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Joint Tissues Amplify Inflammation and Alter Their Invasive Behavior via Leukotriene B4 in Experimental Inflammatory Arthritis

Mei Chen,*† Bing K. Lam,*† Andrew D. Luster,‡ Simona Zarini,§ Robert C. Murphy,§ Angela M. Bair,* Roy J. Soberman,§ and David M. Lee*†

Mechanisms by which mesenchymal-derived tissue lineages participate in amplifying and perpetuating synovial inflammation in arthritis have been relatively underinvestigated and are therefore poorly understood. Elucidating these processes is likely to provide new insights into the pathogenesis of multiple diseases. Leukotriene B4 (LTB4) is a potent proinflammatory lipid mediator that initiates and amplifies synovial inflammation in the K/BxN model of arthritis. We sought to elucidate mechanisms by which mesenchymal-derived fibroblast-like synoviocytes (FLSs) perpetuate synovial inflammation. We focused on the abilities of FLSs to initiate and amplify synovial inflammation in the K/BxN model of arthritis. We sought to elucidate mechanisms by which mesenchymal-derived tissue lineages in generating immune responses and disease pathology remain poorly understood.

The leukotrienes (LTs) are biologically potent metabolites of arachidonic acid (AA) (1, 2). These lipid mediators are known to modulate innate and adaptive immunity by impacting vascular permeability, inducing adhesion molecule expression on vascular endothelium, and activating adhesion molecules (3, 4). Further, LTs increase leukocyte chemoattraction, neutrophil degranulation, smooth muscle contraction, and cytokine secretion (5, 6).

LT synthesis is initiated in leukocytes when AA is released by phospholipases from the cell membranes of activated leukocytes. Only BM-derived myeloid cells, including neutrophils, macrophages, mast cells, and basophils (7–12), possess the enzyme 5-lipoxygenase (5-LO) that sequentially converts AA to 5-hydroperoxylipoycicosatetraenoic acid and then in a second step to leukotriene A4 (LTA4). LTA4 is metabolized further to either leukotriene B4 (LTB4) or leukotriene C4 (LTC4) by the enzymes LTA4 hydrolase (LTA4H) or LTC4 synthase, respectively. To initiate this process, 5-LO must associate on the nuclear envelope with another myeloid-restricted protein, the 5-lipoxygenase activating protein (11).

Whereas LTA4 synthesis must occur in myeloid cells, its downstream metabolizing enzymes are distributed more widely, with LTA4H expressed in most tissues (10). This distribution of the downstream enzymes affords the opportunity for transcellular biosynthesis (13–17) wherein LTA4 generated in a transcellular manner have been shown to contribute to the pathology of any mouse model of human autoimmune disease, in particular autoimmune arthritis. The K/BxN serum-transfer model of joint inflammation is dependent on polymorphonuclear leukocyte (PMN) 5-LO synthetic

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Abbreviations used in this paper: AA, arachidonic acid; B6, C57BL/6J; BLT, leukotriene B4 receptor; BM, bone marrow; FLS, fibroblast-like synoviocyte; iRAD, irradiated; 5-LO, 5-lipoxygenase; LT, leukotriene; LTA4, leukotriene A4; LTA4H, leukotriene A4 hydrolase; LTB4, leukotriene B4; LTC4, leukotriene C4; PDGF, platelet-derived growth factor; PMN, polymorphonuclear leukocyte; RP, reversed phase; WT, wild-type.

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activity as well as neutrophil recruitment via LTβ. The synovi-
tis in K/BxN arthritis is characterized by pathologic responses in
synovial fibroblasts whose aberrant behavior results in synovial
lining hyperplasia and erosive pannus formation (21, 22). Although
recent studies demonstrate that mesenchymal-derived fibroblast-
like synoviocytes (FLSs) actively contribute to the overall level
of joint inflammation (21), the mechanisms by which FLSs con-
tribute to the amplification of synovial inflammation, their own
recruitment, and the recruitment of PMNs in this model are un-
known. In addition, a role for LTβ in regulating the biology of
these cells has not been investigated.

In this article, we define a novel LTβ-mediated amplification
loop in which FLS promote significant synovial inflammation in
vivo via transcellular synthesis of LTβ. LTβ stimulates FLS
migration and invasion in vitro with comparable efficiency to
platelet-derived growth factor (PDGF) and also drives erosive
pannus tissue behavior in vivo.

