Review

LTB4-induced anti-apoptosis and infiltration of neutrophils in rheumatoid arthritis

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ABSTRACT

Rheumatoid arthritis (RA) is the most common autoimmune disease, resulting in synovitis, joint pain and stiffness, even deformity and disability. The interactions between leukotriene B4 (LTB4) and neutrophils in RA progression have not been elucidated in detail. Our review focuses on the correlation of LTB4 and neutrophils in the development of RA especially in terms of infiltration and delayed life span of neutrophils. In this article, the roles of LTB4 in the anti-apoptosis of neutrophils will be detailed, which is achieved by suppressed pro-apoptotic Bax and up-regulated anti-apoptotic Mcl-1, and several key molecules, as well as signalling pathways and factors relevant to the enhancement of LTB4 production and functions. The mechanisms of LTB4-induced anti-apoptosis and infiltration of neutrophils provide more potential targets in the treatment of RA and recent therapeutic strategies are also discussed.

Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease, which presents massive synovial proliferation with inflammatory cell infiltration and angiogenesis, leading to bone and cartilage erosion, as well as bone synovial hyperplasia (1). The main manifestations include progressive articular damage, functional loss, and associated syndrome in metabolism and vascular system (2). RA can affect people at any age with 1% of the population worldwide, and women are more sensitive than men (3). There are many commons and variations in both disease development and prognosis for patients with RA, which means current therapeutic management cannot fulfill the whole patients and more drug targets are required.

A variety of hypotheses have been developed to explain RA, in which the main aetiological factors are mentioned below, including heredity, environment, infection and immunity (4). With the development of conventional and genome-wide approaches, it is detected that there are more than 100 loci that may participate in the risk and progression of RA, and most of them encode major histocompatibility complex (MHC) class II cell surface receptors that evolves in T-cell recognition, thereby taking effect in immune activation and regulation (5). What is more, many environmental factors contribute to RA, such as smoking, silica exposure, other bronchial stresses, vitamin D deficiency and obesity (6, 7). Numerous infectious agents and their products have been reported to be associated with RA, such as Epstein-Barr virus, Escherichia coli and cytomegalovirus (8).

An army of immune cells and molecules infiltrate in the synovial membrane, such as neutrophils, macrophages, dendritic cells, activated T cells and B cells, human lymphocyte antigen (HLA) class II molecules, and costimulatory molecules (9, 10). These numerous cells, inflammatory factors (adhesion factors and chemoattractants) and various signal transduction pathways unite to induce abnormal inflammatory response to RA (11-13). Among them, it has already been proved that there are strong correlations among LTB4, neutrophils and RA pathogenesis, for there is high concentration of LTB4 and neutrophils in the articular fluid in patients with RA (12). As a pro-inflammatory lipid mediator, LTB4 is originated from arachidonic acid (AA) through serious catalytic reactions of 5-lipoxygenase (pathway) and leukotriene A4 hydrolase (LTA4H) (14, 15). This pathway often occurs in leukocytes and other

immune cells, including eosinophils, neutrophils, monocytes. LTB4 initiates inflammatory signalling cascades by binding its two G protein-coupled receptors: BLT1 with high affinity and wild-distributed BLT2 with low affinity. BLT1 receptor is well known to establish the early stage of RA and plays a role in the synovial chemokine production, activation and recruitment of leukocytes to the synovium, as well as joint inflammation, amplifying inflammatory response, which further leads to inflammation infiltration (16-19). BLT2 receptor is less studied but has been proved to be involved in neutrophil recruitment and bone destruction in the RA pathogenesis (16, 18, 20). To reduce the inflammatory response, many scholars devote themselves to reducing the production of LTB4 by interfering the production of the enzymes in the synthesis of LTB4. Oral LTB4 receptor antagonist BIIL-284 is applied for a long-term treatment of rheumatoid arthritis (21). However, it is disappointing that the high costs and side effects cannot be ignored, particularly for long-term users (22) and some patients still do not achieve good responses even to the drug combination therapy (23). Further exploring the roles of LTB4 in the pathogenesis of RA is expected to provide more targets for RA treatment. The earliest and apparent marker in RA is a large proportion of neutrophils in the RA synovial fluid, for 70% of total leukocytes in peripheral blood for RA patients (24). The impacts induced by neutrophils on the RA pathogenesis largely depend on extended survival and infiltration. LTB4 has a vital role in neutrophil duplication, mediates neutrophil recruitment and enhances neutrophil activity, and the delayed neutrophil apoptosis further connives neutrophils in the RA progression, which is also associated with LTB4.

