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Polyunsaturated Fatty Acids and Inflammatory Diseases

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1. Introduction

Polyunsaturated fatty acids and their metabolites are crucial to the physiologic and pathophysiologic processes in inflammation. The balance between *n-6* and *n-3* polyunsaturated fatty acids in body tissues is a key to regulating the inflammatory reaction and preventing exacerbated inflammation in inflammatory disorders. Altering fatty acid type and their composition in phospholipids through diet supplements for beneficial outcomes in disease has been of major interest to the community. The sources of polysaturated fatty acid include de novo synthesis, essential fatty acids obtained from the diet, elongation and desaturation to obtain fatty acids of longer chain length by tissues.

The types of fatty acids being esterified in membrane phospholipids provide a characteristic fatty acid composition of the phospholipids which can dictate the characteristics of the inflammatory response depending on the types of metabolites of polyunsaturated fatty acids formed through the lipoxygenase (LOX) and cyclooxygenase (COX) pathways, either promoting or inhibiting the inflammatory process, by controlling intracellular signalling pathways, such as PKC, MAP kinases, PI3 kinase etc

While focusing on the use of n-3 polyunsaturated fatty acids in treating rheumatoid arthritis, cardiovascular diseases and asthma, it is highlighted that such treatments are at a 'cross road' because of poor understanding of the field of lipidomics and in supplementation approaches in those with various illnesses, including inflammatory diseases.

2. Sources of arachidonic acid and other fatty acids

Fatty acids in the body can be obtained by *de novo* synthesis in tissues, through the diet or from the hydrolysis of membrane phospholipids. Human beings can synthesize fatty acids up to 16:0 (palmitate) *de novo* from acetyl coenzyme, by a series of cycles of sequential condensation, reduction, dehydration and reduction. The chain is elongated by two carbon atoms per cycle. 16:0 is then elongated to 18:0 (stearate) and desaturated to yield 18:1*n-9*

(oleate). Alternatively, 16:0 is desaturated to 16:1*n-9* (palmitoleate) and elongated to 18:1*n-9*. A variety of longer chain fatty acids can be derived from 18:1*n-9* by a combination of elongation and desaturation reactions. However, mammalian cells are unable to perform these reactions because they do not express the enzymes, Δ12 and Δ15 desaturases to introduce double bonds at carbon atoms beyond C-9. Consequently, mammalian cells cannot synthesise 18:2*n-6* (linoleic) and 18:3*n-3* (α-linolenate). These fatty acids, required by the animal but cannot be synthesised endogenously, are therefore considered as essential fatty acids and are obtained from the diet. The essential fatty acids serve as starting points for the synthesis of longer chain fatty acids such as 20:4*n-6* (arachidonic acid, AA) and the *n-3* fatty acids 20:5*n-3* (eicosapentaenoic acid, EPA) and 22:6*n-3* (docosahexaenoic acid, DHA) by elongation and desaturation through the action of elongases and desaturases (e.g Δ6, Δ5 and Δ4). AA is derived from 18:2*n-6* while EPA and DHA are derived from 18:3*n-3*.
Dietary fatty acids can be obtained from animal meats, fish, green vegetables, and from oils derived from the above. They mainly occur as triacylglycerols. Essential fatty acids are found in abundance in green leafy vegetables and the seeds of most plants. The *n-3* fatty acids EPA and DHA are abundant in marine oils and fish rich diets are another source of these fatty acids. Grain-fed animals are rich in AA (Simopoulos, 1991).

3. Transport and uptake of fatty acids

Following absorption by the intestine, the fatty acids are transported to tissues where they may be utilized immediately or stored. At least four types of vehicles have been shown to be involved in their transportation: (i) chylomicrons, where dietary triacylglycerol is carried in protein-coated lipid droplets and transported to the whole body from the intestine; (ii) ketone bodies (acetoacetate and β-hydroxybutyrate) and (iii) very low density lipoprotein (VLDL), which are responsible for transporting fatty acids, processed by or synthesised in the liver, to either adipose tissue for storage, or to various tissues to be used for cell structure and metabolism and (iv) as non-esterified fatty acid. Triacylglycerol in the blood is enzymatically hydrolysed by lipases, such as lipoprotein lipases, on the surface of endothelial cells. The released free fatty acids become bound to serum fatty acid binding protein eg. albumin, type IV fatty acid transporter, which carries the released fatty acids in the blood stream to appropriate tissue sites. The free fatty acids in the extracellular fluid continuously exchange with the intracellular fatty acids which are released by the action of phospholipase A_2. This process is called intracellular fatty acid turnover (McGarry, 1993).
How fatty acids are taken up by cells remains unclear. It has been proposed that fatty acids firstly become dissociated from albumin and then bind to a fatty acid transporter protein in the plasma membrane or a flip flop mechanism. A fatty acid translocase (FAT) with homology to CD36 has also been reported to be involved in the transport of long chain fatty acids (Bonen et al, 2002). There is evidence that fatty acids can also enter cells by a flip flop mechanism (Kamp et al, 2003). These two modes of fatty acid uptake need not be mutually exclusive. Once inside the cell, fatty acids are transported to various intracellular sites by the cytosolic fatty acid binding protein (FABP, 14-15 kDa) where they interact with appropriate proteins/structures to evoke cellular responses (Spector, 1992; Poirrier et al, 1996). The precise mechanism by which fatty acids are taken up by neutrophils is still poorly defined. However, it has been demonstrated that the ability of a fatty acid to partition into the neutrophil plasma membrane is not sufficient to evoke superoxide production (Steinbeck et al, 1991). Similarly, the observation that saturated fatty acids (lacking biological actions) have a greater ability to partition into the plasma membrane of cytotoxic T lymphocyte than

cis unsaturated fatty acids, is inconsistent with their biological activity being totally caused by membrane partitioning of a fatty acid (Anel et al, 1993).

