

## Pain and bone damage in rheumatoid arthritis: role of leukotriene B4

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### ABSTRACT

Rheumatoid arthritis is a chronic autoimmune disease characterised by unbearable joint pain as well as bone and cartilage destruction. Although RA development is greatly controlled, the pain and bone damage failed to be relieved and managed. Leukotriene B4 (LTB4) has been proved to play an essential role in the induction of pain and bone damage. The nerve injury of RA can promote the production of LTB4, which act on their receptors, leading to the increased release of pro-inflammatory cytokines and ROS to reduce neuron viability and pain threshold. Moreover, LTB4-BLT1 activation can also increase intracellular calcium concentration and neuron excitability as well as NF- $\kappa$ B pathway activation, which further promote the production of MMP-9 and CXCR3R-1. The mutual promotion between LTB4 and neutrophil accumulation accelerates the release of TNF- $\alpha$  and IL- $\beta$ , which enhance both peripheral and central nerve system sensitisation. LTB4 also involve in TrpV1 channel activation and modulation of P2X3 receptor activation. All above mechanisms contribute to the development of RA pain. IL-23, cPLA2 and PI3K increase the production of CD11b+Gr1high myeloid subtype and calcium concentration, which promote the production of LTB4 and further accelerate IL-17 and TNF activation as well as calcium influx to conduce to osteoclastogenesis, resulting in aggregated bone damage. Our review is the first to conclude the signalling pathways and associated molecules in LTB4-induced pain and bone damage.

### Introduction

Rheumatoid arthritis (RA) is a chronic multisystem autoimmune disease characterised by synovitis, and generally

causes bone and cartilage destruction, pain, joints stiffness, function loss, and pathological alterations in psychological domains, metabolism and vascular system (1). The recent pathology of RA is mainly defined by joint inflammation and bone and cartilage destruction, accompanied with the severe pain, resulting in severe damage to various facet of life and diminish life quality (2, 3). It is estimated that approximately 0.5–1% of the population in developed regions are affected by RA and is more prevalent in women than in men (4). The disease can affect individuals at any age groups, but the onset is more frequent in 40s or 50s (5). Much work so far has focused on the onset and progression of RA, which are associated with various aetiologies, including genetics, environment, infection and immunity.

Several excellent reviews describing pathogenesis of RA have been widely detected in multifarious aspects, such as gene, environment, infection, and immune system. With the development of genome-wide approaches and multiple technologies, more than 100 loci have been intensively investigated to be associated with the risk and progression of RA (6), prominent among these loci are major histocompatibility complex-human leukocyte antigen (MHC-HLA) genes which are mainly implicated to immune function (7). A host of environmental factors operate on this genetic background to contribute to disease development, such as smoking, silica exposure, vitamin D deficiency, and obesity (8). Various infectious factors have been relevant to RA, such as Epstein-Barr virus (EBV), Escherichia coli, cytomegalovirus (9). Considerable research efforts have been devoted to proving that both innate and adaptive immune deregulation arbitrate RA. Various immune cells and molecules

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largely accumulate in the synovium of RA patients, such as neutrophils, macrophages, dendritic cells, activated T cells and B cells, and interleukin (IL)-18, IL-6. The imbalance of Th17/Treg cells in RA patients is bias towards Th17 cells (10), which secret IL-17, resulting in cartilage and bone damage (11). Macrophages can be stimulated by tumour necrosis factor (TNF)- $\alpha$  or IL-1 to form osteoclast inducing bone absorption (12).

### Pain and RA

Pain is a predominant problem for RA patients, which starts before the manifestations of disease, then causes damage to psychosocial distress and fatigue (13). Pain in RA can be constant or intermittent and localised or wide-spread (14). The pathological mechanisms contributing to pain in early RA are attributable to chronic inflammation whereas in the developed progression, RA are introduced by not only inflammation but also bone damage and other changes, and the pain progression in RA also proceeds through sequential processes. Moreover, low mood may administer to pain development and inversely pain further worsens mood disturbance (15). Synovial inflammation, such as synovitis, is the main characteristic of RA correlating to pain severity. Noxious stimulation, like inflammation, can activate the nociceptive receptors by low intensity stimulation which normally cannot induce pain. Peripheral sensitised tissues continuously release algogenic substance to stimulate free nerve ending, transmitting impulse to central nerve system to induce pain. The constant peripheral stimulation results in the continual release of neurotransmitter and cytokines by afferent fibres in spinal dorsal horn, inducing the increased excitability, the wider receptive field, and the improved reaction to noxious or non-noxious stimulation of dorsal horn neuron to afferent signals, thus leading to continual pain. Furthermore, pain can trigger more severe outcomes than joint damage in RA patients. The pain thresholds for RA patients are reduced over affected joints and associated with synovitis (16, 17).

