The role of leukotrienes in immunopathogenesis of rheumatoid arthritis

Bahman Yousefi · Farhad Jadidi-Niaragh · Gholamreza Azizi · Fatemeh Hajighasemi · Abbas Mirshafiey

Abstract  Rheumatoid arthritis (RA) is a chronic inflammatory disorder of joints for which there is no strict cure. However, conventional medications can reduce inflammation, relieve pain, and slow joint damage. Leukotrienes are a family of paracrine agents derived from oxidative metabolism of arachidonic acid. Synthesis of lipid mediators and subsequent induction of receptor activity are tightly regulated under normal physiological conditions, so that enzyme and/or receptor dysfunction can lead to a variety of clinical signs and symptoms of disease, such as local pain and tissue edema. In these tissues, immunocompetent cells accumulate at the site of injury, contributing to tissue damage and perpetuation of the disease process. Leukotrienes (often leukotriene B4) as potent chemotactic agents derived from oxidative metabolism of arachidonic acid can provoke most signs and symptoms in rheumatoid arthritis by initiating, coordinating, sustaining, and amplifying the inflammatory response, through recruitment of leukocytes. A number of studies have reported that pharmacological modulation in this field can significantly attenuate clinical manifestations associated with different inflammatory pathologies.

Keywords  Rheumatoid arthritis (RA) · Leukotriene · RA immunopathogenesis

Introduction

Rheumatoid arthritis (RA) is a chronic progressive inflammatory autoimmune disease resulting in joint inflammation that manifests as swelling, pain, functional impairment, and muscle wasting, affecting 0.5–1 % of the adult population worldwide [1]. Although the etiology of RA is not clear, the innate immune system, various cells and humoral factors, such as cytokines, chemokines, cell adhesion molecules, and matrix metalloproteinases, as well as genetic susceptibility to environmental factors have been postulated to play a critical role in the pathogenesis of RA [2]. Lipid-derived mediators are well positioned to play key role(s) as signaling molecules in inflammation because they are small molecules, local acting, rapidly generated, and locally inactivated [3]. Prostaglandins, leukotrienes, platelet-activating factor, lysophosphatidic acid, and sphingosine 1-phosphate, collectively referred to as lipid mediators, are produced by multistep enzymatic pathways. Their production is initiated by de-esterification of membrane phospholipids by phospholipase A2s or sphingomyelinase. Lipid mediators exert their biological effects by binding to cognate receptors, which are members of the G protein-coupled receptor (GPCR) superfamily and play pivotal roles in immune regulation, self-defense, and maintenance of homeostasis in living systems [4]. In inflammatory joint disease, the levels of arachidonate metabolites [leukotriene B4 (LTB4)] are significantly higher in the active stage of the disease compared with values obtained from patients during the inactive stage of the disease and from healthy subjects [5, 6]. In this review, our aim is to study the role of leukotrienes in RA disease.
Leukotrienes

Leukotrienes (LTs) and other lipid mediators such as prostaglandins, thromboxanes, lipoxins, heproxins, and some hydroxyl, epoxy, and hydroperoxy fatty acids are products of arachidonate cascade metabolism. Following several reactions, various lipid mediators derived from two reactions (oxygenation and/or hydroxylation) of polyunsaturated fatty acids (PUFAs) are produced. To present LTs precisely, we first describe the mechanism of arachidonate metabolism in two major metabolism pathways (lipooxygenase and cyclooxygenase); we then explain the lipoxygenase (LOX) pathway, step by step. Following the release of arachidonic acid from membrane lipid bilayer phospholipids by cytosolic phospholipase A2-alpha (cPLA2a), two pathways for arachidonic acid metabolism (lipoxygenase and cyclooxygenase) are open. Cytosolic PLA2a attaches to membrane bilayer phospholipids by means of its N-terminal domain C2 and releases arachidonic acid from membrane lipid bilayer. The cyclooxygenase pathway produces prostanooids, such as prostaglandin I2 (PGI2), PGE2, PGD2, and PGF2. In the LOX pathway, there are different LOX enzymes, but 5-LOX is the major enzyme for production of two groups of LTs, including cysteinyl LTs (cysLTs) and LTD4 [7–9].

The importance of 5-LOX in production of LTs is explained as follows: 5-LOX, the key enzyme in leukotriene biosynthesis, is built of a catalytic C-terminal domain and a regulatory N-terminal C2-like domain. The C2-like domain is the target of many regulatory factors or proteins [10]. 5-LOX generates 5(S)-trans-5,6-oxido-7,9-trans-11,14-cis-eicosatetraenoic acid (LTA4) from arachidonic acid through two reactions. In the first reaction, 5-LOX induces dehydration at C7 and incorporates molecular oxygen at C5 to generate 5(S)-hydroperoxy-6-trans-8,11,14-cis-eicosatetraenoic acid (5-HPETE) from arachidonic acid (oxygenation reaction). In the second reaction, by second dehydration at C10, 5-LOX generates LTA4 [11, 12].

It has been recently shown that a 5-LOX activating protein (FLAP) is associated with 5-LOX and is an essential factor for 5-LOX function. FLAP has a membrane anchor role for 5-LOX attachment to nuclear envelope and also functions as carrier protein for transport of arachidonic acid [13, 14].

In its usual status, 5-LOX is a cytosolic enzyme, but after activation, it binds to nuclear envelope to produce LTs [15, 16].

Several factors are involved in the stimulation, activation, and transfer of 5-LOX to nuclear envelope, such as Ca2+, adenosine triphosphate (ATP), phosphatidyl choline, and reactive oxygen intermediates (ROS) in low (but not high) concentration [17, 18].