4. Arachidonic acid and its metabolites are central to the development of inflammatory reactions

AA is an important promoter of physiologic processes of body tissue and organs. AA and its metabolites can act as an intercellular signalling molecule as well as intracellular secondary signalling molecule (Ferrante et al. 2005). The role of this fatty acid and its products in autoimmune and allergic inflammation has also been abundantly described and appreciated, such that AA and pathways involved in its metabolism have been the targets of medications. The resolution of inflammation initiated through the release of AA from cellular membrane phospholipids is important and where its generation persists, this reaction evolves into a chronic and debilitating condition. The main pathways for its metabolism have been the lipoxygenase (LOX) and cyclooxygenase (COX) enzymes.

AA and its metabolites can be generated at several cellular points. Apart from production at local tissues, be these immune cells or barrier cells such as endothelial cells, epithelial cells etc, the infiltrating cells, particular leukocytes, provide a rich source of these mediators of inflammation. Thus it is not surprising that the lipids influence several key functions of leukocytes which include, chemotaxis, oxygen radical generation, granule enzyme release and cytokine production (Ferrante et al, 2005).

AA may control inflammation through several activities. AA per se has been shown to cause cellular activation independently of its metabolism via the LOX and COX pathways (Ferrante et al, 2005). The generation of several metabolites such as LTB4 and PGE2 gives the system a potent inflammatory potential.

5. Release of AA from the cellular phospholipid pools and the generation of eicosanoids

Cellular activation leads to the activation of Phospholipase A_2 (PLA_2) and the release of AA from the sn-2 position of the phospholipids (Balsinde et al, 2002). This activation can be brought about by many different types of agonists acting usually via a cell surface receptor. This includes intercellular signalling molecules such as cytokines which are involved in inflammatory responses in both the context of physiologic and pathophysiologic states.

Amongst the various structurally different forms of the PLA_2 is the cytosolic ($cPLA_2\alpha$, Group IVA), believed to play an important role in eicosanoids production (Kita et al, 2006; Ghosh et al, 2006). Submicromolar concentrations of Ca^{++} promotes the translocation of $cPLA_2\alpha$ from the cytosol to the perinuclear membrane where it becomes activated by MAP kinases (Lin et al, 1993; Nemenoff et al, 1993). There it causes preferential hydrolysis of AA-containing phospholipids, releasing AA to become available to downstream enzymes including LOX and COX, important in production of leukotrienes and prostaglandins (Fig 1). Its importance has been concluded from studies showing that mice deficient in $cPLA_2\alpha$ were not susceptible to allergy induced brocho-constriction, airway hyper-responsiveness, as well as adult respiratory distress syndrome (Wu et al, 2010). Mice deficient in cPLA2α were also protected from experimental autoimmune encephalomyelitis (Marusic et al, 1995).

