonstrated that such effect is mediated by the MEK/ERK and PI3 kinase/AKT pathways [122,123]. Moreover, the same group showed that both the general LOX inhibitor (NDGA) and the 5-LOX inhibitor (Rev5901) induce apoptosis in four different pancreatic cancer cell lines, emphasizing the participation of 5-LOX in tumor progression [124]. In addition, a follow-up study performed by Tong clearly showed that mitochondria-mediated pathway is also involved in LOX inhibitors-induced apoptosis. Indeed, LOX inhibitors decrease Bcl-2 and Mcl-1, increase Bax expression in human pancreatic cancer cells and also induce cytochrome-c release as well as caspase-9 activation [125]. These studies strongly highlight the relationship between 5-LOX and cell apoptosis in tumor microenvironment. In addition a phase II trial studied the effects of chemotherapeutic Uracil/Tegafur plus Leucovorin and Celecoxib (a common NSAID) combined with radiotherapy in patients with locally advanced pancreatic cancer, showing no improved response compared to controls and resulting in substantial gastrointestinal toxicity [126]. In a phase II trial of gemcitabine/Irinotecan (common chemotherapeutic drugs) and Celecoxib in patients with inoperable pancreatic cancer, the addition of Celecoxib demonstrated an increase of the percentage of patients achieving a one-year overall survival from about 3 months to 9 months and increased overall survival from about 6 months to 18 months [127]. It has been also investigated the effect of Zileuton, a 5-LOX inhibitor, in animal models of pancreas carcinoma. In pancreatic cancer studies using the Syrian hamster model with BOP-induced pancreatic cancer, Zyflo (an extended release formulation of Zileuton) has been found to reduce the incidence and size of pancreatic cancer both alone and in combination with a COX-2 inhibitor [128]. Besides, it has been observed that human pancreatic duct epithelial cancer cells generated increase LTB<sub>4</sub> levels *in vitro* following treatment with omega-6 FA. Human Pancreatic Duct Adenocarcinoma (PDAC) with increased 5-LOX over-expression also shows increased mast

cell infiltration. Since K-ras mutations are observed in the large majority of PDAC patients, *EL-Kras* mouse model of pancreatic cancer has been further investigated. 5-LOX mediates neoplastic lesion development and mast cell infiltration in *EL-Kras mice* through LTB<sub>4</sub> synthesis. Indeed, *EL-Kras/5-LOX<sup>-/-</sup>* mice seem to develop fewer pancreatic lesions and show decreased mast cell infiltration when compared with *EL-Kras/5-LOX<sup>+/+</sup>* mice. Thus, mutant Kras induces pancreatic neoplastic lesions and this effect is dependent on 5-LOX activation. Interestingly, because 5-LOX acts downstream of mutant Kras in mediating inflammation, it may represent a potential chemopreventive and therapeutic target in pancreatic cancer [129].

### *Prostate and Ovary*

Prostate cancer is the most common form of malignancy and the second leading cause of cancer-related deaths in men in the United States [130]. Epidemiological studies and experiments with laboratory animals have repeatedly suggested a link between consumption of high-fat “Western” diets and clinical prostate cancer [131]. Recent analysis points towards a role of omega-6 fatty acids, such as AA, in the promotion and progression of prostate cancer. 5-LOX, which participate to eicosanoids synthesis from AA, is not expressed in normal prostate epithelium, but it is highly expressed both in human and mouse prostate tumor tissues as well as in prostate cancer cell line. Interestingly, it has been observed that inhibition of 5-LOX blocks production of 5-LOX metabolites and induces apoptosis both in androgen-sensitive as well as androgen-independent prostate cancer cells [132]. Moreover, MK591, a specific inhibitor of 5-LOX activity, remarkably inhibits the expression of *c-Myc* mRNA. C-Myc is a basic helix-loop-helix leucine-zipper transcription factor that dimerizes with its partner MAX and associates with gene-promoters to induce downstream genes transcription [133]. Myc is up-regulated in almost all cancer types, and is the subject of intense investigation, because it is involved in a broad spectrum of biological functions including cell proliferation, metabolism, differentiation, sensitization to apoptotic stimuli, and genetic instability, events intimately associated with cancer initiation, promotion and progression [134]. Because of its central role in oncogenesis, c-Myc has emerged as a promising molecular target for therapy of cancers characterized by alteration in this oncogene. Inhibition of 5-LOX dramatically reduces the protein level, nuclear accumulation, DNA-binding, transcriptional activity, and oncogenic function of c-Myc in prostate cancer cells. Interestingly, MK591 does not alter the basal level of c-Myc and its target proteins in normal fibroblasts, which do not express 5-LOX, suggesting that the oncogenic c-Myc function in transformed cells is specifically suppressed by inhibition of the 5-LOX activity. It has been also reported that Myc-driven prostate tumors express high levels of 5-Lox, and Myc-transformed prostate cancer cells depends on 5-Lox activity for their survival as well as metastatic abilities. These findings indicate that the activity of 5-Lox is required for the oncogenic functions of *c-Myc* in prostate cancer cells, and suggest that prostate cancer cells promote their survival and metastasis using AA, a common fatty acid in “modern-diets” via metabolic conversion through the 5-Lox pathway. Since deregulation of Myc represents one of the most common abnormalities in human malignancy and is frequently observed in aggressive cancer cells, these findings suggest the possibility to effectively regulate c-Myc function through inhibition of 5-Lox activity by specific chemical inhibitors, such as MK591 [135]. The effect of down-regulating COX-2 on human ovarian cancer cells growth has been also investigated. Silencing of COX-2 through specific siRNA in ovarian cancer cells may

inhibit VEGF, matrix metalloproteinase (MMP)-2 and MMP-9 protein expression, which correlate with reduced cell motility. Thus, silencing COX-2 leads to inhibition of ovarian cancer cells invasion, indicating the potential of targeting COX-2 as a novel gene therapy approach for preventing metastasis in ovarian cancer [136].

## Conclusion

Lipid metabolism is very complex and regulated by a complex signaling network in cancer cells. The same lipid molecules (via different signaling pathways or under different conditions) can generate different metabolites. Clinical, epidemiological, in vitro and in vivo studies highlights the relationship between inflammation and cancer progression. COXs, LOXs and derived eicosanoids show pro-inflammatory effects and are over-expressed during the early steps of malignant transformation. In addition, they directly contribute to cancer cell proliferation. Thus, future studies about the lipid metabolism pathways in cancer cells are needed. Certainly, a deeper understanding of the complex relationship between inflammation and cancer might offer new rational targets, i.e., eicosanoids, for cancer therapy.

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# Allergic Disease and Mast Cells: The Role of Eicosanoids in the Pathogenesis and Therapy

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## Introduction

### Heterogeneity of Mast Cells

Mast cells are tissue cells that are distributed widely throughout mammalian tissues. The histological appearance of mast cells and basophils has fascinated scientists ever since the innovative experiments of Paul Ehrlich in the 1870s, which showed them to stain metachromatically with aniline dyes. Indeed dyes such as toluidine blue are used routinely today to visualize mast cells and basophils [1].

They are present at both mucosal and serosal surfaces, in lymphoid tissues and connective tissues, and are associated with nerves, blood vessels and tumours. The tissue disposition of mast cells distinguishes them from basophils, which are essentially blood leucocytes and invade tissues only during inflammatory events. Human mast cells occur from CD34<sup>+</sup> pluripotent stem cells in the bone marrow, circulate in the blood as precursors, then home to tissues. They mature under the influence of stem cell factor (SCF), also called c-kit proto-oncogene ligand, local cytokines and other factors. SCF is released as a soluble

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mediator, but it is also expressed on the cell surface of stromal cells [2]. In CD34+ peripheral blood-derived human mast cells, exposure to IFN- $\gamma$  results in upregulation of Fc $\gamma$ RI mRNA and the receptor is observed on the cell surface. Instead, the removal of SCF causes the apoptosis of the mast-cells.[3, 4]. Mast cells are long lived and are reported to proliferate in association with IgE-dependent activation and in the presence of IL-4 [5].

## Preformed Mediators in Granules

The acute reactions that occur as a result of mast cell activation are initiated as a consequence of degranulation and the generation of lipid-derived mediators, whereas more chronic mast cell-mediated symptomology is an outcome of the delayed generation of chemokines, cytokines and growth factors which follow enhanced gene expression. The process of degranulation occurs within seconds of mast cell activation and the initial rapid phase is essentially complete within 5–10 minutes [6]. Although mast cell proteases such as tryptase, chymase and carboxypeptidase, constitute the major components of mast cell granules, histamine is the predominant granule mediator of acute reactions to mast cell activation [7, 8]. Histamine acts through at least four G protein-coupled histamine (H1 through H4) receptors [9]. H1 receptors reside primarily on bronchial smooth muscle, endothelial cells, and certain neurons, and are largely responsible for bronchoconstriction, increased vascular permeability leading to hives and allergic rhinitis, through separation of endothelial cells, itching, and pain. H2 receptors are located on vascular smooth muscle and gastric parietal cells and, thus, mediate vascular dilatation and gastric secretion. Collectively, the H1 and H2 receptors contribute to the wheal and flare reaction in skin. H3 receptors are present primarily in the CNS, whereas H4 receptors are expressed on immunocompetent cells, including basophils, mast cells, and eosinophils, and mediate chemotaxis [10].

The primary effects of histamine release from mast cells and basophils are increased vascular permeability, vasodilatation and bronchial constriction, which are readily reversed by antihistamines.

The sedative side effect of classical antihistamines (H1 blockers) is now attributed to reversal of the normal CNS function of histamine in provoking wakefulness and was obviated by the introduction of antihistamines that do not penetrate the CNS. Antagonists of H3 and H4 receptors are under preclinical and clinical investigation as potential therapeutic agents for various CNS disorders but also for the treatment of allergic rhinitis, asthma, pruritis in atopic dermatitis, and inflammatory pain [11].

In rodent the mast cells contain significant quantities of 5-hydroxytryptamine (5HT, serotonin) within the granules [12].

The proteases of mast cells constitute between 30–50% of the total protein content of mast cells; thus in terms of mass, they represent the major group of mediator released by exocytosis [13]. The mast cell proteases, released from activated mast cells, have been implicated in allergic inflammation and tissue remodeling, however recent evidence suggests that they may also have a protective role help protect against allergic inflammation [14, 15].

## Eicosanoids and Interleukins

The bioactive eicosanoids, leukotriene C<sub>4</sub> (LTC<sub>4</sub>), LTB<sub>4</sub>, prostaglandin D<sub>2</sub> (PGD<sub>2</sub>) and, under specific circumstances, PGE<sub>2</sub>, are generated and released almost simultaneously with the granule-associated mediators. The initiating process in the generations of these molecules is receptor-mediated activation of phospholipase (PL) A<sub>2</sub>, with consequential hydrolyses of arachidonyl-containing phospholipids, primarily phosphatidylcholine, yielding free arachidonic acid [16]. These mediators, often absent in the resting mast cells, are also well produced during IgE-mediated activation, and they are, other the leukotriene, the prostaglandin D<sub>2</sub> (PGD<sub>2</sub>) and the cytokines [17]. Of particular interest in humans is the production of tumor necrosis factor (TNF- $\alpha$ , $\beta$ ), and interleukin (IL)-4, IL-5, IL-6, IL-1 $\beta$  and IL-13 [18].

## Atopic Asthma

Patients with atopic asthma have bronchial and bronchoalveolar T cells displaying a type 2-predominant cytokine profile. It was reported that patients with atopic asthma had leukocytes (mainly T cells and fewer eosinophils and mast cells) in their bronchoalveolar lavage fluid that express a predominance of IL-4 and IL-5 mRNA, with little IFN- $\gamma$  mRNA. However, this classic cytokine pattern is also characterized, by modest increase in IL-2 mRNA levels. Similarly, other Authors found that grass pollen allergen challenge can activate type 2 cytokine production. They derived T-cell clones from bronchial and nasal airway mucosa of patients with atopic asthma and allergic rhinitis [19]. Finally, increased IL-13 mRNA and protein levels were found in the bronchoalveolar lavage fluid of allergen-challenged asthmatic patients, suggesting that this type 2 cytokine may contribute to the allergen-induced inflammatory response of asthma [20].

Other Authors [21] proposed a model for the pathogenesis of atopic asthma in which predominantly type 2 cytokine (IL-4 and IL-5) production by T cells, in response to allergens or viral antigens, leads to bronchospasm and bronchial inflammation. Eosinophils are the primary inflammatory effector cells in this model, in conjunction with mast cells. We would propose that these eosinophils contribute to the type 2 cytokine state by production of IL-4 and IL-5. As noted above, in a type 2 cytokine (IL-4) milieu, CD81 T cells, responding to a respiratory viral infection may switch from production of IFN- $\gamma$  to production of IL-5, thereby inducing the pulmonary eosinophilic inflammation characteristic of atopic asthma [22]. The role of T cells and cytokines in asthma has been evidenced [23]. In particular, the inflammatory nature of the disease and the predominant involvement of IL-4 and IL-5 in atopic asthma, however has been emphasized a potential role for type 1 cytokines in the etiology of a minority of cases of asthma [24]. Recently, the model of type 1 and type 2 cytokines and asthma have been expanded including nitric oxide (NO). Thus NO from airway epithelial cells inhibits IFN- $\gamma$ . This decrease in IFN- $\gamma$  levels results in increased IL-4 and IL-5 levels, which, in turn lead, to increased IgE levels and recruitment of eosinophils into the lung [25, 26]. With relation to that the development of a vaccine against asthma based on modulation of the type 1 and type 2 cytokine profile was proposed [27].

Glucocorticoids are one form of therapy for asthma; however, the reason why some patients do not respond to glucocorticoids is not well understood. The comparison [28]

between patients with steroid-sensitive and steroid-resistant asthma, before and after 1 week of prednisone therapy, demonstrated that the steroid-sensitive group had a decrease in the number of bronchoalveolar lavage derived cells expressing IL-4 and IL-5 mRNA, by *in situ* hybridization, and an increase in the number of cells expressing IFN- $\gamma$  mRNA. In contrast, the steroid-resistant patients had no decrease in the number of cells expressing IL-4 or IL-5 mRNA but did have a decrease in the number of cells expressing IFN- $\gamma$  mRNA. A pathophysiologic mechanism for steroid-resistant asthma was postulated involving a combination of type 1 and type 2 cytokine dysregulation, with impairment of glucocorticoid binding to T cells.

Tissue remodeling is a characteristic feature of asthma and other lung diseases. The mechanisms behind the relationship between mast cells and fibrosis/tissue remodeling are unclear. It has been shown that mast cells may have a substantial effect on tissue remodeling, especially in the airway, leading to smooth muscle hypertrophy and mucus hypersecretion, by releasing proteases, such as tryptase, and growth factors [29]. These cells also have an effect on epithelial damage, as well as, on basement membrane thickening in patients with allergic asthma [30], to airway smooth muscle hypertrophy, compared to healthy controls. Tryptase and other proteases, such as chymase, are abundant in mast cell granules. Therefore, mast cells seem to play a crucial role in airway remodeling by releasing tryptase onto smooth muscle and epithelium. These data suggest multiple mechanisms by which mast cells can regulate tissue fibrosis and repair, and provide evidence for the direct involvement of mast cells in fibrosis and human connective tissue remodeling.

## Allergic Rhinitis

Allergic rhinitis (AR) is the most common allergic disease in the world. It affects up to an estimated 40% of children and 25% of adults. The pathophysiology of AR shares many similarities to allergic asthma and the two diseases are often considered manifestations of 'one airway, one disease' [31].

Mast cells constitutively reside in the nasal mucosa and do not normally venture into the superficial airway epithelium. Mast cells within the subepithelium phenotypically are both tryptase (MCT) positive and tryptase/chymase (MCTC) positive. With allergen exposure, mast cell migration to, and proliferation within, the epithelium occurs [31]. However, these epithelial mast cells predominantly express only tryptase (MCT) and are selectively increased in AR [32, 33].

Mast cell degranulation is evidenced by elevated tryptase, histamine, LTB<sub>4</sub>, LTC<sub>4</sub> and PGD<sub>2</sub> levels in the nasal lavage fluid of individuals with AR following nasal allergen provocation [31-33]. These mediators contribute to the sneezing, pruritus, rhinorrhea and nasal congestion, characteristic of early-phase symptoms of AR. Histamine is a major mediator, inducing vasodilation, increased vascular permeability and increased glandular secretion. In addition, histamine acts on the sensory nerve endings of the trigeminal nerve to cause sneezing. A strong Th2 cytokine expression profile (TNF- $\alpha$ , IL-4, IL-5, IL-6 and IL-13) follows mast cell activation and is believed central to the late-phase reaction. Mast cells induce eosinophilic infiltration through the release of platelet activating factor (PAF) and LTB<sub>4</sub>; and the upregulation VCAM-1 expression on endothelial cells. Eosinophil survival is promoted through mast cell release of granulocyte macrophage-colony stimulating factor

(GM-CSF) and IL-5. Additionally, histamine up-regulates CCL5 and GM-CSF, while IL-4, IL-13 and TNF- $\alpha$  up-regulate CCL11 and CCL17, further contributing to the late-phase eosinophilic/T cell infiltration. Clinically, this leads to an increase in nasal mucosal thickening with increased nasal airway resistance [34]. Of direct relevance is the pathophysiology behind the nasal hyper-responsiveness found in AR. There is evidence that this hyper-responsiveness is the result of exaggerated neural reactivity, with NGF being involved [35].

## Allergic Eye Disease

Ocular allergy occurs in the allergic population (36). The location of mast cells in close proximity to the external environment, in the mucosa of the eye, allows for exposure of these cells to allergen, thereby facilitating crosslinking of membrane-bound IgE, which leads to degranulation and release of inflammatory mediators. The two most common forms of ocular allergy are seasonal and perennial allergic conjunctivitis. Of these two forms, seasonal allergic conjunctivitis, is the more common [37]. Seasonal allergies are triggered by aeroallergens that have a botanical periodicity, such as tree, grass, and weed pollens which abound in spring and late summer/fall [38]. Patients sensitive to those allergens tend to present most frequently during one or more of those seasons. Perennial allergies, by contrast, are triggered by environmental allergens commonly found in the home, such as dust mites, mold spores, or animal dander, which are problematic for patients all year long [38].

Mast cell activation and migration within and around the conjunctival epithelium is one of the histopathologic features of severe chronic allergic conjunctivitis, atopic keratoconjunctivitis (AKC), and vernal keratoconjunctivitis (VKC) (39,40). Possible interactions of mast cells and conjunctival epithelial cells were investigated *in vitro*, using coculture models, and it has found that CCL2 expression in mast cells was upregulated by coculture, suggesting that the degranulation of mast cell, may have a role in the pathophysiology of AKC and VKC [41]. In normal individuals, mast cells are abundant in the conjunctival stroma, with an estimated 50 million cells residing at this environmental interface [42]. In symptomatic allergic patients, an increase in mast cells with evidence of degranulation is seen in conjunctival biopsies [43]. In addition to common mast cell mediators, such as histamine and cytokines, chemokines released from activated mast cells mediate late-phase reactions, by recruitment of additional inflammatory cells. Mast cells residing within the conjunctiva express CCR3 and the use of a CCR3 antagonist in a mouse model of allergic conjunctivitis ablated both the early and late-phase reactions [44].

## Anaphylaxis

Anaphylaxis is an acute, severe, systemic reaction to a foreign stimulus that is often thought to be associated with mast cell activation. The strongest evidence of a role for mast cells in anaphylaxis comes from assessments of serum tryptase levels during anaphylaxis [45]. Serum levels of tryptase, which predominantly arise from mast cell degranulation, peaks 1–2 h following the onset of IgE-mediated anaphylaxis [45]. Classical IgE-dependent anaphylaxis occurs upon exposure to specific antigens including venoms, latex, and

pharmaceutical agents. In addition to IgE-mediated mast cell activation, anaphylaxis may be elicited by certain agents or stimuli that activate mast cells independent of IgE. IgG and complement receptors expressed on mast cells may contribute to these IgE-independent events [46-48]. The anaphylaxis is considered a systemic event; the presence and activation of mast cells in specific organs may play a critical role in the severity. Cardiac mast cells *in vitro* release many of the classic mast cell mediators of anaphylaxis including PAF. This is a critical factor in the development of anaphylactic shock, because induces hypotension and cardiac dysfunction [49]. In the heart, mast cells are located between myocardial fibers, around blood vessels and in the arterial intima. Activation of these critically positioned mast cells may directly contribute to cardiopulmonary failure [50]. It is known that individuals with recurrent anaphylaxis tend to have more dermal mast cells than those without anaphylaxis. Mastocytosis, a disease characterized by the pathologic accumulation of mast cells in tissues, is often associated with spontaneous episodes of hypotension and has served as a unique disease model [51, 52]. However, it is important to note that there are instances of anaphylaxis not associated with tryptase elevation [53].

## Atopic Dermatitis

Mast cells are increased in a variety of chronic inflammatory skin disorders, as atopic dermatitis (AD) [54]. However, the precise contribution of this mast cell presence to the pathophysiology of AD is not understood. Therefore, biopsies of AD lesions demonstrate an increase in mast cell numbers as compared with uninvolved sites [55, 56]. Tryptase and activation of proteinase-activated receptor-2 (PAR-2) may contribute to the pruritus seen in AD, as tryptase is reported to be increased up to fourfold in AD patients and PAR-2 expression is markedly enhanced on primary afferent nerve fibers in skin biopsies from patients with AD [57]. However, the contribution of the histamine of mast cell to cutaneous itching in AD is questionable [58]. A biphasic immunological pattern has been suggested, starting with a Th2-type allergic reaction, being allergen-specific, followed by a Th1-type allergic reaction, non-allergen specific. The Th2-type reaction is important in induction of inflammation, whereas Th1-type reaction is responsible for maintenance and aggravation of the inflammation, representing the chronic phase of AD [58, 59]. In AD, inflammation appears to be mediated by neuropeptides such as substance P, calcitonin gene related peptide, vasoactive intestinal peptide and NGF [60-62].

## Mast Cells Therapeutics

The symptoms of type I type allergies, such as rhinitis, asthma, and urticaria, have traditionally been associated with inflammation mediators of the mast cells. Two of the mediators released by mast cells on activation by different stimuli are histamine and leukotrienes (LTs). H<sub>1</sub>- and H<sub>2</sub>-antihistamines and LTC<sub>4</sub> synthesis inhibitors or receptor antagonists are the drugs commonly used in clinical practice, to antagonize the mast cell mediators. Allergic symptoms have generally been treated with success by these drugs. However, there are numerous patients who do not obtain sufficient symptom-relief, even after administration of high doses of drugs of the above to classes.

Mast cells drugs may be classified into those directed to cell membrane targets (membrane receptors), to intracellular targets (cell signaling, gene expression) or to extracellular targets (released mediators). Cell membrane target drugs include the following therapeutical classes: chromones [63],  $\beta_2$  agonists [64], omalizumab [65], CCR3 antagonists [66],  $\text{Ca}^{++}$  and  $\text{K}^+$  channel antagonists [67], and anti CD63 antibody [68]. Chromones,  $\beta_2$  agonists, omalizumab are in clinical use, CCR3 antagonists have been used in animal,  $\text{Ca}^{++}$  and  $\text{K}^+$  channel antagonists, and anti CD63 antibody are in pre-clinical phase. The mechanism of action of chromones is potential disruption of  $\text{Ca}^{++}$  influx, chloride ion transport and exocytic processes [63]. Beta-2 agonists increase cytosolic cAMP levels through binding of  $\beta_2$  receptors [64]. Omalizumab binds free IgE, resulting in decreased Fc $\epsilon$ RI membrane expression (65). CCR3 antagonists block chemotaxis and degranulation [66].  $\text{Ca}^{++}$  and  $\text{K}^+$  channel antagonists disrupt ions influx, with attenuation of degranulation and chemotaxis [67]. Anti CD63 antibody interfere with cellular adhesion to  $\beta_1$  integrins and blocks Fc $\epsilon$ RI-induced degranulation via impairment of Gab2-PI3k pathway [68].

Intracellular target drugs comprise the following therapeutic class: glucocorticoids [69], Syk kinase inhibitors [70], and MAPK inhibitors [71], 5-LO- inhibitor [72].

Among these only the glucocorticoids are in clinical use, whereas Syk kinase inhibitors and MAPK inhibitors have been evaluated in clinical trials. The mechanism of action of glucocorticoids is transcription regulation of numerous inflammatory genes [69]; Syk kinase inhibitors block the IgE-Fc $\epsilon$ RI-mediated downstream signaling (phosphorylation) [70]; and finally MAPK inhibitors block phosphorylation of multiple intracellular proteins (including transcription factors) that are involved in cellular proliferation, differentiation, survival and chronic inflammation [71]. Among these classes of drugs only 5-LO-inhibitor, CysLTR1 antagonists,  $\text{H}_1$  and  $\text{H}_2$  receptor antagonists are in clinical use, while PDE4 inhibitors,  $\text{H}_3$  receptor antagonist have been evaluated in clinical trials.