Recent therapy is better to aim at both disease treatment and relieving pain symptoms. It is urgently needed to develop better treatment of RA pain.

### Role of LTB4 in RA pain

Various cells (endothelial cells, leukocytes), inflammatory factors (adhesion factors, chemoattractants) and many signal transduction pathways unite to induce this abnormal inflammatory response to RA (2, 18, 19). Among of them, leukotriene B4 (LTB4) plays a vital role in the pathogenesis of RA and administers to joint pain and destruction. It has been proposed that there is high concentrations of LTB4 in the articular fluid in patients with RA (18), suggesting a strong correlation between LTB4 and RA pathogenesis. As a pro-inflammatory lipid mediator, LTB4 originates from arachidonic acid (AA), then experiences a series of enzymatic catalytic reactions involving in 5-lipoxygenase (5-LO) which produces leukotriene A4 (LTA4), and leukotriene A4 hydrolase (LTA4H) which directly hydrolyses LTA4 to LTB4. Another protein, 5-lipoxygenase-activating protein (FLAP), is an integral membrane protein that is also required for leukotriene synthesis (20). Studies demonstrate that, over 45% of the synthetic LTA4 produced by 5-LO active leukocytes, are released into extracellular and absorbed by the cells, and 5-LO inactive leukocytes express LTA4H or leukotriene C4 (LTC4) synthase, for further LTB4 or LTC4 synthesis (20). LTA4H are expressed in endothelial cells, leukocytes and erythrocytes, but 5-LO is only expressed in neutrophils and mononuclear macrophages. The cells without 5-LO can synthesise LTB4 only after provided with LTA4 by 5-LO active cells (21). The pathogenesis of RA mainly involve LTB4, and the synthesis of LTB4 mainly depends on 5-LO and LTA4H. Then, LTB4 initiates inflammatory signalling cascades by binding its two receptors: BLT1 with high affinity and BLT2 with low affinity, resulting in the activation and recruitment of leukocytes to the synovium, which further leads to pain and bone damage (22, 23). The positive effects of 5-LO and LTB4 in hyperalgesia has been stated by pre-

vious studies (24, 25). Cortes-Burgos *et al.* (26) illustrate that the LTB4 concentration in brain in disease groups is three times higher than that in control group, and CJ-13610, an inhibitor of 5-LO, can suppress the synthesis of LTB4 to ameliorate hyperalgesia, which implies leukotrienes and 5-LO pathway are important mediators for pain. Prostaglandins, the metabolites of AA via cyclooxygenase (COX)-pathway, have complementary effects in pain response with leukotrienes, so that co-administration of 5-LO and COX inhibitors, such as zileuton and indomethacin, can result in better anti-hyperalgesia than either alone (27). For example, Me-UCH9, a dual 5-LO/COX-2 inhibitor, can decrease the content of LTB4 in paw oedematous fluid, and inhibits the inflammatory response and pain thus modulating the nociceptive responses and inflammation (25). It has been described by Ben *et al.* that *Ficus platyphylla* (FP) extract has anti-hyperalgesia effect, which may be due to its dual inhibitions on the formation of COX-derived PGs and 5-LO-derived LTs (28).