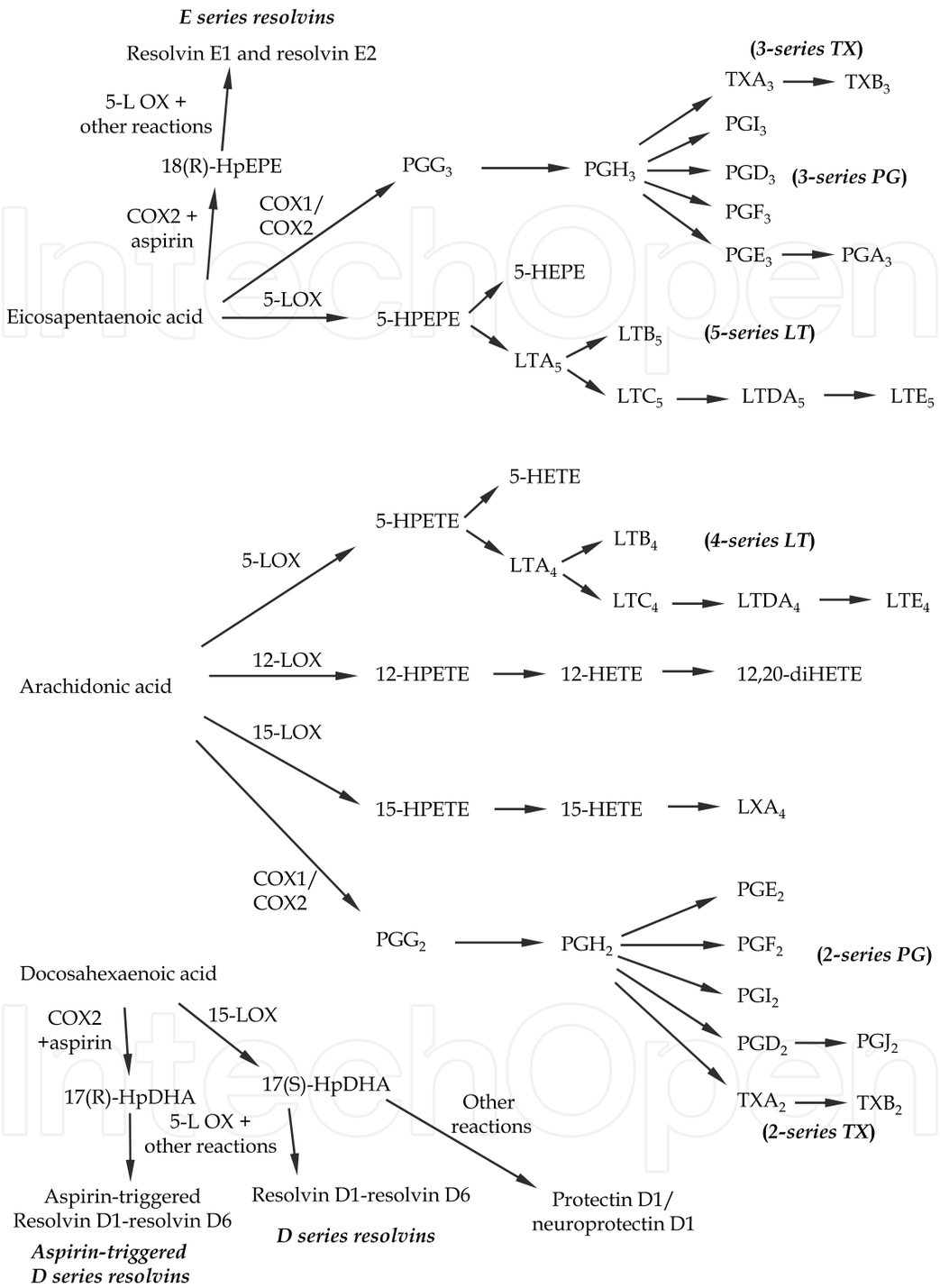

Fig. 1. Outline of the metabolism of arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid by the lipoxygenases and cyclooxygenases. LOX, lipoxygenase; COX, cyclooxygenase; HPETE, hydroperoxyeicosatetraenoic acid; HPEPE, hydroperoxypentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HEPE, hydroxypentaenoic acid; LT, leukotrienes; TX, thromboxane; PG, prostaglandin; LX, lipoxin; HpDHA, hydroperoxyDHA.

Cell activation leads to the generation of AA-derived eicosanoids (Fig 1). The main ones are divided into three groups, leukotrienes (LTs), prostaglandins (PGs) and lipoxins (LXs). Their biological properties give rise to a regulatory network of inflammation, having both an effect on cells of the immune system, macrophages, T cells, neutrophils as well as non-immune cells such as endothelial cells. While the metabolism of AA via the LOX and COX pathways is considered to be inflammatory, it is also evident that some products may exhibit anti-inflammatory properties.

It is clear from several studies that different eicosanoids are generated at different times of an acute inflammatory response (Serhan, 2005; Serhan et al, 2008). Hence, prostaglandins and leukotrienes are rapidly generated, whereas the lipoxins, also produced from AA, are generated later with the onset of the resolution phase. During acute inflammation, activation of cPLA2 results in the conversion of the released AA by 5-LOX and COX to leukotrienes such as LTB4, a potent neutrophil activator and chemoattractant, and proinflammatory prostaglandins which control local blood flow, vascular dilation and permeability changes needed for leukocyte adhesion, diapedesis, and recruitment. The prostaglandins initiate a number of responses relevant in inflammation (i.e., vasoconstriction, vascular permeability changes, pain, vasodilation and edema). However, the production of prostaglandins such as PGE2 and PGD2 which possess proinflammatory properties, also signals the end of the inflammatory response by activating the transcriptional regulation of 15-LOX in neutrophils that initiates an eicosanoid class switch from a proinflammatory profile to a resolving profile, including the generation of EPA- and DHA-derived resolvins and/or protectins. In murine models, resolution of acute inflammation is accompanied by the appearance in exudates of EPA and DHA, which follows the appearance of non-esterified AA. Indeed, leukotrienes (potent chemoattractants) are deactivated and the transcriptional regulation of enzymes required for LX and resolvin production is activated. (Serhan, 2005; Serhan et al, 2008). The combined actions of 5-LOX, 12-LOX and 15-LOX lead to the generation of lipoxins such as LTA_4, resolvins and/or protectins. These mediators have anti-inflammatory and pro-resolving activities.

6. Biological properties of eicosanoids

The cellular actions of the LTB_4 and cysteinyl leukotrienes (cysLTs), LTC_4, LTD_4 and LTE_4, occur through their binding to specific G protein-coupled receptors found on responsive tissues e.g., smooth muscle and inflammatory cells of the immune system e.g., neutrophils. The distribution of receptors for LTs on various cell types are summarised in Table 1 (Okunishi et al, 2011). Two receptors for cysLTs have been well characterised, CysLt1 and CysLt2. LTD_4 has high affinity and LTE_4 has low affinity for these receptors. But other receptors are being recognised for CysLTs which includes a specific receptor for LTE_4 (Maekawa et al, 2008).

LTB_4 has two receptors, a high affinity, BLT1 and lower affinity, BLT2 (Yokomizo et al, 2000). Notably BLT1 is expressed predominantly on leukocytes (Table 1). The biological role of BLT2 requires further studies. LTB_4 is recognised for its potent chemoattractant properties for neutrophils. It also activates other neutrophil responses such as respiratory burst (oxygen radical production) and degranulation. As indicated by the expression of BLT1 on other leukocytes, apart from neutrophils (Table 1), LTB_4 activates also macrophages, eosinophils, T cells and dendritic cells (DCs).