Extracellular target drugs include the following therapeutic class: PDE4 inhibitors [73], tryptase inhibitors [74], CysLTR1 antagonists [75],  $\text{H}_{1-4}$  receptor antagonists [76], PAR-2 antagonists [77], and DP and CRTH-2 receptor antagonists [78]. Finally, tryptase inhibitors,  $\text{H}_4$  receptor antagonist, PAR-2 antagonists, and DP and CRTH-2 receptor antagonists are in pre-clinical phase. The mechanism of action of PDE4 inhibitors is to block the hydrolysis of cAMP to 5'AMP [73], that of 5-LO- inhibitor is to block the conversion of arachidonic acid to  $\text{LTA}_4$ , which subsequently prevents CysLT formation [72], that of tryptase inhibitors is to block the protease activity of tryptase [74], that of CysLTR1 antagonists is to block the binding to and effects of CysLT on target cells [75], that of  $\text{H}_{1-4}$  receptor antagonists is to block the binding to and effects of histamine on target cells [76], that of PAR-2 antagonists is to block PAR-2 receptor signaling following activation by proteases (e.g., tryptase) [77], and finally, that of DP and CRTH-2 receptor antagonists is to block the binding and the effects of  $\text{PGD}_2$  on target cells [78].

## Conclusion

The mast cells clearly have a central role in the pathogenesis of allergic diseases. They contribute to the chronic inflammatory response that causes the airway remodeling. However,

mast cells also have a central role in the onset of the allergic inflammatory response, caused by the synthesis of IgE deriving from B-lymphocytes, induced by Th2 lymphocytes.

Numerous inhibitors have been developed against individual components of signaling pathways that are involved in the activation and degranulation of MCs.

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# **Eicosanoids in Rheumatic Diseases: Patho-physiology and Targets for Therapies**

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## **Abstract**

Eicosanoids have an important role in the pathogenesis of rheumatic diseases and a number of enzymes and receptors involved in eicosanoid biosynthesis or action constitute valuable therapeutic targets. Among the pro-inflammatory eicosanoids, prostaglandin E2 (PGE2) is considered a key mediator of various inflammations; this includes swelling, fever and inflammatory pain. Prostaglandin I2 and thromboxane A2 regulate platelet aggregation and leukotriene B4 is one of the most powerful chemotactic agents. Prostaglandins of the J2 series decreases pro-inflammatory cytokine release and are implicated in the resolution phase of inflammation. In inflammatory rheumatic diseases, including rheumatoid arthritis, spondyloarthritis, osteoarthritis, gout and in autoimmune rheumatic diseases including systemic sclerosis, the arachidonic acid cascade is activated by cytokine-dependent enzyme induction, and the activity and/or expression of components of several pathways. Several enzymes of the arachidonic acid cascade or eicosanoid receptors are well-recognized targets of anti-inflammatory drugs. More simply, they can reduce symptoms of inflammation in rheumatic diseases. A more exciting idea is that PGD2 pathway might possess anti-inflammatory properties which could be utilized in future treatment strategies.

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## Introduction

Eicosanoids are a cluster of lipid autacoids which control many physiological and pathological processes often in opposing directions [1]. Derived from polyunsaturated fatty acids such as arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), these lipids display unique properties. Their role in inflammation is indeed distinct from that of other lipids derived from the same precursor [2]. Prostaglandins (PGs) and thromboxane (prostanoids), formed by cyclooxygenase (COX), leukotrienes (LTs) and lipoxins (LXs) formed by lipoxygenases (LOX) [3, 4], and epoxyeicosatrienic acid (EETs) by cytochrome P450 enzymes [5] are included in this family. These metabolites have opposing effects in inflammation, both proinflammatory and anti-inflammatory. Among the pro-inflammatory eicosanoids, prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) is considered a key mediator of various aspects of inflammation; these include swelling, fever and inflammatory pain [6]. Prostaglandin I<sub>2</sub> (PGI<sub>2</sub> or prostacyclin) and thromboxane A<sub>2</sub> (TXA<sub>2</sub>) regulate platelet aggregation, and leukotriene B<sub>4</sub> (LTB<sub>4</sub>) is one of the most powerful chemotactic agents [6]. Cysteinyl leukotrienes modulate vascular and smooth muscle responses [1]. Prostaglandins of the J<sub>2</sub> series (e.g., PGJ<sub>2</sub>,  $\Delta$ 12,14-PGJ<sub>2</sub> and 15-deoxy- $\Delta$ 12,14-PGJ<sub>2</sub>) via ligation to the nuclear receptor PPAR- $\gamma$  decreases pro-inflammatory cytokine release and are implicated in the resolution phase of inflammation [2, 7]. The diversity of their actions arises due to metabolites being synthesized via discrete enzymatic pathways and because they elicit their response via different receptors. Their biosynthesis is significantly increased in inflamed tissue and they contribute to the development of the cardinal signs of acute inflammation. In inflammatory rheumatic diseases including rheumatoid arthritis (RA), spondyloarthritis (SpA), osteoarthritis (OA), gout and in autoimmune rheumatic diseases including systemic sclerosis the arachidonic acid cascade (part of the eicosanoid pathway) is activated by cytokine-dependent enzyme induction, and the activity and/or expression of components of several pathways (COX, 5-LOX and 15-LOX) are up-regulated. Several enzymes of the arachidonic acid cascade or eicosanoid receptors are well-recognized targets of anti-inflammatory drugs; this can reduce symptoms of inflammation in rheumatic diseases. Non-steroidal anti-inflammatory drugs (NSAIDs) have an eicosanoid-depressing and anti-inflammatory action. NSAIDs are first-line drugs for treatment of inflammation and pain in RA, OA and gouty arthritis. The mechanism of action is based on inhibition of the activity of prostaglandin G/H synthase 1 (also known as COX-1) and prostaglandin G/H synthase 2 (also known as COX-2) (Figure 1). NSAIDs efficiently relieve pain, fever and inflammation owing to suppression of PGE<sub>2</sub> production, but they also cause adverse events such as gastric ulcers and severe cardiovascular events as a result of the inhibition of cyto-protective prostaglandins [8]. Three structurally and biologically different prostaglandin E synthases act downstream of COX-1 and COX-2 to produce PGE<sub>2</sub>: prostaglandin E synthase (also known as microsomal prostaglandin E synthase 1, mPGES-1); prostaglandin E synthase 2 (also known as microsomal prostaglandin E synthase 2, mPGES-2); and prostaglandin E synthase 3 (also known as cytosolic prostaglandin E synthase, cPGES). mPGES-2 and cPGES have been associated with physiological PGE<sub>2</sub> production, whereas mPGES-1 is specific to the inducible production of PGE<sub>2</sub> (Figure 1). New potent inhibitors of the 5-LOX pathway have been identified and characterized in the past few years. 5-LOX inhibitors and antagonists of

cysteinyl leukotriene receptor 1 have been successfully used in the treatment of asthma but they have not been used in patients with rheumatic diseases [9].

## Eicosanoid Pathways in RA

### Evidence from Animal Models

RA is characterized by synovial inflammation and hyperplasia, autoantibody production (rheumatoid factor and anti-citrullinated protein antibody [ACPA]), cartilage and bone destruction, and systemic features, including cardiovascular, pulmonary, and skeletal disorders [10]. Evidence that the COX pathway might be involved in the pathogenesis of RA dates from the 1970s, when elevated eicosanoids levels were reported in synovia from patients with RA [11]. Different studies in experimental models of arthritis have demonstrated critical roles for enzymes in the COX. In fact the expression of cytosolic phospholipase A2 (cPLA2) was observed to be substantially upregulated in the inflamed joints of mice with collagen-induced arthritis (CIA), and inhibition of elevated cPLA2 expression after development of inflammation resulted in a dramatic reduction in inflammation [12]. In CIA models, COX-2 deletion considerably suppresses synovial inflammation and joint destruction, whereas arthritis in COX-1-deficient mice is indistinguishable from that of controls [13, 14]. These findings indicate that COX-2 products have an essential role in the pathogenesis of CIA. A potentially important role for mPGES-1 and PGE2 in the pathogenesis of RA has been suggested by data from multiple studies in experimental models of inflammatory arthritis [15]. However, a major difference from targets upstream of PGH2 generation is that mPGE-1 inhibition should not suppress generation of other prostaglandins and particularly anti-inflammatory lipid mediators 15d-PGJ2, resolvins and protectins (Figure 1). In addition, redirection of the metabolism of PGH2 following mPGES-1 inhibition might lead to a changed prostaglandin profile, for instance towards prostaglandin D2 (PGD2) production which has anti-inflammatory activity [16]. Other prostaglandins, such as the cyclopentenone 15d-PGJ2, a metabolite of PGD2, function as endogenous ligands for peroxisome proliferative-activated receptor  $\gamma$  (PPAR $\gamma$ ) and possess anti-inflammatory properties. Interestingly, 15d-PGJ2 suppresses the IL-1 $\beta$ -mediated induction of cPLA2, COX-2 and mPGES-1 in synovial fibroblasts from patients with RA [17] and ameliorates adjuvant-induced arthritis and CIA [18]. It had long been thought that PGE2 was the primary PG responsible for inflammation during RA. However, new evidences indicate that PGI2 is as important as PGE2 for the progression of CIA [19, 20]. Genetic and pharmacological studies in mice provided strong evidence that the 5-LOX pathway is essential for the development of inflammatory arthritis [21, 22]. These data suggest that the 5-LO/LTB4 pathway is pro-inflammatory in arthritis and consequently constitutes a target for therapeutic intervention.





## Evidence from *In Vivo* Studies

Synovial fluid samples from untreated patients with RA display elevated levels of PGE<sub>2</sub>, PGF<sub>2</sub> $\alpha$ , 6-keto-PGF<sub>1</sub> $\alpha$ , PGD<sub>2</sub> and thromboxane B<sub>2</sub> (TXB<sub>2</sub>), in comparison with patients treated with NSAIDs [23]. In accordance, cPLA<sub>2</sub>, COX-1, COX-2 and mPGES-1 expression was strongly increased in synovial tissue from patients with active RA [24-26]. mPGES-1 is located in the synovial lining cells, sublining cells, mononuclear infiltrates and endothelial cells of some blood vessels. High levels of LTB<sub>4</sub> in synovial fluid [27, 28] and increased capacity for LTB<sub>4</sub> formation by synovial macrophages and to a lesser extent in neutrophils and mast cells [29, 30] in patients with RA have implicated this leukotriene in the pathophysiology of the disease. Some of the beneficial effects of certain antirheumatic drugs are, at least in part, due the suppression of LTB<sub>4</sub> production. Indeed, administration of glucocorticoids [30] and methotrexate [31] substantially reduced the 5-LOX expression in synovial tissue from patients with RA. However, treatment of patients with RA with the 5-LOX inhibitor zileuton or an LTB<sub>4</sub> receptor antagonist failed to show notable clinical efficacy [32]. These disappointing results indicate that LTB<sub>4</sub> is not a major contributor in the inflammatory process in RA or, alternatively, that more efficacious inhibitors of the 5-LOX pathway are required. The roles of 15-LOX and its products in the pathogenesis of RA are poorly understood. 15-LOX in concert with 5-LOX can also generate anti-inflammatory LXA<sub>4</sub> that has been described to inhibit neutrophil chemotaxis, adhesion and migration [2]. Synovial fluid from patients with RA also contains LXA<sub>4</sub> [33]. LXA<sub>4</sub> can bind to functional LXA<sub>4</sub> receptor and inhibits IL-1 $\beta$ -induced production of the inflammatory cytokines IL-6 and IL-8, as well as matrix metalloproteinase (MMP) 3, and stimulates production of tissue inhibitors of matrix metalloproteinases [34]. The presence of both 5-LOX and 15-LOX in the synovium of patients with arthritis [30] suggests that anti-inflammatory eicosanoids might be produced in RA. In support to this concept is the presence of pro-resolving lipid mediators maresin 1, LXA<sub>4</sub> and resolvin D5 in the synovial fluid from patients with RA [35]. These anti-inflammatory lipid mediators might suppress leukocyte infiltration and enhance the ameliorating actions of macrophages, thus limiting further joint inflammation and tissue damage.

## Eicosanoids and Cytokine Network in RA

A variety of different cell populations, including lymphocytes, innate immune cells, synovial fibroblasts and osteoclasts play a role in the development of RA (Figure 2). Th17 cells contribute to the development of arthritis in each of the initiation, inflammatory, and bone destructive phases through the production of autoantibodies as well as the activation of innate immunity and synovial fibroblasts [36-38]. In RA synovium, elevated levels of the proinflammatory cytokines IL-1, IL-6, and TNF- $\alpha$  are produced by macrophages and synovial fibroblasts. These proinflammatory cytokines both directly and indirectly exert their effects through the production of additional proinflammatory cytokines and chemokines as well as eicosanoids and matrix-degrading enzymes, resulting in a cytokine “storm” in the inflamed synovium. Accordingly, stimulation with TNF- $\alpha$  and IL-1 $\beta$  induces the expression of cPLA<sub>2</sub>

in synovial fibroblasts [39, 40]. Multiple mechanisms seem to be involved in the up-regulation of the COX-2/mPGES-1/PGE2 axis. The induction of COX-2 and mPGES-1 expression and the enhanced PGE2 release occur in response to the proinflammatory cytokines IL-1 $\beta$  and TNF $\alpha$  or lipopolysaccharide in synovial fibroblasts and mononuclear cells [41, 42]. Moreover, in RA, PGE2 released from synovial fibroblasts further increases the expression of mPGES-1 in vitro, via an autocrine positive-feedback loop [43]. Additional factors implicated in the induction of COX-2 and mPGES-1 expression in synovial fibroblasts include epidermal growth factor [44], adiponectin [45] and microparticles [46]. In addition, PGE2 promotes immune inflammation through Th1 cell differentiation and Th17 cell expansion [47]. Interestingly, inducers of PGE2 formation (IL-1 $\beta$  and TNF- $\alpha$ ) are able to affect COX-2 (increased) and 15-PGDH (decreased) expression reciprocally, resulting in boosted PGE2 levels [48]. The pathogenic role of LTB4 in inflammatory arthritis is associated with its strong chemotactic properties essential for leukocyte recruitment in the joint [1]. However, some data reveal other critical functions of LTB4 in synovial inflammation. For instance, LTB4 increases the expression of TNF- $\alpha$  and IL-1 $\beta$  in synovial fibroblasts in RA, [49] and promotes synovial fibroblast migratory and invasive behavior [50]. LTB4 also acts as a suppressor of regulatory T cells whilst promoting generation of type 17 helper T cells [51] (Figure 2). LTB4 production by activated mast cells could be important for the recruitment of effector CD8+ T cells to sites of inflammation [52]; this now suggests that mast cells could significantly modify T-cell function not only through chemokine release but also via LTB4 (Figure 2). In fact recently mast cell biology has assumed increasing prominence in the theories of synovitis, providing a potential cellular link between humoral autoimmunity (B cells) and synovial inflammation [53]. Furthermore, LTB4 is capable of inducing formation and activity of mouse osteoclasts in vitro and in a mouse model of bone resorption in vivo [54].

## Eicosanoid Pathways in Spondyloarthritis (SpA)

Ankylosing spondylitis (AS) is characterized by inflammation of the spine and entheses and by erosions followed by bone formation [55]. Although the block of COX in the treatment of AS is used by a long time, the rationale for such therapy is only based on a few experimental studies [56, 57]. In fact, the majority of studies was conducted in the CIA models and in patients with RA. In contrast to RA, where structural changes are of primarily catabolic nature, resulting in a net loss of bone substance in the vicinity of joints, structural changes in AS are dominated by anabolic processes (Figure 3). Bony spur formation, which arises from the cortical bone surface, is a common feature of AS and virtually affects all skeletal compartments that show disease morbidity. In the case of the vertebral column, such lesions are termed “syndesmophytes”. On the other hand, inflammatory lesions in the neighboring bone marrow (osteitis) are considered as a risk factor for syndesmophyte formation, although this association is not complete and bony spurs can also be found at sites where no osteitis seen and vice versa. Bone formation and ankylosis in AS depends on molecular signals, which regulate differentiation and activity of osteoblasts. Several mediators are of importance for osteoblast differentiation and prostaglandins, such as PGE2,

are important local factors. PGE<sub>2</sub> has anabolic effects on bone and promotes proliferation and differentiation of osteoblasts, thereby inducing the expression of bone sialoprotein and alkaline phosphatase [58]. Moreover, PGE<sub>2</sub> can synergize with bone morphogenic protein (BMP)-2, a member of the TGF/BMP protein family in inducing bone formation [59]. These groups of molecules, PGE<sub>2</sub>, BMP and Wnt proteins manage differentiation of mesenchymal precursor cells into bone-forming osteoblasts (figure 3). In the light of data showing no effect on bony proliferation after anti-TNF $\alpha$  therapy [60], it is interesting to note that continuous (daily) therapy with NSAIDs seems to retard new bone formation. Such an inhibitory effect of continuous NSAID therapy has already been suggested from an early observation using phenylbutazone [61], and more recently using anti-COX2 [62].

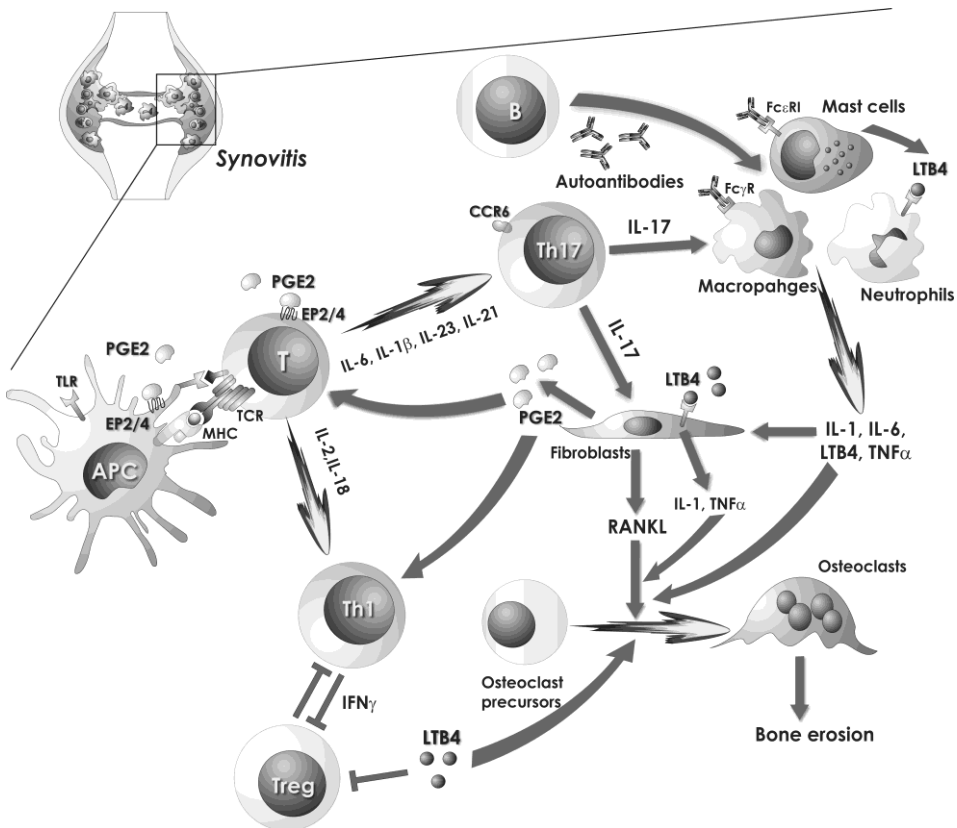


Figure 2. Eicosanoids in RA. The eicosanoids are involved in different stages of the pathogenesis of RA. Cells of the innate immune system such as fibroblasts and the APCs can activate T lymphocytes via PGE<sub>2</sub>. The pathogenic role of LTB<sub>4</sub> in inflammatory arthritis is associated with its strong chemotactic properties essential for leukocyte recruitment in the joint. Furthermore, LTB<sub>4</sub> is capable of inducing formation and activity of osteoclasts and bone resorption (v. text).

## Eicosanoid Pathways in Gout

Gout is a common arthritis caused by deposition of monosodium urate crystals (MSU) within joints after chronic hyperuricaemia [63]. MSU microcrystals induce recruitment and

activation of inflammatory cells in the joint and promote the release of many mediators of inflammation, including eicosanoids [64]. Prostanoids are present in the synovial fluid of patients with gouty arthritis [23] and might account for severe pain, oedema and erythema in the joint associated with crystal-induced inflammation. MSU crystals can also trigger the formation of leukotrienes by human neutrophils and higher LTB<sub>4</sub> levels were detected in synovial fluids from patients with gout than in those with active RA [65]. One of the features of acute gouty arthritis is its self-limiting course; this type of arthritis often spontaneously improves after several days. Anti-inflammatory metabolites of the PGD<sub>2</sub> pathway (PGD<sub>2</sub>, 15d-PGJ<sub>2</sub>) might have a decisive role in the spontaneous resolution of gout [66]. Indeed, NSAIDs/Coxib are the first line of therapy in acute gouty arthritis; are equally efficacious, with coxib showing an improved safety profile [67].

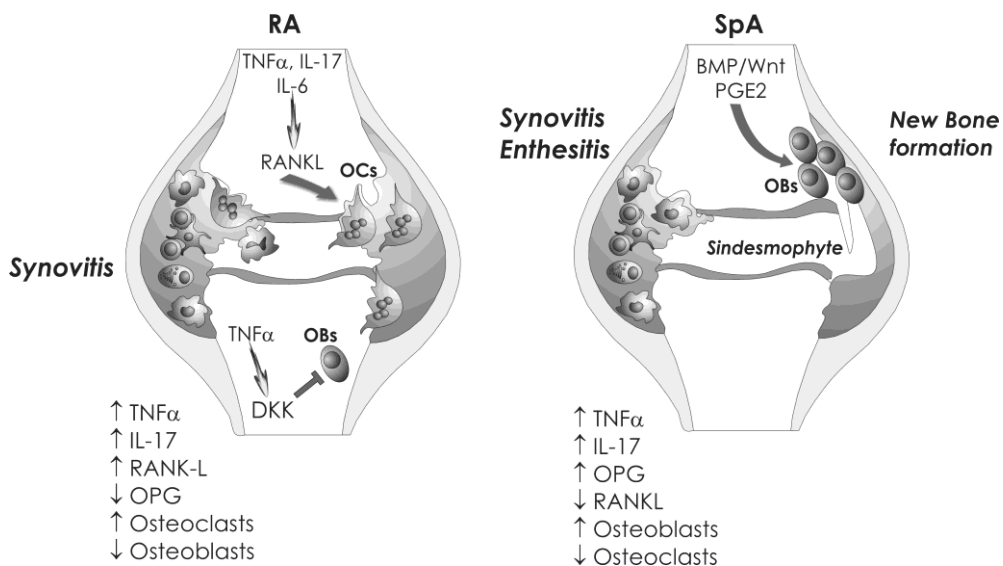


Figure 3. Eicosanoids in spondyloarthritis. In RA, joints undergo progressive resorption, which is mediated by the generation of osteoclasts in the joint. These cells resorb bone and are induced by RANKL, which is activated by TNF $\alpha$ , IL-6 and IL-17. Moreover, osteoblasts are suppressed in RA, which is at least partly mediated by Dickkopf (DKK) proteins. In SpA such as ankylosing spondylitis, bridging of joint and intervertebral spaces by bony spurs is observed. These changes are based on endochondral ossification and may represent a kind of repair strategy of the joint. TNF drives inflammation in SpA similar to that in RA, but there is no connection to increased bone formation driven by osteoblasts, which is based on increased activation of PGE<sub>2</sub>, Wnt (Wnt)- proteins, bone morphogenic proteins (BMPs) and transforming growth factor beta (TGF $\beta$ ).