In the LT generation process there are two pathways: one for production of cysLTs (LTC4, LTD4, and LTE4) and another for generation of LTB4. For production of cysLTs, the first enzyme is LTC4 synthase (LTC4S), which generates LTC4 by conjugating tripeptide reduced glutathione with LTA4. LTC4S is a member of the membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG) enzyme family and expresses in mast cells, eosinophils, basophils, monocytes, macrophages (MQs), platelets, and endothelial cells [19, 20].

Generation of LTD4 from LTC4 is mediated by the function of a LTC4 metabolizing enzyme that is a surface enzyme termed γ-glutamyl transpeptidase [21]. Conversion of LTD4 to LTE4 is performed by dipeptidase enzyme, which occurs in two forms: one on the cell surface and the other in granules (with activation at both granules and cell surface). Despite expression of this enzyme in all leukocytes, MQs have shown the highest activation of dipeptidase. It has also been suggested that activation of dipeptidase is affected by Ca2+ and some metal ions [22, 23]. LTA4 can also convert to LTB4, instead of LTC4. This reaction is mediated by LTA4 hydrolase. LTA4 hydrolase is a bifunctional zinc metalloenzyme with an anion-dependent aminopeptidase function [24].

There is another pathway for biosynthesis of LTs that is termed the transcellular LTA4 metabolism pathway and occurs in cells with complete lack of 5-LOX. This phenomenon occurs by transport of LTA4 from leukocytes to vicinal acceptor cells such as platelets, endothelial cells, smooth muscle cells, chondrocytes, and vascular cells [25, 26].

There are distinct types of receptors for LTs that express in various cells and organs and mediate several functions of LTs. CysLTs have three forms of GPCRs: with cysLT1 receptor (cysLT1R), with cysLT2 receptor (cysLT2R), and GPR17. Among these receptors, cysLT1R has the highest affinity for cysLTs. Also, the affinity of LTD4 for binding to cysLTRs is higher than for other cysLTs including LTC4 and LTE4 [27, 28]. CysLT1R expresses in various cells and organs, such as spleen (the highest), lung, pancreas, small intestine, prostate, smooth muscle cells, neutrophils, eosinophils, MQs, mast cells, basophils, and T cells [29, 30]. It has been reported that cysLT1R expresses on nuclear membrane and contributes to signaling [31]. CysLT2R is a low-affinity receptor for cysLTs and is highly expressed in central nervous system (CNS), brain, and spleen. The other organs and cells which express it consist of adrenal glands, heart, coronary vessels, neutrophils, monocytes, T cells, MQs, mast cells, and endothelial cells [32, 33]. GPR17 is the third receptor for cysLTs that has recently been orphanized and phylogenetically lies between P2Y receptors and cysLTRs and can bind to both cysLTs and some nucleotides. GPR17 is coupled to Gi and thus inhibits
adenylyl cyclase and increases cytosolic Ca\(^{2+}\) concentration. GPR17 mainly expresses in organs with susceptibility to ischemia such as brain, heart, and kidney [34]. Among all the GPCRs, P2Y receptors are the GPCRs with the highest homology to cysLTRs [35]. P2Y receptors divide into two subtypes; one subtype includes P2Y\(_{1,2,4,6,11}\), and the other subtype includes P2Y\(_{12,13,14}\) [36]. Adenine nucleotides and uracil nucleotides [uracil diphosphate (UDP)-glucose] are the main ligands for P2Y receptors. P2Y receptors mainly express on CNS, microglia, vascular cells, epithelial cells, blood cells, osteoclasts, and MQs [37, 38]. LT4 has also three receptors with different binding affinity. BLT1 is a high-affinity receptor for LT4 and expresses on neutrophils, monocytes, T cells, eosinophils, MQs, smooth muscle cells, and mast cells [39, 40]. BLT2 is a low-affinity receptor for LT4 that expresses on CD8+ T cells, CD4+ T cells, and monocytes, particularly [41]. Peroxisome proliferator-activated receptors (PPARs) are the third group of receptors for LT4 which express in nucleus and function as lipid homeostasis factors and controllers of inflammatory responses [42]. Moreover, it has been demonstrated that all of the LTRs can match to bond Gi and Gq proteins and give rise to decrease in cyclic adenosine monophosphate (cAMP) and increase in cytosolic Ca\(^{2+}\) [43]. It has been shown that, in signaling processes, cysLTs robustly provoke Ca\(^{2+}\) influx, and mitogen-activated protein kinase–kinase-1 (MEK-1) and extracellular signal-regulated kinase (ERK) phosphorylation [44].

Table 1  Main cell sources of LTs, as well as affinity and cell distribution of receptors