LTB4 involvement in the anti-apoptosis of neutrophils

Neutrophils as the first line immune cells have a defense response to the unfriendly components to induce innate and adaptive immunity (25). By releasing lypase enzyme, reactive oxygen species (ROS) and nitrogen species, neutrophils eliminate pathological stimuli and induce cell necrosis or apoptosis. The functions on modification and recruitment of other immunological cells also exist. In normal conditions, as neutrophils have a short half-life in a terminally differentiated form, it has no need to worry about the damage to the normal tissues. But if the life span of neutrophils is extended, it could lead to chronic pathological autoimmune diseases such as RA. It has been investigated that the anti-neutrophilsapoptotic effect can be prevented if LTB4 is blocked (26).

NADPH derived ROS from neutrophils by LTB4 regulating nuclear factor kappaB (NF-κB) pathway to control neutrophil cell fate

Either delay or shortening neutrophil survival can also be influenced by many other factors, among which, low local oxygen tensions in the synovial fluid are predominant in controlling LTB4-induced neutrophil survival (27). Oxidative stress contributes to the RA pathogenesis and there is evidence that abnormal antioxidant system, active serum and synovial fluid lipid peroxidation exist in RA. Oxidative stress is induced by elevated ROS, which results from the imbalance between the generation and detoxification. This kind of imbalance can be due to immune disturbance, stress and so on (28). ROS, free active oxygen radicals (29), are produced by neutrophils in the process of cellular oxidative reaction, which mainly refers to mitochondria-derived and the NADPH oxidases complexes (NADPHox) activity, which are associated with LTB4 (30) in the pro-inflammatory pathogenesis of RA. ROS production via NADPH oxygen complex 2 (NOX2) (31) in neutrophils is widely studied and most effective intracellularly and extracellularly (32). NADPH passes electrons from its cytosolic region to oxygen in the extracellular region to produce O^{2-}, and eventually ROS is synthesised (33, 34). As mentioned above, the pathway of leukotriene production involves AA metabolism, which also participates in the ROS production (30). ROS play a dual role in the physiological action. It can not only participate in the anti-microbial and pathogenic immunological response, but is also considered as a regulator in low concentration for signal transduction and gene expression to control cell fate, including growth, differentiation and cell death (32). The latter function may be related to the prevention of neutrophil apoptosis. Low levels of ROS upregulate the pro-survival mechanism and inhibit the spontaneous neutrophil apoptosis to achieve the prolongation of the neutrophil life-span, and evolves in the initial stage of disease and continues to aggravate RA mainly by releasing very toxic ROS (35-38). However, if there is imbalance of synthesis and deoxidation, long-term and high level of ROS can induce cellular death and tissue damage.

LTB4 can also activate NF-κB, and the evolvement of NADPH-derived ROS generated by LTB4 is discovered in the NF-κB pathway, which can delay neutrophil apoptosis. NF-κB is a transcription factor of proinflammatory genes in the inflammatory response (39). LTB4 triggers the degranulation of neutrophils to release NADPHox enzyme complexes, which can generate ROS in low concentration to activate inhibitor of kB kinases (IKKs), then induce the phosphorylation and degradation of inhibitor of κBα (IκBα), encouraging NF-κB to enter the nucleus affecting the neutrophil outcome (40). NF-κB evolves in encoding pro-survival genes to control cell outcome, so it can prolong the neutrophil life-span (41).