Materials and Methods

Mice

Six- to 10-wk-old mice were used for these studies. Control wild-type (WT) C57BL/6J (B6) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). The 5-LO−/− mice and LTβ−/− mice (kindly provided by Dr. Beverly H. Koller) and LTβ4 receptor (BLT1)−/− mice (all N10 back-
crosses on the B6 background) (23–25) were maintained as inbred homozygotes. K/BxN mice were maintained as described (26). All of the procedures were approved by the Dana-Farber Cancer Institute Insti-
tutional Animal Care and Use Committee.

Generation of BM chimeras

BM transplantation into irradiated recipient mice was performed as described previously (27). Briefly, BM cells were flushed from donor tibia and femur bones with RPMI 1640 (Invitrogen, Grand Island, NY) con-
taining 5% FCS, and marrow plugs were disrupted by trituration to gen-
erate a unicellular suspension. The entire BM preparation from one donor
was resuspended in 0.5 ml 1× PBS. For transplantation, recipient mice were
irradiated with a split dose (500 and 450 cGy) of gamma irradiation,
and mice were subsequently supported with oral antibiotic (Baytril; Bayer,
MgCl2 (0.5 mM). The cells then were stimulated with A23187 (1

Histological examination

For histomorphometric analysis, ankle tissues were fixed for 48 h in 4%
formaldehyde in PBS and decalcified with modified Kristensen’s so-
lution for 48–72 h (27, 32, 33). Tissues were then dehydrated, embedded
in paraffin, sectioned at a thickness of 5 μm, and stained with H&E. Arthritis
changes in joint tissues were graded based on a pathological scoring system
as described previously (27). To confirm the presence of neutrophils, after Ag
removal per the manufacturer’s protocol, sections were stained with the
NIMP R14 Ab (Abcam, Cambridge, MA) or with control rat IgG, followed
by detection with a Vectastain ABC HRP kit and diaminobenzidine substrate.
NIMP R14 is a neutrophil-specific Ab that recognizes the surface protein
Ly6G/Gr-1. Tissue was subsequently counterstained with Gill’s II hema-
toxylin and mounted with Crystal/Mount (Biomeda, Foster City, CA) (33).

Isolation and culture of synovial fibroblasts

Synovial fibroblasts were obtained from donor mouse ankle tissues as de-
scribed (27). Briefly, after careful removal of skin tissue, the ankle and
midfoot was disarticulated distal to the tibia and proximal to the metatarsal
by ligament and tendon bisection. Dissected ankle tissues were infiltrated
and incubated in type IV collagenase (Worthington Biochemical, Lake-
wood, NJ) suspended at a concentration of 1 mg/ml in DMEM (Invitrogen)
at 37 ºC for 3 h. Cells in suspension were passed through a sterile 100 μm
mesh and washed extensively in media. After overnight culture, nonadherent
cells were washed away, and adherent cells were maintained in DMEM
supplemented with 10% heat-inactivated FCS (Gemini Bio-Products,
Woodland, CA), 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml strepto-
mycin, and 50 μM 2-ME. After the fourth passage, no CD45+ BM-
derived lineage cells were identified by cytofluorometric staining (data not shown), and cells displayed a fibroblast morphology (21).

Ex vivo conversion of LTA4 to LTβ4 by FLSs

After the growth medium was removed, FLSs were cultured in HBSS with
1% BSA (Sigma-Aldrich, St. Louis, MO). LTA4 hydrolyzed from LTA4
methyl ester (Cayman Chemical) per the manufacturer’s protocol was
added to the cell cultures (8 × 104 cells per milliliter) and incubated for 20

BM transplantation into irradiated recipient mice was performed as described previously (27). Briefly, BM cells were flushed from donor tibia and femur bones with RPMI 1640 (Invitrogen, Grand Island, NY) containing 5% FCS, and marrow plugs were disrupted by trituration to generate a unicellular suspension. The entire BM preparation from one donor was resuspended in 0.5 ml 1× PBS. For transplantation, recipient mice were irradiated with a split dose (500 and 450 cGy) of gamma irradiation, and cells displayed a fibroblast morphology (21).