<table>
<thead>
<tr>
<th>Leukotriene</th>
<th>CysLTs (LTC4, LTD4, LTE4)</th>
<th>LT4</th>
</tr>
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<tbody>
<tr>
<td><strong>Cell source</strong></td>
<td>Mast cell, eosinophil, basophil, macrophage, epithelial, and endothelial</td>
<td>Innate immune cells such as neutrophil, macrophage, mast cell, and dendritic cell</td>
</tr>
<tr>
<td><strong>Receptors</strong></td>
<td>CysLT(_1)R</td>
<td>CysLT(_2)R</td>
</tr>
<tr>
<td>LTR expression in immune cells</td>
<td>Neutrophil</td>
<td>Neutrophil</td>
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<td></td>
<td>Eosinophil</td>
<td>Mast cell</td>
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<td>Fibrocyte</td>
<td>Pregranulocytic</td>
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<td><strong>LTR affinity</strong></td>
<td>High</td>
<td>Low</td>
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receptor-2 (LTB4-R2) [49]. These two receptors, despite the difference in their affinity and specificity for LTB4, have different tissue distribution as presented in Table 1, and the exact role of each receptor remains elusive [53]. The BLT1 receptor is only expressed in inflammatory cells [54] and shows a high degree of specificity for LTB4, with \( K_d \) of 0.15–1 nM [45, 55]. The receptor BLT1 was found to be necessary to elicit the physiological effects of LTB4 (e.g., chemotaxis, calcium mobilization, and adhesion to endothelium) and important for recruitment of leukocytes in an in vivo model of peritonitis. A second G-protein-coupled 7TM receptor for LTB4, BLT2, was recently identified [55–57]. This receptor is homologous to the BLT1 receptor but has a higher \( K_d \) value for LTB4 (23 nM) and a different ligand specificity and binding profile for various BLT antagonists [54, 55]. In an autoantibody-induced inflammatory arthritis model, BLT2(-/-) mice showed reduced incidence and severity of disease, including protection from bone and cartilage loss. Reciprocal bone marrow transplant experiments identified that loss of BLT2 expression on a bone-marrow-derived cell lineage offers protection against severe disease. Thus, BLT2, a unique receptor for 5-lipoxygenase- and cyclooxygenase-1-derived lipid mediators, represents a novel target for therapies directed at treating inflammation associated with arthritis [58]. To evaluate the role of LTB4 receptors in inflammatory arthritis, Hashimoto et al. investigated the expression of BLT1 and BLT2 messenger RNA (mRNA) as detected by reverse-transcription polymerase chain reaction (RT-PCR) in synovial tissues of patients with RA and osteoarthritis and in situ hybridization in synovial tissues from patients with RA and osteoarthritis (OA). BLT2 (the low-affinity receptor for LTB4) showed stronger expression than BLT1 (the high-affinity receptor) in actively inflamed synovial tissue from patients with RA. BLT2 mRNA was strongly expressed in the synovial lining cells, which also expressed 5-lipoxygenase, an enzyme that synthesizes LTB4. BLT1 and BLT2 mRNA expression in synovial tissues was stronger in RA than in OA. In contrast, leukocytes infiltrating synovial fluid predominantly expressed BLT1 mRNA in patients with RA. The two receptors for LTB4 have quite different pharmacological effects and different tissue distribution, and BLT2 is the main receptor mediating the effects of LTB4 in synovial tissues of patients with RA; this suggests the possibility of developing a new therapy to block LTB4 in inflammatory arthritis [59]. In contrast to the BLT1 receptor, which is predominantly found in leukocytes, BLT2 is ubiquitously expressed in various tissues. On the cell surface, LTB4 binds to BLT1 and BLT2, but can also enter the nucleus to bind PPAR\( \alpha \).

PPARs are the third group of receptors for LTB4 which are expressed in nucleus and function as lipid homeostasis factors and regulators of inflammatory responses [42, 60]. Although PPAR\( \alpha \) regulates oxidative degradation of fatty acids and their derivatives, like this lipid mediator, a feedback mechanism is proposed that controls the duration of an inflammatory response and clearance of LTB4 in the liver. Thus, PPAR\( \alpha \) offers a new route to the development of anti- or proinflammatory reagents [42].

In particular, three molecules, the receptor activator of NF-kappaB (RANK), its ligand RANKL, and the decoy receptor of RANKL, osteoprotegerin (OPG), have attracted the attention of scientists and pharmaceutical companies alike. Genetic experiments revolving around these molecules established their pivotal role as central regulators of osteoclast function. Not only does RANK–RANKL signaling activate a variety of downstream signaling pathways required for osteoclast development, but crosstalk with other signaling pathways also fine-tunes bone homeostasis in both normal physiology and disease [61]. LTB4 can directly stimulate osteoclast differentiation independent of RANKL. To determine whether LTB4 could indirectly stimulate human osteoclast differentiation through increasing RANKL expression of RA fibroblast-like synoviocytes, Chen et al. utilized a coculture model of RA fibroblast-like synoviocytes and monocytes, which were stimulated in the presence of macrophage colony-stimulating factor (M-CSF) in the control group, M-CSF + LTB4 in experimental group a, and M-CSF + M LTB4 + OPG in experimental group b. There were almost no osteoclast-like cells in the control group or experimental group b, but there were many osteoclast-like cells in experimental group a. These results indicated that LTB4 is capable of inducing osteoclast differentiation by a RANKL-dependent mechanism [62].