## Eicosanoid Pathways in OA

OA is characterized by the degeneration and loss of joint cartilage, abnormal bone remodeling and osteophyte formation [68]. Prostaglandins have an important role in homeostasis of human cartilage and pathophysiology of OA; are known to affect bone formation and resorption, regulate the extracellular matrix metabolism, and mediate pain and inflammation [68]. By contrast, the role of leukotrienes in OA is less explored; however,

LTB<sub>4</sub> has been shown to propagate production of IL-1 $\beta$  and TNF $\alpha$  involved in the pathophysiology of OA [69, 70]. Human cartilage explants spontaneously produce 6-ketoPGF<sub>1</sub> $\alpha$ , PGF<sub>2</sub> $\alpha$ , PGE<sub>2</sub>, TXB<sub>2</sub>, PGD<sub>2</sub> and LTB<sub>4</sub>. Spontaneous production of PGE<sub>2</sub> and LTB<sub>4</sub> was higher in OA cartilage explants than in healthy cartilage samples and further enhanced in response to IL-1 $\beta$  [71]. Proinflammatory cytokines IL-1 $\beta$ , TNF $\alpha$  or IL-17 strongly induce expression of mPGES-1, and enhance PGE<sub>2</sub> production in OA chondrocytes and synovial fibroblasts [7]. Besides these proinflammatory cytokines, several other factors are implicated in the induction of COX pathway in the OA joint, including hypoxia inducible factor 1 $\alpha$ , advanced glycationend products, mechanical loading and the adipokines leptin and visfatin [72]. In OA PGD<sub>2</sub> pathway suppresses inflammation and down regulate IL-1 $\beta$ -induced production of MMP1 and MMP13 by chondrocytes [73]. These data suggest that modulation of L-PGDS activity and/or PGD<sub>2</sub> levels in the joint might have therapeutic potential in the prevention of cartilage degradation. Treatment with COX inhibitors effectively reduces pain and immobility associated with OA; in addition, evidence exists that inhibition of the COX pathway in human OA cartilage caused accumulation of the end product of the 5-LOX pathway LTB<sub>4</sub> [71]. For these reasons, dual inhibitors that block 5-LOX and COX pathways might be particularly attractive for the treatment of patients with OA. In this context, dual inhibitors of mPGES-1 and 5-LOX might be promising candidates for the development of safer and more effective anti-inflammatory drugs in OA [74, 75].

## Eicosanoid Pathways in Systemic Sclerosis (SSc)

SSc is an autoimmune disorder of connective tissue characterized by dysregulation of endothelial function and progressive tissue fibrosis. Fibroblasts release prostaglandins, among other inflammatory mediators, in response to cytokine stimulation. In both tissue repair and fibrosis PGE<sub>2</sub> production by fibroblasts is enhanced [76]. Although the levels of PGE<sub>2</sub> are sometimes elevated in patients with SSc, COX-2 deficiency has been implicated in the pathogenesis of pulmonary fibrosis associated with SSc and other etiologies [77-79]. Stable synthetic analogues of PGI<sub>2</sub> are widely used for the treatment of complications related to SSc vasculopathy. The PGI<sub>2</sub> analogue iloprost is effective in ameliorating digital ulcers, which affect a high proportion of individuals with SSc [80]. In addition, iloprost displays *in vitro* antifibrotic properties [81] and may have beneficial effects on the course of SSc in a subgroup of patients [82, 83]. Studies using bronchoalveolar lavage have revealed that there is an overproduction of proinflammatory and profibrotic leukotrienes in the lungs of patients with scleroderma, and that leukotriene levels correlate with inflammatory indices within the lungs [84, 85]. Moreover, the increased levels of leukotrienes in these patients are not balanced by an upregulation of anti-inflammatory and antifibrotic lipoxins [86] (Figure 4). Unopposed actions of leukotrienes might, therefore, induce chronic inflammation and fibrosis in the lungs of SSc patients. Accordingly, pharmacologic correction of a leukotriene/lipoxin imbalance using leukotriene inhibitors or lipoxin analogs might be a new approach to the treatment of scleroderma pulmonary fibrosis [87]. Arterial pulmonary hypertension (PAH) is another severe complication of SSc and one of the major causes of mortality in SSc [88]. In SSc-PAH, the interplay among endothelial cells, smooth muscle cells, inflammatory cells,

fibroblasts and platelets present in pulmonary vessels wall, is regulated by several mediators, including eicosanoids, produced by these cells. This interaction contributes to the pathophysiologic features of PAH (Figure 5) and can be reflected by a reduction in vasodilators/growth inhibitors like NO and PGI<sub>2</sub> and by an increase in vasoconstrictors/co-mitogens like endothelin-1 and TXA<sub>2</sub>. PGI<sub>2</sub> signalling is a major pathway in the pathophysiology of PAH [89]. The prostacyclin synthase and its metabolites are reduced in PAH patients [90]. Prostacyclin is mostly produced by endothelial cells and acts in a paracrine manner with a very short half-life as a potent pulmonary vasodilator; at the same time, prostacyclin is a potent antithrombotic, antiproliferative, antimitogenic and an immunomodulatory factor [91]. Nowadays different stable prostacyclin analogues are available for the treatment of PAH.

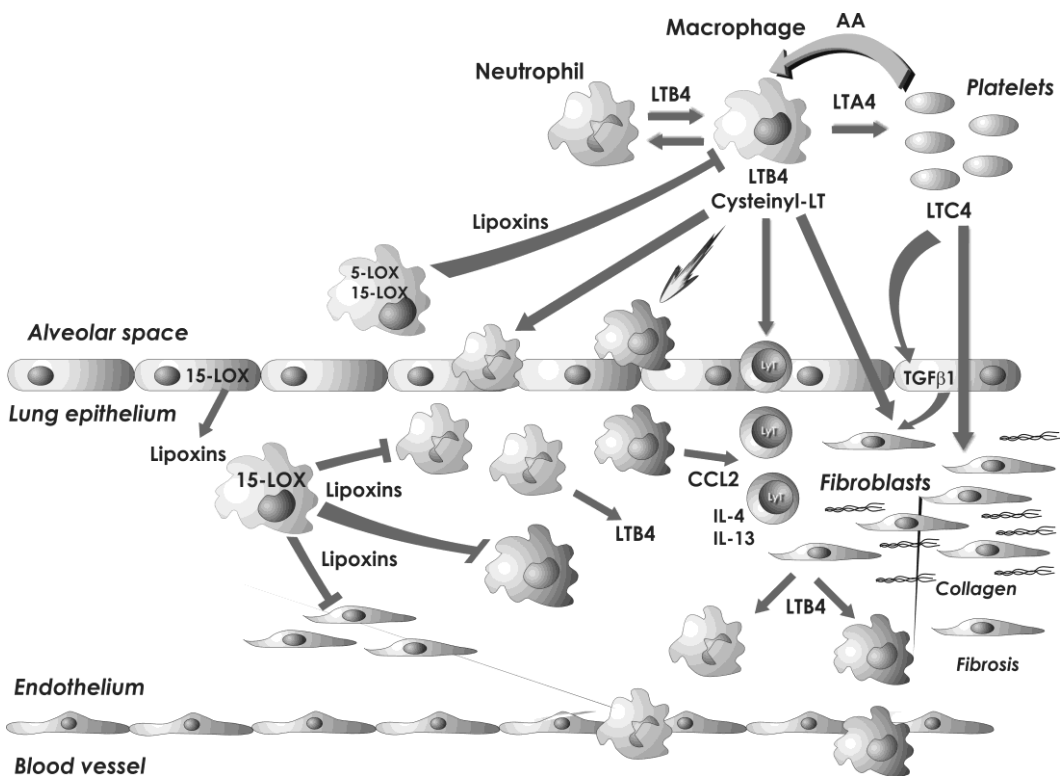


Figure 4. Eicosanoids in SSc pulmonary fibrosis. Leukotrienes are produced by activated leukocytes and lung fibroblasts. In addition, activated platelets exacerbate the production of leukotrienes by metabolizing LTA<sub>4</sub> to LTC<sub>4</sub> or by releasing free arachidonic acid, which serves as a substrate for leukotriene synthesis. LTB<sub>4</sub> induces chemotaxis of leukocytes and fibroblasts, thereby contributing to the development of inflammatory infiltrates and fibroblast accumulation within the lungs. Cysteinyl leukotrienes directly stimulate collagen synthesis. Both LTB<sub>4</sub> and cysteinyl leukotrienes stimulate monocytes and macrophages to produce CCL2, which further induces mononuclear cell chemotaxis. Moreover, CCL2 stimulates T lymphocytes to produce IL-4 and IL-13 which, in turn, enhances fibrosis by upregulating collagen synthesis in fibroblasts. When 15-LOX is expressed, the synthesis of 5-LOX-derived eicosanoids switches from leukotrienes to lipoxins, which, in turn, inhibit the synthesis and counteract the actions of leukotrienes. Abbreviations: 5-LOX, 5-lipoxygenase; 15-LOX, 15-lipoxygenase; CCL2, CC-chemokine ligand 2; IL-4, interleukin 4; IL-13, interleukin-13; LTA<sub>4</sub>; leukotriene A<sub>4</sub>; LTB<sub>4</sub>, leukotriene B<sub>4</sub>; LTC<sub>4</sub>, leukotriene C<sub>4</sub>.

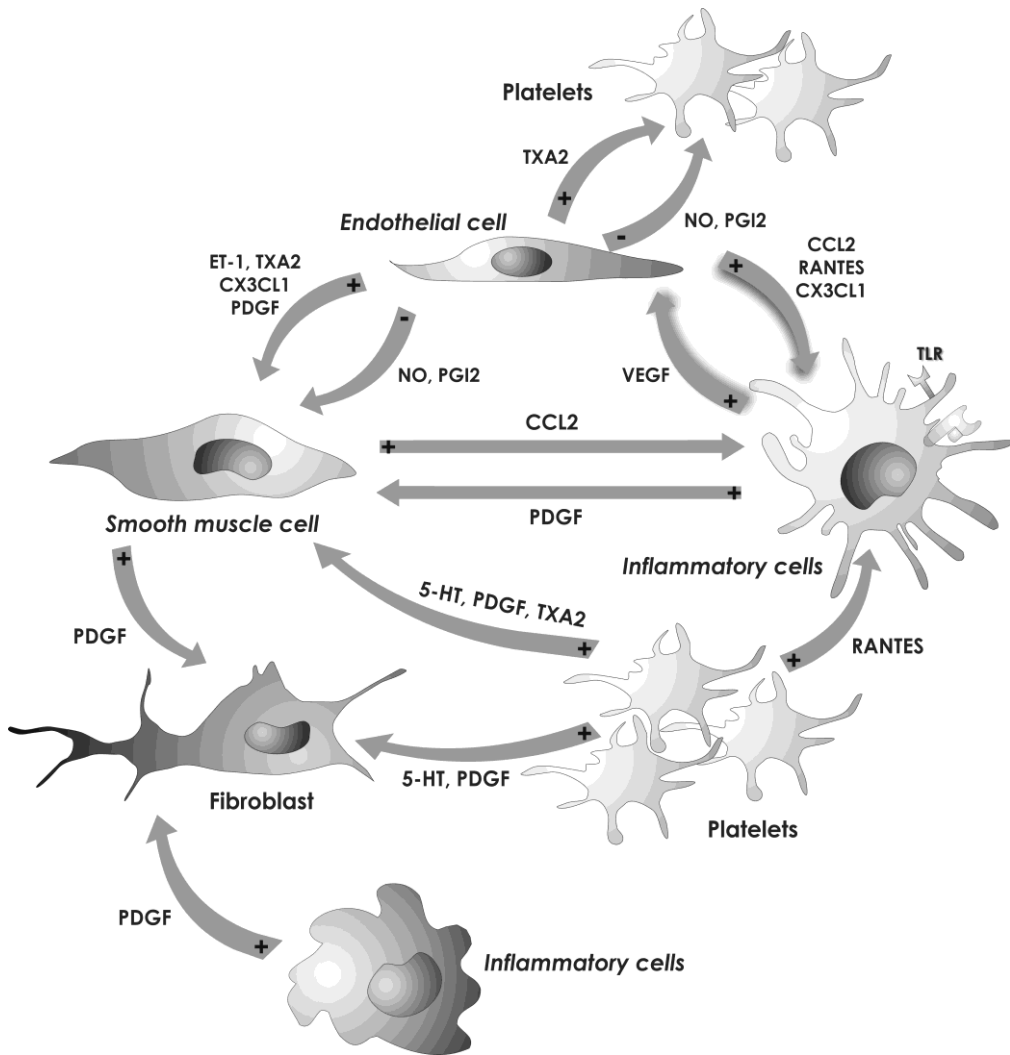


Figure 5. Eicosanoids in SSc pulmonary hypertension. Interplay between endothelial, smooth muscle, inflammatory cells, platelets and fibroblasts in pulmonary arterial hypertension. + arrows show the interactions mediated by several mediators associated with vasoconstriction, proliferation, migration and platelet aggregation. - arrows denote the interaction between endothelial cells, platelets and smooth muscle cells, mediated by NO and PGI<sub>2</sub>, which leads to vasodilator, anti-proliferative and anti-platelet aggregation effects.

## Conclusion

Eicosanoids have an important role in the pathogenesis of rheumatic diseases, and a number of enzymes and receptors involved in eicosanoid biosynthesis or action constitute valuable therapeutic targets. mPGES-1 has been shown to be involved in several inflammatory diseases and inhibition of mPGES-1 is hypothesized to result in the same anti-inflammatory effects as traditional NSAIDs. Redirection of PGH<sub>2</sub> into anti-inflammatory pathways might be an intrinsic part of the mechanism of action of the mPGES-1 inhibitor that

enables increased anti-inflammatory action beyond PGE<sub>2</sub> inhibition. Combination of mPGES-1 inhibition with leukotriene inhibitors might also improve the therapy of certain disorders. Whether inhibitors of 5-LOX or FLAP, or antagonists of the LTB<sub>4</sub> receptor have to be preferred, remains to be elucidated. Early treatment of rheumatic diseases and the use of safe drugs that in combination with other antirheumatic drugs can better maintain remission with less ongoing subclinical inflammation and bone destruction remains an unmet clinical need. Although the evidence of anti-inflammatory eicosanoid pathways in rheumatic diseases has to be further elaborated, is particularly attractive the principle that the PGD<sub>2</sub> pathway might possess anti-inflammatory properties which could be utilized in future treatment strategies.

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# Eicosanoids and Nonalcoholic Fatty Liver Disease

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## Abstract

Nonalcoholic fatty liver disease (NAFLD) is regarded as the hepatic phenotype of insulin resistance, and affects about 20%-30% of the general population. In this context, it is possible to distinguish a condition of simple fatty liver, where the only histological finding is the presence of steatosis, from a state of non-alcoholic steatohepatitis (NASH), characterized by hepatocellular injury/inflammation with or without fibrosis and capability to evolution towards end-stage liver disease and hepatocellular carcinoma. Both genetic background and environmental factors drive a low-grade chronic inflammatory condition which may be considered the main actor of liver injury, especially in NASH patients. In this section, we discuss the most recent experimental and clinical evidences on the link between NAFLD/NASH and eicosanoids, i.e., all biomolecules derived from arachidonic acid, including “classical” (leukotriens, prostaglandins, thromboxanes) and “non-classical” (lipoxins resolvens, protectins, endocannabinoids) eicosanoids, together with their precursors, particularly focusing on the balance between polyunsaturated omega-3 and omega-6 fatty acids. In this setting, the importance of such compounds as potential therapeutic targets should be emphasized.

## Introduction

Nonalcoholic fatty liver disease (NAFLD) is regarded as the hepatic phenotype of insulin resistance (IR), affects about 20%-30% of the general population, and can be considered a spectrum of disorders characterized by predominantly macrovesicular hepatic steatosis

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presenting in individuals in absence of significant alcohol consumption. In this context, it is correct to discriminate between a condition of simple fatty liver, where the only histological finding is the presence of steatosis, and a state of non-alcoholic steatohepatitis (NASH), featured by hepatocellular injury and inflammation, with or without fibrosis [1]. It should be pointed out that a considerable proportion of NAFLD subjects (20%-30%) develop NASH, and that this condition, as opposed to simple fatty liver, is a potentially progressive hepatic disorder that can lead to end-stage liver disease and hepatocellular carcinoma [2]. In addition, several lines of evidences clearly demonstrated that all NAFLD/NASH patients are at high risk of cardiovascular diseases, cardiac and cerebrovascular events, type 2 diabetes, kidney failure and colorectal cancer [3]. In this complex scenario driven by genetic background and environmental factors, a low-grade chronic inflammatory condition can be considered the main effector of liver injury, especially in NASH patients. Accordingly, several studies suggested that pro-inflammatory cytokines and eicosanoids, together with a reduced formation of antiinflammatory cytokines and inflammation-resolving bioactive lipids, seem to play a key role in the pathobiology of NAFLD/NASH [4].

In this section, we discuss the most recent experimental and clinical evidences on the link between NAFLD/NASH and eicosanoids, i.e., all biomolecules derived from arachidonic acid (AA), including “classical” (leukotriens, prostaglandins, thromboxanes) and “non-classical” (lipoxins resolvins, protectins, endocannabinoids) eicosanoids, together with their precursors, particularly focusing on the balance between polyunsaturated omega-3 and omega-6 fatty acids. Figure 1 resumes mechanisms potentially linking eicosanoids with NAFLD pathogenesis.

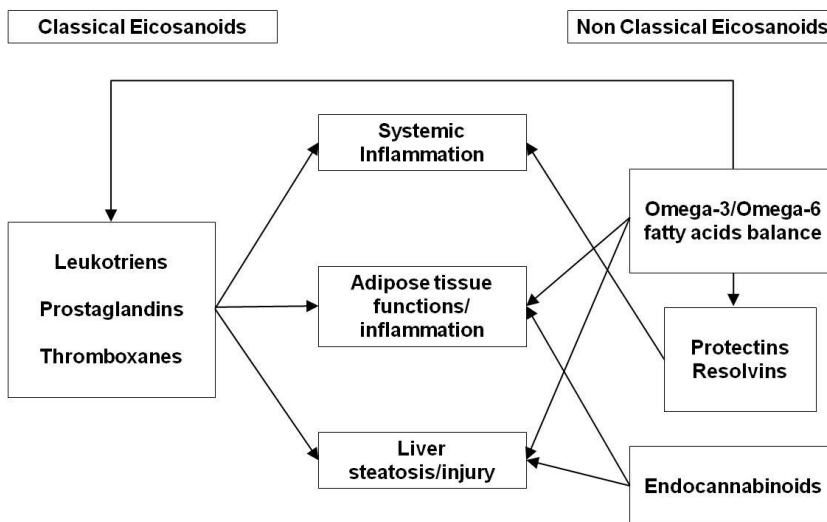


Figure 1. Scheme showing the potential pathogenic role of classical and non-classical eicosanoids in NAFLD. NAFLD can be regarded as a low-grade systemic inflammatory condition mainly expressing in adipose tissue and liver. Proinflammatory classical eicosanoids and endocannabinoids are able to modulate systemic inflammation, adipose tissue functions and inflammation, and hepatic fat accumulation and injury. Omega-3 and omega-6 fatty acids may exert a direct role on liver and adipose tissue functions. In addition, their balance is crucial in this setting, since omega-6 fatty acids produce 2- and 4-series proinflammatory prostaglandins, leukotriens, and thromboxanes, whereas omega-3 fatty acids produce Eicosapentaenoic acid -derived eicosanoids, resolvins and protectins, which are bioactive lipids with antiinflammatory properties.



## “Classical” Eicosanoids

Several evidences highlighted how the single components of the metabolic syndrome (MS), including NASH, are exacerbated by the presence of a chronic low grade inflammatory condition arising from the adipose tissue. This inflammatory state results in an increased production of proinflammatory adipokines (i.e., IL-6, TNF- $\alpha$ , and MCP-1) accompanied by a reduction in the anti-inflammatory and insulinsensitizing adipokine, adiponectin [5, 6]. In addition to adipokines, adipose tissue inflammation is also driven by the activation of “classical” proinflammatory pathways such the 5-lipoxygenase/leukotrienes (LTs) pathway [7]. Interestingly, adipokines and lipid mediators released by adipose tissue have a direct influence on the homeostasis of other organs and tissues, including the liver, as exemplified by the fact that the circulating fatty acid pool derived from visceral fat is the main contributor to hepatic steatosis [8]. In this line, linkage analysis studies have characterized 5-lipoxygenase as a gene with pleiotropic actions on adipose fat accumulation [9], and the presence of all enzymes necessary for the synthesis of 5-lipoxygenase products (5-lipoxygenase, five lipoxygenase-activating protein, LTA4 hydrolase, and LTC4 synthase), as well as all receptors involved in LTs signaling, has been reported in adipose tissue of both lean and obese mice with NAFLD [10]. In particular, the 5-lipoxygenase product LTB4 seems able to enhance the nuclear translocation of p50 and p65 NF- $\kappa$ B subunits and, therefore, to induce the activity of this proinflammatory transcriptional factor in visceral fat, as demonstrated by tissue models [10]. Furthermore, the 5-lipoxygenase pathway may play a relevant role in the modulation of the flux of free fatty acids from adipose tissue, since the increased lipolysis generated by inflamed fat elevates the circulating levels of free fatty acids, thus contributing to obesity-associated IR and liver steatosis [10]. These experimental findings are reinforced by the *in vivo* observation that inhibition of the 5-lipoxygenase pathway with a selective five lipoxygenase-activating protein inhibitor reduced circulating free fatty acid concentrations, IR and hepatic steatosis in mice with dietary obesity [10]. In addition to 5-lipoxygenase, recent studies have shown that also the 12/15-lipoxygenase pathway is associated with the pathogenesis of metabolic disorders: rodent studies demonstrated that a high-fat diet induces 12/15-lipoxygenase overexpression, whereas gene deletion of 12/15-lipoxygenase reduces inflammatory cytokine production and makes mice resistant to diet induced obesity and IR [11, 12]. Furthermore, it is possible to hypothesize that lipoxygenases may also contribute directly to the severity of NAFLD/NASH. Apolipoprotein E deficient (ApoE-/E) mice spontaneously develop a hyperlipidemic phenotype with a liver profile characterized by severe steatosis, increased oxidative stress, and induction of proinflammatory and profibrogenic genes, displaying in particular a significant hepatic overexpression of the genes coding for 5-lipoxygenase and 12/15-lipoxygenase [13]. Interestingly, in mice double knockout for ApoE and 5-lipoxygenase, as well as in mice double knockout for ApoE and 12/15-lipoxygenase, the genetic disruption of lipoxygenases genes resulted in protection against necroinflammatory injury associated with a high-fat diet, improvement of insulin signaling via reduction of hepatic JNK-phosphorylation, and reduction of steatosis [14, 15]. Taken together, all these findings are in agreement with the potential role of lipoxygenases in liver injury emphasized by antisteatotic, anti-inflammatory, and antifibrotic effects exerted by 5-lipoxygenase inhibition also in experimental models [16], and are in line with a human lipidomic study [17] which showed

that the progression from normal liver to NASH goes in parallel with an increased production of 5-lipoxygenase and 12/15-lipoxygenase products.

Experimental studies have demonstrated a potential role of proinflammatory prostaglandins (PGs) on hepatic fat deposition and hepatocellular damage. Group IVA PLA2 (IVA-PLA2) is regarded as the key enzyme responsible for the release of AA, the precursor of PGs. IVAPLA2-knockout mice showed a decrease in hepatic triglycerides content and a lower serum level of PGE2 compared with wildtype mice, suggesting that the circulating level of PGE2 may be related to the levels of intracellular triglycerides in the liver and adipose tissue [18]. Similarly, stimulation of rat hepatocytes with PGE2 and the administration of PGE2 to rats increased the levels of triglycerides in the cells and the liver, respectively [19, 20], suggesting that IVA-PLA2 mediates fat deposition in the liver and adipose tissues through the generation of proinflammatory PGs. Furthermore, a deficiency of IVA-PLA2 alleviated fatty liver damage caused by high-fat diets [21] as a result of the lower generation of PGE2 and other PGs. All the above quoted experimental evidences on the proinflammatory effect of PGs find further support from prospective data on 300,504 men and women aged 50 to 71 years in the National Institutes of Health-AARP Diet and Health Study cohort [24]. In this study the authors showed that NSAIDs Aspirin, in particular, when used exclusively or with other nonaspirin NSAIDs had consistent protective effect related to both hepatocellular carcinoma incidence and chronic liver disease mortality, regardless of the frequency or exclusivity of use [24]. This data take into account the ability of aspirin to modulate the risk of inflammation by inhibiting the COX enzymatic pathways necessary for synthesis of prostaglandins [25].