Cell-type	BLT1	CysLT1	CysLT2
Macrophages	+	±#	±
Dendritic cells	+	+	?
B cells	?	+	?
CD4 + T cells	+	+	?
CD8 + T cells	+	?#	?
Neutrophils	+	+	+
Eosinophils	+	+	+
Basophils	+	+	+
Mast Cells	+	+	+
Airway smooth muscle cells	+	+	?
Endothelial cells	+	+	+

Table 1. Leukotriene receptor expression. (+), positive; (±), negligible; (?), not determined; (#) up-regulation upon cell activation (adapted from Okunishi et al, 2011)

The action of PGs is through the rhodopsin-like 7-transmembrane-spanning G protein-coupled receptors. These prostanoid receptors subfamily consists of 8 members EP1/EP2/EP3/EP4 bind PGE_2, DP/CRTH2 bind PGD_2, FP binds $PGF_2\alpha$, IP binds PGI_2, TP binds TXA_2 (Table 2). The CRTH2 receptor is expressed on Th2 lymphocytes and a member of the fMLF receptor superfamily (Ricciotti and FitzGerald, 2011).

7. Eicosanoids and inflammation

7.1 Leukotrienes

Inflammation, whether physiologic or pathophysiologic, is manifested by the accumulation of leukocytes and plasma leakage into the tissue site. The leukotrienes form a major family of the inflammatory mediators generated which significantly contributes to the process. LTB_4 through its chemotactic properties for several cell types including neutrophils and T cells, causes infiltration and accumulation of leukocytes at inflammatory foci. LTB_4 increases vascular permeability of post capillary venules. The ability of LTs to activate DCs has indicated a role in antigen presentation and T cell sensitisation. Relevant to this action is the finding that LTs are produced through the innate immune response, where the various pattern recognition receptors (e.g., Toll-like receptors, TLRs) are expressed on different cell types (Alvarez et al, 2010). Thus LTs may play important roles in the adaptive immune response at an early phase.

Prostaglandin Class	Receptor subtype	Type of cells/tissues
PGE_2	EP1, EP2, EP3, EP4	Brain, kidneys, VSMCs, platelets
PGD_2	DP, CRTH2	Mast cells, brain, airways, Th2 lymphocytes
PGF_{2a}	FPA, FPB	Uterus, airways, VSMCs, eyes
PGI_2	IP-IP, IP-TP_a	Endothelium, VSMCs, platelets, kidney, brain
TxA_2	TP_a, TP_b	Platelets, VSMCs, macrophages, kidney

Table 2. Expression of prostanoid receptors (adapted from Ricciotti and FitzGerald, 2011). VSMC ; vascular smooth muscle cells

The role of LTs has been established as a key mediator in several inflammatory diseases, both in experimental and clinical settings. This includes asthma and allergic rhinitis (Dahlen,

2006; Peter-Golden & Henderson, 2007). Their importance extends to the pathophysiology of atherosclerosis and progression of cancer.

7.2 Prostaglandins

PGE_2 is involved in all the signs of inflammation; redness, swelling and pain (Ricciotti & Fitzgerald, 2011), through its effects on arterial dilation, increased permeability of the microvasculature, peripheral sensory nervous and central sites within the spinal cord and brain. PGE_2 acts via one of its receptors, EP1 to EP4. Using KO mice deficient in these receptors, it has been appreciated that they play important roles in hyperalgesia (PGE_2-EP1), paw swelling in collagen-induced arthritis (EP2, EP4), Carrageenan-induced paw oedema/pleurisy (EP2, EP3), IL-6 production and joint destruction in RA and anti-inflammatory effects of PGE_2 as seen in allergic inflammation.

The regulation of various cell types by PGE_2 occurs through the expression of different EP receptors. PGE_2 exerts an anti-inflammatory effect on functions of neutrophils, macrophages and natural killer cells, the cell-types which underpin the innate immune response (Harris et al. 2002). PGE_2 also exerts regulatory actions on macrophages, DCs, T and B cells which may manifest itself as either inflammatory or anti-inflammatory actions. Examples are regulation of DC cytokine profiles and Th1 or Th2 lymphocyte development (Egan et al, 2004). Engagement of EP4 by PGE_2 in DCs and T cells promotes differentiation to Th1 and also Th17 lymphocytes (Yao et al, 2009). Other ways that PGE_2 regulates the immune response is through its role in the development of DCs with a migratory phenotyping to promote homing to drain lymph nodes (Kabashima et al, 2003; Legler et al, 2006), as well as upregulating the expression of co-stimulatory molecules on these cells (Krause et al, 2009). In an anti-inflammatory manner, PGE_2 suppresses Th1 cell development, B cell function and IgE-driven inflammatory response (Roper et al, 1995; Harris et al, 2002**)**.

PGI_2 has potent vasodilator actions and is known to be important in regulation of cardiovascular homeostasis. The eicosanoid is an inhibitor of platelet aggregation, leukocyte adhesion and vascular smooth muscle cell proliferation (Gryglewski, 2008; Kawabe et al, 2010). Mice deficient in receptors for PGI_2 show an abrogation of the ability of PGI_2 to potentiate the bradykinin-induced microvascular permeability and a substantially reduced carrageenan-induced paw oedema (Murata et al, 1997). Other actions of PGI_2 involving the IP receptors is an allergic inflammation where it may suppress the Th2-mediated lung inflammation (Jaffar et al, 2002).