Antigen-experienced CD8 T cells divided into at least two populations in mice, namely central memory CD8 T cells (Tcm; CD62Lhi, CCR7hi), which traffic primarily to lymphoid tissues, and effector memory CD8 T cells (Teff; CD62Llo, CCR7lo), which migrate to nonlymphoid tissues [63]. Recent studies using BLT1-deficient mice have demonstrated an important role for the BLT1 receptor and LTB4 in recruitment of T cells to an inflammatory site [64]. In autoimmune inflammatory diseases, migration of T cells to an inflamed region in the body is essential for a proper response of the adaptive immune system. More specifically, it was demonstrated that recruitment of a specific population of circulating CD8+ effector T cells depends on selective activation of BLT1 receptors and rapid integrin-mediated cell attachment. Circulating wild-type CD8+ effector T cells more efficiently migrated to the inflamed peritoneal cavity than BLT1 receptor-deficient CD8+ effector T cells [65]. The investigators obtained evidence for an essential role of BLT1 receptor signaling and CD8+ effector T cells in the development of increased airway hyperresponsiveness (allergic asthma) [66]. These results strongly emphasize the importance of both BLT1
receptor function and LTB4 with the function of T cells in the etiology of certain chronic inflammatory diseases [67]. Ott and colleagues [64] presented an elegant series of experiments suggesting that mast-cell-dependent LTB4 may subserve CD8 Teff recruitment to tissues. Mast cell biology has assumed increasing prominence in theories of synovitis, providing a potential cellular link between humoral autoimmunity (B cells) and synovial inflammation [68]. The present observations provide a novel molecular mechanism for interactions between mast cells and T cells [64]. Using a Transwell migration assay, Ott and colleagues [64] observed that murine CD8 Teff cells but not Tcm cells migrated in response to soluble factor(s) released by FcεRI activated, but not resting, bone-marrow-derived mast cells. Importantly, migration occurred within minutes of mast cell coculture, suggesting release of a preformed or rapidly synthesized factor [64]. This now suggests that mast cells could significantly modify T-cell function not only through chemokine release but also via LTB4. Indeed, since LTB4 is also a potent inducer of neutrophil migration, these effects may have broader functional importance in synovium [69, 70]. Commensurate with a functional role for LTs, the 5-lipoxygenase-activating enzyme inhibitor MK-886 inhibited mast-cell-induced Teff migration, and purified LTB4, but not LTC4, directly induced Teff directional migration with a bell-shaped dose–response curve typical of many chemokines. In contrast, centrally derived (lymph node) CD122hi Tcm cells were unable to migrate to LTB4 unless first activated via the T-cell receptor in the presence of interleukin (IL)-2 to promote a Teff phenotype. Using the inhibitor CP-105696, LTB4-induced Teff migration was shown to be dependent on BLT1 (high affinity) rather than BLT2 (low affinity). Finally, addition of pertussis toxin inhibited migration further, implicating BLT1 via activation of Gi-type G proteins. Together, these data strongly suggest that a novel function of tissue-activated mast cells could be to rapidly recruit Teff cells to tissues during the early phase of innate inflammatory responses [71].

BLT1 and BLT2 are expressed at low levels in an apparently silent state in human umbilical vein endothelial cells (HUVEC). Qiu et al. demonstrated that treatment of these cells with lipopolysaccharide (LPS) leads to a >10-fold increase in the levels of BLT1 mRNA without any significant effects on BLT2 mRNA. In parallel, LPS also increases the amounts of BLT1 protein. Tumor necrosis factor-alpha (TNFα) increases the expression of BLT2 mRNA. Interleukin-1 causes variable and parallel increases of both BLT1 and BLT2 mRNA. The natural ligand LTB4 also increases BLT1 (but not BLT2) mRNA and protein expression. Along with induction of BLT1 and/or BLT2, HUVEC acquire the capacity to respond to LTB4 with increased levels of intracellular calcium, and these signals can be blocked by isotope-selective BLT antagonists, CP-105696 and LY-255283. In addition, treatment of HUVEC with LTB4 causes increased release of both nitrite, presumably reflecting NO and monocyte chemotactic protein-1 (MCP-1). Accumulating data indicate that expression of functional BLT receptors may occur at the surface of endothelial cells in response to LPS, cytokines, and ligand, which in turn may have functional consequences during early vascular responses to inflammation. Moreover, the results point to BLT receptors as potential targets for pharmacological intervention in LT-dependent inflammatory diseases such as asthma, rheumatoid arthritis, and arteriosclerosis [40].