Finally, several “classical” eicosanoids seem to be involved in the regulation of intrahepatic vascular resistance in livers with cirrhosis due to NASH. It has been reported that high-fat diets and hyperleptinaemia can enhance oxidative stress, which in turn plays a relevant role in increasing intrahepatic vascular resistances in cirrhosis, acting both directly and indirectly via the stimulation of the release of AA [24-28]. Indeed, several animal models have demonstrated that PGI2, thromboxane A2 (TXA2) and cysteinyl leukotrienes are all involved in increasing intrahepatic vascular resistance [29-31]. In NASH-cirrhotic rats, in particular, such increase was mainly mediated by Kupffer-cell-derived TXA2 release [32]. Interestingly, Ajamieh and colleagues, using mice with simple steatosis or NASH exposed to 60 min of partial hepatic ischemia/24-hours reperfusion, demonstrated that atorvastatin exerts major hepatoprotection against ischemia-reperfusion injury in fatty and NASH livers independently from cholesterol removal. Indeed, statins are able to downregulate Toll-like receptor 4 (TLR4) to prevent NF- $\kappa$ B activation, with consequent suppression of adhesion molecules, chemokines/cytokines, and thromboxane B2 production [33], thus emphasizing the role of AA-derivates in the regulation of hepatic microcirculation.

## Endocannabinoids

The term cannabinoids refers to a class of several compounds able to activate the cannabinoid receptors (namely CB1, located throughout the body, but mostly on central nervous system, and CB2, primarily expressed in the peripheral cells of the immune system), including phytocannabinoids (found in cannabis and some other plants), synthetic

cannabinoids (produced chemically by humans), and the endocannabinoids (ECs - produced naturally in the body, including molecules such as Anandamide, 2-arachidonyl-glycerol, noladine, virodhamine and others) [34]. These latter are endogenous mediators derived from AA and synthesized from membrane phospholipids. In the normal liver, the expression of CB1 and CB2 receptors is modest, although ECs system is activated during chronic liver diseases, mostly in stellate cells, hepatic vascular endothelial cells and in monocytes [35-37]. The craving effect of marijuana is widely documented in the literature, and regulated through complex central and peripheral mechanisms involving the activation of CB1 receptors and the interplay with other hormones, such as leptin, insulin, and ghrelin [38]. Animal models revealed that the ECs anandamide and 2-arachidonyl-glycerol increase food intake when administered orally, subcutaneously, or centrally and that this effect is antagonized by CB1 blockade [39, 40]. Nevertheless, reduction of food intake only cannot fully explain anti-obesity effects of CB1 antagonists. Investigators showed that CB1 knockout mice were resistant to diet-induced obesity, although their caloric intake was similar to that of wild-type mice, which became obese on the same diet, and that the use of Rimonabant, a CB1 antagonist, induced a transient reduction of food and a sustained reduction of body weight and adiposity of wild-type animals [41]. These observations led to hypothesize that ECs may influence weight changes, and steatosis occurrence, also via peripheral metabolic effects. In this line, animal models showed that also hepatocytes are able to express CB1, and that its stimulation induces the lipogenic transcription factor sterol regulatory element-binding protein (SREBP)-1c and its target enzymes, Acetyl coenzyme-A carboxylase-1 and fatty acid synthase, thus increasing *de novo* fatty acid synthesis [42]. Other than experimental evidences, the role of ECs has been demonstrated in clinical studies: obese individuals displayed higher serum levels of ECs than lean individuals, and a strong association between high plasma ECs levels and high triglycerides, low HDL cholesterol, high IR, and visceral obesity has been highlighted both in obese and in type 2 diabetes mellitus patients [43, 44]. Specifically, the link between ECs and steatosis has been analyzed in a recent study, which demonstrated that the human fatty liver takes up 2-arachidonyl-glycerol and overproduces triacylglycerols containing saturated fatty acids, an observation which is consequence of the increased *de novo* lipogenesis induced by ECs [45]. This steatogenic role of ECs in humans is furtherly reinforced by the clear evidence that exogenous phytocannabinoids affect the severity of steatosis. In a study of 315 untreated patients with chronic hepatitis C, daily cannabis smoking was identified as an independent predictor of severe steatosis [46], whereas in another cohort of chronic hepatitis C patients, hepatic CB1 expression was associated with the severity of steatosis, although this association was highly significant for genotype 3 only [47]. As above mentioned, Rimonabant is a selective CB1 antagonist which has been tested over 6600 humans by the four "Rimonabant in obesity" (RIO) studies [48-51]. These studies revealed a positive effect of Rimonabant on weight reduction, abdominal obesity, insulin sensitivity, liver steatosis, HDL cholesterol, triglycerides and LDL cholesterol levels. Unfortunately, an increased incidence of severe psychiatric disorders related to Rimonabant use was observed, and the drug was never approved for the treatment of obesity.

In contrast to CB1, the potential role of CB2 receptors in the development of fatty liver is poorly investigated. Animal models demonstrated that the administration of JWH-133, a CB2 agonist, enhanced hepatic triglycerides content and IR, and increased fat inflammation in mice treated with a high-fat diet [52]. Conversely, the genetic or pharmacological inactivation

of CB2 receptors decreased adipose tissue macrophage infiltration and protected mice from both age-related and diet-induced IR [53]. In humans, CB2 receptors are expressed in patients with both simple steatosis and NASH, whereas biopsies from normal livers showed absence of CB2 expression [54]. Taken together, it is likely that CB2 receptors have a potential role on liver steatogenesis and fat inflammatory response associated with IR, even if the mechanisms underlying these phenomena are still unclear. Interestingly, there is also some experimental evidence of a potential anti-fibrogenic role of CB2 receptors: the activation of hepatic CB2 receptors reduced hepatic collagen content and enhanced regenerative response to acute liver injury [55], while the use of CB2 agonist JWH-133 reduced the injury and accelerated liver regeneration in rats with pre-existing cirrhosis [56].

## **Omega-3 and Omega-6 fatty Acids**

Omega-3 fatty acids (main compound: alfa-linolenic acid - ALA), together with omega-6 fatty acids (main compound: linolenic acid - LA), are regarded as essential fatty acids belonging to the family of polyunsaturated fatty acids. In the liver,  $\Delta 6$  and  $\Delta 5$  desaturases are the essential enzymes for the biotransformation of dietary LA and ALA into their respective long-chain metabolites through a competitive metabolism. In summary, omega-6 fatty acids produce, mainly via the production of AA, 2- and 4-series PGs, LTs, and thromboxanes, whereas omega-3 fatty acids produce, via Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), not only 3- and 5-series PGs and LTs, but also other bioactive lipids with anti-inflammatory properties, such as resolvins and protectins [57]. The optimal ratio between omega-6 and omega-3 fatty acids intake should be 1-4:1. In a Western diet, however, omega-6 fatty acid consumption is significantly higher than omega-3 fatty acid intake [58]; as a result, this ratio can increase to 10:1 or even 20:1. It has been suggested that an alteration in this balance and the consequent increase of AA-derived eicosanoids may be important in the pathogenesis of NAFLD/NASH and MS. Indeed, high levels of AA-derived eicosanoids lead to the synthesis of 2- and 4-series PGs, LTs, and thromboxanes, all of them enhancing the production of proinflammatory cytokines. Conversely, EPA-derived eicosanoids have anti-inflammatory effects compared with AA-derived eicosanoids [59]. Furthermore, omega-3 fatty acids serve as substrates for a novel group of lipid mediators, namely resolvins and protectins, with potent anti-inflammatory effects, as they regulate the circulation and activation of inflammatory cells, including macrophages, granulocytes, and lymphocytes [60]. In addition to these anti-inflammatory systemic properties, omega-3 fatty acids regulate hepatic lipid metabolism through several mechanisms. Indeed, they decrease triglycerides levels through the suppression of hepatic VLDL apoB production. In this line, an Australian study [61] showed a decrease of triglycerides plasma levels, accompanied by a significant reduction in triacylglycerol synthesis and an increase in fatty acid mitochondrial oxidation, in obese subjects after six weeks of treatment with high doses of fish oil capsules containing EPA and DHA. Furthermore, omega-3 fatty acids downregulate the expression of several genes involved in lipogenesis, mainly via inhibition of SREBP-1c [62], and upregulate lipid oxidation via activation of PPAR $\alpha$  [63]. In hepatocytes, both omega-3 and omega-6 fatty acids may downregulate the activity of SREBP-1c and its lipogenic function [64], although it is DHA the main compound with specific effects on the expression of SREBP-1c gene.

Interestingly, by stimulation of PPAR $\gamma$ , omega-3 fatty acids can also increase adiponectin levels [65]. This observation is very relevant considering the well-known key role of adiponectin in NAFLD/NASH pathogenesis [1], and thus the potential capability of omega-3 fatty acids in reducing the progression from simple steatosis to NASH. Finally, recent evidences demonstrated that omega-3 fatty acids are also ligands of farnesoid X receptor (FXR). This interaction affects lipid metabolism [66] increasing primary bile acid synthesis and bile acid excretion from the liver. All these considerations served as substrate for several clinical studies aiming to investigate the role of omega-3 fatty acids as potential therapeutic targets in NAFLD/NASH. An Italian study [67] tested the effects of daily EPA supplementation for a period of 12 months in patients with NAFLD, recording an improvement of steatosis, measured by ultrasound, and a reduction of AST/ALT and triglycerides serum levels in the treatment group compared with controls [67]. Another study performed in patients with NAFLD [68] compared the effects of omega-3 fatty acid supplementation combined with American Heart Association (AHA) diet recommendations versus the effects of AHA diet recommendations only. Similarly to the previous study, there was an improvement of liver steatosis evaluated by ultrasound, and a reduction of levels of ALT, TNF- $\alpha$ , triglycerides, and IR in the omega-3 fatty acids group [68]. Furthermore, Tanaka and colleagues [69] observed a reduction of fatty liver content in patients with biopsy-proven NASH after 12 months of EPA supplementation. A recent meta-analysis on this topic [70] suggested that omega-3 long-chain fatty acid supplementation may decrease liver fat, although there was a great heterogeneity among studies. Nevertheless the above quoted promising results, and the evidence that not only circulating omega-3 are associated with lower total cholesterol and cardiovascular mortality [71], but also that their supplementation reduces risk of fatal CHD [72], negative results arise from an RCT on NASH. Specifically, results from a multicenter phase 2b double-blind RCT of EPA ethyl ester 2700 mg vs. 1800 mg vs placebo for 12 months in 240 patients with biopsy-proven NASH showed that experimental treatment had no effects on liver histology, neither on lipids and insulin resistance [73]. However, it should be pointed out that the optimal dose of omega-3 fatty acids is not known, and that probably larger randomized controlled trials are surely needed to better define their potential therapeutic role.

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## **Eicosanoids in Tissue Regeneration, Repair and Injury Healing**

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### **Abstract**

Tissue repair and regeneration are essential processes in maintaining tissue homeostasis in response to injury or stress. During the progression of tissue repair process, localized progenitor cells, as well as newly recruited circulating progenitor cells, proliferate and assume a differentiated phenotype for re-establishing tissue's integrity and functional organization.

Arachidonic acid-derived eicosanoids are ubiquitous mediators of cell proliferation and differentiation, and they regulate the induction and resolution of inflammation associated with the tissue response to injury. The major number of eicosanoids generated in almost every organs potently mediate brief tissue responses during physical and chemical injury, infections, and other intense challenges.

In the present chapter, we describe eicosanoids as key mediators of physiological functions and homeostasis as well as pathological processes in several tissues and organs, such as liver, bone, skin, kidney, lung, skeletal muscle and gastro-intestinal epithelium. According to these observations, lipid mediators or their derivatives may represent promising targets to develop future treatments for several diseases, but further studies are certainly needed to translate this knowledge into clinical applications.

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## Introduction

Tissue repair and regeneration are essential processes in maintaining tissue homeostasis, especially in response to injury or stress. Tissue regeneration is evoked when a tissue is significantly damaged or (either partially or completely) removed, and its tissue's original cell types are structurally and functionally restored [1, 2].

In organ regeneration, the lost tissue does not typically grow back, but rather, the remaining mass expands, a process termed "compensatory growth". In comparison, tissue repair is characterized by epithelial proliferation in the site of injury, fibrosis, and extracellular matrix deposition [1].

An active, coordinated program of resolution initiates in the first and few hours after the evocation of an inflammatory response. Injury and the ensuing inflammatory response cause the release from fibroblasts and endothelial cells of a wide variety of soluble factors, including members of the epidermal growth factor and fibroblast growth factor families (TGF- $\beta$ , KGF, HGF), chemokines (MCP-1), interleukins (IL-1, IL-2, IL-4, IL-13), and eicosanoids, which contribute to repair mechanisms.

Several key signaling pathways are important in regulating these processes, including sonic hedgehog, Rho GTPases, MAP kinase pathways, STAT3, and Wnt.

During the progression of the wound repair process, localized progenitor cells, as well as newly recruited circulating progenitor cells, proliferate and differentiate for re-establishing tissue's integrity and functional organization, by releasing growth factors and cytokines.

Eicosanoids are ubiquitous mediators of cell proliferation and differentiation and they regulate the induction and resolution of inflammation associated with tissue injury response.

After their recruitment in damaged tissues, immune cells, mainly granulocytes, promote phospholipase A<sub>2</sub> (PLA<sub>2</sub>)- dependent release of arachidonate which switch to prostanoids (prostaglandins and thromboxanes), leukotrienes and lipoxins.

These mediators are involved in proliferation and differentiation of stem cells which can be introduced into organs or tissues to replace diseased or damaged cells. In fact, current approaches can be used for regenerative medicine as stem-cell based therapy.

## **Eicosanoids: Key Mediators of Tissue Repair and Regeneration in Multiple Organ Systems**

Eicosanoids are implicated in the physiological regulation of hematopoiesis with pleiotropic effects on hematopoietic stem cells (HSCs) and various classes of lineage restricted progenitor cells. HSCs are defined by two fundamental characteristics: the ability to self-renew (i.e., the ability to form new HSCs), and the ability to differentiate through multilineage and lineage restricted hematopoietic progenitor cells (HPC) into all mature blood lineages [3,4,5].

HSC transplantation potentially is a curative therapy for a variety of malignant and non-malignant blood disorders, and this treatment depends on the homing and differentiation of transplanted HSCs.

Several studies showed that E-type prostaglandins (PGE), especially PGE<sub>2</sub>, stimulate hematopoietic stem cell proliferation [6,7] and promote HSC homing and survival [8].

PGE<sub>2</sub> has a regulatory role during myeloid differentiation, erythropoiesis and stromal cell homeostasis in murine bone marrow. Additionally, hematopoietic lineage regeneration is impaired in cyclooxygenase-2(COX-2)-deficient mice. These data indicate that PGE<sub>2</sub> plays a critical role in HSC induction as well as maintenance and function in the adult organism.

Moreover, proliferation of stem cells or progenitor cells can be promoted by apoptotic cells through activation of molecules, such as caspases 3 and 7, which are essential for mobilizing tissue stem and progenitor cells and promoting tissue regeneration [9]. Indeed, these factors activate Calcium-independent phospholipase A<sub>2</sub> (iPLA<sub>2</sub>) resulting in increased PGE<sub>2</sub> production. PGE<sub>2</sub>, in turn, modifies the Wnt pathway, which regulates HSC self-renewal [10]. These data suggest that executioner caspases not only contribute to the death process, but also participate in the production of paracrine signals promoting tissue regeneration.

Many eicosanoids generated in almost every organs potently mediate brief tissue responses to physical and chemical injury, infections, and other intense challenges.

## Liver

The liver has the most profound ability to regenerate, making it one of the most widely studied systems of tissue regeneration.

Hepatic damage induced by hepatic stress is mediated by various factors, including vasoactive agents, proinflammatory cytokines, reactive oxygen species and eicosanoids. The action of these mediators results in microcirculatory dysfunction, leukocyte infiltration, damage of cellular membranes, development of fibrosis, and stasis of biliary flow[11,12].

Various studies using partial hepatectomy (PH) in animal models have indicated that this process is precisely regulated in its initiation, duration, and termination, with the regenerative response proceeding only until the liver weight of the animals has been restored. Moreover, regeneration occurs while the liver continues to perform its critical functions including glucose homeostasis, protein synthesis, bile secretion, and toxin degradation.

Unlike other regenerating tissues (e.g., skin, gastrointestinal epithelium, and bone marrow) the liver does not require a stem cell population for regeneration. Instead, liver regeneration can proceed by stimulation of existing, normally quiescent, mature cellular populations to re-enter the cell cycle [13].

The entire process of liver healing is highly complex, and numerous soluble mediators and growth factors have been implicated in its regulation.

COX activation and generation of prostanoids after partial hepatectomy are critical steps for hepatocyte proliferation.

Dual inhibition of COX-1 and COX-2 markedly impairs liver regeneration. Exactly, selective inhibition of COX-1 delays regeneration, while selective inhibition of COX-2 partially inhibits regeneration [14].

Hepatocytes respond both *in vivo* and *in vitro* to the major number of the stimuli that positively regulate COX-2 expression in other cell types, including lipopolysaccharide (LPS), IL-1 $\beta$ , TNF- $\alpha$ , and reactive oxygen intermediates. However, adult hepatocytes failed to induce COX-2 expression regardless of the proinflammatory factors used and only Kupffer cells or immortalized mouse liver cells retain the ability to express COX-2.

Using pharmacological inhibitors of COX-2 and mice with a disrupted COX-2 gene, it has been observed that PGs, including prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and prostacyclin (PGI<sub>2</sub>), are important molecules for the early steps of liver regeneration, with respect to DNA synthesis and cell proliferation after PH [15,16]. In addition, several studies show that COX-2 has hepatoprotective effects [17,18] against hepatotoxicity elicited by acetaminophen and carbon tetrachloride (CCl<sub>4</sub>) [19,20].

Another prostanoid, thromboxane A<sub>2</sub> (TxA<sub>2</sub>), plays a pivotal role in producing hepatic injury during various forms of stress. Kupffer cells, resident hepatic macrophages, may be a major source of stress-induced thromboxane, although other cell types in the liver, such as sinusoidal endothelial cells and hepatocytes, may also produce this eicosanoid. Thromboxane induces hepatic damage through vasoconstriction, platelet aggregation, induction of leukocyte adhesion, up-regulation of proinflammatory cytokines, and induction of other vasoconstrictor release. These effects are explicated by binding to a G-protein-coupled receptor, the thromboxane prostanoid (TP) receptor.

In animal and human hepatectomy cases, it has been showed that circulating TX levels are remarkably increased during and after hepatic resection and inhibition of TXA<sub>2</sub> synthesis substantially reduces hepatic damage [21,22]. Nevertheless, it has also been showed that TXA<sub>2</sub> is responsible for liver regeneration after partial hepatectomy. Indeed, TP receptor signaling facilitates liver recovery following CCl<sub>4</sub>-induced hepatotoxicity by affecting the expression of hepatotrophic growth factors, and through the recruitment of macrophages [23]. However, the mechanisms involved in TP receptor signaling after a severe toxic insult are still not well understood and remain unclear.

## Bone

Bone regeneration is a complex with well-orchestrated physiological processes which occur during normal fracture healing, and are involved in continuous remodelling throughout adult life.

Prostaglandins stimulate bone formation mediated by osteoblasts and are critical mediators of bone resorption, a process whereby osteoclasts break down bone and release minerals, resulting in a transfer of calcium from the bone to the blood [24].

PGE<sub>2</sub> is an important regulator of local bone metabolism, inducing osteoclast differentiation from hematopoietic precursors and inhibiting bone resorption in mature osteoclasts [25]. Exactly, PGE<sub>2</sub> induces the cAMP pathway in osteoblasts, leading to release of cytokines, such as interleukin-1 and interleukin-6, which in turn induce the osteoclast activity [25,26].

The complex PGE<sub>2</sub> actions on bone metabolism depend on its interaction with different types and subtypes of G protein-coupled receptors (EP1- EP4) [27]. Among these, three are involved in modulation of cAMP levels. Activation of the EP2 and EP4 receptor subtypes results in the elevation of intracellular cAMP levels, whereas activation of the EP3 receptor results in a reduction of intracellular cAMP levels [28]. Thus, in bone, PGE<sub>2</sub> has an anabolic action that has been linked to an elevated level of cAMP, thereby implicating the EP2 and/or EP4 receptor subtypes in bone formation.

Moreover, prostaglandins stimulate angiogenesis – the growth of new blood vessels – which plays a pivotal role in bone growth and repair [29]. Mainly, PGE<sub>1</sub> induces hypoxia-

inducible factor 1 (HIF-1) activation and vascular endothelial growth factor (VEGF) gene expression in vascular-derived cells, through activation of multiple signal transduction pathways downstream of EP1 and EP3 receptors [30].

Instead, the role of leukotrienes in bone regeneration has been less extensively studied than the cyclooxygenase-derived metabolites. Arachidonic acid is converted to leukotrienes by 5-lipoxygenase. It was shown that genetic loss of 5-LO activity alters bone morphology and increases cortical bone thickness [31].

Leukotrienes produced via 5-LO (namely 5-HETE and the peptido-leukotrienes LTC<sub>4</sub>, LTD<sub>4</sub> and LTE<sub>4</sub>) can stimulate osteoclast activity, suggesting that the increased cortical thickness found in 5-LO knockout mice may be associated with reduced osteoclast activity. These observations suggest the possibility that 5-LO metabolites might act as negative regulators of bone formation [32].

## Skin

Skin wound healing is an highly ordered process, which determines a rapid closure of the wound site and a subsequent tissue regeneration after injury. The cutaneous wound healing process is known to differ between fetal and adult skin. Wound repair in adult skin begins with an acute inflammatory phase and ends with the formation of a permanent scar. In contrast, early gestation fetal wounds (first and second trimester) heal in a near perfect fashion, rapidly and without the production of a scar.

Activated macrophages are central to wound repair, as they amplify the initial inflammatory response by release of a variety of signaling molecules, and, moreover, provide growth factors for fibroblast and endothelial cell proliferation and contribute to extracellular matrix degradation. After a short lag period of several hours after injury, the re-epithelialization is marked by keratinocytes of the cut site starting to migrate.

The factors involved in intercellular communication during repair are only in part known. Among these, eicosanoids are key bioactive lipids, intimately involved in skin biology, inflammation and immunity.

Especially, the role of PGE in skin, was analyzed in rat cell cultures and it was shown that PGE<sub>1</sub> have a protective effect on radiation-induced inhibition of cell proliferation in keratinocytes but not in fibroblasts.

X-irradiation in rats induced epilation, minor erosions or skin ulcers and PGE<sub>1</sub> administration prior to irradiation reduced these irradiation injuries. An elegant study by Takikawa and co-workers demonstrates that proportions of apoptotic keratinocytes in the X-irradiated skin of PGE<sub>1</sub>-administered rats were significantly lower than for those in the skin of rats which did not receive PGE<sub>1</sub>. In this study, wound healing was significantly delayed by X-irradiation but PGE<sub>1</sub> administration prior to irradiation led to a significantly shorter delay in wound healing compared with controls. In addition, it was observed that this effect was correlated with concentration of PGE<sub>1</sub> administered [33]. These results suggest that PGE<sub>1</sub>-administration may potentially alleviate the radiation-induced skin injury.

Moreover, cyclooxygenase-derived mediators are critical to mediating the balance between perfect tissue repair and scar formation. An alteration in the normal process of skin repair can lead to the formation of hypertrophic scars.

Adequate prostaglandins formation is necessary for healing, indeed, altered levels of PGE can contribute to the development of hypertrophic scars [34].

It is known that high COX-2 expression and PGE<sub>2</sub> production contribute to the uncontrolled proliferation of tumor cells, so it is possible to speculate that higher COX-2 expression in fibroblasts could stimulate their own activation, migration, and/or proliferation, augmenting scar tissue production. It has been suggested that the mitogenic effects of PGE<sub>2</sub> on fibroblasts is mediated by signaling through the EP1 receptor.

In addition, it has been known that COX-1-coupled PG biosynthesis is favored over COX-2 directed synthesis in the presence of high AA concentrations, whereas COX-2 driven PG pathways predominate at lower concentrations of AA [35]. Thus, it is reasonable to speculate that normal skin tissue drives COX-1-coupled PG synthesis, as constitutively expressed cytosolic PLA<sub>2</sub> is present in skin keratinocytes. Involvement of PGE in skin repair was also demonstrated in COX-1-deficient mice, showing down-regulation in production of PGE<sub>2</sub>/D<sub>2</sub>. Thus, in addition to COX-2, COX-1 is also involved in skin repair and COX-1 coupled PGE<sub>2</sub>/D<sub>2</sub> biosynthesis plays a crucial part in the regulation of the cutaneous wound healing process [36].

## Kidney

Eicosanoids are also involved in renal physiology and pathophysiology. Indeed, embryonic disruption of COX-2 induces renal dysgenesis, such as glomerular hypoplasia and loss of subcapsular tubules [37,38].

In particular, eicosanoids play a role in the regulation of renal blood flow and glomerular filtration rate. However, their involvement in kidney repair and regeneration is still less understood.

In almost organs, COX-2 expression is inducible and associated with inflammatory reaction, while COX-1 is constitutively expressed, but in the kidney, the expression of both COX-1 and COX-2 is constitutive [39]. Precisely, COX-1 seems not to be essential for renal development because no alterations were observed in renal structures in COX-1 deficient mice. On the contrary, COX-2 deficient mice exhibit abnormal renal development [40,41].