Western blotting

FLSs from WT, 5-LO−/−, or LTβ−/− mice were harvested and lysed in
lysis buffer containing 50 mM Tris-HCl (pH 7.2), 275 mM NaCl, 55 mM
KCl, 1.0 mM CaCl2, 1% Triton X-100, and 0.5% Nonidet P-40 with
protease inhibitor mixture (Sigma-Aldrich), then centrifuged at 12,000
rpm for 30 min (36). Samples then were separated on 10% SDS-PAGE and
probed for LTA4 (Cayman Chemical) as described.

Isolation of BM neutrophils

Isolation of mature neutrophils from WT B6, 5-LO−/−, or LTβ−/− mice
BM was performed as described previously (27). Briefly, BM was flushed
from donor tibia and femur bones with HBSS (Invitrogen), and marrow
plugs were disrupted by trituration to generate a unicellular suspension.
The entire BM preparation was resuspended at a concentration of 5 × 109
cells per milliliter in HBSS. Mature neutrophils were separated via cen-
trification over a discontinuous Percoll gradient at 500 × g for 30 min at
room temperature. The three-step discontinuous Percoll gradient consisted
of 55, 65, and 75% (v/v) Percoll (Amersham Biosciences, Piscataway, NJ)
in PBS. Mature neutrophils were recovered at the interface of the 65 and
75% fractions. Neutrophil purity (>97%) was determined morphometri-
cally by Diff-Quick staining (IMEB, San Marcos, CA) and by cytofluor-
ometric expression of Gr-1 (27).

Coculture of FLSs and PMNs

After FLSs (8 × 104 cells per milliliter) were cultured for 12 h in DMEM/1%
FCS, the media was aspirated and replaced with HBSS with CaCl2 (2 mM)
and MgCl2 (0.5 mM). The cells then were stimulated with A23187 (1 μM) or
either alone or in the presence of neutrophils (8 × 106 cells per milliliter) for
30 min at 37ºC, 5% CO2. Cell supernatants were collected. The concen-
tration of LTβ in the cell supernatants was determined as described above.

65% fractions. Neutrophil purity (>97%) was determined morphometri-
cally by Diff-Quick staining (IMEB, San Marcos, CA) and by cytofluor-
ometric expression of Gr-1 (27).
Immunoﬂuorescence staining

FLSs were grown on coverslips in DMEM/10% FCS for 24–48 h. After 12 h of incubation in DMEM/1% FCS, cells were stimulated by LTB4 at the indicated concentration at 37°C. PGDF was used as a positive control. Thirty minutes after stimulation, the cells were ﬁxed in 2% paraformaldehyde in PBS for 15 min and permeabilized in 0.5% Triton X-100 (Electron Microscopy Sciences, Hatﬁeld, PA) in PHEM buffer (60 mM PIPES, 25 mM HEPES, 10 mM EGTA, and 2 mM MgCl2) for 5 min. Coverslips then were blocked for 1 h in blocking buffer (PHEM buffer supplemented with 5% FBS). After being washed twice with blocking buffer, cells were incubated with Alexa Fluor 488-conjugated phallolidin (1:2000 in blocking buffer; Molecular Probes, Eugene, OR) for 1 h at room temperature. Preparations then were washed three times with blocking buffer, mounted, and analyzed by confocal microscopy (ECLIPSE TE2000-U; Nikon, Melville, NY).

Invasion assays

Invasion assays were performed using 12-well transwell ﬁlters coated with growth-factor-reduced Matrigel (BD Biosciences, San Jose, CA). WT or BLT1−/− FLSs were plated in the top chamber. Cell invasion was quantiﬁed by applying LTB4 or PDGF in the lower chamber and counting the cells on the lower side of the membrane. Invasion values are expressed as the average number of cells per microscopic ﬁeld. Three microscopic ﬁelds per membrane in triplicate experiments were counted. For the pharmacological study, BLT1-speciﬁc antagonist CP-105,696 (37) was provided by Pfizer Pharmaceuticals (New York, NY). WT FLSs were pretreated with 5 μM CP-105,696. Thirty minutes later, LTB4-induced invasion assays were performed in the presence of 5 μM CP-105,696 as described above.