LTB4 activates PI3K and extracellular signal-regulated kinase (ERK) signalling pathway to extend neutrophil life span

With the help of BLT1, LTB4 can induce the activation of both phosphatidylinositol 3-kinase (PI3K) and ERK pathway, which is one of mitogen-activated protein kinase (MAPK) cascades, further promoting the production of Mcl-1 and inhibiting Bax, finally merges into the NF-κB pathway to determine the cell fate (42, 43). Although Mcl-1 and Bax both belong to the antiapoptotic B cell lymphoma 2 (Bcl-2) family, they have absolutely different

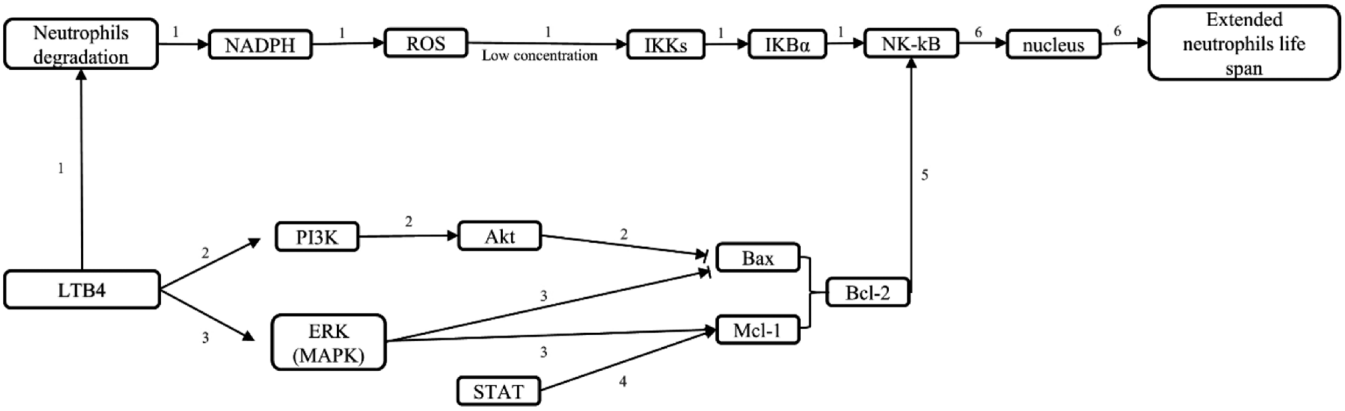

Fig. 1. The mechanisms of LTB4 induced anti-apoptosis of neutrophils.
1 and 6: LTB4 induces the degranulation of neutrophils to release NADPHox enzyme complexes, which generate ROS. ROS in low concentration active IKKs, then IκBα is phosphorylated and degraded, allowing NF-κB to enter into nucleus as a transcription factors of pro-survival genes, extending neutrophils life span (40, 41).
2, 5 and 6: LTB4 activates PI3K and downstream target Akt, controlling the concentrations and entry of Bax into mitochondria and maintains mitochondria stability to determine neutrophils life span (41-43, 45).
3, 5 and 6: LTB4 actives ERK pathway, promoting anti-apoptotic factor Mcl-1, but inhibiting pro-apoptotic factor Bax, and emerging into NF-κB signalling pathway to control cell fate (42, 43).
4, 5 and 6: Mcl-1 level can be also upregulated by STAT (50).
LTB4, leukotriene B4; NADPHox, NADPH oxidases complexes; ROS, reactive oxygen species; IKKs, inhibitor of κB kinases; NF-κB, nuclear factor kappa B; IκBα, inhibitor of κBα; PI3K, phosphatidylinositol 3-kinase; ERK, extracellular signal-regulated kinase.

functions. Mcl-1, as anti-apoptotic factor, can extend cell survival (44). PI3K, as well as its downstream target serine/threonine kinase AKT, are responsible for modulating the neutrophil apoptosis by controlling the concentrations and entry of Bax into mitochondria (45). In the K/BxN serum transfer model, gene deletion or selective PI3K δ inhibition on the p110 δ isomer (PI3K δ) of PI3K, can relieve joint damage and reduce LTB4-induced neutrophil migration (46). It is also reported that NADPHox-dependent degradation of Bad via ROS inhibits the translocation of Bcl-2 members into the mitochondrial outer membrane (47-49) and pro-apoptosis factors into cytosol, maintaining mitochondrial stability (41). Mcl-1 expression is regulated by signal transduction through ERK, and by transcriptional activation through STAT molecules (50). NF-κB also help strengthen the expression of anti-apoptosis factor, Mcl-1 (42).