Kidney do not have the intrinsic ability to regenerate, but it shows the ability to repair the tubular epithelium following injury. The proliferation of glomerular epithelial cells may be accompanied by a release of PG and TXA<sub>2</sub>. The role of PGE<sub>2</sub> in renal lesions was examined using rat renal epithelial cell line (NRK-52E) and it was observed that the administration of PGE<sub>2</sub> or 11-deoxy-PGE<sub>1</sub> (EP4 receptor agonist) to NRK-52E increased the cell number, indicating the effects of PGE<sub>2</sub> in renal epithelial regeneration by EP4 receptor activation.

Thus, PGE<sub>2</sub> may regulate renal epithelial regeneration via EP4 receptor through inhibition of apoptosis and epithelial-mesenchymal transition but its actions in renal lesions remain to be clarified [42].

## Lung

Similar to other organ systems, prostaglandins have been implicated in mechanisms of airway repair and protection. Lung injury can occur in both proximal and distal airways, via a



variety of mechanisms, including physical trauma, infection, inflammation and exposure to toxins.

The migration of fibroblasts is believed to play a key role in both normal lung wound repair and abnormal tissue remodeling.

The arachidonic acid cyclooxygenase metabolite prostaglandin E<sub>2</sub> is a potent regulator of fibroblast functions, including chemotaxis [43,44], proliferation [45], matrix production [46,47], and remodeling [48]. PGE<sub>2</sub> is a major product of lung fibroblasts [49] and alveolar macrophages [50] and an increase or a decrease in its concentration, exerts physiologic effect on lung fibroblasts [51].

PGE<sub>2</sub> modulates human lung fibroblast chemotaxis through interaction with multiple EP receptors that can either stimulate or inhibit chemotaxis. The activity of these individual receptors was demonstrated by the use of selective agonists and antagonists. Especially, two EP receptors, EP2 and EP4, inhibit both chemotaxis and migration of pulmonary fibroblasts. In contrast, both the EP1-selective and EP3-selective agonists stimulated cell migration of human lung fibroblasts. These effects suggest that PGE<sub>2</sub> can regulate human lung fibroblast migration in complex ways. They also suggest that altered PGE<sub>2</sub> signaling, which could result from differences in receptor expression or function, may lead to alterations in fibroblast recruitment [52].

Reduced levels of PGE<sub>2</sub> were observed in the lungs of patients with idiopathic pulmonary fibrosis (IPF), a chronic, progressive interstitial lung disease characterized by alveolar epithelial cell injury, fibroblast accumulation, and differentiation to myofibroblasts. This disease is characterized by a loss in production of prostaglandins, including PGE<sub>2</sub>, due to a down-regulation of COX-2 [53]. Because PGE<sub>2</sub> has potent inhibitory effects on fibroblast proliferation and collagen production, this decreased capacity to synthesize PGE<sub>2</sub> may affect fibroblast function and contribute to the pathogenesis of pulmonary fibrosis.

Moreover, others effects contribute to increased proliferation and collagen production by fibroblasts from IPF lung [54]. Usually, in normal human lung fibroblasts, PGE<sub>2</sub> increases apoptosis (via Fas/Fas ligand) which is important in the resolution phase of the normal wound healing response, while IPF lung fibroblasts are more resistant to apoptosis increasing cellularity and also formation and deposition of extracellular matrix [55]. The proapoptotic effect of PGE<sub>2</sub> on fibroblasts depends on inhibition of the phosphorylation of Akt, an important prosurvival protein kinase.

In addition to repair and resolution, the lung may also undergo compensatory growth in response to loss of lung tissue [56,57]. Indeed, it was observed that left pneumonectomy elicits compensatory growth of the remaining right lung to total lung mass in rodent model. Anyway, eicosanoids have not been investigated in this process, but they may be involved also in this model of lung regeneration.

## Skeletal Muscle

Muscle regeneration is a strictly controlled process in which activated myoblasts proliferate, differentiate and fuse to damaged myofibers [58].

Inflammation is an important phase of skeletal muscle healing and largely involves macrophages, TGF-β1, and the COX-2 pathway. Macrophages can affect myofibers healing by inducing the production of TGF-1 and PGE<sub>2</sub> in different muscle cell types. TGF-β1 is a

multifunctional cytokine with fibrogenic properties, indeed, it accelerates the deposition of extracellular matrix (ECM) by increasing the synthesis of ECM proteins on the one hand and acting to inhibit their degradation on the other [59-60].

It was also observed that TGF- $\beta$ 1 may increase the production of PGE<sub>2</sub> by COX-2, and PGE<sub>2</sub> may inhibit fibroblast proliferation and collagen production to balance the fibrotic effect of TGF- $\beta$ 1 [61].

Prostaglandins appear to be involved in others aspects of muscle regeneration after injury. In particular, PGF<sub>2 $\alpha$</sub>  increases myotubes size by stimulating the secondary fusion between nascent myotubes and more single myogenic cells. These results were observed using a muscle-laceration injury model in both wild-type (Wt) and COX-2 gene-deficient (COX-2<sup>-/-</sup>) mice. In particular, it was showed that, in COX-2<sup>-/-</sup> mice, the fusion of myogenic precursor cells (LP cells) was compromised just before that nascent myotubes (formed by the fusion of 2 myogenic cells) fuse with more single myogenic cells to increase in size and become fully mature. The addition of PGE<sub>2</sub> and PGF<sub>2 $\alpha$</sub>  significantly improved the fusion of the COX-2<sup>-/-</sup> LP cells, so COX-2<sup>-/-</sup> mice exhibited impaired skeletal muscle healing after laceration injury. Thus, COX-2 pathway is important in skeletal muscle healing and PGE<sub>2</sub> and PGF<sub>2 $\alpha$</sub>  mediate its effects [62].

Studies in other cell types, have linked PG signaling to a reduction in apoptosis. Control of apoptosis is critical for tissue development and maintenance. Exactly, PGF<sub>2 $\alpha$</sub> inhibits apoptosis and promotes muscle cell survival by up-regulating the apoptosis inhibitor BRUCE (Baculoviral inhibitor ofapoptosis repeat-containing 6 gene) [63], in this way, reducing cell death during myogenesis increases the pool of myoblasts available for fusion and, thus, enhances myotube growth.

The 5-lipoxygenase (5-LOX) pathway is also implicated in muscle regeneration by producing leukotriene B<sub>4</sub>, which accelerate myoblasts proliferation and differentiation. Indeed, it was showed that LTB<sub>4</sub> treatment expedite the expression of differentiation markers, such as MyoD and M-cadherin, in this cell model, resulting in accelerated proliferation and differentiation of satellite cells and contributing to muscle regeneration [64].

## Gastro-intestinal Epithelium

Endogenous prostaglandins (PGs) play an important role in modulating the mucosal integrity and various functions of the alimentary tract. Upon injury, the intestinal epithelium undergoes a wound healing process, which dependent on the precise balance of migration, proliferation, and differentiation of the epithelial cells adjacent to the wounded area.

PGE<sub>2</sub> shows a healing-promoting effect on intestinal lesions via the activation of EP receptors. Indeed, PGE<sub>2</sub> regulates the proliferation of intestinal stromal cells, which are located next to intestinal stem cell compartment and express prostaglandin-endoperoxidase synthase 2 proliferative factor. These stromal cells are called prostaglandin-expressing stromal cells [PSCs] andstimulate the healing of small intestinal lesions by the generation of PGE<sub>2</sub>, which (as above mentioned) induces angiogenesissthrough EP4 receptors activation and VEGF upregulation [65].

It is also known that prostaglandins generated by COX-2 can accelerate ulcer healing by stimulating the gastric mucosa to release of growth factors such as epidermal growth factor

(EGF), hepatocyte growth factor (HGF), and basic fibroblast growth factor (bFGF), which accelerate ulcer healing enhancing the microcirculation around the ulcer [66].

## Conclusion

Tissue repair and regeneration in different damaged organs are essential processes to ensure survival and represent complex processes regulated by several common mechanisms and molecules. Arachidonic acid-derived eicosanoids (mainly PGE<sub>2</sub>) are important factors involved in regulation of physiological functions and homeostasis, but they also seem to be involved in various pathologies onset. Thus, lipid mediator or its derivatives would be used in the treatment of several diseases. However, further studies are still needed to translate this knowledge into clinical applications.

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# **Eicosanoids As Health and Disease Biomarkers**

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## **Abstract**

Eicosanoids have been identified in all major phyla and play important roles in inducing complex and crucial physiological processes. Their biosynthesis in animals is widely reported and they are present in all tissues and body fluids of mammals. In the course of evolution, the Arachidonic Acid (AA) metabolic system has adapted to respond to the selective pressure generated by environmental conditions. During the inflammatory process a great amount of AA derived mediators are produced. This gives rise to a complex network of signals whose function is very important primarily during childhood in response to environmental aggressions. Moreover, later in life the beneficial effect of the inflammatory response decreases as it is established a low-grade chronic inflammation characterized by a two- to fourfold increase of mediators, triggered by various stimuli many of which are closely related to the lifestyle of the industrialized countries.

## **Introduction**

Eicosanoids are a large family of compounds that derive from the omega-6-polyunsaturated fatty acid (PUFA) arachidonic acid. The most prominent members are molecules such as prostaglandins, thromboxane and leukotrienes which play multiple physiological roles. Since their initial identification, however, hundreds of lipid biomolecules

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have been recognized so far and the whole subject of study, known as lipidomics, is aimed today at the characterization of such molecules in a particular biological system, at cell or tissue level, in addition to the elucidation of their role and function. AA is an essential fatty acid, because it cannot be synthesized *de novo* in animals but linoleic acid from the diet is required as a precursor, necessary for cell membrane integrity and fluidity; as eicosanoids have a potent biological activity, the availability of free AA is often a self-limiting step in eicosanoid generation [1]. After the membrane mobilization the generation of eicosanoids is achieved by four different pathways specifically cyclooxygenase, lipoxygenase, cytochrome P-450, and oxygen-species-triggered reactions occur. So far, a great amount of compounds have been discovered [2] acting through specific receptor on the plasma membrane in a paracrine or autocrine manner. Many of them are known since long time as being implicated in physiological or pathological processes while the modern mass-spectrometry approaches [3] have given a great contribution to lipidomics in that they have made possible the discovery of an increasingly number of molecules, whose functions are only partially understood, within a biological context and in association with protein and gene expression studies. Following these approaches it has been possible to put in evidence a more dynamic role of such biomolecules in the cell behavior so that today it is widely accepted that an imbalance of lipid metabolism could be the causative agent of a number of high-prevalence disorders of developed countries such as cardiovascular diseases, obesity, diabetes and cancer. This review will focus on the physio-pathological relevance of inflammation and AA activation and the consequent production of AA mediators which can influence health and disease.

## **Inflammation, Biomarkers and Eicosanoids**

Inflammation arises from sustained activation of the innate immune system (neutrophils, macrophages and fibroblasts) as well as the adaptive immune system (B and T cells). The inflammatory response to persistent infection or environmental insults is characterized by a large number of circulating inflammatory mediators, including chemokines, proinflammatory cytokines, anti-inflammatory cytokines, growth factors, angiogenesis factors, and metabolic markers.

The inflammatory response can arise from a large number of different stimuli and, depending on the nature of inducing agent or on the extent of activation, the reaction can be localized at tissue level or trigger a cytokine-regulated systemic reaction characterized by leukocytosis, fever, somnolence, anorexia, activation of hypothalamic-pituitary-adrenal axis, increased levels of glucocorticoid and synthesis of acute phase proteins in the liver. This network of factors operate to a large extent via the activation of NF- $\kappa$ B a cytoplasmic sensor that can be activated by specific immunological stimuli or by broad danger signals. Moreover the activation of an inflammatory process is also regulated by variation arising from genetic heterogeneity, i.e., single nucleotide polymorphisms (SNPs) which can affect the production of such mediators [4,5]

Inflammation of course has been widely investigated over the last years in a great number of degenerative diseases but what has emerged thanks also to the advancing technologies, which allow the simultaneous evaluation of a wide range of compounds in the same

biological sample, is that often it is possible to find few molecules that play an important pathogenetic role in a particular disease despite the very complex network of molecules or pathways to which they belong. Under this respect many studies have been carried out aimed at identifying some biological parameters that can predict a disease state, measure the progression of a disease or the effects of a treatment. Such parameters that can be used as indicators, are described as *biomarkers* and play a very important role in modern medicine; they are characteristic biological molecules that should be detected and measured easily by sampling little amounts of a biological fluid such blood, or by removal a small amounts of cells or tissues. Despite the term biomarker has been recently introduced, biomarkers have been already used in preclinical research or clinical diagnosis: in fact they have been primarily physiological indicators such body temperature, hearth rate or blood pressure.

Cytokines as previously reported, are very important mediators of inflammation and immune response; their role is to modulate the activity of a large number of cells and, as they operate as molecules that can act in synergism, antagonism, pleiotropy and redundancy, their concentration is often unpredictable in biologic fluids. In conditions of hyper inflammatory activation, some cytokines are released into the circulation giving rise to the acute phase reaction. Thence the presence of increased levels of one or more of these molecules in serum of patients makes them potential candidates as serum biomarkers for disease studies. An expanded panel comprised of multiple cytokines, chemokines, growth factors, which individually may show correlation with disease status, might provide higher diagnostic power if used in combination [6]. Furthermore, these biomarkers may provide insights into mechanisms of immune activation and inflammation, when correlated with clinical outcome.

Hereditary angioedema (HAE) for example, is a rare autosomic dominant disorder characterized by a C1 esterase inhibitor deficiency (C1INH) which causes crisis of episodic swellings of subcutaneous tissues, bowel walls (with intestinal swellings associated to abdominal pain, nausea, vomiting or diarrhea) and upper airways. C1INH has a regulatory activity in many biological cascades and in HAE the key mediator responsible for the symptoms of the disease is bradykinin whose activation indeed is no more regulated by C1INH. In HAE only few papers have been reported on the involvement of cytokines, though cytokines are important modulators of inflammatory processes and are also involved in C1INH synthesis in various cell types. In this disease a pathogenic role for IL-17 has emerged: in fact this molecule is the only cytokine constantly increased in serum samples of patients in absence of crisis. In addition other Th1 and Th2 cytokines show statistically different concentrations in the basal state in comparison with crisis stressing however the very important role of cytokines as disease inflammatory biomarkers [7]

Cytokine and eicosanoids represent the key regulators of inflammatory response; besides cytokines there are other clinically relevant biomarkers of cellular inflammation such as high sensitivity CRP (hs-CRP) [8, 9]. This laboratory parameter, however, may not to be a very selective one and the reason for this is that it can raise its level in presence of simple infections. It is noteworthy that “classical” biomarkers of inflammation, cytokines and acute phase proteins, have been correlated with serum fatty acid to better describe the profile of the involved mediators in diseases characterized by low-grade inflammation. From this point of view a very selective biomarker of cellular inflammation is the ratio of two fatty acid present in the blood: the omega-6 fatty acid AA and the omega-3 fatty acid eicosapentaenoic acid (EPA) from which anti-inflammatory eicosanoids are generated. It has been evidenced, in

fact, that the higher the AA/EPA ratio in the blood, the greater the level of cellular inflammation likely to be present in various organs [10, 11].

## **The Inflammatory Response and Its Metabolites in Metabolic Disorders**

Cardiovascular diseases, obesity, metabolic syndrome and diabetes represent multifactorial conditions resulting from a complex interplay of genetic and environmental factors. Metabolic syndrome, more specifically can be considered as the preliminary stage of these conditions because it confers a 5-fold increase in the risk of type 2 diabetes mellitus (T2DM), 2-fold the risk of developing cardiovascular disease (CVD), 2- to 4-fold increased risk of stroke and 3- to 4-fold increase of risk of myocardial infarction [12,13]. The factors that may be considered clinical markers of such a heterogeneous syndrome are atherogenic dyslipidemia, hypertension, glucose intolerance, proinflammatory state, and a prothrombotic state. The inflammatory state in particular has been revealed to play an important role since the continuous, long-term production of inflammatory mediators could allow other factors to develop and progress, in other words it represents a favorable environment for tissues and organ dysfunction[14].

The most significant example of this is represented by the influence of inflammatory mediators on insulin signaling pathway. This has been put in evidence empirically in the past years by several observations reported in the medical literature, pointing at restoring partially normal levels of glycemia and improving insulin resistance in patients treated with high doses of anti-inflammatory drugs [15, 16]. Insulin resistance, which has an important role specially in T2DM [17], is characterized by impaired sensitivity to insulin in its main target organs. After insulin production and release by pancreatic beta cells, it binds to its receptor and helps the liver, muscle, and fat to take up glucose from the blood, storing it as glycogen in the liver and muscle. Insulin also increases fatty acid synthesis and inhibits gluconeogenesis and lipolysis [18]. Early in the development of T2DM, a compensatory mechanism of insulin hypersecretion maintains normal levels of glycemia, but progressive insulin resistance which may be accompanied by beta cell dysfunction can lead to overt hyperglycemia. Low-grade inflammation contributes to insulin resistance [19] and accumulating evidence from rodents to large-scale studies of populations has demonstrated that AA-derived eicosanoids regulate inflammatory cytokines and chemokines and contribute to the pathogenesis of insulin resistance and diabetes-related complications.

The health adipose tissue, whose normal function is the storage of triglycerides in the body, controls the blood levels of AA and has, as a consequence, a central position in controlling chronic inflammation: another important point to be considered in this complex scenario is thence represented by the dysfunction of adipose tissue which is not necessarily associated with obesity but which depends on excessive triglyceride storage in adipocytes, due to weight increase, which in turn leads to changes such as adipocyte insulin resistance (resulting in higher lipolytic activity), decreased adiponectin production, increased TNF- $\alpha$ , IL-6 production and increase in several other adipocytokines [20]. Moreover an altered adipokines profile appears to be related to cardiometabolic risk indexes [21]. The excessive triglyceride storage affects primarily the cellular response to insulin signaling probably due to

the generation of pro-inflammatory eicosanoids derived from AA, that disrupt the insulin signaling pathway inside the fat cell as previously described. The adipose tissue function is compromised and consequently free fatty acids are released into the circulation [22] which, in fact, represent the hallmark of classical insulin resistance and the coupled hyperinsulinemia. Moreover, the increase of AA inside the fat cells may lead to cell death and necrosis causing macrophage migration and infiltration and consequent secretion of additional inflammatory cytokines (TNF- $\alpha$ , IL-1, IL6) by these newly recruited cells [23, 24]. These cytokines act locally triggering NF- $\kappa$ B activation in the nearby cells but may also reach the circulation and promote CRP synthesis by the liver giving rise to a systemic response. Thus the percentage of normal fat cells in the adipose tissue decreases over time until, following the instauration of insulin resistance to other cellular districts, lipotoxicity occurs, that is the deposition of small lipid droplets in smooth muscle cells, cardiac cells, liver cells and beta cells of the pancreas [25]. Clinically lipotoxicity marks the onset of the metabolic syndrome underlined by clinical markers such as high TG/HDL ratio and, as reported before, by hyperinsulinemia while quite recently a strong association with the levels of AA in the adipose tissue has been evidenced [26]. This is thence an AA driven inflammation supporting the idea that metabolic syndrome and its associated conditions, such as diabetes, CVD, stroke or myocardial infarction are strongly influenced by chronic inflammation; this has suggested that anti-inflammatory drugs, which as known act through the inhibition of the enzymes responsible of eicosanoids synthesis, can be administered to slow or even prevent the systemic involvement characteristic of this kind of conditions. The most common anti-inflammatory drugs in fact inhibit COX and LOX enzymes responsible respectively for the synthesis of prostaglandins and leukotrienes. The most common drugs used in the clinical practice to block COX enzyme are aspirine and non steroidal anti-inflammatory drugs (NSAID); the first one acts as suicide inhibitor that inactivates PGH<sub>2</sub> synthase. NSAID have a different mode of action because they act on the same enzyme but in a competitive manner. Both drugs have little or no effects on the LOX enzymes. Corticosteroids inhibit both COX and LOX enzyme by preventing the release of AA from the cell membrane. Unfortunately high doses of these drugs are required to reach this goal giving rise to the generation of very toxic side effects. In conclusion it must be emphasized that the levels of AA are entirely controlled by the diet and as a consequence the diet induced inflammation is dependent on eicosanoids production from AA. The employ of anti-inflammatory drugs concern the next steps downstream cell membranes AA utilization. The real target to be reached to block the spread of metabolic syndrome and its related diseases in developed countries is thence reducing the intake of refined vegetable oils rich in omega-6 fatty acids specially linoleic acid which is rapidly converted in AA, and decreasing the consumption of refined carbohydrates that significantly increase the glycemic load of the diet which in turn results in the increased secretion of the insulin necessary to lower the resulting rise in blood glucose [27].

## **Eicosanoids in Cancer Inflammation**

Inflammation and its related mediators play a crucial role in cancer development and progression. This has been widely assessed in the last years: it has been evidenced in fact that inflammation may contribute directly, through an intrinsic mechanism due to the alteration

(mutations, chromosomal rearrangement or amplifications) of genes involved in inflammation or not directly by an extrinsic mechanism in which a coexisting inflammatory process contribute to augment the risk of developing or maintain cancer [28]. For this reason inflammation has been recently added as a “hallmark of cancer” [29]. Moreover, as previously accounted for metabolic disorders also in cancer there is evidence that non-steroidal anti-inflammatory drugs (NSAID) reduce the risk of developing certain cancers (such as colon and breast cancer) by reducing tumor associated inflammation [30], and reduce the mortality caused by these cancers. Despite epidemiological data (20% of cancer deaths are linked to unabated inflammation and chronic infections) and experimental evidence support the causal relationship between cancer and inflammation [31] however the underlying molecular mechanism has been only in part elucidated. The inflammatory cancer microenvironment has been extensively studied and many interesting features have been evidenced; macrophages, as known, are very plastic cells and tumor associated macrophages (TAMs) become capable of modifying cancer cell behavior promoting tumor angiogenesis, invasion, intravasation and metastasis in animal models [32-33]. One of the mechanism taking place involves for example the secretion of epidermal growth factor (EGF) produced by TAMs that increases the invasiveness and migration of neighbouring breast cancer cells expressing the EGF receptor (EGFR). Cancer cells in turn express colony stimulating factor 1 (CSF1), which acts as a potent chemoattractant for CSF1 receptor (CSF1R) expressed on TAMs, triggering in this way a paracrine loop [34-35]. On the other hand also tumor suppressing TAMs exist and the equilibrium among different cellular subsets over the course of tumor progression may take place. Apart macrophages other inflammatory cells are able to profoundly influence the tumor microenvironment such as neutrophils, myeloid-derived suppressor cells and dendritic cells. However it is also to be kept in mind that completely blocking inflammation may be unbeneficial so inflammation can be considered as a double edged sword in this ambit. After these considerations it becomes very interesting to understand how AA metabolites influence cancerogenesis. The COX pathway of AA has a very important function in tissue homeostasis; COX-2 is a critical enzyme in the regulation of inflammation and plays also an important role in the cancer-associated inflammation, tumor progression and metastasis. It is up-regulated, in fact in human colorectal adenomas and adenocarcinomas [36] having also a key role in inflammatory bowel disease and colorectal cancer [37]. In general the survival rate is diminished in cancer patients in which tumors express high levels of COX-2 [38]; furthermore PGE2 is the most common prostanoid whose level is up-regulated in many human cancers as lung, colon, breast, head and neck cancer: the up-regulation is associated with a poor prognosis [39], while a decrease of serum levels of PGE2 is observed after a successful anti tumor treatment [40]. The PGE2 support on some colon carcinomas has been demonstrated also in many mouse models of cancer [41]. One of the pro-tumorigenic mechanism of PGE2 is exerted through the local influence on immune response [42]. In fact, PGE2 inhibits the activation of locally recruited inflammatory cells as T cells, macrophages and natural killer cells [43]. In addition PGE2 is an inducer of the myeloid-derived suppressor cells (MDSC) expressed in different types of cancers which have the capability of suppressing the anti-tumor immune response [44]. One of the strategies used to block the harmful potential of PGE2 is the inhibition of the eicosanoid receptors or the administration of PGE2 receptor antagonists: this approach has been revealed successful in some mouse models [45-46]. Concerning PGD2 another member of Cox pathway, an anti-

tumorigenic activity has been evidenced for this metabolite in addition to the pro-tumorigenic one, in various experimental models [43].