Statistical analysis

Results are presented as the mean ± SEM. The statistical signiﬁcance for comparisons between groups was determined using the Student unpaired two-tailed t test or two-way ANOVA, followed by Bonferroni correction using the Prism software package (version 4.00; GraphPad Software, San Diego, CA). The p values <0.05 were considered signiﬁcant.

Results

Both transcellular and conventional generation of LTB4 are sufﬁcient to drive K/BxN serum-transfer arthritis

We have shown previously that 5-LO expressed by BM-derived cells controls the synthesis of neutrophil-dependent LTB4 that is central to the pathogenesis of K/BxN serum-transfer arthritis (27). To understand how LTB4 regulates synovial tissue function in this model, we tested the concept that LTB4 generated via transcellular metabolism of neutrophil-derived LTA4 contributes to the pathology of joint inﬂammation. We initially generated radiation chimeras in which irradiated (iRAD) 5-LO−/− mice were reconstituted with LTA4H−/− BM (LTA4H−/− → iRAD 5-LO−/−). In these mice, transcellular synthesis comprises the only route of LTB4 generation. More speciﬁcally, because LTA4H is generated by the enzymatic action of 5-LO on AA in BM-derived cells (Ref. 27 and shown as a control in Fig. 1A, green line), the tissues of the chimeric animals (radioresistant cells) were incapable of generating substrate LTA4 because they lacked 5-LO (Supplemental Fig. 1). However, because they express LTA4H, they retain the capacity to synthesize LTB4 from LTA4 supplied by inﬁltrating BM-derived neutrophils. Similarly, because they lack LTA4H, BM-derived leukocytes are incapable of generating LTB4. As seen in Fig. 1A, the severity of arthritis observed in the chimeric mice did not differ from that observed in WT controls (red line). We next generated chimeric mice by transplanting irradiated LTA4H−/− mice with WT BM (WT → iRAD LTA4H−/−). Because synovial tissue lacks LTA4H, these mice are incapable of generating LTB4 from transcellular LTA4. These mice demonstrated the same severity of arthritis as the LTA4H−/− → iRAD 5-LO−/− chimeras (Fig. 1A, blue line), indicating that both conventional and transcellular routes of LTB4 generation are sufﬁcient to drive an equivalent degree of clinical and histological synovial inﬂammation.

Transcellular LTB4 is a major contributor to synovial pannus-mediated cartilage erosion

To determine whether comparable levels of tissue LTB4 were produced by transcellular metabolism in these BM chimeras, we assayed the concentration of LTB4 in their joint tissues (Fig. 1B). In LTA4H−/− → iRAD 5-LO−/− chimeric mice, where transcellular metabolism comprised the only route of LTB4 generation, we observe 55.3% of WT LTB4 (Fig. 1B, red bar). Histological examination revealed equivalent increases in tissue inﬂammation, tissue neutrophil inﬁltration, synovial hyperplasia, and erosion activity in WT → iRAD WT, WT → iRAD LTA4H+/−, and LTA4H−/− → iRAD 5-LO−/− groups (Fig. 1C, 1E). These results indicate that the amounts of synovial LTB4 generated in WT mice are well in excess of that needed to propel the observed clinical and histological damage. Consistent with this observation, the numbers of Gr-1+ neutrophils that inﬁltrate arthritic joint tissues from LTA4H−/− → iRAD 5-LO−/− mice (Fig. 1D, 1F) were equivalent to WT → iRAD WT mice and to WT → iRAD LTA4H+/− mice, indicating that transcellular LTB4 biosynthesis is sufﬁcient to drive maximal neutrophil recruitment to the inﬂamed synovium.

FLSs convert exogenous LTA4 to LTB4

Having identiﬁed that transcellular biosynthesis of LTB4 by radioresistant cells in joint tissue is sufﬁcient to promote inﬂammatory arthritis in vivo, because FLSs are the major cell lineage in synovial tissue, we focused on their ability to accomplish transcellular LTB4 biosynthesis. Initially, we conﬁrmed that FLSs express LTA4H but not 5-LO (Fig. 2A, Supplemental Fig. 1). We then showed that FLSs can generate substantial amounts of LTB4 from exogenous substrate LTA4 in a concentration-dependent manner (Fig. 2B). The role of FLS LTA4H was veriﬁed by employing FLSs from LTA4H−/− mice that were shown to be incapable of generating LTB4 (Fig. 2C).