### LTB4 induced neutrophils accumulation in hyperalgesia

Neutrophils play an important role in the development of both inflammatory pain and neuropathic pain. LTB4 can activate and accumulate neutrophils, and subsequently increase exudate from venules, resulting in hyperalgesia (29) (Table I). Guerrero *et al.* (30) demonstrate that neutrophils are involved in LTB4-induced pain in the zymosan-induced arthritis (ZIA) rat model. Meanwhile, in human ZIA model, the LTB4-induced hyperalgesia is relevant to neutrophil accumulation. LTB4 can act on the BLT1 receptors on neutrophils activating and accumulating neutrophils, leading to the release of TNF- $\alpha$ , which binds the receptors on neurons to depolarise neurons membranes, producing action potentials, which are then transformed to central nerve system to induce pain. There are other multiple products of neutrophils such as IL-2, IL-6 and ROS, can result in the reduction of neuron viability (31). Neutrophils can also transport leukotriene

**Table I.** The role of leukotrienes in RA pain.

Pathogenesis	Leukotrienes	Associated receptors	Associated effects	Pain	References
Nerve injury; neutrophil accumulation	LTB4↑	BLT1 activation ↑	Intracellular Ca <sup>2+</sup> ↑	↑	(24)
			Neuron excitability↑	↑	(24)
			IL-2, IL-6, ROS ↑    Neuron viability↓	↑	(31)
			TNF-α, IL-β↑    Peripheral sensitisation↑ CNS activation↑	↑	(31)
			NF-κB activation↑    MMP-9 and CXC3R-1↑	↑	(36)
		TrpV1 channel activation ↑	Nerve fibres activation↑	↑	(45)
Nerve injury	CysLT↑	CysLTR↑	Pro-inflammatory cytokines↑	↑	(32)
			Modulate P2X3 receptor activation↑	↑	(50)
			ROS↑    Pain threshold↓	↑	(40)

A4 (LTA4) to other cells to synthesise Cysteinyl leukotrienes (CysLT), which can act on their receptors on glial cells aggravating pain development (32). So activated inflammatory cells can release inflammatory factors with hyperalgesia effect, such as IL-β and TNF-α, acting on the receptors on neurons to induce peripheral sensitisation (33) (Table I).

#### *LTB4 activated NF-κB pathway in pain*

Leukotrienes can be effective through their G-protein-coupled receptors to activate nuclear factor-kappa B (NF-κB) pathway, enhancing the combination of NF-κB and DNA, as well as improving the expression of various inflammatory factors (Table I). Serezani *et al.* have reported that, the activation of the MyD88-dependent NF-κB pathway requires LTB4-BLT1 recognition (34), and in the cultured monocytes, LTB4 activates NF-κB through MAPK and ROS -dependent mechanism (35) (Table I). After NF-κB pathway is activated, neurons upregulate the expressions of MMP-9, chemokine CX3C receptor 1, and pro-inflammatory cytokines to directly modulate the neuron activation or indirectly activate microglia to promote pain development (36).

#### *MAPK activated 5-LO/LTB4 pathway in neuropathic pain*

Nerve injury can trigger activation of mitogen-activated protein kinases (MAPK), which consists of extra-cellular signal-regulated kinase 1/2

(ERK1/2), p38 MAPK, and c-Jun NH2-terminal kinases (JNK1/2) in spinal glial cells, and MAPK inhibitors can reverse injury-induced hyperalgesia (37-39). Through p38 MAPK activation, 5-LO as the first step of LTs pathway can be activated by nerve injury in spinal microglia, resulting in the increase of mechanical hypersensitivity (24). Injury in peripheral nerves induces the activation of microglia in spinal cord, and promotes 5-LO expression through p38 protein kinase signalling pathway, then 5-LO catalyses AA metabolism to produce LTB4 and CysLT. LTB4 can directly act on the BLT1 in dorsal root ganglion (DRG) to increase intracellular calcium concentration and neuron excitability, leading to nociceptive response (24) (Table I). And CysLT by their receptors activates microglia to release pro-inflammatory factors, leading to nociceptive sensitisation (24). After nerve injury, spinal cord microglia produce ROS, contributing to descending pain threshold (Table I), which is supported by the study of Kim *et al.* (40) that NADPH oxidase 2 (NOX2)-derived microglial ROS can induce neuropathic pain after spinal nerve transection (SNT), and the SNT-induced thermal hyperalgesia is reversed in NOX2-deficient mice, in which TNF-α and IL-1β expression in the spinal cord is reduced, suggesting these pro-inflammatory cytokines are involved in the development of neuropathic pain and these cytokines expression require NOX2-derived ROS generation. And

the administration of sulforaphane can reduce microglial reactive oxygen species (ROS) level, thus attenuating thermal hyperalgesia in SNT-injured mice, indicating its strong analgesic effect on neuropathic pain and important therapeutic implications (40).