Concerning lipoxygenase (LOX) pathway an involvement in tumor progression and survival has been reported too [47]. In humans the principal LOX are 5-LOX, 12-LOX and 15-LOX with at least two forms type 1 and type 2; 5-LOX and 12-LOX stimulate tumor growth and angiogenesis [48], 15-LOX1 has both anti-and pro-tumorigenic activity while 15-LOX2 has anti-tumorigenic function [47]. Interestingly inflammation-associated cancers express both 5-LOX and COX2 proteins [49]. The combined use of COX2 and 5-LOX inhibitors has been proven to be more potent in several tumor models than inhibiting either pathway alone as demonstrated by decrease of tumor growth and downregulation of PGE2 and LTB4 [50]. On the contrary lipoxins (LX), synthesized also by the LOX pathway act in the late phase during the resolution of inflammation. They are considered to be anti tumorigenic: LXA4 reduces cell proliferation, tumor cell invasion and suppresses tumor growth in experimental models [51].

## **Protectins and Resolvins: A Novel Class of Mediators Involved in the Termination Process of Inflammation**

Early studies of bioactive lipids have introduced the concept that inflammation is orchestrated by potent bioactive eicosanoids such as prostaglandins and leukotrienes, assuming that these same mediators were formed and used in the initiation and termination of inflammation, as well as in the transition from acute to chronic inflammation. However other class of mediators exist having an important role in the resolution of inflammation such as lipoxins, PGD2, PGJ2, TGF beta. The isolation and identification of new families of local-acting mediators termed resolvins and protectins has provided evidence that the resolution of inflammation is an active rather than a passive process [52]. These new families of compounds are biosynthesized from the major omega-3 PUFA, the essential fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) employing the same metabolic pathways, although with different modalities, that lead to the synthesis of the well known inflammatory compounds. The same factors that initially generate the inflammatory response also signal the termination of the reaction by switching the production of lipid mediators from the omega-6 PUFA AA to the omega-3 PUFAs leading to the synthesis of resolvins and protectins [53].

These act as “stop signals” favoring an immunomodulatory and anti-inflammatory milieu driving, for example, macrophage differentiation to phagocytosing cells, which remove dead cells [54]. Moreover, dietary supplements enriched in omega-3-PUFAs favors the formation of these endogenous anti-inflammatory and pro-resolution mediators.

## Conclusion

The examples discussed so far indicate that altered AA metabolism has a profound impact on the pathogenesis of several diseases. The AA cascade has been largely investigated in the last years also with the help of the emerging technologies that have allowed the characterization of eicosanoid biosynthetic pathways in conjunction with protein and gene expression studies; these approaches have identified NF- $\kappa$ B as one of the key signal transducers within the AA cascade. Deregulated AA metabolism, deriving from a chronic low-grade state of mild inflammation, creates an imbalance in the tissue homeostasis concerning proliferation, regeneration and repair, and host defense, contributing to sustained inflammatory processes. Through the lipidomics approach, it will be possible to evaluate additional eicosanoid pathways for their potential impact on diseases or for therapeutic approaches. Further studies will be needed to determine if targeting products of the arachidonic acid cascade (using eicosanoid receptor antagonists or manipulating the biosynthetic pathways) or modulating endogenous lipid mediators production (resolvins and protectins) can be used to control chronic inflammation associated diseases.

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## Therapeutic Potential of Lipoxins in the Prevention and Treatment of Inflammatory Disorders

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### Abstract

Naturally occurring lipoxins and aspirin-triggered lipoxins exert potent counter-regulatory actions on cells involved in the immune-inflammatory response. The biological action of LXA4 has previously been demonstrated in severe infections. Indeed, certain pathogens can stimulate supraphysiological amounts of lipoxins to evade the host response. Therefore, this section provides firstly an overview about the role of lipoxin in several disease which originate from parasite, bacteria or virus infection. Then, we focused on the link between lipoxins and cancer. Indeed, people with chronic inflammatory diseases are at increased risk to develop cancer of the respective inflamed tissue and it was recently observed that lipoxins are implicated in reducing the development of several cancer types. Moreover, decreased formation of lipoxins were seen when pancreatic  $\beta$  cells are exposed to toxic agents, resulting in  $\beta$  cell dysfunction or destruction and Diabetes Mellitus onset. Thus, these findings suggest the leading role of lipoxins in the resolution of infections and other inflammatory disease. A deeper understanding of their effect may allow the development of useful therapeutic strategies against several inflammatory disease.

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## Introduction

The inflammatory response is necessary for effective host defense. However, prolonged inflammation can be dangerous and it contributes to the pathogenesis of many diseases. Indeed, compromised resolution has been proposed as an underlying mechanism in many prevalent chronic diseases, such as arthritis, diabetes, and atherosclerosis [1].

It is now recognized that resolution of inflammation is an active regulated program, accompanied by a switch in the mediators in exudates [2]. Inflamed tissue generates local pro-inflammatory stimuli to drive inflammation. This is also accompanied by a systemic and local production of endogenous anti-inflammatory mediators which counterbalance these pro-inflammatory events. These mediators including cytokines, chemokines, and lipid mediators such as the lipoxins (LXs), resolvins, and protectins [3].

Interestingly, the signaling pathways initially involved in the production of prostaglandin (PG)<sub>E2</sub> and PGD<sub>2</sub> formation, may actively switch in the production of pro-resolving mediators, such as LXs, protectins, and resolvins [4].

Each of these potent agonists families control the duration and magnitude of inflammation, stimulating and accelerating tissue resolution [5-7].

They are also potent chemoattractant molecules, which use a via a non-inflammatory mechanism. For example, lipoxins activate mononuclear cell recruitment without stimulating release of pro-inflammatory chemokines or activation of pro-inflammatory gene pathways [8]. They also activate macrophage phagocytosis of microorganisms and apoptotic cells [9] and stimulate expression of antimicrobial defence mechanisms.

Lipoxins, represented by the “lipoxygenase interaction products”, are mainly generated via trans-cellular biosynthesis during cell-cell interactions among neutrophils, platelets, and epithelial cells [10] and possess anti-inflammatory and pro-resolving properties both in vitro and in vivo.

The principal LXs found in mammals include the naturally occurring lipoxin A<sub>4</sub> (LXA<sub>4</sub>) and its positional isomer lipoxin B<sub>4</sub> (LXB<sub>4</sub>), and the aspirin-triggered lipoxins (ATLs) 15-epimeric lipoxin A<sub>4</sub> and 15-epimeric lipoxin B<sub>4</sub>.

Lipoxin A<sub>4</sub> is a dual acting mediator, binds the membrane G protein-coupled receptor formyl peptide receptor 2 (FPR2/ALX), and activates specific cellular pathways via FPR2/ALX to elicit both anti-inflammatory and pro-resolution effects [11].

LXs are formed in various inflammatory conditions. For example, LXA<sub>4</sub> is produced by polymorphonuclear leukocytes (PMN) from asthmatic patients [12] as well as during the resolution of acute inflammation [13] and in murine spleens after microbial challenge.

## Infection Disease

Resolution of inflammation and restoration of normal tissue function are critical events following the clearance of an infectious agent, to prevent the development of complications due to an excessive inflammatory response.

Cytokines, such as IL-12, IFN- $\gamma$ , and TNF, are critical for host resistance to many intracellular pathogens. Because of its potential toxicity when produced in excess, IL-12 production is known to be carefully regulated by a remarkably large number of different

mechanisms, some of these involving the eicosanoids. Especially, the impact of lipoxins in maintaining the equilibrium between effective host defense and homeostasis is remarkably illustrated by the fact that over-production of LXs changes host defense to pathogens.

The biological action of LXA<sub>4</sub> has previously been demonstrated in severe inflammation. Indeed, the absence of LXA<sub>4</sub> leads to unbridled inflammation and elevated mortality in animal models of infection. Along these lines, certain pathogens can stimulate supraphysiologic amounts of LXA<sub>4</sub> as part of their highly evolved mechanism to evade the host response [14].

The bioactions and signaling of lipoxins in infectious diseases have been largely elucidated.

Chagas' disease is an infection produced by the parasite *Trypanosoma cruzi* and represents a serious health problem in large parts of Mexico and Central and South America, where it is a major cause of morbidity and mortality. There is no curative treatment against Chagas' disease. However, in a mouse model of *Trypanosoma cruzi* infection, it has been observed that aspirin administration increases survival and decreases peak parasitaemia. Moreover, exogenous administration of aspirin-triggered lipoxin, 15-epi-lipoxin A<sub>4</sub>, has been found to decrease parasitaemia peaks, decrease cardiac inflammation and increase survival in the same model of infection. This beneficial effect of aspirin on infected mice as a result of the production of 15-epi-lipoxin A<sub>4</sub> [15] seems to suggest a potential role of lipoxins as anti-inflammatory molecules in the acute phase of Chagas disease.

*Plasmodium* infection induces severe neurological complication known as Cerebral Malaria. This infection is still one of the primary contributors to childhood mortality and obstetric complications in the developing world.

The pathogenesis of cerebral malaria involves the sequestration of parasitized red blood cells in the brain microvasculature, the accumulation of mononuclear cells in brain tissue, and the increased expression of pro-inflammatory cytokines, including IFN- $\gamma$  [16,17].

Experimental cerebral malaria (ECM) can be induced by *Plasmodium berghei* ANKA infection of C57BL/6 mice, which represent the most widely used inbred mouse model and is characterized by susceptibility to diet-induced obesity, type 2 diabetes, atherosclerosis and macrophages resistance to the effects of anthrax lethal toxin. This mice model of ECM has been useful in identifying host factors involved in disease pathogenesis and displays many features of the human disease. A balance between host pro-inflammatory and anti-inflammatory response is a key determinant for the pathogenesis of cerebral malaria. The anti-inflammatory actions of LXs play a host-protective role during the pathogenesis of ECM. Endogenously generated LXA<sub>4</sub> protects mice against ECM by inhibiting IL-12 production and accumulation of IFN- $\gamma$ -producing cells in the brains of infected mice. In addition, administration of 15-epi-LXA<sub>4</sub>, a more stable endogenous epimer of LXA<sub>4</sub>, prolongs survival and dampens pro-inflammatory response in mice wild type infected by *Plasmodium*. Therefore, lipoxins appear to increase host survival in a mouse model of cerebral malaria by limiting inflammation, not by enhancing control of parasite levels. These observations provide a proof-of-concept for a potential new therapy for cerebral malaria in humans (HCM) [18].

Lipoxin A<sub>4</sub> appears also to regulate IL-12 generation by dendritic cells exposed to a *Toxoplasma gondii* extract. *Toxoplasma gondii* is an intracellular parasitic protozoan which induces acute and chronic inflammation (toxoplasmosis) in several mouse models. Type 1

cytokines, IL-12 and IFN- $\gamma$  are essential for an effective immune response during both phases of toxoplasmosis [19].

It was well demonstrated that, in mice, *Toxoplasma gondii* induces an early splenic dendritic cell activation, which is characterized by a T cell-independent induction of IL-12 production that involves stimulation of the chemokine receptor CCR-5 on dendritic cells. This early innate immune response wanes within 1 day and is followed by a state of dendritic cell “paralysis,” in which dendritic cells lose their ability to generate IL-12 for several day [20].

Induction of lipoxin A<sub>4</sub> biosynthesis by *T. gondii* *in vivo* has been reported and appears to represent a host defense mechanism.

It has been showed that, in a mouse model of *Toxoplasma gondii* infection, serum levels of lipoxin A<sub>4</sub> rise during infection and remain high once chronic infection onset [21]. Transgenic mice unable to produce lipoxin A<sub>4</sub> suffered increased mortality following infection, compared with wild-type control animals. In the lipoxin-deficient mice, increased serum levels of IL-12 and IFN- $\gamma$  were seen when compared with wild-type mice [22]. Therefore, it likely seems that the increased mortality of the lipoxin-deficient mice is attributable to cytokine-mediated tissue damage, despite better parasite control.

Administration of a lipoxin analogue rescued the lipoxin-deficient mice from this fatal phenotype and lowered IL-12 and IFN- $\gamma$  levels (to wild-type levels in the case of IFN- $\gamma$ ). As mentioned above, interleukin-12, produced by dendritic cells following CCR-5 stimulation, has an important role in host control of intracellular pathogens, but in excess can cause immunopathology. In mice, it has also been showed that lipoxin A<sub>4</sub> analogues suppress IL-12 production by dendritic cells stimulated with *T. gondii* extract [22]. Thus, suppression of dendritic cell function, induced by *T. gondii*, is mediated, in part, via formation of remarkably high levels of LXA<sub>4</sub>, which induces down-regulation of dendritic cell CCR5 and reduces IL-12 production *in vivo*. Although this effect may be a host-driven response to curb excessive inflammation, induction of lipoxin production could be a strategy adopted by pathogens to modulate host immunity and perhaps facilitate chronic infection by reducing tissue damage.

Similarly to *Toxoplasma gondii*, *Mycobacterium tuberculosis* infection induces lipoxin A<sub>4</sub> up-regulation. Indeed, high levels of lipoxin A<sub>4</sub> are detected in mice following aerosol infection with *Mycobacterium tuberculosis* [23]. Pulmonary endothelial cells and macrophages are the key players of this response. Transgenic mice deficient in 5-lipoxygenase were able to control *M. tuberculosis* infection better than wild-type mice. Extensive inflammation and areas of necrosis were seen in wild-type mice whereas transgenic mice had a lesser degree of inflammation and little evidence of necrosis. Importantly, the transgenic mice unable to produce lipoxins enjoy enhanced survival in this model of *M. tuberculosis* infection and show increased pulmonary levels of IL-12, IFN- $\gamma$  and inducible nitric oxide synthase, which are known to have a protective role in host control of *M. tuberculosis* infection [24]. Oral administration of a lipoxin A<sub>4</sub> analogue reverse the enhanced control of infection in the transgenic mice [23].

The contrasting roles of lipoxins in these two models of infection may relate to the dynamics of the specific pathogen–host interactions. *Toxoplasma gondii* replicates more quickly than *M. tuberculosis* and the risk of excessive inflammation and ensuing immunopathology may therefore be greater [23]. By preventing this response, lipoxins are beneficial to the host, and so enhance survival. *Mycobacterium tuberculosis* induces a weaker T helper 1 cell response in mice than *T. gondii* and replicates more slowly, therefore the enhanced inflammatory response seen in the lipoxin-deficient mice may be advantageous, by



‘topping-up’ existing host control. The reduced tissue damage seen in lipoxin-deficient mice may be a result of better control of *M. tuberculosis* replication.

Lipoxins also up-regulate the expression of bactericidal/permeability-increasing protein (BPI) and promote its localization to the membrane surface in several cell lines and in diverse mucosal tissue. BPI is an innate immune defence molecule that exerts multiple anti-infective actions against Gram-negative bacteria, including cytotoxicity through damage to bacterial inner-outer membranes, neutralization of bacterial lipopolysaccharide (endotoxin), as well as serving as an opsonin for phagocytosis of Gram-negative bacteria by neutrophils. It was demonstrated that epithelial cell culture pre-exposed to lipoxin analogue ATLa and then incubated with *Salmonella typhimurium*, showed a concentration-dependent increased bacterial killing compared with non-pre-treated cultures. Therefore, regulated expression of BPI by ATLa provides additional clues to the potent nature of these anti-inflammatory agents [25].

In addition, Serhan and co-workers investigate the effects of lipoxin in a rabbit model of periodontitis. Transgenic rabbits overexpressing arachidonate 15-LO type I show, upon challenge, a phenotype that leads to enhanced endogenous anti-inflammation. Of interest, the isolated PMN from these transgenic rabbits showed a marked reduction in their ability to release granule-associated enzymes upon exposure to secretagogue compared with non-TG rabbits. The overexpression of 15-LO type I also leads to reduced LTB<sub>4</sub> levels and dampened LTB<sub>4</sub>-dependent PMN activation as well as recruitment.

Excessive recruitment of PMN to the periodontium contributes to the progression of periodontal disease and to the destruction of periodontal tissue. Therefore, overexpression of 15-LO in 15-LO type I transgenic rabbits can dramatically alter the outcome and pathogenesis for a focal inflammatory event and has a protective effect against periodontal disease *in vivo* [26].

Lipoxins are also involved in regulating host resistance to several respiratory viruses. Alveolar macrophages, the major resident inflammatory cell of the respiratory tract, produce and release lipoxins following infection with a range of respiratory viruses (murine parainfluenza virus type 1, rat coronavirus, pneumonia virus of mice and mouse adenovirus).

Host transcriptional responses to infection with Influenza A virus has been firstly studied, comparing reconstructed 1918 H1N1 virus to avian H5N1 virus. H5N1 avian virus is highly pathogenic and more virulent in humans than the 1918 H1N1 virus, with a case-mortality rate of 60% compared with 2-5%. H5N1 infection has been associated with up-regulation of inflammation related genes and down-regulation of genes involved in mediating the pro-resolution effects of lipoxins on leucocyte recruitment and counter-regulation of pro-inflammatory cytokine induction. In particular, H5N1 virus inhibits the expression of the suppressor of cytokine signalling (SOCS) 2 gene, the product of which is activated by lipoxins and represent an essential intracellular mediator of lipoxin’s effects on inflammatory cell trafficking and cytokine induction [27].

Loss of lipoxin’s pro-resolution effects may be associated with greater influenza A virus virulence, by suggesting a protective role for lipoxin in this infection, possibly related to the suppression of pro-inflammatory cytokines. However, avian H5N1 virus is less successful at spreading between human hosts compared with other influenza A virus strains. A successful pathogen minimizes damage to its host prolong the availability of its replicative niche, so the high case-mortality seen with infection by this H5N1 strain indicates that it is not well

adapted to humans. Reduction of lipoxin-mediated pro-resolution effects may contribute to the virus's poor adaptation to humans.

Respiratory syncytial virus (RSV) is the most significant cause of serious lower respiratory tract infection in infants and young children worldwide. It is known that "Alternative Macrophage Differentiation", that induce anti-inflammatory cytokine expression and drive tissue repair, would mediate resolution of RSV-induced lung injury. Of note, mice deficient in 5-lipoxygenase, an enzyme required for lipoxin production, failed to elicit alternative macrophage differentiation following respiratory syncytial virus infection while treatment with lipoxin A<sub>4</sub> partially restored the alternatively activated macrophage phenotype in the 5-lipoxygenase-deficient mice. These observations support a pro-resolution role for lipoxins in viral respiratory tract infection [28]. These data suggest that lipoxin induced alternative macrophage differentiation may represent a new treatment strategies for RSV infection.

The role of lipoxin in Human immunodeficiency virus (HIV) infection has been also studied. In a cell culture model of HIV central nervous system infection, it has been observed that TNF- $\alpha$  and IL-1 $\beta$  were produced and their production correlated with synthesis of large amounts of leukotriene B<sub>4</sub>, leukotriene D<sub>4</sub> and lipoxin A<sub>4</sub> [29]. Although this study clearly demonstrates that lipoxin A<sub>4</sub> is produced in direct response to viral infection, the exact role of lipoxin in HIV infection has not been understood. Therefore, further studies are needed in order to better understand the role of these lipid mediators in HIV infection.

## Cancer Disease

Inflammation in the tumor microenvironment is now recognized as one of the hallmarks of cancer. People with chronic inflammatory diseases are at increased risk to develop cancer of the respective inflamed tissue [30,31]. Although experimental evidence supports the causal relationship between inflammation and cancer, the molecular mechanisms and pathways linking inflammation and cancer remain not well understood. Endogenously produced eicosanoids play a central role in inflammation and tissue homeostasis, and recently they have been implicated in cancer.

Conversely, anti-inflammatory drugs decrease the risk of developing certain cancer types. Eicosanoid generating enzymes are over-expressed in several neoplasia, including breast, lung, and pancreas cancer [32].

The cyclooxygenase pathways have been extensively studied in cancer. In contrast, the role of lipoxygenase pathways remains to validate, even if they have an important role in tumor progression and survival [33]. The principal lipoxygenases expressed in humans are 5-lipoxygenase (5-LOX), 12-lipoxygenase (12-LOX), and 15-lipoxygenase (15-LOX) with 2 main forms Type 1 and 2. 5-LOX and 12-LOX stimulate angiogenesis and tumor growth [34,35], while 15-LOX-2 has an anti-tumorigenic role and 15-LOX-1 can have both pro-tumorigenic and antitumorigenic activity [33]. Both leukotrienes and lipoxins have an emerging role in preventing cancer development [36].

LXA<sub>4</sub> and LXB<sub>4</sub> represent the two principal lipoxins generated during the resolution of inflammation and might be a useful strategy for certain cancer treatments. In effect, a novel approach to cancer therapy is the administration of endogenous anti-inflammatory lipid

mediators such as lipoxins. Specifically, LXA<sub>4</sub> reduces cell proliferation, inhibits tumor cell invasion, and suppresses experimental tumor growth [37]. It was demonstrated that LXA<sub>4</sub> exhibits anti-inflammatory actions in human astrocytoma cells by inhibiting pro-inflammatory chemokine IL-8 and the adhesion molecule ICAM-1 [38].

In addition, lipoxins are lipid mediators with a potent inhibitory effect on angiogenesis in different biological models *in vivo* and *in vitro*. ATL-1, a synthetic analog of LXA<sub>4</sub>, inhibits various actions stimulated by vascular endothelial growth factor (VEGF). However, LX actions on endothelial cells in tumor-related contexts are still little known. It has been demonstrated that ATL-1 has a potent inhibitory effect on the permeability induced by VEGF. This pharmacological effect could be used to block tumor extravasation across endothelial barriers, with a possible prospect of reducing the haematogenic spread of cancer cells [39].

Furthermore, pioneering studies demonstrate that soluble epoxide hydrolase is a therapeutic target for inflammation. Soluble epoxide hydrolase inhibitors lead to an increase in endogenous pro-resolution lipoxin levels [40,41] which regulate inflammation. It might be an useful strategy for certain cancer treatments. Several studies showed that administration of a soluble epoxide hydrolase inhibitor in a mouse colitis model result in decreased ulcer incidence [42]. Based on the anti-inflammatory effects of soluble epoxide hydrolase inhibitors, these agents may have anti-cancer effects solely to their anti-inflammatory activity. However, soluble epoxide hydrolase inhibitors exhibit pro-angiogenic activity [43]. Since tumor growth is angiogenesis-dependent [44], soluble epoxide hydrolase inhibitors may have a bi-phasic effect in tumorigenesis. Therefore, further studies are needed to well understand the role of this enzyme in tumor growth.

## Diabetes Mellitus

The general purpose of lipoxins and similar compounds generated from AA, is not only to suppress the production of pro-inflammatory prostaglandins, thromboxanes, leukotrienes and isoprostanes limiting inflammation, but also enhance wound healing, resolve inflammation and thus, restore cell, tissue and organ function to normal.

Diabetes mellitus (DM) is a metabolic disorder of glucose homeostasis characterized by destruction or dysfunction of pancreatic  $\beta$ -cells and correlates with an inflammatory disorder. Decreased formation of lipoxins has been detected when pancreatic  $\beta$ -cells are exposed to toxic agents, such as alloxan, IL-6, TNF- $\alpha$  and macrophage migration inhibitory factor (MIF), resulting in  $\beta$  cell dysfunction or destruction and diabetes onset. In this context, it is noteworthy that anti-inflammatory cytokines IL-4 and IL-10 trigger the conversion of AA to lipoxins, suggesting a mechanism by which they are able to suppress inflammation. IL-4 up-regulate 15-LO gene expression in human leukocytes, suggesting that IL-4 promote anti-inflammatory actions by enhancing LXA<sub>4</sub> formation [45].

Based on the preceding discussion, it has been suggested that a deficiency of LXA<sub>4</sub>, having anti-inflammatory actions, may perpetuate inflammation in DM. Hence, it was proposed that decreased production of LXA<sub>4</sub> may lead to infiltration of leukocytes and macrophages to pancreas that ultimately produce damage to  $\beta$ -cells and the onset of type1 diabetes mellitus. Furthermore, patients with type 2 DM have low AA content in their plasma [46]. These evidences suggest that deficiency of AA may predispose both experimental

animals and humans to develop type 1 (alloxan-induced) and type 2 DM (WNIN/Ob and WNIN/GR-Ob rats and humans).

Moreover, Wei and colleagues studied diabetes onset in fat-1 transgenic mice, which are characterized by cellular increase of  $\omega$ -3 PUFAs and reduction of  $\omega$ -6 PUFAs (including AA).

Fat-1 mice injected with streptozotocin, which is known to induce diabetes in rats and mice, did not develop hyperglycemia compared with wild-type mice. Additionally, streptozotocin-treated fat-1 mice show enhanced insulin secretion stimulated by glucose, in isolated pancreatic islets and enhanced islets resistance to cytokine-induced cell death. This effect has been associated with no or reduced production of TNF- $\alpha$  and IL-1 $\beta$ , decreased NF- $\kappa$ B and increased I $\kappa$ B pancreatic protein expression, resulting in anti-inflammatory phenotype. In this context, it was noteworthy that fat-1 mice show decreased production of prostaglandin E<sub>2</sub> which in turn contribute to elevated anti-inflammatory lipoxin A<sub>4</sub> levels and increased insulin secretion [47].