PGD2 has inflammatory and homeostatic properties. Thus while in the brain it regulates sleep and various other central nervous activities, it has inflammatory function particularly in atropic conditions. The eicosanoid promotes broncho- constriction and airways neutrophil infiltration, typical of allergic asthma (Hardy et al, 1984; Emery et al, 1980; Fujitani et al, 2002). The DP1 and DP2/CRTH2 receptors bind PGD2 with similar high affinity and both are responsible for the pro-inflammatory responses. Effects caused by PGD2 are dictated by the differential expression of these receptors in tissues such as the expression of DP1 receptors in bronchial epithelium is believed to promote the production of cytokines and chemokines which recruit eosinophils and lymphocytes in airway inflammation and hyperreactivity associated with asthma (Kabashima & Narumiya, 2003). Using mice deficient in DP1 receptors the role of PGD2 and the receptor in airway hyperreactivity and Th2-mediated lung inflammation has been documented in animal models (Matsuoka et al. 2000). The use of DP1 antagonists in animal models supports a role

for the receptor in antigen-induced microvascular permeability and ovalbumin (OVA)-induced airway hyperreactivity. Reports have also suggested that the DP2/CRTH2 receptors contribute to inflammatory reactions by controlling cell traffic and the effect of function of leukocytes in which it is expressed, Th2 cells, mast cells and eosinophils. An increase in its expression has been shown to be associated with some forms of atopic dermatitis. However inflammation in other contexts may be inhibited by PGD2-DP1 receptor where inhibition of DC migration affects T cell proliferation and cytokine production (Hammad et al, 2003).

TXA_2 is highly unstable and its activity is mediated mainly through the TP receptor. Studies regarding the role of this receptor in physiologic and pathophysiologic responses show that its involved in platelet adhesion and aggregation, activation of endothelial inflammatory response and contraction/proliferation of smooth muscle cells (Nakahata, 2008).

8. Arachidonic acid and metabolites in inflammatory disorders

AA and its metabolites have been shown to play major roles in the pathogenesis of several inflammatory conditions (Table 3). Three inflammatory conditions, asthma, rheumatoid arthritis and atherosclerosis, will be used to demonstrate the interest and the importance of AA and its metabolites in such diseases/conditions.

8.1 Asthma

Arachidonic acid and eicosanoids have been long recognised to play a key role in asthma pathophysiology. The hallmark of this condition is airway inflammation and hyperresponsiveness. The importance of the enzyme responsible for releasing AA from membrane phospholipids, PLA_2 has been established using pharmacological inhibitors and more recently genetically modified mice. Bronchoalveolar lavage fluid of asthmatics contains increased amounts of secretory phospholipase A_2 ($sPLA_2$) and increased activity compared to controls (Triggiani et al, 2009; Chilton et al, 1996; Bowton et al, 1997). Particular interest has centred on group X $sPLA_2$ as this was responsible for most of the activity (Hallstrand et al., 2011). Levels of this $sPLA_2$ correlated with eicosanoid release, severity of asthma and airway inflammation (Hallstrand et al., 2011). Further studies have suggested that group X $sPLA_2$ functions in asthma pathogenesis through the release of cysLTs which are involved in airway inflammation and hyperresponsiveness. Mice genetically deficient in group X $sPLA_2$ showed a marked reduction of asthma induced with OVA, manifested as decreased interstitial oedema, and the accumulation of eosinophils and T cells into the lung (Henderson et al, 2007). The Th2 cytokine levels were also decreased in the deficient mice as were the eicosanoids, PGE_2, PGD_2, LTB_4, and cysLTs (LTC_4, LTD_4, LTE_4) (Henderson et al, 2007). Using knock-in mice with human group X sPLA2, it was demonstrated that this could restore the airway inflammation (Henderson et al., 2011). The important role of the high affinity LTB_4 receptor BLT1 also supports the role of LTs in pathogenesis of asthma (Watanabe et al, 2009).

8.2 Rheumatoid arthritis

AA derived eicosanoids have been shown to play a major role in the pathogenesis of RA. In a murine model of arthritis, a role for cPLA2α was examined using the inhibitor, pyrroxyphine (Tai et al, 2010). There was an increase in $cPLA_2\alpha$ activity which correlated

with arthritis severity. Both bone destruction and incidence of arthritis was reduced by the inhibitor. Such effects correlated also with an inhibition of the production of eicosanoids and COX-2 induction.

The importance of eicosanoids in inflammatory arthritis can also be seen from studies with mice which were deficient in either the BLT1 receptor or the low affinity BLT2 receptor. In the collagen-induce arthritis model, BLT1 was found to mediate the disease. Thus, BLT1$^{-/-}$ mice were completely protected and so were the BLT1$^{-/-}$/BLT2$^{-/-}$ double knockout mice (Shao et al, 2006). Because the BLT1$^{-/-}$ mice were completely protected, the authors were not able to determine the contribution of BLT2. This was addressed by Mathis et al (2010) who reported that BLT2$^{-/-}$ mice showed reduced incidence and severity of disease in an autoantibody-induced arthritis model. Note that while the BLT2 receptor is a low affinity receptor for LTB_4, it is in fact a high affinity receptor for the COX-1 derived 12(S)-hydroxyheptadeca-5Z, 8E, 10E-trienoic acid. BTL2 is considered a model target for treating this inflammatory condition.

Prostaglandins levels are elevated in synovial fluid and synovial membranes of RA patients and are believed to play a role in fluid extravasations and pain in synovial tissue, as well as articular cartilage erosion. COX-2 is present in high and COX-1 in smaller amounts in RA synovial tissue. Selective COX-2 inhibitors (e.g. celecoxib, valdecoxib and rofecoxib) have been used to treat inflammation in RA patients, although some (e.g. valdecoxib and rofecoxib) have been withdrawn owing to increased risk of heart attack and stroke in users (James et al, 2007). The presence of the metabolites of AA in RA patients' synovial fluid has been outlined (Grignani et al, 1996). When compared with patients with artherosis (degenerative joint disease), the levels of LTB_4, LTC_4 and 6-keto-PGF1 were significantly higher in RA patients.

