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Targeted Disruption of Leukotriene B4 Receptors BLT1 and BLT2: A Critical Role for BLT1 in Collagen-Induced Arthritis in Mice

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Leukotriene B4 mediates diverse inflammatory diseases through the G protein-coupled receptors BLT1 and BLT2. In this study, we developed mice deficient in BLT1 and BLT2 by simultaneous targeted disruption of these genes. The BLT1/BLT2 double-deficient mice developed normally and peritoneal exudate cells showed no detectable responses to leukotriene B4 confirming the deletion of the BLT1/BLT2 locus. In a model of collagen-induced arthritis on the C57BL/6 background, the BLT1/BLT2−/− as well as the previously described BLT1−/− animals showed complete protection from disease development. The disease severity correlated well with histopathology, including loss of joint architecture, inflammatory cell infiltration, fibrosis, pannus formation, and bone erosion in joints of BLT1/BLT2−/− animals and a total absence of disease pathology in leukotriene receptor-deficient mice. Despite these differences, all immunized BLT1−/− and BLT1/BLT2−/− animals had similar serum levels of anti-collagen Abs relative to BLT1/BLT2−/− animals. Thus, BLT1 may be a useful target for therapies directed at treating inflammation associated with arthritis. The Journal of Immunology, 2006, 176: 6254–6261.

Rheumatoid arthritis (RA) is a chronic inflammatory disease involving multiple joints and remains an autoimmune disease of unknown etiology (1). Collagen-induced arthritis (CIA) is a model for RA that is induced in susceptible mouse strains by intradermal immunization with collagen type II (CII) emulsified in a complete adjuvant (2, 3). The significance of this model is that CII is the major constituent protein of cartilage in diarthrodial joints, the predominant site of inflammation in RA. In addition, the pathogenesis of CIA is in many ways similar to that of RA as both RA and CIA are characterized by an intense synovitis accompanied by erosions of cartilage and subchondral bone by a pannus-like tissue (4). Susceptibility to CIA is considered to be MHC class-linked (H-2q and H-2r) as only DBA/1 (H-2q) and B10.RIII (H-2r) mice, among the most commonly used strains, are susceptible to CIA (5). However, recently Campbell et al. (6, 7) modified the immunization procedure and showed that clinically and histologically similar CIA may be induced in C57BL/6 (B6) mice. In addition to serving as a valuable tool to study immunity to CII, the CIA model has proven equally useful to investigate inflammatory joint injury and led to the development of novel TNF-based therapies for human RA (8, 9).

Leukotriene B4 (LTB4; cis-8,10-trans-eicosatetraenoic acid) is one of the most potent chemoattractants of leukocytes (10). LTB4 promotes inflammation by stimulating CD11b up-regulation and adhesion of leukocytes, emigration of leukocytes from the bloodstream, neutrophil activation leading to respiratory burst, degranulation, and release of enzymes (10). In addition, LTB4 can alter transcriptional profiles resulting in proinflammatory amplification circuits (11, 12). These processes have been implicated in the pathogenesis of a variety of diseases such as atherosclerosis, asthma, allergic encephalomyelitis, psoriasis, and inflammatory bowel disease (13, 14). A role for LTB4 in RA was suggested by several observations over the past two decades. LTB4 levels in synovial fluids from patients with active RA were 5-fold higher relative to synovial fluids from osteoarthritis (15, 16). Neutrophils from RA patients undergoing methotrexate therapy displayed both acute and chronic suppression of LTB4 synthesis ex vivo (17). LTB4 receptor antagonists were found to inhibit CIA in mice (18, 19). Mice deficient in 5-lipoygenase-activating protein (FLAP), and as a consequence in LTB4 synthesis, were partially protected from developing CIA (20).

Two distinct G protein-coupled receptors, BLT1 and BLT2, likely mediate the effects of LTB4 in different cell types (21, 22). BLT1 is a high-affinity receptor expressed in a variety of leukocytes including neutrophils, monocyte/macrophages, eosinophils, mast cells, and activated T lymphocytes. BLT2 is a low-affinity LTB4 receptor more widely expressed in human tissues. Of interest, high levels of BLT2 mRNA expression were observed in actively inflamed synovial tissue from patients with RA where as leukocytes infiltrating synovial fluid predominantly expressed BLT1 mRNA in these patients (23). Previously described BLT1−/− mice allowed the determination of a critical role for BLT1 in atherosclerosis and airway hyperresponsiveness (11, 24, 25). However, the physiological role of BLT2 is unknown. The genes for BLT1 and BLT2 are adjacent to each other in both mouse and human genomes and are separated by only 4 kb of intergenic region. Moreover, the promoter of the BLT1 gene is within the coding region of BLT2 (26).