Therefore, despite increased expression of fat-1 gene, leading to low amounts of tissue AA and significantly high amounts of  $\omega$ 3 PUFAs, when Fat-1 mice were treated with streptozotocin, the pancreatic tissue showed high amounts of LXA<sub>4</sub> and did not develop diabetes. Anyway, this finding is in tune with the proposal that deficiency of AA and/or LXA<sub>4</sub> occurs in diabetes mellitus and when the tissue levels of AA and/or LXA<sub>4</sub> are normal, pancreatic  $\beta$  cells will be protected from the cytotoxic action of alloxan and streptozotocin.

## Conclusion

Given the leading role of lipoxins in several models of human disease, deficiencies in resolution pathways may contribute to many diseases and offer exciting new potential for future therapeutic strategies based on stimulation of resolution. Indeed, the effects of lipoxins in the resolution of infections and other inflammation-associated disease represent an interesting emerging theme for medical research. As above described, the effects of lipoxins are complex and may vary depending on the specific infection and pathology. Thus, in order to achieve this aim it certainly is imperative a profound knowledge of their actions.

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# **Therapeutic Potential of Resolvins and Docosatriene in the Prevention and Treatment of Inflammatory Disorders**

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## **Abstract**

Acute inflammatory response is composed of two distinct steps: initiation and resolution. Actually, it is now well accepted that inflammation is a self-limited process which encompasses in its terminal step the repair of injured tissues and the return to homeostasis. The omega-3 fatty acids EicosaPentaenoic Acid (EPA) and DocosaHexaenoic Acid (DHA) have long been held to display anti-inflammatory and pro-resolution properties. The omega-3 fatty acids represent the substrates for the synthesis of several molecules, such as resolvins, protectins and maresins collectively termed as Specialized Pro-resolving Mediators (SPMs). The action mechanism of SPMs generally bases on binding to specific receptors and are basically finalized to promote both the interruption of inflammatory response and the promotion of tissue repair. Several studies demonstrate the action of resolvins in different districts of the body, including blood vessels, airway, skin, kidneys, eyes and nervous system. Moreover, the benefic role of SPMs has been also demonstrated in metabolic diseases and infective processes. Thus, there is a growing interest towards the role of n-3 derivatives in different pathological conditions and further studies could allow their use as new and more effective therapeutic tools.

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## Introduction

Inflammation is a protective response involving host cells, blood vessels, proteins and other mediators. This immune response has as crucial role the elimination of the initial cause of cell injury, as well as the clearance of cellular debris in damaged tissues and the induction of tissue repair. Indeed pathologists divide the acute inflammatory response into initiation and resolution. During the initial phase of inflammation, leukocytes traffic from the circulation, forming inflammatory exudates [1]. Polymorphonuclear cells (PMNs), particularly neutrophils, exit from the venules following chemotactic gradients and reach the inflammation site. This process involves different molecules, like pro-inflammatory prostaglandins, acting on both endothelium and blood cells. The integration of signals coming from prostaglandins, leukotrienes and pro-inflammatory cytokines leads to an initial amplification of the inflammatory response [2]. Ideally, inflammation is a self-limited process that encompasses, in its terminal step, the repair of injured tissues and the return to homeostasis. The cessation of PMN influx and macrophage clearance of debris, including apoptotic neutrophils, represents the main event in the resolution of inflammation [3, 1].

The omega-3 fatty acids EicosaPentaenoic Acid (EPA) and DocosaHexaenoic Acid (DHA) display anti-inflammatory properties and compete with arachidonic acid by reducing pro-inflammatory eicosanoids [4]. The omega-3 fatty acids represent the substrates for the synthesis of several molecules, such as resolvins, protectins and maresins collectively termed as Specialized Pro-resolving Mediators (SPMs). Resolvins (RVs) derive from EPA and DHA metabolism and are classified in D and E resolvins. Three different E-type and six D-type resolvins have been discovered until now. E-series RVs derive from EPA metabolized by cyclooxygenase-2 (COX2) or cytochrome-P450 in the endothelium and by Lipoxygenase 5 (5-LOX) in neutrophils. On the other hand, the D-series RVs can be obtained metabolizing DHA, activity induced by 15-lipoxygenase (15-LOX) and 5-LOX. Another pathway involving the D-series RVs is based on the presence of Aspirin, which triggers COX-2 to metabolize DHA, in turn metabolized by 5-LOX to create Aspirin-Triggered RV-D (AT-RvD) [5]. Specifically, aspirin and NSAIDs respectively are irreversible, and reversible inhibitors of prostanoid biosynthesis. In particular, aspirin acetylates COX-2 catalytic domain and blocks PG-biosynthesis, but the production of 15R-HETE, 18R-HEPE and 17R-HDHA remains unchanged, respectively from Arachidonic acid, EPA and DHA, giving rise to aspirin-triggered resolvins and protectins [6-8]. Protectins have been discovered for the first time by Bazan and colleagues in the brain. Subsequently, it has been found that protectins derive from DHA conversion by 15-LOX to 17S-H(p)-DHA and can occur in several cells, such as leucocytes, retinal cells and microglial cells. The 17S-H(p)-DHA intermediate 16(17)-epoxide is then transformed into 10,17-dihydroxy docosatriene (PD1) [9].

SPMs exert their biological effect through the binding to specific receptors, such as ChemR23, enhancing macrophage phagocytosis via phosphoprotein-mediated signaling without intracellular Ca<sup>2+</sup> mobilization [10,11]. Particularly, RvE1 is also able to bind Leukotriene B<sub>4</sub> receptor 1 (BLT1) and promotes apoptosis of PMNCs and their clearance by macrophages counteracting BLT1-LTB<sub>4</sub> interaction [10,12]. Furthermore, another mechanism employed by SPMs is provided by the modulation of some MiRNAs, such as Mir208, involved in the regulation of the inflammatory process [13].

All these molecules have opened a new interesting research field in resolution pathways involved in the regulation of homeostasis. SPMs are able to stimulate key cellular events in resolution of inflammation, particularly in the cessation of PMNCs infiltration and enhancement of macrophage efferocytosis [3,1] in pre-clinical animal models [14]. Identification of omega-3 derived mediators consent to clinicians to consider the resolution of inflammation as an active programmed response. The potent *in vivo* actions of resolvins are reported in different districts of the body, including blood vessels [15], airway [16], skin, kidneys, eyes [14]. Exogenous 17-HDHA and RvD1 increase the production of IgM and IgG in human B lymphocytes. In particular, 17-HDHA induces differentiation of B cells toward CD27<sup>+</sup> CD38<sup>+</sup> Memory phenotype [17].

Moreover, PD1 is released by T helper 2-skewed mononuclear cells, limiting T cell migration and promoting apoptosis [18]. It has been also seen that RvE1 and PD1 induce CCR5 expression on leukocytes, facilitating their clearance and promoting the resolution of inflammation [19]. RvD1 is also able to block the increment in cytosolic calcium and consequently suppresses Ca<sup>++</sup>/Calmodulin-dependent protein Kinase (CaMKII) activation. Specifically RvD1 promotes 5-LOX cytoplasmic localization with consequent production of LXA4, endowed with anti-inflammatory properties, in a CaMKII dependent manner [20]. CaMKII seems to be involved in endoplasmic reticulum-stress-induced macrophages apoptosis [21], which plays a key role in several pathologies, such as atherosclerosis and certain autoimmune diseases [22, 23].

Therefore, EPA and DHA derivatives play a leading role in different pathological contexts. In fact they reduce the risk of cardiovascular disorders, prevent some forms of mental illness and insulin resistance [24, 25].

## Bone

Bone remodeling is a concerted process, involving osteoblast (bone-forming cells) and osteoclast (bone-resorption cells) activity. In particular, osteoclastogenesis is regulated by nuclear factor kappa- $\beta$  (NF- $\kappa$ B), activated by RANKL, through the binding to its receptor RANK, leading to the inhibition of osteoclasts apoptosis [26]. Several studies on patients with rheumatoid arthritis highlight the crucial role of osteoclasts in the pathogenesis of bone erosions. It has been observed that omega-3 fatty acids, DHA and EPA, increase bone formation and are beneficial for bone metabolism [27]. In Rheumatoid Arthritis (RA), it has been showed that  $\omega$ -3 fatty acids act by changing the biosynthesis of different eicosanoids. Indeed, diets rich in omega-3 fatty acids counteract the effects of PGE2, which stimulates osteoclast activity, resulting in reduction of secondary osteoporosis in RA patients [28].

Furthermore, RvE1 induces expression of the decoy receptor osteoprotegerin (OPG) without provoking change in receptor activator of NF- $\kappa$ B ligand (RANKL) levels. These results indicate that RvE1 not only shows anti-inflammatory and pro-resolving action in bone, but it also directly modulates osteoclast differentiation and bone remodeling increasing OPG expression [29].

## Cornea

The complete comprehension of molecular mechanisms underlying the action of anti-inflammatory and pro-resolution mediators in ocular tissue might provide a potential therapeutic weapon to ameliorate signs and symptoms of ocular inflammatory disorders, even leading to a complete resolution. Recently, there has been a large amount of interest in using poly-unsaturated fatty acids (PUFAs) as a treatment for ocular surface inflammatory disease [30]. It has been observed that alpha-linolenic acid (ALA), an omega-3 poly-unsaturated fatty acid, exerts potent anti-inflammatory effects on stimulated human corneal epithelial cells. Both protein and mRNA levels of several pro-inflammatory cytokines dramatically decrease following treatment with ALA [31]. Furthermore, the anti-inflammatory effects of Resolvin-D1 (RV-D1) and its mechanism of action in human corneal epithelial (HCE) cells have been also investigated. In particular, RV-D1 acts as a potent anti-inflammatory agent in ocular surface inflammation, with effects comparable to those of Dexamethasone and mediated by NF- $\kappa$ B signal transduction [32]. Treatment of HCE with RV-D1 significantly reduces the inflammatory reaction induced by Poly I:C (TLR3 agonist) and dramatically reduces the production of the pro-inflammatory cytokines TNF- $\alpha$ , IL-6, IL-1 $\beta$  and IL-8 [33]. RV-E1 seems to share the anti-inflammatory effects of RV-D1 [10], and thus, its effector activity has been investigated on different ocular pathologies in various studies. The resulting data seem to suggest that RV-E1 decreases inflammation and neovascularization through reduction of both leukocyte infiltration and release of pro-inflammatory mediators as TNF- $\alpha$  and VEGF-A [34]. Moreover, topical administration of RV-E1 promotes a healthy epithelium, increases tear production by 60% and induces migration of superficial corneal epithelial cells to the dry areas. In addition, RV-E1 decreases COX2 expression and macrophage infiltration in a mouse model of DES (Dry Eye Syndrome) [35]. However, Rv-D1 differs from Rv-E1 not only for their molecular origin and structures, but also for some features in their mechanism of action. For example, it was showed that RvD1 significantly decreased PGE2 levels in choroid-retinal endothelial cells after IL-1 $\beta$ - stimulation, whereas RvE1 had little effect [36]. Furthermore, in the context of conjunctival allergic disorders RvD1 also directly reduces histamine-mediated conjunctival goblet cells secretion acting as a pro-resolving agent [37]. It has been evidenced that initial therapeutic approaches employed RvD1 analogue as protective agent in corneal allograft. In particular, it has been demonstrated that after implantation of cornea tissues from C57BL/6 (H-2b) mice in BALB/c (H-2d) mice, the intravenous administration of RvD1 analogue in recipient mice effectively enhances graft survival, decreasing both direct and indirect allo-sensitization, as well as inhibiting both hemangiogenesis and lymphangiogenesis [38]. Finally, RvD1 also mitigates the response to C5a and C3a by diminishing chemotaxis of CD11b<sup>+</sup> leukocytes as well as CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes within the eye in uveitis [39].

## Kidney

AKI (Acute Kidney Injury) consists of a severe reduction in renal function due to a considerable damage against renal tubules [40]. Kidney damage results from a persistent inflammatory environment, leading to kidney failure [41]. It has been well demonstrated that EPA and DHA exert pro-resolution actions and might also provide new therapeutic

approaches against kidney inflammatory disorders. It has been seen that Aspirin-triggered RvD1 (AT-RvD1) exerts reno-protective effects in a mouse model of AKI induced by LPS. AT-RvD1 gives rise from aspirin-acetylated COX-2, that produces 17(R)- hydroxy docosahexaenoic acid, an intermediate metabolized finally by 5-LOX [42].

AT-RvD1 treatment down-regulates endothelial ICAM-1 and VCAM-1, by lowering PMNCs infiltration and reduces the release of pro-inflammatory cytokines in the injured tissue. In addition, it has been also observed in LPS-treated mice a down-regulation of Claudin-4, which participates in paracellular anion transport in kidney [43]. This effect has been counteracted by AT-RvD, promoting the integrity of tight junctions during AKI. LPS also up-regulates IL-6, an effect reverted by AT-RvD1. Moreover, ERK, STAT3 and NF-kB signaling, known to be all involved in tissue damage during AKI, results less activated in AT-RvD1 treated mice [44].

## Lung

Inflammation plays a key role in the pathogenesis of Acute Respiratory Distress Syndrome (ARDS). RvD1 shows biphasic activity with both anti-inflammatory and pro-resolution properties by binding G protein-coupled receptors (GPCRs), and it takes part in the regulation of the inflammatory process in lung [45,46]. RvD1 enhances the apoptosis of macrophages, reduces PMNCs activation and release of TNF- $\alpha$ , IL1 $\beta$ , IL-17 and IL-13, and increases IL-10 production [47,48]. In addition, RvD1 blocks adhesion molecule integrin  $\beta$ -2 expression, leading to PMNCs chemotaxis inhibition and therefore relieving acute inflammation response [49,50]. RvD1 synthesis has been studied in rat models, showing a reduction trend during the early stage of pathology onset with increased resolvins concentration 10 days after LPS administration [44]. Furthermore, increased levels of IL-6, which exacerbates the severity of lung injury in ARDS patients [51], have been observed 12 hours after LPS-administration. These findings may allow the development of new therapeutic strategies for the treatment of ARDS patients. The effect of RvD1 in modulating alveolar fluid clearance (AFC) has been also investigated on LPS-induced acute lung injury. RvD1 administration induces  $\alpha$ ,  $\gamma$  subunits of ENaC and  $\alpha$ 1 of Na, K-ATPase expression and activity in primary rat alveolar type II epithelial cells. The mechanisms underlying these effects involve ALX/cAMP/PI3K axis [52]. The expression of COXs in lung seems to follow a specific kinetic depending on the action of RvD1. In particular, the latter induces a peak in COX2 expression at 6 hours and 48 hours, leading respectively to an increase in PGE2 and PGD2 release [53]. PD1 has been also detected in exhaled breath condensates at lower levels compared to healthy state in allergic disorders, such as asthma. Indeed, in asthma murine models the administration of PD1 before sensitization with allergens reduces considerably the recruitment of eosinophils and T lymphocytes showing potential use of this compound in allergic diseases [54].

## Heart

The relationship between the consumption of saturated fatty acids and coronary-heart disease is nowadays well established [55]. Epidemiological studies show a beneficial effect of the Mediterranean (rich in olive oil) and seafood-rich diets, containing substantial amounts of poly-unsaturated n-3 fatty acids, like EPA and DHA, on cardiovascular risk [56]. Recently, the effect of EPA-derived RvE1 has been investigated in a rat model of myocardial ischaemia-reperfusion. The resulting data show that RvE1 administration, just prior to reperfusion, significantly limited infarct size, through activation of PI3K/Akt pro-survival pathway [57]. Since chronic low-grade inflammation and leucocyte infiltration play a determinant role in heart failure onset [58], it may be intriguing to further study the effects of these lipidic mediators in heart diseases. Unexpectedly, overexpression of 15-lipoxygenase in transgenic-rabbits sharply reduces atherosclerotic lesions [59]. RvD1 enhances macrophage uptake of apoptotic cells, resulting in a protective and anti-atherogenic role of this pathway [60, 61].

## Gut

RvD1 also influences the outcome of inflammatory disorders in the peritoneal cavity resulting in a lowered PMNCs recruitment in a dose dependent manner [62,63]. In particular, it has been demonstrated that AT-RvD1 inhibits leucocytes infiltration in mouse models of peritonitis with a better efficacy compared to RvD1 [64,65]. Furthermore, RvD3 appears to affect the late resolution phase in mouse peritonitis through reducing PMNCs number in peritoneal cavity [66]. It has been also observed that eosinophils in turn exert a relevant pro-resolution role in mouse peritonitis reducing leucocytes infiltration and modulating macrophages activity through RvE3 and PD1 synthesis [67,68].

## Nervous System

Excessive inflammation is widely known to represent an important feature in neurological diseases. The discovery of SPMs, biosynthesized from omega-3 essential fatty acids, underlines that resolution is a finely regulated process finalized to “switch off” inflammation. In the central nervous system, resolvins and protectins modulate cytokine expression in human and murine microglial cells [62, 69]. Data coming from studies conducted on murine models of ischemic stroke indicate that aspirin can trigger resolvins and protectins in microglia. Thus, down-regulating leucocyte infiltration, COX2 induction, IL-1 $\beta$  and NF- $\kappa$ B activity, neural damage result less widespread [70]. The intrathecal administration of RvD1 and RvE1 in murine models results beneficial in the treatment of neuro-inflammatory pain, with better results compared to morphine or COX-2 inhibitor [71, 72].

RvD1, RvD2 and RvE1 improve formalin-induced flinching, capsaicin-induced nocifensive behavior and Complete Freund’s adjuvant induced thermal and mechanical hypersensitivity in mice, post-operative tactile hypersensitivity and hyperalgesia in rats if

injected in spinal cord. In particular, the obtained results are reached through the suppression of Transient Receptors Potential (TRPs) in primary afferents [72,73]. The omega-3 derivative 10,17-dihydroxy-protectin, also called neuroprotectin D1, has been found actively synthesized in neural tissue and retinal epithelial cells by Bazan and colleagues. In this context neurotrophins seem able to modulate NPD1 production [74]. Administration of 17R-NPD1 to Sprague-Dawley rats 2 hours after middle cerebral artery occlusion results in a decreased brain edema in the penumbra and subcortical lesion size improving neurological scores [75].

In a rat model of peripheral inflammation induced by carrageenan, it has been observed an increased mechanical sensitivity that was attenuated by 17(R)-RvD1 intrathecal supply. The noticed effect seem to be due to a minor TNF- $\alpha$  secretion in CSF after 17(R)-RvD1 treatment. In the same study primary astrocytes derived from treated rats produce more TNF- $\alpha$  after LPS induction and this cellular response is counteracted by 17(R)-RvD1, which also diminished ERK activation induced by TNF- $\alpha$  [76]. NPD1 and SPM-receptors are known to be diminished in brain from Alzheimer's disease (AD) patients [77]. Moreover, reduction in RvD1 was reported in cerebrospinal fluid and hippocampus of AD subjects, correlating to mini-mental state examination scores [78].

RvD1 reduces polarization of macrophages towards the M1 phenotype increasing meanwhile their phagocytic activity on beta-amyloid deposits. These findings substantiate the hypothesis that the anti-inflammatory effects of resolvins in the brain of AD subjects are also due to an enhancement in beta-amyloid clearance resulting in reduction of inflammation [79].

The modulation of resolvins synthesis can be carried out by nervous system through specific neuro-mediators, such as acetylcholine. Indeed, vagotomy reduces production of RvD1 and this process involves axonal guidance of netrin1 [80].

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder that involves the death of motor neurons and is characterized by stiff muscles, muscle twitching and gradually worsening weakness due to muscle wasting. Since inflammatory background is a characteristic noticeable by the appearance of reactive microglial and astroglial cells, Liu and coworkers investigated the role of RvD1 in ALS patients. The study of macrophages in post-mortem ALS spinal cords, revealed that neurons were ingested by IL-6- and TNF- $\alpha$ -positive macrophages. Treatment with RvD1 on macrophages derived from ALS patients decreased dramatically the production of pro-inflammatory cytokines [81]. Thus, these data prompt researchers to deepen the molecular mechanism underlying resolvins effects to elaborate new therapeutic tools for the treatment of nervous system pathologies.

## Metabolic Disorders

Resolvins are known to exert pleiotropic effects from the induction of anti-inflammatory mediators to the modulation of insulin sensitivity and this led researchers to investigate their involvement in metabolic disorders such as obesity, type 2 diabetes mellitus, and non-alcoholic fatty liver disease (NAFLD). The latter disease is linked to obesity and insulin resistance [82]. The resulting hepatic steatosis is consequent to endoplasmic reticulum (ER) stress, typical of this NAFLD. In animals and humans with NAFLD, stress on the hepatic endoplasmic reticulum (ER) plays a crucial role in metabolic dysregulation that results in

hepatic steatosis [83]. SREBP is a transcription factor activated by ER stress and promotes triglycerides intake and cholesterol synthesis in human hepatocytes [84].

It has been shown that RvD1 can decrease hepatic steatosis, which represents characterizing condition of NAFLD patients. In addition, experiments conducted on HepG2, hepatocarcinoma cell line, clearly showed that pretreatment with RvD1 is able to attenuate ER stress-induced apoptosis through inhibiting caspase 3 activity. The analysis of HepG2 lipid content revealed that RvD1 strongly decreases tunicamycin-induced triglycerides accumulation as well as SREBP-1 expression [85]. Furthermore, PD1 ameliorates necro-inflammatory liver injury by decreasing COX2 expression and, consequently, PGE2 release by hepatocytes, protecting DNA from oxidative stress damage [86]. Thus, these evidences suggest that future therapies could take advantages of RvD1 and PD1 in order to ameliorate liver functionality in NAFLD patients. Low-grade inflammation is a feature shared by metabolic disorders since adipose tissue resident macrophages can release pro-inflammatory mediators which regulate adipokine secretion by adipocytes. In particular, it has been seen a reduction in anti-inflammatory adiponectin, and an increase in pro-inflammatory adipokines compared to healthy people [87].

In addition, decreased levels of anti-inflammatory RvD1 and PD1 have been detected in adipose tissue of obese subjects [88]. Administration of RvD1 to leptin receptor-deficient obese and diabetic (db/db) mice augmented glucose tolerance by increasing insulin-stimulated Akt phosphorylation. At the same time a reduction in crown-like structures formation, macrophages rich structures, was detected in adipose tissue [89]. RvD1 modulates gene expression in adipose tissue resident macrophages, inducing the expression of typical M2 subset markers (IL-10, CD206, RELM- $\alpha$ , arginase 1 and Ym1) and lowers Th1 lymphocytes activation [90]. In particular, RvD1-induced M2 macrophages are characterized by CD11b<sup>low</sup> phenotype and are able to engulf a larger amount of apoptotic bodies in adipose tissue [91]. Studies conducted on obese and diabetic mice revealed that RvE1 induces adiponectin release and therefore promotes insulin sensitivity through increasing GLUT4 (glucose transporter 4) and IRS-1 (insulin receptor signaling 1) expression, conferring protection against hepatic steatosis [92].

Together these findings emphasize the benefic effects of n-3 FA-derivatives in metabolic disorders and future studies could allow their use as new therapeutic tools.

## Infective Processes

The role of SPMs was also investigated in infectious diseases. For example, in a rabbit model of periodontitis induced by *Porphyromonas gingivalis* topical administration of RvE1 seems reduce the severity degree [94].

Interestingly, in a mouse model of cecal ligation puncture induced sepsis, RvD2 shows potent protective actions through enhancing bacterial killing and phagocytosis [95]. Moreover, PD1, RvD5 and RvD1 levels increase in a mouse model of *Escherichia Coli* infections and promote resolution program [96]. RvD1, RvD5 and PD1 have also been tested during *Staphylococcus Aureus* infection in mouse dorsal skin pouches, revealing able to potentiate the bactericidal effect of vancomycin, reduce neutrophil infiltration and determine bioburden reduction [96]. Kurihara and colleagues also demonstrated that neutrophils isolated



from burned rats exhibit reduced motility towards chemoattractants, accumulating in healthy tissues and determining tissue damages. RvD2 administration restored neutrophil chemotaxis to almost normal and improved rat survival after intravenous injection of LPS [97].

Furthermore, in vitro studies on human lung epithelial cells infected with *H1N1 influenza A virus*, showed that PD1 significantly reduces viral replication [98].