Leukotrienes and RA

The joints of patients with RA are characterized by infiltration of immune cells into the synovium, leading to chronic inflammation, pannus formation, and subsequent irreversible joint and cartilage damage (Fig. 1) [72]. RA synovium is known to consist largely of macrophages (30–40 %), T cells (30 %), and synovial fibroblasts, and also of B cells, dendritic cells, other immune cells, and synovial cells such as endothelium [72, 73]. RA synovial fluid has been shown to contain a wide range of effector molecules that interact with one another in a complex manner that is thought to cause a vicious cycle of proinflammatory signals resulting in chronic and persistent inflammation, including proinflammatory cytokines (such as IL-1β, IL-6, TNFα, and IL-18), chemokines (such as CXCL8, IP-10, MCP-1, MIP-1, and RANTES), matrix metalloproteinases (MMPs) (such as MMP-1, -3, -9, and -13), and metabolic proteins [such as cyclooxygenase (COX)-1, COX-2, and inducible nitric oxide synthase (iNOS)] [72]. Although the innate arm of the immune system aims to protect the host in response to trauma or infection, its incompatible activation often leads to pathologic accumulation of leukocytes in involved organs. A diverse network of chemotactic signals is capable of recruiting leukocytes to sites of inflammation, including chemokines, bacterial peptides, proteolytic fragments of complement, and lipids. One of these mediators, LTB4, is a highly potent chemoattractant lipid, which is produced and released within minutes by neutrophils, macrophages, and mast cells as a key element of the immediate inflammatory reactions [74]. LTs and PGs are inflammatory mediators with potent biological activities in the pathogenesis of many diseases and play major roles in various inflammatory reactions. The release of these mediators, by cells recruited to or present at the site of inflammation, modulates and influences the magnitude of the inflammatory response [75]. The production of a variety of lipid
mediators is enhanced in bone-receptive diseases such as osteoporosis, rheumatoid arthritis, osteoarthritis, and periodontitis [76]. In inflamed joints of patients with RA, elevated levels of LTB4 correlate with disease severity [77]. Moreover, synovial fluid leukocytes can highly express BLT1 [59], suggesting that this receptor–ligand pair contributes to the characteristic synovitis of RA by recruiting leukocytes to the inflamed joint [52]. The production of different LTs from arachidonic acid is dependent on the expression of 5-LOX, an enzyme that regulates the first step in the synthesis of LTs. LTs are well-known mediators of acute inflammatory and immediate hypersensitivity responses whose increased effects are due to expression of adhesion molecules such as P-selectin, E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), which are responsible for the interaction between neutrophils and endothelial cells [78, 79]. The elevated tissue levels of LTs in K/BxN serum transfer arthritis. In contrast to wild type, 5–10 null control mice were remarkably resistant to development of K/BxN serum-induced inflammatory arthritis [45]. Other arachidonic acid-metabolizing enzymes, including human 15-lipoxygenase 1 (15-LO-1) and its murine ortholog 12/15-LO, have been involved in the generation of a subclass of eicosanoids, including the anti-inflammatory lipoxin A4 (LXA4). Nevertheless, the impact of 12/15-LO on chronic inflammatory diseases such as arthritis has remained elusive. Using two experimental models of arthritis (K/BxN serum-transfer and a TNF transgenic mouse model), Kronke et al. showed in 2009 that deletion of 12/15-LO leads to uncontrolled inflammation and tissue damage. Moreover, 12/15-LO-deficient mice showed enhanced inflammatory gene expression and decreased levels of LXA4 within their inflamed synovia [80, 81]. Consistent with these findings, Serhan et al. [82] indicated that overexpression of 15-LO and LXA4 were associated with depressed polymorphonuclear leukocyte (PMN)-mediated tissue degradation and bone loss, suggesting that enhanced anti-inflammatory status is an active process. Likewise, in isolated macrophages, addition of 12/15-LO-derived eicosanoids blocked both phosphorylation of p38 MAPK and expression of a subset of proinflammatory genes. Conversely, 12/15-LO-deficient macrophages displayed significantly reduced levels of LXA4, which correlated with increased activation of p38 MAPK and enhanced inflammatory gene expression after stimulation with TNFα [80].

In an opposite manner, Wen et al. in 2007 reported that 12/15-LO products stimulated mRNA and protein expression of IL-6 and TNFα in a dose-dependent manner. Therefore, this report suggests a potentially important mechanism linking 12/15-LO activation to chronic inflammation [83]. The most potent LTs, LTB4 and cysLTs, are synthesized primarily by stimulated leukocytes and to a lesser extent by other types of cells including epithelial cells and endothelial cells at the site of inflammation [57]. LTB4 is a potent chemoattractant that is primarily involved in inflammation, immune responses, and host defense against infection [55] by activating its high-affinity receptor BLT1, which can be localized on neutrophils, eosinophils, monocytes, and T cells [84].

Expression of cathelicidin (LL-37) is upregulated in similar inflammatory diseases. The novel interactions between LTB4 and human LL-37 can enhance PMN activities that could be critical to the function of innate immune responses. Further investigations into the mechanisms and consequences of LTB4 and LL-37 signaling circuits may provide new insight into LT- and cathelicidin-dependent immune responses, and identify novel targets for treatment of inflammatory reactions. LTB4-induced LL-37 release is mediated by the BLT1 receptor, and protein

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**Fig. 1** Interactions of inflammatory cells and LTB4 in RA pathogenesis. In inflamed joints, LTB4 is produced by innate immune cells such as neutrophils, mast cells, macrophages, and dendritic cells, leading to leukocyte infiltration to the site of inflammation. The infiltrated leukocytes producing cytokines, chemokines, tissue-destructive enzymes, antibodies, and complement cascade activators lead to chronic inflammation, cartilage damage, and bone erosion. RA, rheumatoid arthritis, MMP, matrix metalloproteinase, iNOS, inducible nitric oxide synthase, MCP-1, monocyte chemotactic protein-1, Ab, antibody, IC, immune complex.
phosphatase-1 (PP-1) inhibits the release by suppressing BLT1-mediated exocytosis of PMN granules. Conversely, LL-37 elicits translocation of 5-lipoxygenase (5-LOX) from the cytosol to the perinuclear membrane in PMNs and promotes synthesis and release of LTB4. Since, in human PMNs, positive feedback circuits exist between LL-37 and LTB4 that reciprocally stimulate the release of these mediators with the potential for synergistic bioactions and enhanced immune responses [85].

Using genetic and pharmacologic approaches in the K/BxN serum transfer model of arthritis, it has been shown that autoantibody-driven erosive synovitis is critically reliant on the generation of LTs, and more specifically on LTB4. These experiments demonstrate a nonredundant role for LTB4 in inflammatory arthritis and define a neutrophil mediator involved in orchestrating synovial eruption [86]. Neutrophils serve as a front line of the acute innate immune response to invading pathogens [86]. Trafficking of immune cells to inflamed joints is the hallmark of rheumatoid arthritis, and the abundant neutrophils in rheumatoid joints suggest that these cells have potential to inflict tissue damage by secretion of oxidants and proteases [87]. RA is generally characterized by significant infiltration of neutrophils into the synovial space from the joint tissue, and the confirmed presence of CXCL1 and CXCL5 in both tissue and fluid would suggest that they can act as key mediators in recruitment of neutrophils. It has previously been shown that LTB4 is increased in the synovial fluid of RA patients [77, 86], suggesting that CXCL1 and CXCL5 induce production of LTB4 by neutrophils in the synovial fluid of RA patients, providing robust evidence for a mechanism that links this potent neutrophil chemotactic factor with chemokine mediators. Therefore, this would suggest that CXCL1 and CXCL5 released by synovial cells stimulate neutrophil migration and LTB4 release by the migrating neutrophils. LTB4 amplifies the neutrophil recruitment process through autocrine secretion. These findings contribute to a better understanding of the mechanisms involved in promoting synovitis and suggest that CXCL1 and CXCL5 may be effective targets for therapeutic intervention in RA [88].