The LTB4-dependent anti-apoptotic effect of neutrophils is still obscure, for there are some conflicts which still need an explanation. For example, the report by Murray (51) illustrates that there is no association between LTB4 and dexamethasone, GM-CSF and LPS-induced retardation of neutrophil apoptosis, however, this contradicts tthe conclusions made by Lee (52) and Stankova (26). To further explore and confirm more mechanisms, more studies are needed.

Inhibitions on LTB4 induced anti-apoptosis of neutrophils

All in all, the conversion of neutrophils in limited life into extensive survival existence leads to much more tissue damage, induces the onset of RA pathogenesis and interrupts the therapeutic effects. Therefore, suppressions on any pathways evolving in anti-apoptotic manner or directly accelerating neutrophil apoptosis becomes our therapeutic target. Macrolides, including azithromycin, tilmicosin, tulathromnycin and erythromycin, have both antimicrobial and mediation of host immunity and inflammation functions. Azithromycin and tilmicosin can induce neutrophil apoptosis and suppression of LTB4 generation. Tulathromycin with satisfactory clinical treatment efficacy, can facilitate neutrophil apoptosis and downregulate LTB4 concentration in inflamed synovial joints by suppressing IkBα phosphorylation and translocation of NF-κB p65 subunit into nuclear to abrogate NF-κB pathway, thus further interrupting the expression of proinflammatory product, CXCL8 (53). Erythromycin also takes effect based on the NF-κB pathway but more detailed mechanisms are not identified. ROS are generated from the process of LTB4 production associated with 5-LO metabolite via NOX stimulation. Hesperidin, flaveanone glycoside, can suppress the production of ROS and reactive nitrogen species (RNS), protecting cellular membrane and preventing the progression of RA (54). It has also been reported that low dose methotrexate administered to patients with very early rheumatoid arthritis can obtain a very good clinical effect (55) (Fig. 1).

Roles of LTB4 in neutrophil chemotaxis

Recruitment of neutrophils from blood to injury tissue sites is a hallmark of early innate immune responses, and it has been widely defined that this process relies on various molecular events (56). Neutrophil snormally act to protect the host when the acute innate immune system responds to injury inflammation, but its excessive accumulation and activation also damage the surrounding organs (16). There is strong evidence demonstrating a large cluster of leukocytes in the inflammatory synovial fluid, among which are predominantly neutrophils (57). Neutrophil infiltration is a very complete process because of the evolvement of abundant cytokines, chemokines and

inflammatory cells, and plays an important role in the few early months of RA pathogenesis, which determines the onset of RA, leading to further development and aggravation (58). Some studies have demonstrated that the inhibited neutrophil infiltration can attenuate secondary injury (59). To participate in the pathological inflammatory process, neutrophils continuously produce large amounts of pro-inflammatory cytokines, among them, leukotrienes, which are first secondary chemoattractants, are largely expressed in injury to the synovial membrane (57).

LTB4 as signal-relay molecule guide neutrophil chemotaxis in a direct way

N-formyl-methionine-leucine-phenylalanine (fMLP) -induced LTB4 secretion in autocrine and paracrine manner enhanced and stabilised neutrophil polarisation, and amplified neutrophil chemotaxis in a signal-relay way. LTB4 plays a critical role for intercellular signal relay among neutrophils, amplifying local cell death signals to enhance directed neutrophil recruitment (60). In the inflammatory sites, various primary chemoattractants, such as formyl peptides released during bacterial infection, and complement fragments released during tissue injury, are produced to stimulate the surrounding immune cells, especially neutrophils, to release secondary chemoattractants (61, 62). After neutrophils response to primary inflammatory signal and enter the inflamed tissues, the continued secondary chemoattractants attract neutrophils towards the inflammation sites. Then, the sequential primary chemoattractants further guide neutrophils to the centre of the inflammation (61). Moreover, neutrophils can in turn continue to produce more secondary chemoattractants once they reach the centre of an inflammatory site, guiding more neutrophils to the inflammatory sites and then the inflammation becomes aggravated (63).