8.3 Cardiovascular disease and atherooscleroses

The 5-LOX/LT pathway has been implicated in the development of cardiovascular disease (CVD) based on studies of human genetic variation (polymorphisms in the genes that code for 5-LOX or its activating protein, 5-LO-activating protein (FLAP)) and in animals, leading to the hypothesis that this pathway promotes atherosclerosis, abdominal aortic aneurysm, and myocardial infarction/reperfusion injury. Much of this is based on the known effects of LTs on leucocyte chemotaxis, vascular inflammation and enhanced permeability, and subsequent tissue/matrix degeneration. Data from a series of studies that involved genetic or pharmacological inhibition of either LT biosynthesis (5-LOX, FLAP, LTA_4 hydrolase, LTC_4 synthase) or the LT receptors, have painted a complex picture of 5-LOX/LT participation in cardiovascular disease, which is further complicated by marked differences between mice and humans (reviewed by Poeckel and Funk, 2010). Added to this is another layer of complexity imposed by the cytokine network specific to a particular pathological condition which impacts on the expression level and hence, the contribution of 5-LOX to the overall disease state. Nevertheless, current data suggest roles for 5-LOX in the early/acute stages of atherosclerosis in mice and humans, but only in the advanced stage of the human pathology. Hence, LTB_4 and CysLT are likely to play critical roles in the early phase of atherosclerosis through their influence on leukocyte recruitment, smooth muscle cell proliferation, migration of endothelial cells (properties of LTB_4) and inflammatory cell recruitment, coronary artery constriction and endothelial cell activation (properties of CysLT). In the advanced phase, LTB_4 may affect plaque stability, and the expression of other

components of the 5-LOX pathway such as BLT_1, BLT_2, FLAP, LTA_4 synthase, $CysLT_1$ and $CysLT_2$ are up-regulated (reviewed by Poeckel and Funk, 2010).
The action of LTs in various stages of atherosclerosis development has been reviewed (Back, 2009). The role of LTB4 in the lipid retention and modification stage is seen by the finding that targeting BLT1 receptor decreases the accumulation of lipids and the infiltration of foam cells. The early development of intimal hyperplasia appears also to be influenced by cysLTs and LTB4. Targeting their receptors most likely inhibits intimal hyperplasia with respect to endothelial dysfunction. Cys-LTs have been suggested to play a role in both endothelium relaxation and constriction. The discovery, that endothelial cells express the BLT1 receptor during atherosclerosis (Back et al., 2005) has also implicated LTB4 in causing the changes on the endothelium. The disease progresses into recruitment of leukocytes in the vascular wall and the formation of atherosclerotic plaque. Most likely the macrophages continue to be the centre point of this development, through their ability to produce LTs , such as LTB4 and exacerbate the inflammation at atherosclerotic lesions. This draws in T cells which can stimulate macrophages to generate more LTB4, leading to a vicious cycle, perhaps halted by anti-LT treatments (Back, 2008). Upon rupture of the fibrous cap there is exposure to the blood elements with the consequences of platelet activation and thrombotic occlusion. To date there is only indirect evidence for a role of LTs where it has been shown that 5-LOX is located in areas where matrix metalloproteinases are present and which are responsible for rupture. Plaque in the coronary cerebral arteries will lead to myocardial ischemia and cerebral ischemia, respectively. In myocardial ischemia the role of LTs has been controversial from suggestions of a major, no role and even to possibly protective role (Back, 2009; Adamek et al., 2007). In contrast, in cerebral ischemia, the importance of LTs has been acknowledged. LT synthesis inhibitors and cysLT receptor antagonists limited the damage in animal models.

9. Omega 3 polyunsaturated fatty acids

Supplementation with n-3 fatty acids is of interest because of its potential benefits in treating a range of human diseases and conditions (Table 3). In particular, it is well appreciated that increasing the ratio of *n-3* over *n-6* polyunsaturated fatty acids in membrane phospholipids in patients experiencing inflammation has benefits (Simopoulos, 1991). Thus, fatty acid diet manipulations have been used in treating a wide variety of diseases/conditions including those which have an autoimmune and allergic base. While the mechanisms governing the beneficial effects of certain types of polyunsaturated fatty acids in different types of diseases is likely to vary, it is well appreciated that altering the types of polyunsaturated fatty acids in diets can modify the immune response. This is thought to be the major mechanism by which polyunsaturated fatty acids exert their protective effects in inflammatory and autoimmune disorders (Calder, 2010).
High *n*-3 fatty acid intake for four months significantly increased the general score and sigmoidoscope score of active ulcerative colitis patients compared with a placebo diet (Simopoulos, 1991; Greenfield et al., 1993). The effects were maintained for three months after the fatty acid treatment was discontinued. In a human gingival inflammation model, 28-day treatment with EPA and DHA (1.8g/day) markedly reduced the gingival index in interdental papilla (Campan et al., 1996). Dietary supplementation with fish oil fatty acids in conjunction with conventional treatment (cyclosporin) in psoriasis patients has been shown to improve the skin lesions and decrease the nephrotoxicity of cyclosporin (Simopoulos, 1991). In the treatment of RA, the beneficial effects of fish oil are pronounced and

Conditions
Rheumatoid arthritis
Atherosclerosis
Acute coronary events
Allergic diseases
Asthma
Psoriasis
Inflammatory disease
Multiple sclerosis
Systemic lupus erythematosus
Cystic fibrosis
Type 1 diabetes
Chronic obstructive pulmonary disease