Mast cells are present in limited amounts in normal human synovium, but in RA and other inflammatory joint diseases this population can expand to constitute 5 % or more of all synovial cells [89]. They produce an array of proinflammatory mediators, tissue-destructive proteases, and cytokines, most prominently TNFα, which is one of the key cytokines in the pathogenesis of RA [90]. Therefore, mast-cell-derived mediators induce edema, destroy connective tissue, and are involved in lymphocyte chemotaxis and infiltration and in pathological fibrosis of RA joints. Moreover, mast cells are involved in angiogenesis during RA, and also their proteolytic activity results in cartilage destruction and bone remodeling [91]. An important consequence of mast cell activation may be the mobilization of adaptive immunity through recruitment of memory CD4+ and CD8+ T cells, which can then be activated locally by mast cells presenting phagocytosed peptides via both major histocompatibility complex (MHC) class II and MHC class I molecules [65]. Mast cells might also potentiate de novo antigen-specific responses by promoting migration of dendritic cells to lymph nodes and recruiting circulating naive T cells to these nodes by means of TNF and MIP-1β [92]. Collectively, the ultimate physiological importance of each of these defensive capabilities remains to be clarified [89].

IL-1 and TNFα have been identified as important mediators of chronic inflammatory disease states. TNFα reportedly plays a pivotal role in the pathogenesis of RA, especially its ability to regulate interleukin-1β expression, this being important for induction of prostanooid to and matrix metalloproteinase production by synovial fibroblasts and chondrocytes [93]. LTB4 can significantly increase mRNA expression of TNFα and IL-1β [94]. Therefore, in addition to leukocytes, RA synovial fibroblasts (RASF) produce a broad array of inflammatory mediators to recruit, retain, and activate immune cells and resident mesenchymal cells in joints to promote inflammation and tissue destruction, suggesting that LTB4 contributes to RA by regulating expression of TNFα and IL-1β. In a study where RASF from synovial tissues were cultured in the presence of exogenous LTB4, this treatment remarkably increased expression of TNFα and IL-1β at both mRNA and protein level. In contrast, MK-886 and bestatin significantly inhibited their expression. These data suggest that LTB4 contributes to RA by regulating expression of TNFα and IL-1β in RASF; also, BLT2 is probably the major receptor mediating such effects [95].

**NF-κB, TLRs, LTs, and RA**

On the other hand, toll-like receptors (TLRs) are a family of pattern-recognition receptors that play a crucial role in detection of invading pathogens, and individual TLR subtypes initiate activation of NF-κB and of MAPKs, resulting in subsequent immune responses [96]. There is increasing evidence that these TLRs are involved in the inflammatory response and arthritis [97]. NF-κB is known to be activated in the pathogenesis of RA. The trigger molecule that starts this process is unknown, but could include molecules on the surface of T cells (TCRs), endogenous or exogenous ligands for the TLR family (TLR ligands), or other unknown molecules [98].

NF-κB is an inducible transcription factor that tightly regulates the expression of a large cohort of genes,
TLR2 ligands augment cPLA2 activation and subsequent LT biosynthesis. Therefore, release. Moreover, TLR2 mediates augmented cPLA2 activity and production of proinflammatory molecules in vivo and in vitro [96, 100]. However, it is unknown whether 5-LOX metabolites can influence and are in fact necessary for TLR responses, and which specific 5-LOX metabolites can exert such actions is not well understood [101]. Indeed, TLR ligands have been shown to promote LT biosynthesis [96, 100]. On the other hand, both LTB4 [100] and cysLTs have been reported to activate NF-κB in specific cells and contexts.

The extra-domain A (EDA)-containing fibronectin isoform is generated under pathologic conditions such as RA, as an endogenous TLR4 ligand, so that Lefebvre et al. aimed to investigate the putative effects of EDA on LT biosynthesis and PMN migration through TLR signaling. rhEDA efficiently primed LTB4 biosynthesis in PMN and monocyte suspensions. This effect could provide new insight into TLR-dependent mechanisms of regulation of LTB4 biosynthesis and PMN infiltration in inflammatory joint diseases [97].

TLR2 ligands elicit time-dependent activation of p38 MAPK and ERK1/2 pathways, which lead to phosphorylation of cPLA2 at Ser505. Simultaneous inhibition of p38 MAPK and ERK1/2 pathways could prevent the increase of cPLA2 phosphorylation and the augmentation of AA release. Moreover, TLR2 mediates augmented cPLA2 activation and subsequent LT biosynthesis. Therefore, TLR2 ligands augment cPLA2 activity and lead to enhanced LT release in human monocytes [96]. A potential mechanism for LT production may involve Rac-1-dependent activation of phosphatidylinositol 3-kinase, which has recently been proposed as an important pathway in TLR2 signaling [96].