A positive dual directions between LTB4 production, IL-1 family and tumour necrosis factor α (TNF-α)

The lipid-cytokine-chemokine cascade pattern enhances neutrophil recruitment, which means that, LTB4 via BLT1 promotes neutrophils to secrete cytokines, such as IL-1, which further induces chemokine production such as TNF-α from synovial cells, promoting neutrophil activation and infiltration to inflammatory sites. Following leukocyte recruitment, this pathway is consistently positively repeated, amplifying the sequential actions, thus aggravating RA (64). For example, a member of the IL-1 cytokine family, IL-18 has been reported to be highly expressed in RA synovial fluids, indicating its participation in inflammatory disease development (65). IL-18 stimulates numerous cell types, such as neutrophils, macrophages, and $CD4^+$ T cells, to produce large amounts of TNF-α, which induce the synthesis of LTB4 to recruit neutrophils to the inflammatory site where acute and chronic inflammation significantly develop, suggesting that IL-18-induced neutrophil recruitment is LTB4-dependent (66). It was found that in a CIA model, inhibited LTB4 suppresses the TNF-α production, indicating that LTB4 induces the synthesis of TNF-α, and in turn, TNF-α also stimulates synovial fibroblasts to produce more IL-18 (66, 67), suggesting a positive feedback among the productions of LTB4, IL-18 and TNF-α in RA.

IL-17 and IL-23 induce production of LTB4

IL-17 and IL-23 work together to induce LTB4 production, facilitating neutrophil infiltration. IL-23/IL-17 axis is vital in the neutrophil influx during the RA pathogenesis (68). IL-17 is well-known as the regulator of neutrophil recruitment (69) and produced by Th17 cells which are activated by TGF-β, IL-6 and IL-23 (70). In the pathway of IL-23-induced neutrophil infiltration, IL-17 is required to promote the production of TNF-α, LTB4 and CXC chemokines. CXC chemokines act on the neutrophils in autocrine and enhance the direct effect of IL-17 on neutrophils (71). This pathway is also influenced by the communications between the postaglandin E_2 (PGE_2) and IL-23/IL-17. Cyclooxygenase 2 (COX-2) derived prostaglandin, such as PGE_2, suppress the productions of IL-12 and interferon γ (IFN-γ) to expand IL-17, promoting IL-23-LTB4 induced neutrophil infiltration to the knee and peritoneal joints (71). PGE_2 can be increased by both IL-23 and IL-1 by upregulating COX-2 gene transcription to further enhance neutrophil migration (72).

It has been found that blocking IL-17 can suppress neutrophil infiltration and chemokine production, particularly LIX and C-X-C motif chemokine 1, and achieve bone protection (68, 73). In addition, it has been discovered that the mechanism of Hyaluronidase treatment on RA acts on TNF-α, IL-8, LTB4 and leukotriene C4 (LTC4), suppressing their synthesis except PGE_2 and PGD_2, to prevent the adhesion of leukocytes to the endothelial cells and neutrophil trafficking (74). Glucocorticoids, leflunomide, methotrexate and anti-TNF-α mAbs are commonly utilised to inhibit neutrophil migration (75-78). More research needs to be done on other drugs to explore the above -mentioned.mechanisms

C-X-C chemokine ligand 1 (CXCL1) and 5 (CXCL5) promote neutrophils to synthesise LTB4