FLSs convert neutrophil-derived LTA4 to LTB4

Our previous studies revealed that neutrophils can supply 5-LO activity sufﬁcient to promote arthritis in the K/BxN serum-transfer model (27). Further, neutrophils comprise a major population inﬁltrating the arthritic joint in both K/BxN arthritis and in human rheumatoid arthritis. In addition, it is well established that neutrophils can generate and release LTA4 in substantial excess to their capacity to form LTB4 (14). Therefore, to gain insight into the physiology of synovial conversion of LTA4 to LTB4, we examined whether interacting neutrophils and FLSs are competent to generate signiﬁcant amounts of LTB4. As anticipated, monocultured neutrophils stimulated with a calcium ionophore generated substantial LTB4, whereas FLSs, which lack 5-LO expression, generate no LTB4 (Fig. 3A). However, in neutrophil/FLS coculture, LTB4 biosynthesis substantially exceeded that observed with neutrophils alone (34 ± 2.6 pg/ml versus 18 ± 2.0 pg/ml, p < 0.05), which is consistent with FLS conversion of neutrophil-derived LTA4 to LTB4. That neutrophils are an obligate source of LTA4 was conﬁrmed in coculture experiments using 5-LO−/− neutrophils where no LTB4 was generated (Fig. 3B). To demonstrate that LTB4 production resulted from transcellular movement of LTA4 from neutrophils to FLSs, we coincubated FLSs with LTA4H−/− neutrophils, which then were stimulated with ionophore. FLSs, which provide the only source of LTA4H in this system,
synthesized LTB₄ with the same efficiency as WT neutrophils (Fig. 3C).

**FLS production of LTB₄ is stimulated by TNF**

Aside from its self-limiting property via suicide inactivation (15, 38), little is known about the molecular regulation of LTA₄H epoxide hydrolase activity. Most current models assume that LTA₄H activity is regulated simply by enzyme quantity and the amount of substrate LTA₄ available (12, 39), however, most studies of LTB₄ production have been performed in myeloid lineage cells. Studies in fibroblasts have used transformed cells and found that increased LTB₄ production correlated with elevated LTA₄H mRNA and protein levels (40). We investigated whether primary FLS production of LTB₄ from exogenous LTA₄ could be modulated by
inflammatory stimuli. Because TNF plays a significant role in disease pathophysiology in rheumatoid arthritis and in the K/BxN model of arthritis (41, 42), we exposed FLSs to TNF (10 ng/ml) for 2 h prior to supplying exogenous LTA4 and observed a significant (∼3-fold) increase in concomitant LTB4 in the supernatant of these stimulated FLSs (Fig. 4).

To gain further insight into the molecular basis for the observed increase in LTB4 synthetic capacity in FLSs, we assessed induction of LTA4H protein expression over time in response to TNF stimulation (Supplemental Fig. 2). Interestingly, FLSs treated with TNF for up to 2 h did not show a change in immunoreactive LTA4H protein levels. Because previous studies document that increased mRNA levels correlate with increased LTA4H expression, we assessed FLS LTA4H protein levels in FLSs exposed to transcription inhibitor actinomycin D (5 μg/ml) or RNA polymerase II inhibitor 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (50 μM). As shown in Supplemental Fig. 2, no change in expression of LTA4H was apparent in FLSs whose transcriptional capacity was inhibited, confirming that the stimulation-induced LTB4 synthetic capacity in FLSs proceeds in a manner independent of modulation of LTA4H enzyme levels.

BLT1 expression on radioresistant cells contributes to arthritis severity and pannus tissue invasion into cartilage

Having observed tissue capacity to generate LTB4 in a transcellular fashion, we next investigated a role for LTB4 in modulating tissue behavior in inflammatory arthritis. To establish an impact from LTB4 on tissue behavior in vivo, we transferred WT BM into irradiated mice lacking BLT1 to generate radiation chimeric mice wherein BLT1 remains absent in radioresistant (tissue) cells. In these mice, we find a partial amelioration of clinical signs of arthritis (Fig. 5A). Of particular interest, examination of joint tissues from mice lacking tissue BLT1 expression reveals a striking decrease in synovial pannus erosion into cartilage, whereas other measurements of tissue inflammation and bone erosion were only partially impacted (Fig. 5B). Because pannus erosion into cartilage is thought to be predominantly driven by behavior of FLSs, these in vivo results correlate nicely with our in vitro demonstration that LTB4 directly modifies behavior of this mesenchymal lineage.