#### *LTB4 activated ion channels/receptors*

Numerous pain-producing agents identified in the RA synovial fluids can modify the functions of ion channels, such as Transient receptor potential-vanilloid 1 (TrpV1), to excite peripheral nociceptors (41). Other multifarious cytokines, growth factors, and chemokines have been detected at high level in RA synovial fluids, such as IL-1β, IL-6, and TNF-β, nerve growth factor, vascular endothelial growth factor (VEGF) and C-C motif chemokine receptor ligand 2 (CCL2) (42, 43), and can sensitise peripheral nerves through specific cell-surface receptors. In the CFA-induced arthritis, increased expression of TrpV1 is involved in both peripheral and central mechanism on RA pain onset, and increased purinergic P2X3 receptor is related with the peripheral mechanism (44). Trpv1, one of transient receptor potential channels, is a non-selective calcium channel, mainly located in small and medium nociceptive sensory nerve, as well as central nerve system. The structure of LTB4 is similar to Capsaicin, which can directly activate TRPV1 receptors, and this means LTB4 can have a similar effect (45). In the DRG of rats, LTB4 can

increase intracellular calcium concentration at the same level of Capsaicin, and BLT1 receptors are expressed on the Capsaicin-sensitive C-fibres, which means BLT1 receptors may have a similar internal-activity with TRPV1 (46). Through TRPV1 channel or BLT1, LTB<sub>4</sub> can activate nociceptive nerve fibres to induce pain (Table I). Purinergic receptor X<sub>2</sub> (P<sub>2</sub>X) as an ATP-gated ion channel, is found on both neurons and glial cell (47). The metabolites such as ATP and ADP can increase the synthesis of leukotrienes through the P<sub>2</sub>X receptor on glial cell surface (48). P<sub>2</sub>X<sub>3</sub>, a member of P<sub>2</sub>X family, selectively expresses on nociceptors, and involves in the transmission of pain signals from peripheral nerve to spinal cord (49). Masamichi *et al.* have proposed that together with P<sub>2</sub>X<sub>3</sub> and TRPV1 receptors, CysLT<sub>2</sub> receptor often express on unmyelinated and non-peptidergic neurons. LTC<sub>4</sub>, a CysLT<sub>2</sub> receptor agonist, enhances the hyperalgesia produced by agonist of P<sub>2</sub>X<sub>3</sub> receptor rather than by itself, suggesting CysLT<sub>2</sub> receptors in the DRG neurons act as a modulator of P<sub>2</sub>X<sub>3</sub> receptor to contribute to the pain behaviours (50) (Table I).

### Role of LTB<sub>4</sub> in bone and cartilage damage of RA

Except for pain induced in RA, one of the predominant symptoms for RA patients are the bone destruction and cartilage degradation, which occur at the beginning stage of RA and aggravate as RA develops. Bone erosion starts in the surface of cartilage and inflamed synovium with huge amounts of osteoclast. Elevated differentiation and activation of osteoclasts specialised in the bone absorption of RA result in bone erosion and bone destruction. Another vital cells, fibroblast-like synoviocytes (FLS), which are the main component of synovial tissue, maintain the stable environment inside joint and produce pro-destructive proteases, as well as cytokines to induce pannus, which also facilitates destruction of bone and cartilage (51). So far, the conventional drugs disease-modifying anti-rheumatoid drugs (DMARDs) are supplied for improved inflammatory synovitis and meliorate joint damage (52, 53), but var-

ied adverse events are also stated (54), which alarms us to explore more candidate targets to relieve bone damage.