In an antigen-induced-arthritis model, Grespan *et al*. (79) found that, LTB4, CXCL1 and CXCL5 all express at high levels. CXCL1 and CXCL5 are released from synovial tissues *in vitro*. The neutrophils stimulated by CXCL1 and CXCL5 in RA synovial fluids, can produce substantial amounts of LTB4 (79). So it can be concluded that CXCL1 and CXCL5 induce neutrophil recruitment by driving LTB4 production from neutrophils involved in the pathogenesis of RA (Table I). It is reported that C-X-C motif chemokine receptor 1 (CXCR1)/ CXCR2 inhibitors can not only suppress neutrophil recruitment to inflammatory sites but also block LTB4 synthesis in joint tissues. In addition, C-C motif chemokine receptor 1 (CCR1) and CXCR2 are proved to be involved in the leukocytes recruitment (80). It has been found that LTB4 - IL-1β - chemokine ligands of CCR1 and CXCR2 cascade induce neutrophil recruitment into joint. Since LTB4 and IL-1β are mainly produced by neutrophils, it implies that neutrophils are not only the effector cells but also the main

Table I. The roles of leukotriene B4 on neutrophils infiltration in rheumatoid arthritis.

Pathogenesis	Target cells or associated molecules	Effects	Neutrophil infiltration	References
Primary chemoattracta-nts ↑	Immune cells	Release of secondary chemoattractants, including LTB4↑	↑	(61)
LTB4↑	Neutrophils: IL-18↑ → neutrophils, macrophages, CD4+ T cells: TNF-α↑	LTB4 production↑; Synovial fibroblasts: IL-18↑ → LTB4 production↑	↑	(64, 66, 67).
IL-23, IL-6, TGF-β↑	Th17 cells: IL-17↑	LTB4 production↑	↑	(71)
CXCL1, CXCL5 ↑	Neutrophil activation↑	LTB4 production↑	↑	(79)
LTB4↑	As LTB4- IL-1β - chemokine ligands of CCR1 and CXCR2		↑	(58)
LTB4↑	IL-β and TNF-α synthesis↑	LTB4 production↑	↑	(93, 95, 98, 99)
RhED-A↑	BLT1 receptor	LTB4 production↑	Polymorphonuclear neutrophils (PMN) migration↑	(81)
LTB4↑	ROS↑ and Caspase-1-dependent IL-β↑ → NLRP3 inflammasome↑	LTB4 production↑	↑	(85, 86, 91)

inducer of inflammation (58). CCR and CXCR are indirectly utilised by LTB4 to induce neutrophil infiltration.

LTB4 receptors involve in LTB4 induced neutrophil infiltration

Leukotrienes and their receptors, especially LTB4 and its receptor BLT1, play a crucial role in neutrophil recruitment. In the K/BxN mouse model, BLT1 deficiency mice exhibit greatly relieved phenotype, with reduced productions of inflammatory cytokines and chemokines (16), which illustrates that BLT1 in neutrophils is involved in chemokines production and RA pathogenesis, and the selective inhibitors of BLT1 could reverse them. Furthermore, the injection of BLT1 positive neutrophils into BLT1 deficiency mice promotes the migration of BLT1-deficiency neutrophils into joints, finally leading to the amplification of the response to antibody-induced arthritis, which further confirms the essential effects of BLT1 on LTB4 induced neutrophil infiltration. In several mouse arthritis models, it has emerged that large amounts of extra domain A (ED-A, a type of endogenous TLR-4 ligand) containing fibronectin present in RA synovial fluids, and recombinant human ED-A (rhED-A) has a positive effect on LTB4 synthesis and polymorphonuclear neutrophil (PMN) migration (16, 18, 57). Another study (81) found that BLT1 receptor antagonist can inhibit the rhED-A-induced PMN migration, suggesting that rhED-A induced PMN migration is dependent on the involvement of rhED-A and LTB4 biosynthesis. This means that rhED-A contributes to LTB4 synthesis and induces PMN migration via BLT1. BLT1 antagonists, such as amelubant, which can act as novel drugs for RA (82) (Table I). Compared with BLT1, BLT2 is ubiquitously expressed in a more wider range of immune cells. Although, it is already known that BLT2/LTB4 axis induces intracellular calcium mobilisation, ERK activation and keratinocyte migration (83), but the applications of BLT2 in neutrophil infiltration is poorly reported. Recently, Mathis *et al*. discovered that for a BLT2 $^{-/-}$ mouse line with unaltered 5-LO and BLT1 activity, the incidence and the progression of inflammatory arthritis are interrupted (20), and in the K/BxN arthritis model, mice without BLT2 were protected from inflammatory disease (20). These findings suggest that BLT2 is associated with the inflammatory disease pathogenesis and BLT1 alone without BLT2 fail in arthritis development, and together with the previous acknowledgement that initially responding neutrophils must present BLT1 and produce abundant LTB4. However, the recruitment of subsequent synthesised neutrophils was not necessary to depend on BLT1, which may be due to ligand-induced internalisation at high levels of LTB4 (16, 57, 84). People wonder whether BLT2/LTB4 axis is also involved in neutrophil recruitment. And the absence of 12(S)hydroxyheptadeca-5Z, 8E, 10E-trienoic acid (12-HHT), an agonist with high affinity for BLT2 still results in arthritis development, which shows that 12-HHT is not enough to fully activate BLT2 and LTB4 is responsible for that. Since low affinity of LTB4 to BLT2, it also indicates neutrophil chemotaxis preferentially depends on BLT1 but not BLT2 (20). Therefore, recent experiments demonstrate that BLT2 is indeed utilised in inflammatory disease progression, but whether it participates in neutrophil infiltration is not certain.