Table 3. Inflammation-based conditions which appear to benefit from n-3 polyunsaturated fatty acid or fish oil supplementation (adapted from Calder et al, 2010)

reproducible in both animal models and in human trials. It has been shown that feeding mice with EPA and DHA, reduces the incidence and severity of type II collagen-induced experimental arthritis (Leslie et al, 1985). Another study demonstrated that n-3 fatty acids given in the form of krill oil or fish oil caused a significant reduction in arthritis score and hind paw swelling in a collagen-induced arthritis model (Ierna et al., 2010). Dietary supplementation with *n-3* polyunsaturated fatty acids in RA patients shows significant relief of joint pain and swelling, and the duration of morning stiffness. This has led to a reduction in requirement for nonsteroidal anti-inflammatory drugs (NSAIDs), with some patients able to discontinue NSAIDs while receiving *n-3* fatty acid supplements (Kremer et al., 1995; Sperling, 1991). A recent clinical study of γ-linolenic acid (GLA, 18:3*n-6*) dietary manipulation in RA patients also showed evidence of the alleviation of disease activity (Zurier et al., 1996). Many recent studies have shown that production of immunological/inflammatory mediators can also be regulated by polyunsaturated fatty acids. Diets rich in *n-3* polyunsaturated fatty acids significantly reduce the production of the pro-inflammatory cytokines, tumour necrosis factor, interleukin-1β and interleukin-2, as well as the lipid mediator, platelet activating factor (Sperling, 1991; Endres, 1989; Williams et al., 1996) and this could account for some of their anti-inflammatory properties. However it has recently been appreciated, through meta analysis, that the use of these fatty acids in the human disease is uncertain (James et al., 2010). These investigators have proposed that fish oil may be of benefit in RA because it may overcome the cardiovascular risk of non-steroidal anti-inflammatory agents.

Supplementation with *n-3* fatty acids also decreases the symptoms of dysmenorrhoea, a prostaglandin-mediated condition in adolescents. After a two month treatment with fish oil, a significant reduction in the Cox Menstrual Symptom Scale was found compared with a placebo diet, probably due to alteration of the prostanoid profile by the high *n-3* fatty acid intake (Harel et al., 1996; Deutch, 1995). Essential fatty acids play an important role in brain and retinal development which mainly occurs during the latter half of pregnancy and the postnatal stage. The growth of fetal brain acquires approximately 21g/wk of DHA during the last trimester of pregnancy. Fatty acids are transported from the maternal circulation across the placenta and fetal blood-brain barrier into the central nervous system. A

deficiency of essential fatty acids during pregnancy leads to a reduced level of DHA in the newborn, which is related to a reduction in slow-wave sleep and impaired vision in these infants. Dietary supplementation of *n-3* fatty acids to pregnant women and increasing the amount of DHA in infant formula are beneficial for early neurological development and improve the visual recognition in preterm and term infants (Gibson et al., 1996; Connor et al., 1996; Uauy et al., 1996). It has also been shown that diets rich in *n-3* fatty acids can prevent premature labor and preeclampsia (Olsen & Secher, 1990). Dietary *n-3* fatty acids also reduce the severity and frequency of relapses in patients suffering from multiple sclerosis (Bates, 1990).

Recent work has shown that n-3 fatty acid supplements modify allergic disease development in young children. A systematic review of reports on the effects of *n*-3 fatty acid supplementation during pregnancy and lactation on the risk of developing childhood allergic diseases and asthma, concluded that supplementation during pregnancy but not during lactation decreases childhood asthma and allergy (Klemens et al, 2011; Kremmyda et al, 2009). Thus, fish oil supplements when given to pregnant women with a history of allergic diseases gave rise to significant protection against infant allergy development. This was associated with reduced cord blood IL-13 (Klemens et al, 2011). The difference between supplementation during pregnancy versus in infancy and childhood and the development of eczema, hay fever and asthma has also been highlighted (Calder et al, 2010). Interestingly both the susceptibility and the protection afforded by the n-3 supplements were associated with the levels of protein kinase C (PKC)ζ in cord blood T cells and how those cells mature into Th1 and Th2 cytokine pattern producers. Low PKC ζ expression in the cord blood T cells of an infant increases the risk of development of allergic diseases in childhood (Prescott et al, 2007). In another population study of bronchial inflammation induced by grass pollen allergy challenge showed that the ratio of n-3: n-6 fats were significantly lower for the asthmatics than in healthy subjects (Kitz et al, 2010).

Obese adolescents appear to benefit from n-3 fatty acid supplementation, shown to improve vascular function and cause a reduction in vascular inflammation (Dangardt et al., 2010). Furthermore EPA has been shown to be incorporated into advanced atherosclerotic plaques (Cawood et al, 2010). The higher EPA content in the plaque is associated with reduced plaque inflammation and increased plaque stability (Cawood et al, 2010, Calder & Yaqoob, 2010). There was a reduction in foam cells, T cells and expression of metalloproteinases. In an animal model of atherosclerosis, employing the apoE-deficient mouse, combination of extra virgin oil and fish oil gave rise to protection by a mechanism of anti-thrombotic, anti-hypertriglyceridemic and anti-oxidant (Eilertsen et al., 2011)

The long chain, n-3 polyunsaturated fatty acids, EPA and DHA, are also substrates for the LOX and COX**.** It is evident that the metabolites of EPA and DHA display several means by which they can contribute to the regulatory network of lipid mediators; by replacing the highly inflammatory products of AA; having anti-inflammatory activity *per se* and displaying inflammation resolving abilities by having cell protection activity. The ability of the LOX and COX systems to generate fatty acid metabolites with substantially lower proinflammatory activity than the AA-derived eicosanoids has provided the basis for classical strategies to manipulate the inflammatory reaction. For example, increasing the ratio of *n*-3 to *n*-6 in membrane phospholipids of leukocytes reduces the production of inflammatory eicosanoids in favour of metabolites with markedly reduced or those which lack proinflammatory activity. Thus diets which contain high levels of the *n-3* fatty acids,