Leukotriene-based therapeutic strategies in RA

The current therapies used to treat RA (Fig. 2) include four main available treatments: (1) disease-modifying antirheumatic drugs (DMARDs), used as first-line therapy for all newly diagnosed cases of RA, including methotrexate, hydroxychloroquine, sulfasalazine, and leflunomide; (2) nonsteroidal anti-inflammatory drugs (NSAIDs), used for management of pain and inflammation; (3) biological-response modifiers, targeted agents that selectively inhibit specific molecules of the immune system; (4) glucocorticoids and other antirheumatic drugs also used to treat RA [72]. The anti-inflammatory effects of NSAIDs cannot be entirely explained by their inhibition of prostaglandin synthetase and may, in part, be due to other direct effects upon inflammatory cell activation [102]. The biological-response modifiers include infliximab, etanercept, and adalimumab (inhibitors of TNFα), anakinra (a recombinant inhibitor of interleukin-1), abatacept (the first co-stimulation blocker), rituximab (a chimeric anti-CD20 monoclonal antibody), anti-interleukin-6-receptor monoclonal antibodies, and antibodies against some critical proteins for B cell function and survival [72]. Alternative methods targeting critical pathways could include, for example, Blys inhibition (atacicept). Finally, in recent years, small molecules have received increasing attention, along with some of the protein kinases in treatment of RA [103].

Leukotriene modifiers

Innovative strategies, particularly those based on new concepts in the immunobiology of rheumatoid arthritis, are being developed to target cellular inflammatory mechanisms in order to prevent disease progression. Since LTs can exert a critical role in the maintenance of inflammatory processes, prohibition of LT activity may result in anti-inflammatory action. To date, many LT modifier agents have aimed at either inhibiting biosynthesis of LTs or blocking receptors of LTs. LT modifiers are classified into two classes: LT inhibitors, which inhibit the formation of both LTB4 and the cysLTs, and LT antagonists, which selectively block the action of LTs at one of their receptors (Fig. 2) [52]. Several compounds capable of blocking either LT synthesis or LT receptors have recently been developed and clinically tested in different experimental models. Although further studies and optimization of this kind of treatment are required, a possible clinical role of LT antagonists as anti-inflammatory therapy for selected diseases may be imagined [104].

Leukotriene inhibitors

Several selective cyclooxygenase 2 (COX-2) inhibitors or nonselective nonsteroidal anti-inflammatory drugs (NSAIDs) have been evaluated alone or in combination with LT synthesis inhibitors in the collagen-induced arthritis (CIA)
The results suggest that induction of the disease in CIA is mediated by products of the COX-2 enzyme and LTB4 production, and that blockade of both pathways is required to prevent CIA [105]. Inhibition of LT biosynthesis can be achieved by inhibition of phospholipases releasing the precursor AA, direct inhibition of 5-LOX, and inhibition of FLAP [52]. Rainsford and coworkers demonstrated that certain 5-lipoxygenase inhibitors regulate the formation of those interleukins involved in joint cartilage destruction as well as those seen in inflammatory joint diseases [52, 106]. Some agents, such as inhibitors of 5-lipoxygenase-omega-3 fatty acid and zileuton may be most useful in treatment of milder disease manifestations such as moderate synovitis [107].

In a study, Chen et al. used a pharmacologic inhibitor of 5-LOX, and oral administration of the 5-LOX inhibitor could effectively prevent induction of arthritis. Histologically, 5-LOX inhibitor-pretreated mice display little evidence of leukocytic infiltrate, synovial hyperplasia or joint erosions, whereas control mice displayed marked arthritic activity [52].

Boswellic acids, the biologically active agent of the gum resin of *Boswellia serrata*, have been shown to be a specific inhibitor of 5-lipoxygenase and have been used to treat IBD, RA, and asthma successfully [52]. Boswellic acids inhibit LT biosynthesis in neutrophilic granulocytes by nonredox, noncompetitive inhibition of 5-lipoxygenase. The effect is triggered by boswellic acids binding to the enzyme. In clinical trials, promising results were observed in patients with RA [108]. Recently, a novel boswellia formula, std. to 90 % AKBA, has become available. It shows increased clinical effectiveness over the older formula. We now know that AKBA exerts its anti-inflammatory effects by a multitude of mechanisms: nonredox inhibition of 5-LOX; impairment of leukocyte infiltration; nearly complete suppression of the complement pathway; inhibition of mast cell degranulation, NF-κB pathway, MMPs, and adhesion receptors, IL-2 and IL-1β, human...
involved in joint cartilage destruction [106]. 5-lipoxygenase inhibitors may be usefully employed in human synovial tissue. The results suggest that some thesis inhibitors (tepoxalin) on the production of IL-1 by (MK-886, L-656,224) compared with standard IL-1 syn-

Rainsford et al. investigated the effects of 5-LOX inhibitors drugs that act by blocking LTB4 receptors [59]. Thus, patients with RA; this suggests the possibility of using new inflammatory effects of LTB4 in the synovial tissue of the inflammatory process in RA and mediate the pro-
The LTB4 receptors play an important role in the genesis of the inflammatory process in RA and mediate the pro-
inflammatory effects of LTs and thus inhibit their pathophysiological effects. Several compounds were identified as potent 5-LOX inhibitors; however, such compounds cause serious side-effects [113]. Compounds containing hydroxamic acids or N-hydroxyurea groups that chelate the iron in the active site of the enzyme have been found to be potent and more selective inhibitors of 5-LOX [52].