LTB4 initiates NOD-like receptors containing pyrin domain (NLRP3) inflammasome to induce neutrophil infiltration

The pathogenesis of RA is associated with NLRP3 inflammasome. It is reported that dysregulated activity of NLRP3 inflammasome causes uncontrolled inflammation underlying many chronic diseases, including RA (85-87). NLRP3 inflammasome originated from pro-caspase-1 engaged activated

NLRP3 through adapter molecule ASC, is involved in maturation and secretion of IL-1β and IL-18 initiating inflammation (88, 89). IL-1β with an irreplaceable role in the pathogenesis of RA, is mainly produced by macrophages and induces synovial cell proliferation and cartilage degradation (90). It has been reported that monosodium urate monohydrate (MSU)-induced neutrophil influx is CXCR2-dependent and MSU-induced leukocyte recruitment is NLRP3/ASC/Caspase-1/IL-β/MyD88-dependent. Moreover, CXCR2 is activated by chemokine CXCL1, which relies on IL-β and NLRP3 inflammasome. Therefore, NLRP3 inflammasome also induces CXCL1-CXCR2-MSU-induced neutrophil influx. MSU crystal-activated macrophages can produce LTB4, which stimulates ROS and caspase-1-dependent IL-β production and further activates the ROS-dependent-NLRP3 inflammasome. The above data imply that LTB4 participates in NLRP3 inflammasome induced neutrophil recruitment towards inflammatory joints, and the association between NLRP3 inflammasome and LTB4 can be considered as a novel potential therapeutic target for the treatment of RA (91) (Table I).