EPA and DHA or their precursors have been used as ways of decreasing inflammatory reactions and relieving the symptoms of these diseases (Simopoulos, 1991).
The EPA metabolised through COX and LOX pathways leads to the generation of 3-series PGs and TXs, and 5-series LTs (Fig 1). These products have some thousand fold less inflammatory activity than the 2-series PGs and 4-series LTs derived from AA. Thus the release of EPA from the sn-2 position of the membrane phospholipids will lead to an increased production of 3-series PGs and 5-series LTs following cell activation. This is believed to be a major mechanism of the beneficial effects of EPA/fish oil supplementation of patients with inflammatory diseases such as RA (James et al., 2010). In another development in the lipid mediator network, more recent work has characterised the generation of another class of inflammation regulators, the aspirin-triggered resolvins. This involves COX-2 aspirin triggered metabolism of EPA and further action of the 5-LOX, leading to the generation of the E-series resolvins, E1 and E2 (Fig 1) (Serhan et al., 2008). These molecules are highly potent in inhibiting neutrophil infiltration and in resolving the inflammatory reaction. Aspirin also triggers the generation of the D – series aspirin-triggered resolvins, RvD1, D2, D3, D4, D5, D6 which are generated from DHA, involving COX-2 and the 5-LOX. DHA metabolism via the 15-LOX and other reactions leads to the release also of these resolvins (Fig 1). DHA can also be oxidised via the 15-LOX to protectin D1. This metabolite inhibits neutrophil and T cell migration, airway inflammation, NF-κB activation, COX-2 induction and TLR macrophage activation. Protectin D1/neuroprotectin D1 is known for its neuroprotective properties (Serhan et al., 2008). It reduces brain ischemia and reperfusion injury, kidney ischemic injury and has anti-fibrotic activity.

10. Concluding remarks

Polyunsaturated fatty acids play critical roles in physiologic and pathophysiologic processes involving the immune system. While these have the ability to alter cellular responses as free fatty acids, most interest is on the properties of the array of metabolic products which they generate. These form a regulatory network which either down- or up-regulates the inflammatory reaction. A major effort continues to be made on the need to achieve an appropriate balance of n-6:n-3 fatty acids in tissues. The levels and ratios of these fatty acids is considered to regulate immune cell function through an effect on cell membrane fluidity, cell membrane structure, the expression of functional cell surface receptors and the types of oxidized products formed. Eicosanoid generation appears to be central to the inflammatory process by influencing the activities of many cell types, T cells, macrophages, neutrophils, eosinophils, DCs, endothelial cells, epithelial cells and smooth muscle cells. The characteristics of the inflammatory response is dependent on the concentrations and types of eicosanoids generated as well as the types of eicosanoid receptors expressed on the cell, which can vary dramatically from cell-type to cell-type. Both exogeneous and endogenous stimuli promote the activation of phospholipase A_2, the release of the polyunsaturated fatty acids from the phopholipids and their metabolism via the LOX and COX pathways. While most of the products formed from AA metabolism are highly proinflammatory, a number of these can also have a dampening effect on the inflammatory response. However, it is evident that the elicitation of a highly pro-inflammatory reaction is offset by the presence of n-3 fatty acids, EPA and DHA. These are metabolised into the low inflammatory, 5-series LTs and 3-series PGs, and anti-inflammatory products, the E-series resolvins/D-series

resolvins and neuroprotective lipids, protectins. Because of the diversity of the array of eicosanoids and other fatty acid products generated, there is still much to be discovered on how this network of fatty acid metabolites promote and inhibit the inflammatory response. The time-dependent production of the different types of eicosanoids during various phases of the reaction could explain the types of inflammatory reactions we see, for example resolving and not resolving. The free fatty acids and their metabolic products also govern the synthesis of inflammatory mediators such as cytokines, which are targets for anti-inflammatory medications. Current evidence underscores the importance of phospholipase A2 and eicosanoids in the pathogenesis of inflammatory conditions, including asthma, rheumatoid arthritis and atherosclerosis. In contrast to the view of AA as a promoter of inflammation, n-3 fatty acids are considered as an attractive approach for anti-inflammatory therapy. However, while there is convincing in vitro data and data from animal models of an anti-inflammatory action of n-3 fatty acids in these diseases, results from human studies remain unconvincing or at best only small benefits are achieved. This is likely to reflect still our poor understanding of the actions of these fats when taken as dietary supplements and obviously further and more appropriate clinical trials have been suggested (Fritsche, 2006).

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12. References

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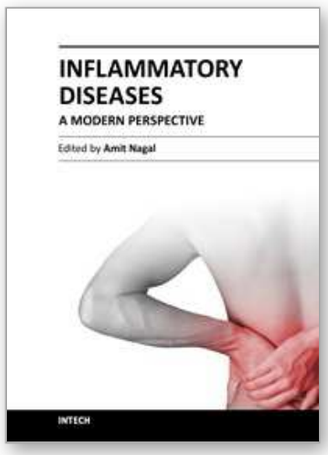

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"Inflammatory Diseases - A Modern Perspective" represents an extended and thoroughly revised collection of papers on inflammation. This book explores a wide range of topics relevant to inflammation and inflammatory diseases while its main objective is to help in understanding the molecular mechanism and a concrete review of inflammation. One of the interesting things about this book is its diversity in topics which include pharmacology, medicine, rational drug design, microbiology and biochemistry. Each topic focuses on inflammation and its related disease thus giving a unique platform which integrates all the useful information regarding inflammation.

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