LTB<sub>4</sub> activates intracellular calcium and augments human osteoclastogenesis via its two receptors to participate in bone and cartilage destruction (55). To understand the mechanisms evolved in the bone erosion, the origination of osteoclasts is better to be specified (Table II) (56). Osteoclastogenesis is a multistep process consisting of proliferation, differentiation, cell fusion, and multi-nucleation (57). The differentiation of multinucleated giant osteoclasts from monocytes and macrophages are controlled by macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor- $\kappa$ B ligand (RANKL). In abnormal condition, B10 cells aberrantly produce RANKL to contribute to the development of bone erosion in RA (58). As a member of TNF superfamily, RANKL binds to his receptor RANK in monocyte-macrophages induced osteoclast precursor cells, mature osteoclasts and dendritic cells to take effects. It has been investigated that by knocking out *Rankl* or *Rank* gene, a very rare complication in RA, osteopetrosis results, which suggests osteoclastogenesis is impaired, and osteoclasts are reduced. And the knockout of osteoprotegerin (OPG), a competitor with RANKL by binding to RANK suppressing the activity of RANKL, shows osteoporosis, which indicates that there are high concentrations of osteoclasts. Combining these two studies, it is easy to conclude the vital roles of RANK and RANKL in the production of osteoclasts. After fully differentiated and functional osteoclasts are synthesised from (c-fms<sup>+</sup>)/RANK<sup>+</sup> monocytes or macrophages precursor cells by recognising M-CSF and RANKL (59), osteoclasts target at the bone surface and secrete tartrate resistant acid phosphatase (TRAP), cathepsin K and matrix metalloproteinase 9 (MMP 9) to induce the bone absorption (60). It is reviewed that MM9 activity, which was associated with TNF- $\alpha$ -308A allele, was higher in erosive RA than in non-erosive RA (58). Therefore, inhibition of osteoclastogenesis becomes our destination to prevent bone damage.

Osteoclastogenesis and synovial inflammatory can be enhanced by the RANKL signalling pathway and proinflammatory cytokines, such as IL-23 (61-63). IL-23 can participate in the inductions of Th17 cells and pro-osteoclastogenic cytokines including IL-17, RANKL and TNF to promote the inflammatory bone loss and osteoclast formation (55). It has been unmasked that IL17 levels in RA patients were higher than normal with more expression of IL17F rs763780, IL17Ar2275913, and IL17Ar3819024 polymorphisms (58). IL17 and TNF act on the G protein coupled receptors (GPCRs), particularly BLT1 and BLT2 of leukotrienes. IL-23 can also stimulate the production of CD11b<sup>+</sup>Gr1<sup>high</sup> myeloid subtype which plays a main role in the production of LTB<sub>4</sub> (63, 64), to further enhance the impacts on LTB<sub>4</sub> induced osteoclastogenesis. In addition, not only can IL-23 play a key part in the facilitation of LTB<sub>4</sub> production and osteoclasts differentiation from the macrophages without RANKL participation, but also aggravate the inflammatory by activating neutrophil (65) (Table II).

It is purported that macrophages are equipped with the two BLT receptors and key LTB<sub>4</sub> synthetases, including 5-LO, LTA4H. These two receptors also exist in osteoclasts, which means LTB<sub>4</sub> can determine macrophages and osteoclasts cellular fate by activating nuclear factors and regulating the transcription of the osteoclast-related genes (65). It must also be mentioned that LTB<sub>4</sub> activates phospholipase C (PLC) and calcium release activated channel (CRAC) to allow calcium influx via BLT1 and BLT2 on the macrophages (65). BLT1 receptor is not only required in the production of monocytes, neutrophils and LTB<sub>4</sub> from the myeloid cells via IL-23 overexpression, but also accelerates the osteoclast differentiation by calcium and NF- $\kappa$ B signalling induced by RANKL. Intracellular calcium concentrations have a strong impact on the osteogenesis as well as enhance the LTB<sub>4</sub> synthesis because of calcium-dependent cytosolic phospholipase A<sub>2</sub> (PLA<sub>2</sub>). The activation of cPLA<sub>2</sub> can be also achieved by the direct effect of IL-23 and PI3K, which facilitates the

**Table II.** The mechanism of LTB4 induced osteoclastogenesis and bone damage.

Pathogenesis	LTB4 production	LTB4 effects	Osteoclastogenesis	Bone damage	References
IL-23 → production of CD11b+Gr1 <sup>high</sup> myeloid subtype <sup>↑</sup>	↑	IL-17 and TNF activation <sup>↑</sup>	↑: TRAP, Dathepsin K and MMP9 <sup>↑</sup>	↑	(55, 60, 63-65)
cPLA2, PI3K and IL-23 → Ca <sup>2+</sup> ↑	↑	PLC and CARC <sup>↑</sup> → calcium influx <sup>↑</sup>	↑: TRAP, Dathepsin K and MMP9 <sup>↑</sup>	↑	(60, 65)
IL-β and TNF-α	↑	IL-β and TNF-α synthesis <sup>↑</sup>	↑: TRAP, Dathepsin K and MMP9 <sup>↑</sup>	↑	(60, 65)

LTB4 production via IL-23 receptor SH2 expressed in the macrophages (65) (Table II).