The other main class of LT inhibitors is FLAP (an essential factor for 5-LOX function) inhibitors, which inhibit the actions of 5-LOX indirectly. These compounds are synthesized from either indole or quinoline structures. MK886, as an indole, inhibits FLAP [114] and production of 5-LOX metabolites potently in leukocytes. However, it is less effective in blood and fails to inhibit the actions of purified 5-LOX and 5-LOX activity in broken cell prepar-
ations [52]. Since LTB4 significantly increases mRNA expression of TNFα and IL-1β. Harris and coworker demonstrated that synoviocytes with addition of MK-886 and bestatin were inhibited from secreting LT4 dose-depen-
dently, following the marked downregulation of TNFα and IL-1β expression at mRNA level [94]. Furthermore, Rainsford et al. investigated the effects of 5-LOX inhibitors (MK-886, L-656,224) compared with standard IL-1 syn-
thesis inhibitors (tepoxalin) on the production of IL-1 by human synovial tissue. The results suggest that some 5-lipoxigenase inhibitors may be usefully employed in regulating the production of prostaglandins and LTs involved in joint cartilage destruction [106].

Leukotriene antagonists

The LT4 receptors play an important role in the genesis of the inflammatory process in RA and mediate the pro-
inflammatory effects of LT4 in the synovial tissue of patients with RA; this suggests the possibility of using new drugs that act by blocking LT4 receptors [59]. Thus, antagonism of LT4 has been proved to be of clinical utility in some inflammatory diseases. The majority of LT4 functions are mediated through BLT1, which is primarily expressed in leukocytes. Some of these have been evaluated as immunosuppressive agents for preventing allograft rejection, depending on which receptor is activ-
ated [53]. SC-41930, a first-generation LT4 receptor antagonist, inhibited the chemotactic actions, and also the second-generation LT4 receptor antagonist, SC-53228, inhibited LT4-primed membrane depolarization of human neutrophils, which may well have application in medical management of some inflammatory diseases such as asthma, RA, and IBD in which LT4 and/or 12(R)-HETE are implicated as inflammatory mediators [115]. BIIL 284 is a new, orally active LT4 receptor antagonist that is able to inhibit LT4-induced macrophage adhesion ligand-1 (Mac-1) expression in leukocytes of RA patients. Therefore, longer treatment with BIIL 284 may result in clinical improvement of patients with RA [52]. Griffiths and coworkers examined the therapeutic potential of inhibiting BLT1 using CP105,696, a potent, specific BLT1 antagonist that inhibits LT4-induced neutrophil chemotaxis; additionally, it could effectively decrease synovial hyperplasia. BLT1 inhibition dramatically suppressed synovial neutrophil infiltration by 99.9 and 81.3 % in the preventative and delayed treatment groups compared with the untreated group, indicating a primary role for BLT1 in recruiting neutrophils to the joint. Although delayed inhibi-
tion of BLT1 was capable of limiting ongoing arthritis, its effectiveness as a protective agent suggests that BLT1 plays an important role in the early stages of disease [116]. Zafirlukast is one of the oral selective antagonists of LT receptors. It competitively inhibits the actions of cysLTs C4, D4, and E4 and is used for preventing and managing chronic asthma [117].

Discussion

Synthesis of lipid mediators and subsequent induction of receptor activity are tightly regulated under normal phys-
iological conditions, and enzyme and/or receptor dys-
function can lead to a variety of disease conditions [4]. During acute inflammation, lipid mediators such as PGs and LTs play pivotal roles in orchestrating the hemody-
amic changes required and also serve as potent chemo-
acceptants to elicit neutrophils and call them into the tissues [3]. In inflammatory joint disease, the levels of arachidonate metabolites (serum LT4) were found to be significantly higher in the active stage of the disease when compared with inactive stage and healthy subjects [5] are higher and more directly correlated with degenerative joint disease [6]. Studies into expression of 5-LOX and the
5-LOX activating protein (FLAP) genes in osteoarthritis and RA have shown that 5-LOX products participate in inflammatory processes leading to joint destruction in RA [118].

Therefore, recent evidence suggests that LTs may play an increasingly important role in the pathophysiology of inflammatory disorders, particularly those events that involve leukocyte activation and regulation of proinflammatory cytokines by LTB4 and suggesting that this receptor–ligand pair contributes to recruitment of leukocytes to the inflamed joint [77]. In RA synovium, leukocytes, immunocomplex, and rheumatoid factor are related to the level of LTB4 [77], and LTs, particularly LTB4, have also been implicated in RA bone remodeling [76]. Proinflammatory PGs and LTB4 control local blood flow, vascular dilation, and permeability changes needed at the site for leukocyte adhesion, diapedesis, and recruitment [3]. To date, 5-LOX-inhibiting drugs have been approved for bone diseases [52]. Since LTB4 is also a potent inducer of neutrophil migration. This effect may have broader functional importance in synovium [69].

In neutrophils, LTB4 exerts chemokinetic and chemotactic migration effects, adhesion to the endothelium, degranulation, aggregation, as well as release of reactive oxygen species and neutrophil-derived elastases and cathepsin species via binding to a specific LTB4 receptor, BLTR [19]. LTB4 also increases vascular permeability and induces expression of adhesion molecules, for example, Mac-1 (CD11b/CD18), on polymorphonuclear leukocytes, as a prerequisite of polymorphonuclear leukocyte adherence to endothelial cells. LTB4 can potentially contribute to accumulation of not only neutrophils but also macrophages, T lymphocytes, and eosinophils at the site of inflammation. Moreover, effects on various immunological phenomena involving T cells, such as release of cytokines [IL-1, TNF, interferon (IFN)]-phenomena involving T cells, such as release of cytokines

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Conflict of interest None.

References