LTB4 promotes synovial fibroblast migratory and invasive behavior

Because the synovial pannus is comprised predominantly of synovial fibroblasts (12, 22) and the tissue extension observed in...
pannus erosion into articular cartilage is thought to implicitly require the ability of FLSs to migrate and invade through extracellular matrix to accomplish tissue remodeling in synovial pannus. We then proceeded with direct examination of the capacity for LTB4 to impact these aspects of synovial fibroblast behavior. After confirming their expression of BLT1 mRNA (Supplemental Fig. 3), we proceeded with functional studies assessing LTB4-stimulated activities in primary FLSs. In contrast to measurements of cytokine (IL-6) production or proliferation, where we observe no impact from administration of LTB4 (Supplemental Figs. 4, 5), we find that LTB4 promotes lamellipodium formation to a degree equivalent to that seen with PDGF, a potent activator of FLSs (21) (Fig. 6A, 6B). We then assessed capacity for LTB4 to promote FLS invasive activity in extracellular matrix-coated transwells and observed a dose-dependent stimulation of FLS invasion again to a degree comparable to that driven by PDGF (Fig. 6C). To confirm the direct activity of LTB4 on primary FLSs, we examined the invasiveness of BLT1−/− and WT FLSs and observe lack of LTB4-driven FLS invasive activity in BLT1−/− FLSs (Fig. 6D). These observations were confirmed via pharmacologic inhibition of BLT1 using the BLT1-specific antagonist CP-105,696 (37) (Fig. 6E).

Discussion

In contrast to our detailed understanding of proinflammatory leukocyte effector pathways, there remains a relative paucity of mechanistic insight into how tissues participate in the generation of pathologic inflammatory reactions. Most studies of inflammation have characterized secondary mesenchymal responses to paracrine signals emanating from infiltrating immune cells rather than focusing on the role of mesenchymal tissues per se. Examples of these diverse responses include mesenchymal cell proliferation, rearrangement of extracellular matrix (e.g., fibrosis), and vascular hyperplasia. However, there is growing evidence that tissue elements are not simply passive responders in immune or autoimmune reactions. In the context of synovitis, prominent among the growing list of FLS effector pathways elicited in response to immune-generated signals are elaboration of cytokines (e.g., IL-6) (43–45), chemokines (e.g., IL-8 and MCP-1) (36, 46, 47), and eicosanoids (e.g., PGE2 and PG12) (28, 48, 49). From an amplification standpoint, because their generation can be accomplished via transcellular metabolism of precursor substrates, the eicosanoids provide an ideal context to examine tissue modulation of inflammatory responses. Our observations demonstrate that radioresistant joint tissue and its primary constituent, the mesenchymal synovial fibroblast (FLS), can participate in the pathophysiology of inflammatory arthritis in a primary manner by potently and dynamically metabolizing substrate LTA4 supplied via a transcellular route to generate LTB4. Further, the predominant activity studied for proinflammatory mediators is their impact on BM-derived cells. In this study, we show that the behavior of mesenchymal tissue lineages are impacted directly by LTB4, a mediator typically viewed as a leukocyte chemoattractant. LTB4 promoted tissue migration and invasion—activities central to joint destruction in inflammatory arthritis. Thus, in the context of the complex physiology of autoimmune arthritis, these findings provide an example of a primary inflammation-amplifying capacity for tissue via generation of and response to LTB4, a mediator typically ascribed to leukocytes.