It is noteworthy that, since RANKL is a pro-osteogenic factor, the combination effect on osteoclast differentiation between RANKL and LTB4 cannot be ignored. However, LTB4 can also set in motion the calcium influx without the involvement of RANKL-RANK pathway in macrophages and promote osteoclast formation in the absence of RANKL as well (65). These various pathways in the osteoclastogenesis and bone loss can become our alternative therapeutic options in the RA treatment (66).

IL-β and TNF-α promote LTB4 production, which in turn increase IL-β and TNF-α concentration (67-70). These positive interactions between productions of LTB4, and IL-β and TNF-α not just participate in neutrophil infiltration, but also induce damage on bone and cartilage. IL-β and TNF-α have a destructive function on reconstruction of bone and cartilage in RA. TNF-α plays a vital role in early RA and long-term inflammation. It sets in motion collagenase production by synovial cells, especially collagenase II, and damages cartilage matrix, furthermore stimulating synovial cells to produce PGE2, as well as promoting adhesions of neutrophils onto endothelial cells to contribute to synovial inflammation (71, 72). IL-β mainly induces local inflammation, which enhances the absorption of articular bone and cartilage, thus interfering with bone reconstruction. It is well known that the production of LTB4, IL-β and TNF-α in the inflammation conduces to the excessive leukocytes accumulation in the synovial fluids, leading to joint damage (73) (Table II).

#### LTB4 induced bone damage: therapeutic approaches

Andrographolide can prevent the RANKL induced bone loss by upregulating NF-κB and ERK/MAPK signaling pathway to achieve bone resolution (65). It interferes the phosphorylation of IκBα induced by binding of RANK and TRAF6, which normally initiates TAK1 kinase activity, and further activates NF-κB pathway (74, 75). The mechanism of Ameloxanox is evolved in the inhibition of RANKL on three spheres: suppressing osteoclastogenesis especially for onset and late progression by inhibiting activated NF-κB and MAPK, as well as associated c-Fos and nuclear factor of activated T cells c1 (NFATc1) expression, and interrupting mature osteoclast activity in attachment and bone resorption, as well as influencing bone marrow mesenchymal stem cells (BMSCs) activity and gene expression for osteoclasts differentiation, such as TRAP, matrix metalloprotein (MMP9), NFATc1 and Cathepsin K bound by NFATc128 (57). C-Fos activates the transcription factor NFATc1 for osteoclasts differentiation (76, 77). And signs of bone loss in the ovariectomized (OVX) mouse model are also improved. HU-308, a synthetic cannabinoid, whose suppression on osteoclastogenesis, inactivate NF-κB ligand (RANKL) activity to bone marrow-derived osteoblasts and stromal cells (78), as well as improving bone resorption are also proved. Denosumab can effectively slow down the development of erosion by inhibiting RANKL, but the suppression of cartilage destruction is failed, which needs to be cleared by more researches (79).

#### Conclusions

Although recent progress has been made in the disease treatments, pain

and deformity remains the most two distressing symptoms for most people with RA. Inflammation, peripheral and central pain mechanisms and altered gene expressions and joints structure all can conduce to RA pain. And both LTB4 and CysLTs take effect in the onset and development of RA hyperalgesia and neuropathic pain leukotrienes by inducing nerve injury, neutrophil activation, as well as stimulating associated signalling pathways and ion channel/receptors. In the terms of bone damage, induced joints stiffness and deformity are currently resolved in the orthopaedic surgery. If the progression of joint injury is slowed down based on involved mechanisms, patients are unnecessary to connive it until compelled to accept surgery, and alleviate no matter physical suffering or mental surgery. Further research is urgently required to define optimal novel therapeutic strategies with greater efficacy and less adverse reactions, against RA, especially its symptoms pain and bone damage.

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