Inflammatory factors involving LTB4 effects in RA

The dual regulations between LTB4 production and IL-β, TNF-α synthesis

The production of LTB4, and synthesis of IL-β and TNF-α are controlled by each other, and are involved in neutrophil infiltration by activating downstream and enhancing upstream signalling pathways and molecules. Exogenous and endogenous LTB4 significantly increase the expressions of IL-β and TNF-α at both mRNA levels and protein levels. It has been found that the interfered spinal p38 MAPK activity can prevent neutrophils from entering inflamed knee joints, achieving therapeutic effects on knee joint inflammation, and MAPK is limited to decrease production of TNF and IL-1β, which illustrates the involvement of TNF and IL-β on neutrophils infiltration (92). IL-β and TNF-α, as cytokines not chemotactic molecules, can promote the transcription of monocyte chemoattractant protein-1 (MCP-1) at a high level in RA patients by activating transcription factors such as NκB in different cells. Cysteinyl leukotriene 1 (CysLT1) receptor inhibitor montelukast inhibits 5-LO, resulting in a subsequent suppression of CysLT1 and LTB4 production, which further decreases pro-inflammatory cytokines such as TNF-α (93), suggesting that LTB4 can regulate the expression of TNF-α to contribute to the process of RA (94). Mostly exogenous and partly endogenous, LTB4 expanse cells outside by binding their receptors on monocytes or neutrophils, then stimulate G-proteins, PI3K, cytosolic phospholipase A2 (cPLA2) and 5-LO to further activate upstream signalling pathways, such as NκB, MEK1, JNK and ERK1/2 (43, 95), and NκB receptors are mainly associated with the synthesis of IL-β and TNF-α (96, 97). Therefore, LTB4 is supposed to directly stimulate NκB receptors to regulate the synthesis of IL-β and TNF-α, allowing the participation of IL-β and TNF-α to neutrophil infiltration. And in turn, TNF-α and IL-1β can also regulate the production of LTB4. It has been found that TNF-α and IL-1β do not just increase the relative abundance of LTCs, LTAH and 5-LO, but also enhance LTB4 and LTC4 secretion from inflammatory endometrial epithelial cells (98, 99). Previous studies have testified that suppressed TNF-α production can prevent the release of LTB4 induced nitric oxide (100). In AIA model, a mediator cascade in neutrophil migration following antigen simulation has been found, identified as MIP2 → MIP1α → TNF-α → LTB4. In the human keratinocytes, LTB4 can activate NF-κB through the BLT1/G(i/o)/PI3K/ERK pathway, and enhances TNF-α to induce CCL27, suggesting a mutual enhancement relationship between TNF-α and LTs (93). Therefore, LTB4 and pro-inflammatory cytokines including TNF-α and IL-1β can reciprocally work as cooperation partners in the induction of RA inflammation, which can be targeted at multiple points for treatment. Malleable protein matrix, whey-fermented product of lactic acid, cannot only prevent LPS-induced neutrophil infiltration, but also decrease multiple cytokines and chemokines, including TNF-α, IFN-β, IL-1β, IL-2, IL-6 and IL-18, to prevent neutrophil infiltration with 50% efficacy (101) (Table I).

Others

LTB4 can not only activate and recruit neutrophils but also promotes the expression of inflammatory cytokines and chemokines to achieve stronger pathological inflammatory response. LTB4 can attract T cells to the synovial fluid and induce them to secrete TNF and interleukin, leading to the destruction of bone and cartilage. It has been demonstrated in the CIA model rats study that LTB4 causes the overexpression of IL-32, IFN-γ and chemokines MCP-α and MCP-1α in synovial cells and induces the apoptosis of synovial cells (102). Among them, IL-1 and TNF-α can promote the recruitment of leukocytes into the synovial joints. IL-32 can attract TNF-α and other inflammatory cytokines to the synovium. IFN-γ can affect the expression of genes in the immunity response to induce TNF-α, IL-2 and IL-10 to further stimulate inflammatory response. MCP-1 shows various concentrations with RA development. MCP-1α, as an inflammatory chemokine, evolves in the recruitment of many inflammatory cells (103).

Conclusion

Extended neutrophil life span and neutrophil infiltration mediated by LTB4 are two essential aspects for neutrophils to take effect on either onset or the progression of RA. LTB4-induced neutrophil infiltration requires the contributions of multiple signalling pathways, inflammatory cells and molecules, in an extremely complicated network. Enhanced neutrophil activity inversely strengthens LTB4 production, which further reinforces the pathological progression. Upregulating pro-survival and suppressing apoptosis allow neutrophil survival with alterations in genetic transcription, as well as damaged membrane integration and permeability. Anti-apoptosis of neutrophils augments its contributions in RA pathogenesis. Once these two sides are inhibited, disease remission

can be greatly achieved. Actually, not only single-target drugs are needed to be explored, but drugs against multiple targets at several signalling pathways in one aspect or multi-aspects of LTB4 or neutrophil activity, are also of great interest and significance. Tanshione IIA is an anti-inflammatory drug targeted at neutrophils with therapeutic effects on the bone erosion and neutrophil infiltration, decreasing IL-6 and TNF-α, as well as inducing neutrophil apoptosis in the adjuvant-induced arthritis murine model (104). Bone degradation can be indirectly improved by pentocifylline (PTX) by targeting TNF-α to prevent neutrophil infiltration, which suggests that many therapeutic managements on neutrophil migration can resolve bone damage (105) and more possible candidates are in great need of investigation.

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