The capacity for FLSs and other cells of tissue lineages to interface with leukocytes in a common effector function provides a basis for local fine-tuning of immune responses. For generation of LTB4 there exist several control points for modulating production. Previous studies, confirmed herein, document that LTA4H is rate-limiting in leukocyte LTB4 production (7–10, 12, 35), because excess LTA4 can diffuse from cells for transcellular metabolism. LTA4H undergoes “suicide inactivation” as a consequence of catalyzing the formation of LTB4 (12, 15), further limiting leukocyte capacity for sustained LTB4 generation. Because stimulated neutrophils secrete abundant LTA4 into their local milieu, the capacity for tissue to act as a synthetic reservoir thereby dramatically extends this leukocyte activity—to the point of profound tissue inflammation in the absence of leukocyte LTA4H in our experiments. Interestingly, FLS LTA4H activity can be upregulated by TNF and likely other inflammatory cytokines. This property affords an opportunity for regulated amplification of the highly constrained leukocyte production of LTB4 via dynamic local tissue mechanisms.
The precise mechanism whereby modulation of FLS generation of LTB4 by external stimuli is regulated remains to be elucidated; it is noteworthy that changes in the levels of LTA4H and therefore de novo synthesis of LTA4H are not involved. This contrasts with other mechanisms governing other examples of stimulation-dependent transcellular eicosanoid generation, such as IL-1β-induced overexpression of cyclooxygenase 2 that modulates transcellular biosynthesis of thromboxane A2 between HUVECs and platelets (50). These observations point to as-yet-unappreciated mechanisms regulating LTB4 production in FLSs. Included among the potential mechanisms contributing to this phenotype are regulation of LTA4 substrate accessibility, modulation of LTA4H enzymatic activity, or alteration of LTB4 release from FLSs.

Of equal importance to the observation that radioresistant tissues amplify LTB4 generation is the finding that the behavior of inflamed parenchymal synovial tissue can be modified by LTB4. In the context of inflammatory arthritis, the synovium undergoes profound changes driven by mesenchymal lineage FLSs via mechanisms that remain poorly understood (51–54). The development of synovial pannus, which is primarily responsible for cartilage tissue invasion and which participates in tissue invasion into bone, is a predominant FLS-dependent pathophysiologic event in disease. Our results underscore the regulated nature of the behavior of joint tissues in response to inflammation and provide new molecular insight by identifying LTB4 as a mediator that promotes the behavior of FLSs in these processes. In the interpretation of our results, it is noteworthy that our studies focus on activities elicited by LTB4 stimulation of BLT1. There exists a second known receptor for LTB4 named BLT2. Xu et al. (55) recently reported a predominance of BLT2 over BLT1 mRNA expression in synovium from patients with rheumatoid arthritis. This observation raises the possibility of further LTB4 function elicited in FLSs distinct from the functions that we identify attributable to BLT1 but does not impact on our conclusions. Although further examination of pathways stimulated in FLSs by LTB4 exposure remains warranted, our in vivo data demonstrate that tissue-specific responses to LTB4 influence both the degree of inflammation and the organized pathologic response of the synovial pannus.

Our studies provide a novel approach for understanding how tissues participate locally in autoimmune inflammation pathophysiology via transcellular generation of LTB4. It is distinct from...
and expands upon the conclusions of previous studies describing the production of eicosanoids by transcellular metabolism in vitro and in vivo. Fitzpatrick et al. (56, 57) reported that LTA4 could be transferred into RBCs and converted to LTB4 by LT4H present in the cytosol of RBCs. Thereafter, transcellular generation of LT4B was demonstrated in a number of cell combinations in vitro (13–18, 58–61). In vivo transcellular biosynthesis of LT4B or LTC4H in response to zymosan injection or to exogenous administration of substrate LTA4H has also been shown (19, 20).

Human inflammatory arthritides, such as rheumatoid arthritis, psoriatic arthritis, gouty arthritis, and others, are characterized by dramatic elevations of neutrophils in synovial fluid and leukocytic tissue infiltration with neutrophil turnover rates estimated at a billion cells per day in a single joint (62–64). Further, LT4B is elevated markedly in the synovial fluid of patients with rheumatoid arthritis (63). Previous studies by our group have documented a role for neutrophil-derived 5-LO activity as a synthetic source for LT4B that promotes synovitis in the K/BxN model (27). Our studies now reveal that LT4B production is substantially more dynamic than previously realized. Consideration of therapeutic inhibition of LT4H will need to account for activity beyond that present in circulating leukocytes. Our findings also suggest that the therapeutically effective blockade of other mediators, such as TNF, may function in part by impacting joint tissue generation of LT4H.

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Disclosures

M.C. is now employed by Pfizer, Inc. D.M.L. is now employed by Novartis Pharma AG. The other authors have no financial conflicts of interest.

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