It has been also demonstrated that RvE1 and PD1 decrease CD4<sup>+</sup> T cell infiltration, stimulate IL-10 production and determine an anti-inflammatory effect when topically administered in a mouse model of stromal keratitis induced by *Herpes simplex* infection [99,100]. The role of SPMs has been also investigated in yeast infections. For example, RvE1 has been shown to enhance *Candida albicans* killing in mice [101]. Together, these findings provide evidences that new therapeutic approaches against different infectious agents could exploit n-3 metabolites to reduce inflammation and enhance pathogen clearance.

## Conclusion

The importance of  $\omega$ -3 PUFAs as precursors for novel potent bioactive mediators has been widely demonstrated in several studies conducted on different pathological conditions. These findings may enable the development of novel drugs specifically designed on SPMs receptors. On the other hand, recent pre-clinical studies encourage the application of pharmacological treatments based on SPM-agonists for inflammatory diseases. This approach might permit to promote healing process and protect organs from collateral effects. Indeed, actual therapeutic protocols generally use immunomodulators resulting in a nonspecific immunosuppression. SPMs could represent a valid alternative to promote tissue regeneration without classical complications characteristic of conventional therapeutic approaches in several pathological conditions.

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# Genetic and Epigenetic Factors and Modulation of Eicosanoid Function: Translation in the Personalized Medicine

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## Abstract

Eicosanids mediate both physiological actions and pathological effects modulated by genetic variants in enzyme or receptor signaling pathways genes. This results in eicosanoids acting as *enhancers or inhibitors*. In addition, the expression of their genes is regulated by miRNA and other epigenetic mechanisms which may modify the intensity of mediated actions of eicosanoid pathways. In turn, this determines a modulation of chronic inflammatory-related tissue damage and the consequent onset and progression of multi-factorial diseases. The epigenetic mechanisms are regulated by a complex gene-environment interplay; lifestyle conditions, principally including the diet and physical activity have a crucial importance. Fine knowledge of the mechanisms involved might consent the development of new strategies of prevention for chronic and multi-factorial diseases. The these major aspects are described in this chapter.

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## Introduction

Eicosanoids constitute a large and expanding family of bioactive lipids synthesized from polyunsaturated fatty acids (PUFA) to either pro-inflammatory omega-6 arachidonic acid (AA) or anti-inflammatory omega-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In these last cases, two essential fatty acids (FAs),  $\omega$ -6 linoleic acid (C18:2n6) and  $\omega$ -3 linolenic acid (LA) (C18:3n3) utilized as substrates and a series of desaturase and elongase enzymes are essential for their production (see Chapter 1). Eicosanoids derived by AA operate as potent mediators able to act in a complex network and to mediate different functions. Indeed, different physiological activities are induced by AA-eicosanoids, such as smooth muscle tone, vascular permeability, platelet aggregation, bronchoconstriction/dilation, intestine motility, inhibition of gastric acid secretion, uterus contraction, kidney filtration and renal blood flow, increase in hypothalamic and pituitary hormone secretion (as described in Chapter 4). Their action is mediated through the binding to specific G-protein-coupled membrane receptors which can triggers an increase or decrease in the rate of cytosolic second messenger generation (cAMP or Ca<sup>2+</sup>), activation of a specific protein kinase or a change in membrane potential (see Chapter 1). Different cellular types are involved in their production from classical inflammatory cells, including polymorphonuclear leukocytes, macrophages (important producers) and mast cells to dendritic cells, which represent both a source and target of AA-derived eicosanoids. In addition, human activated T and B cells produce a significant amount of eicosanoids, particularly prostaglandins, such as PGD<sub>2</sub> and PGE<sub>2</sub>. This propriety might be principally correlated to several functions of B cells (see Chapter 2). However, AA-derived eicosanoids also constitute the optimal modulators of the immune/inflammatory responses by mediating their effects on macrophages, mast cells, dendritic cells, lymphocytes and natural killer cells. Thus, they are also able to exert both the evocation of immune responses and immune-modulation.

AA-derived eicosanoids also involve in inducing immunopathological processes ranging from inflammatory responses to chronic tissue remodeling, obesity, insulin resistance, diabetes, atherosclerosis, allergic diseases, liver diseases, cardiovascular complications (i.e., coronaropathies, aneurysm, etc.), cancer, rheumatoid and autoimmune disorders ( as illustrated in the chapters 6-13). A genetic basis has been postulated for susceptibility to each of these diseases. Each of these pathological conditions is syndromic and it is caused by more than 1 molecular defect. On the other hand, they are multifactorial diseases. Recently, it has also been suggested the role of genetic variants in eicosanoid pathway genes in the risk of these diseases. Accordingly, we describe in the next paragraph the role of polymorphisms PTGS2 (COX-2) and 5-Lo in some chronic inflammatory diseases [1-4].

## Capacity of Genetic Variants in Eicosanoid Pathway Genes in Modulating Their Functions and Translational Data

Genetic variants in eicosanoid pathway genes show significant associations with a high risk of chronic inflammatory diseases. As result, they might be used as promising biomarkers

in a pre- and post-treatment clinical setting of these diseases. Indeed, their identification may be translated in the realization of a personalized medicine. On the other hand, personalized medicine includes the concept that each disease in each person is unique in causes, rate of progression and responsiveness to classical therapies. Genetic variants in eicosanoid enzyme and receptor signaling pathway genes may also operate in combination to create a 'risk profile' and modify the disease onset, progression and the effectiveness of therapies. On the other hand, this concept also illustrates as the physiological effects mediated by each component of eicosanoids are diverse and complex. This diversity is due to variability in genetics background of each individual determining a diverse molecular and structure composition of eicosanoid enzymes, target receptor pathways and GPCR signaling. This influences their activities, acting as *enhancers* or *inhibitors* of both physiological processes and several chronic inflammatory diseases.

In our recent studies we evidenced that polymorphisms (-765G/C;rs20417) in PTGS2 (COX-2) (NM-000963) and (-1708 G/A) 5-Lo (NM000698) genes are able in modulating the susceptibility of prostate cancer, Alzheimer disease and myocardial infarction, creating a risk profile (see Table 1) [1-4]. In the light of these data, we propose the following working hypothesis for the therapeutic treatment of subjects with severe risk factors for these diseases before the clinical illness appearance. In particular, concerning the PTGS2 and 5-LO genes, the presence of high responder alleles in a subject suggests the possibility of preventive treatment with specific inhibitors of eicosanoids or their enzymes. For people who do not respond or handle NSAIDs therapy, other more sophisticated possibilities might be used, such as monoclonal antibodies directed versus receptor eicosanoid pathways, acting as antagonists or antagonists. In addition, we suggest other possible therapeutic and nutrition interventions and life-style modifications, in order to develop potential strategies against chronic inflammation and its pathological complications. In particular, we emphasize the *use of a Mediterranean diet, or rich in polyphenols and resveratrol, curcumin, probiotics and prebiotics, and physical activity* [3, 5]. These potential strategies might modulate/inhibit the inflammatory eicosanoid effects and contemporarily improve and potentiate the synthesis and actions of those eicosanoid molecules having anti-inflammatory effects, such as lipoxins and resolvins (see Chapters 16, 17).

These suggestions are in agreement with the current research on innovative parameters capable of identifying pro-inflammatory genetic risk profile of individuals and developing new translational therapeutic treatments for diagnosis, prevention, and therapy of multifactorial inflammatory diseases. These continuous efforts are, indeed, focused on improving the knowledge of the mechanisms involved in pharmacogenomics [3, 4, 6,7]. The pharmacogenomics analyzes the role of genetic variability in determining inter-individual (between individual) variability in responses to a pharmacological therapy [8-10]. Pharmacogenetics represents a gene-by-environment interaction whereby variation in a gene interacts with an exposure to a drug (the "environment") to alter a measurable phenotype related to drug efficacy or toxicity. The ultimate goal of pharmacogenetics research is the development of personalized medicine through genetic markers (individual genetic profiles), which would accurately predict as individuals with a particular condition would respond to a specific medical therapy, not respond to a therapy, or experience adverse effects [8-10]. Genetic variants in eicosanoid pathway genes represent potential candidates. On the other hand, the characterization and annotation of the human genome, coupled with the development of resequencing and genotyping technologies, have led to an exponential

increase in the number of published genetic association studies on the role of polymorphisms in eicosanoid pathway genes in multi-factorial inflammatory diseases. Such studies contribute to understand the complex interplay between the genetics and environmental contribution to disease, and the identification and characterization of pathways essential to the pathophysiology of the disease of interest, such as eicosanoid pathways [8-10]. In an ideal situation, when the gene of interest is pivotal to the development of the disease, identification of the genes (and the proteins they encode) that lead to disease would allow the rational development of pharmacotherapy directed at disease pathogenesis, and potentially lead to disease prevention strategies. This might be the case of eicosanoid pathway genes [3,4,6,7]. This concept additionally leads to consider that in the complex interaction of genetic background with environmental factors, a crucial role is mediated by epigenomics mechanisms. Epigenomics is the systematic study of the global gene expression changes due to epigenetic processes, but not to DNA base sequence changes [11-13]. Epigenetic processes consist in heritable modification that result in a selective gene expression or repression and consequently in phenotype changes, such as those correlated to disease onset, and hence of pathways essential to the pathophysiology of a disease. These changes include nucleosome positioning, post-translation histone modifications, action of small RNAs, DNA replication timing, heterochromatinization, and DNA methylation [11-13]. Among the environmental factors, the diet affects epigenetic modifications. Nutrients can be active on specific sites. For example, vitamin B12, vitamin B6, riboflavin, methionine, choline and betaine, well known as folates, regulate levels of S-adenosylmethionine and S-adenosylhomocysteine, donor of -CH<sub>3</sub> group and methyltransferase inhibitor respectively [14-16]. Curcumin, resveratrol, polyphenols and flavonoids, phytoestrogen, and lycopene are also considered key nutritional factors both for regulation of enzymes involved in acetylation and deacetylation mechanism and one-carbon metabolism. A diet rich in vegetables and fruit, such as Mediterranean diet, may contain these nutrients. On the other hand, we observed that these effects are significantly associated with the longevity, and they are particularly characteristic of Sicilian centenarians, which observe this kind of diet, as we reported [17]. Sicilian centenarians, as all centenarians, are equipped to reach the extreme limits of human life span and, most importantly, to show relatively good health, being able to perform their routine daily life and to escape fatal inflammatory age-related diseases, such as cardiovascular diseases, Alzheimer disease and cancer (see Table 1) [18]. Since genetic and environmental factors contribute to longevity, this may suggest that epigenetic events associated with the diet-induced modifications are very important for successful ageing processes [18]. Furthermore, several literature data reported a possible link between epigenetic and several age-related diseases, such as cancer, metabolic syndrome, diabetes and neurodegenerative disorders [19,20]. Stable propagation of gene expression from cell to cell during disease pathogenesis is regulated by epigenetic mechanisms [19,20]. For example, during the diabetes onset epigenetic changes act on insulin and insulin metabolism by regulating the gene expression [21-23]. In particular, a recent study has demonstrated that human insulin gene and mouse insulin 2 gene expression are under control of epigenetic changes in CpGIs. In insulin non expressing cells, methylation mechanisms act on the promoter region of insulin coding gene, while in insulin expressing cells demethylation conditions act in the same site resulting the insulin gene expression [21-23]. Another study on monozygotic twin has demonstrated that insulin resistance also is under control of DNA methylation [21-23]. Alterations in insulin pathway are known to be involved in metabolic diseases, such as metabolic syndrome, insulin resistance and type 2

diabetes [21-23]. Recent data also support the existence of a relationship between these alterations and Alzheimer's disease [21-23]. Accordingly, emerging evidence is reporting epigenetic effects on genes encoding eicosanoid pathways.

In the light of these observations, future studies will be need to evaluate the weight of epigenetic changes in expression or inhibition of eicosanoid pathway genes.

**Table 1. Data of Allele frequencies of SNPs in Cox-2 and 5-Lo genes of our studies [1-4, 18]**

Genes	Alleles of SNPs or genetic variant	Centenarians	Young controls (<55 years)	MI patients (< 55 years)	P
		N=96 males	N= 170 males	N=140 males	
Cox-2	-765 G	122 (63.5%)	240 (70.6%)	232 (82.8%)	0.000007
	-765 C	70 (36.5%)	100 (29.4%)	48 (17.2%)	
5-Lo	-1708 G	180 (93.7%)	302 (88.8%)	244 (80%)	0000003
	-1708 A	12 (6.3%)	38 (11.2%)	56 (20%)	
	21 C	176 (91.7%)	299 (88%)	255 (80.4%)	
	21 T	16 (8.3%)	41 (12%)	55 (19.6)	
Genes	Alleles of SNPs or genetic variant	Centenarians	Young controls (<55 years)	PC patients (< 55 years)	P
		N=55 males	N= 125 males	N=50 males	
Cox-2	-765 G	67 (61%)	176 (70%)	77 (77%)	0.05
	-765 C	43 (39%)	74 (30%)	23 (23%)	
5-Lo	-1708 G	104 (95%)	223 (89%)	77(77%)	0.0007
	-1708 A	6 (5%)	27 (11%)	23 (23%)	
Genes	Alleles of SNPs or genetic variant		Age matched controls (73.21±8.24 years)	AD patients (74.88±8.44 years)	P
			N=190	N=341	
Cox-2	-765 G		568 (83.3%)	292 (76.8%)	0.01
	-765 C		114(16.7%)	88 (23.2%)	
5-Lo	-1708 G		589(86.4%)	349 (91.8%)	0.007
	-1708 A		93(13.6%)	31 (8.2%)	

## Emerging Data on the Role of MicroRNA

Of help might also be investigations on microRNA correlated to eicosanoid pathway genes. MicroRNAs (miRNAs) are small noncoding RNAs that take part in post-transcriptional regulation either by arresting the translation or by cleavage (degradation) of mRNA targets. MiRNA regulation is performed by pairing the miRNA to sites in the messenger RNA of protein coding genes. miRNAs have been thought to be involved in many biological processes (i.e., cell proliferation, death, differentiation, tissue degeneration) and are believed to regulate the expression of approximately one-third of all human genes.

Mature miRNAs bind to their target mRNAs by complete or incomplete complementation of their 50-end nucleotides 1–8 (seed sequences) with a binding site in the

30- or 50-untranslated regions of target transcripts or in the coding sequences. This process results in direct cleavage of the targeted mRNAs or inhibition of translation.

Currently, nearly 1700 human miRNAs have been identified [24, 25]. In recent years, miRNAs are emerging as possible diagnostic and prognostic biomarkers for different pathologies, including chronic inflammatory diseases, and thereby improving aspects of disease management (diagnosis, prevention, treatment and prognosis) [24, 25]. Accordingly, emerging data are suggesting the role of miRNAs in the regulation of eicosanoid pathways and their enzymes, and hence able to control the inflammatory processes. In the last years, several miRNAs (miRNA-101, miR199a, miR26b and miR-146a) have been reported to be involved in the regulation of Cox-2 expression [26]. In addition, a set of miRNAs including hsa-miR-21, hsa-miR-146b and hsa-miR-219 has been demonstrated to regulate the biosynthesis of leukotrienes [26].

This indicates that the miRNAs are involved in the control of key enzymes of inflammatory signaling. A growing number of miRNA have been identified in regulating the key player Cox-2 of eicosanoid production. Furthermore, other miRNAs controlling enzymes of the leukotriene branch have been detected suggesting that further steps of the signaling cascade may also be targeted by miRNAs. The extent and importance of miRNA-mediated regulation in inflammatory processes remain to be discovered. A better understanding of the role of miRNAs in lipid signaling will be of interest not only for basic research, but it may also represent a way for discovering interesting novel therapeutic targets. A decade after the realization that miRNAs are important cellular regulators, miRNA inhibitors, so-called antagomirs, antisense molecules specifically binding to the miRNA, have been developed [27]. Antagomirs are nucleic acids that are significantly modified in their backbones to avoid degradation, for example as locked nucleic acids (LNA). They have been tested in a pioneering non-human primate study, which revealed that the antagomirs are specific, stable and non-toxic when administered intravenously. An example is due by Miravirsen (SPC3649), the first miRNA-targeted drug, which has entered in clinical trial studies [28]. However, further investigations will be important for the clinical application of miRNAs and their inhibitors, the antagomirs. These applications may open a new avenue for the development of new therapeutic concepts also for treatment of inflammation.

## Conclusion

As described above, physiological and pathological effects of eicosanoids are modulated by genetic variants in genes encoding the enzymes or receptor signaling pathways involved in their metabolism. This influences the action of eicosanoids as enhancers or inhibitors. In addition, the expression of their genes is regulated by miRNA and other epigenetic mechanisms which may modify the intensity of mediated effect of eicosanoid pathways associated with chronic inflammation and their pathological complications, such as multifactorial diseases. The epigenetic mechanisms are regulated by a complex gene-environment interplay, where lifestyle conditions, including the diet and physical activity have a crucial importance. Fine knowledge of the mechanisms involved might consent the development of new strategies of prevention for chronic and multi-factorial diseases (Figure 1).

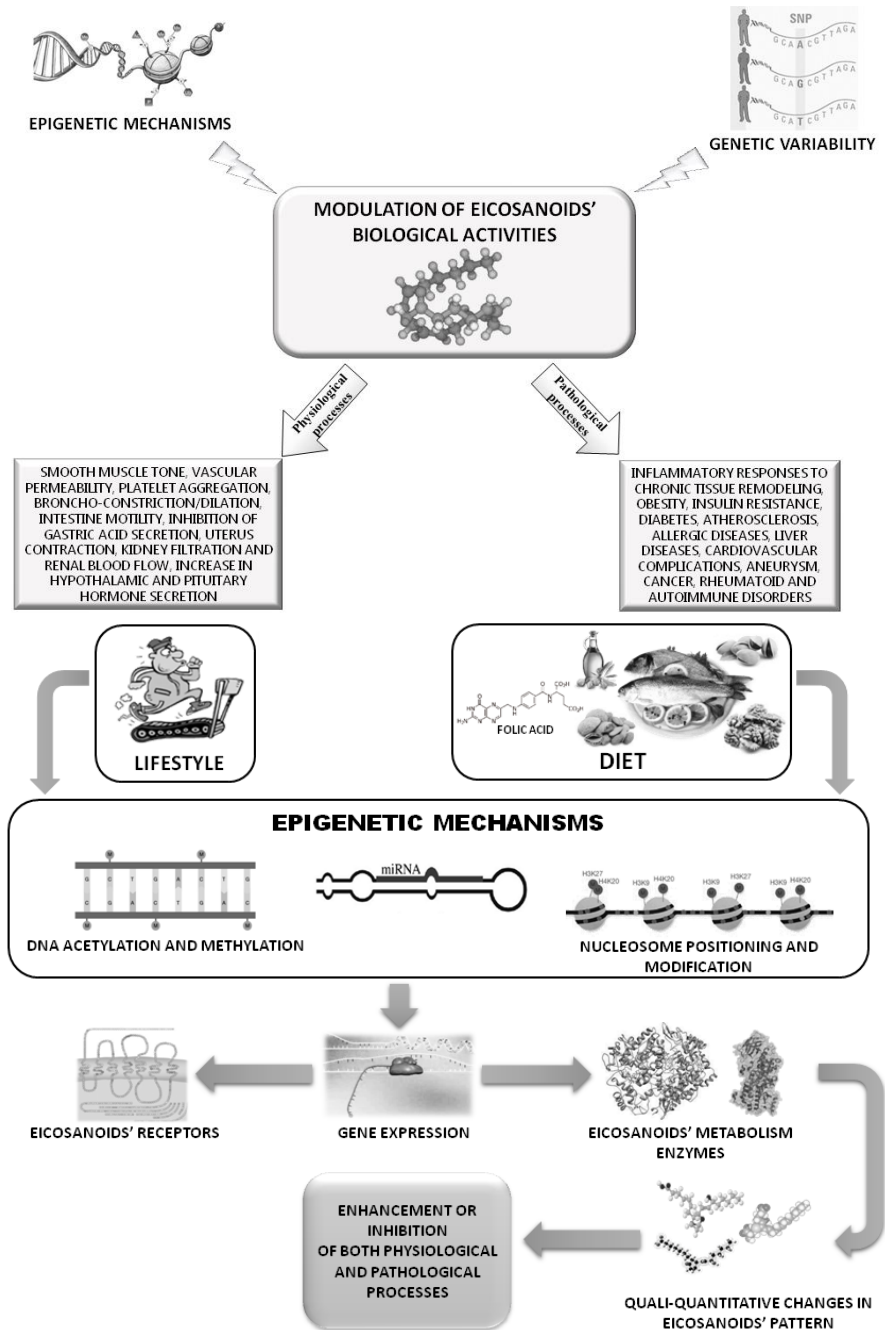


Figure 1 (A and B). Epigenetic mechanisms and variability genetics in the modulation of eicosanoid biological activities. Eicosanoids mediate both physiological actions and pathological effects, modulated by genetic variants in enzyme or receptor signaling pathways genes. This results in a action of eicosanoids as enhancers or inhibitors. In addition, the expression of eicosanoid pathway genes is regulated by miRNA and other epigenetic mechanisms which may modify the intensity of mediated effect of eicosanoid pathways associated with chronic inflammation and their pathological complications, such as multifactorial diseases. The epigenetic mechanisms are regulated by a complex gene-environment interplay, where lifestyle conditions, including the diet and physical activity have a crucial importance.

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## Editor's Biography

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**Carmela Rita Balistreri** received is PhD in Pathobiology in 2007. She is actually an Assistant Professor of Clinical Pathology and a member of the Department of Pathobiology and Medical Biotechnologies, University of Palermo, Italy. In the first phases of her studies, Dr. Balistreri has been actively involved in the study on the immunological mechanisms of senescence, such as apoptosis, interleukin production, activity of natural killer and granulocyte cells in aged individuals. After this stage, her main field of scientific interest has been the study of the association between the haemochromatosis gene mutations and longevity and age related diseases, such as acute myocardial infarction and sporadic Alzheimer's disease. Subsequently, her principal interest has been focused on biogerontological studies, case control studies having the key aim to analyze the role of candidate immune-inflammatory genes in longevity and age related diseases. In these studies a particular approach has been used: the centenarians as a second control group, the "supercontrol" group, since they have escaped major age-related diseases. In particular, these studies demonstrated the role of antiinflammatory polymorphisms of genes involved in

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inflammatory and innate immunity responses (i.e., TLR4, TLR2, CCR5, CRP, Connexin37, MMP9, PECAM-1/CD31, CD14, Cox and 5LO) to increase the chance to achieve longevity, in a modern environment with reduced pathogen load and improved control of severe infections by antibiotics. Furthermore, these data also evidenced the opposite role of these polymorphisms in longevity and age-related diseases, including myocardial infarction, prostate cancer and Alzheimer's disease. In the specific field of Alzheimer's disease, she has published with her group several papers on the role of genetics on inflammation in AD pathophysiology. Recently, Dr. Balistreri is also focusing her studies on identifying the cellular, molecular and genetic mechanisms involved in the pathophysiology of thoracic aorta aneurysms and mitral valve diseases, and suggesting diagnostic, prognostic and preventive biomarkers and targets for personalized treatments. International recognition of her scientific achievements is reflected in frequent citations of her papers (<http://www.scopus.com>) and the referee activity for the following journals: Human Immunology; Diabetic Medicine; Expert Review of Neurotherapeutics; Human Genetics; Ageing Research Reviews, Circulation, Immunity and ageing; PLOSone. Her h-index for the 83 papers (1998-2014) is 23, (<http://www.scopus.com>). To improve her scientific cultural level and to compare her data with those of other groups, she has attended 78 congresses focused on following topics: Age-related diseases, Immunosenescence, Longevity, Model system, Aging & Wellness and Regenerative Medicine, Cardiovascular diseases. In several congresses she has attended as speaker. Dr. Balistreri received several awards and honors: award for 5 years during Postgraduate in Clinical Pathology and 3 years during the doctoral degree; in October 2011 awards "Alvise Cornaro" for researchers, Alvise Cornaro Center, University of Padova; in June 2010 fellow for participation of Advanced Lecture Course "*Protein maintenance and turnover in ageing & diseases*" - FEBS/SFRR-E/IUBMB Societies, Spetses island, Greece; in September 2007 fellow for participation of *MiMage and Link-age joint Summer-school, Les Diablerets-Switzerland*; in September and November 2006 winner of "travel grant" in *Ageing research in immunology: the impact of genomics* congress, Paris, France and European Conference on Ageing, Innsbruck, Tyrol, Austria; in May 2004 award during Italian Society of Clinical Pathology, 54<sup>o</sup> National Congress, AIPAC, Rome; in September 2001 winner of "Genetics Premium", VIII National Congress AIBT, Udine. Dr. Balistreri is a member of Italian Society of Pathology.

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