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3 Abbreviations used in this paper: RA, rheumatoid arthritis; CIA, collagen-induced arthritis; CII, collagen type II; LTB4, leukotriene B4; 5-LO, 5-lipoxygenase; FLAP, 5-lipoygenase-activating protein; ES, embryonic stem; m, murine; CIDE-B, cell death-inducing DFF45-like effector B; ORF, open reading frame; PAF, platelet-activating factor; COX2, cyclooxygenase 2; WT, wild type.
Because crossing of single knockout animals cannot be used to create a double knockout of BLT1 and BLT2, we have generated BLT1/BLT2 double-deficient mice by directly targeting both receptors. In this study, we tested the BLT1−/− and the BLT1/BLT2−/− mice in the CIA model. The results showed that disruption of BLT1 alone is sufficient to offer complete protection of mice from developing arthritis, whereas anti-CII Ab levels in BLT1−/− and BLT1/BLT2−/− were similar to the BLT1/BLT2+/+ mice.

Materials and Methods
Targeting construct and generation of BLT1/BLT2 double-deficient mice
The previously described BLT1 targeting construct was modified to target both BLT1 and BLT2 genes (27). The BAC clone containing the mouse BLT1 and BLT2 genes was mapped by restriction analysis, and a 4.5-kb fragment 10 aa upstream of the BLT2 coding region was subcloned into the NotI-XhoI sites of the pBluescript vector. The EGFP expression cassette was PCR amplified from pEGFP-N2 (BD Clontech) and inserted in-frame after the first 10 aa of the BLT2 coding region at the XhoI site of the 4.5-kb fragment. This fragment with EGFP included was used to replace the long arm of the previously described targeting construct (27). The resulting final construct pW5GB was 16 kb in length. The mock construct which served as a positive control for PCR screening of the embryonic stem (ES) cell clones was the same as previously described (27). AK7 (129S4/SvEvToj) ES cells (107) were electroporated with 25 µg of NotI-linearized W5GB DNA. The resulting ES transfectants were grown in DMEM with 200 µg/ml G418 and 2 × 10−4 M ganciclovir for 10 days. Surviving clones were tested for recombination using a neomycin-encoding sequence primer—tggcagcatcattcttgag—and a primer from the 3′ end of BLT1 gene external to the knockout construct—gtctgggtgctataacagctcagcctct. Of the eight clones, four were expanded and the genotypes were confirmed by Southern blot analysis with 10–20 µg of DNA using the 0.9-kb probe (BglII/BamHII fragment) external to the 3′ end of the knockout construct. Two undifferen-
tiated clones were individually microinjected into C57BL/6 blastocysts and transferred into pseudopregnant C57BL/6 mice. Chimeric mice generated from two individual cell clones resulted in immediate germline transmission and the F1 (C57BL/6 and 129 SvJ) offspring were used to establish the mouse colonies. Genotyping was performed using Southern blotting with the same probe indicated above or more routinely using a three primer PCR with the primers 1) atgtctgtctgctaccgtcc, 2) aggtgcagca and 3) tggcagcatcattcttgag. All mice were housed in a specific pathogen-free barrier facility. Mice were 8–12 wk old at the time of use. All studies and procedures were approved by the Animal Care and Use Committee of University of Louisville Research Resources Center.

Generation and analysis of 300.19 cell lines expressing murine BLT1 and BLT2
Murine (m) BLT1 and BLT2 were stably expressed to similar levels in a mouse pre-B cell line, 300.19 (28). Hemagglutinin-tagged mBLT1 or mBLT2 CDNAs (20 µg) in the eukaryotic expression vector pRK-5 were transfected into 300.19 cells by electroporation, selected for G418 resistance, and stained with 12CA5 Ab and cells expressing the hemagglutinin epitope on the surface were sorted by flow cytometry. Cells expressing similar levels of the receptor were analyzed for functional receptor expression. Reverse-transcribed RNA obtained from 3 × 106 cells was washed and loaded with 1.0 µM Indo-1 AM for 30 min at 37°C as previously described (29). Calcium traces were recorded in a Hitachi fluorescence spectrometer (model F-2500) with an excitation wavelength of 355 nm and an emission wavelength of 405 nm. Calcium mobilization in 4-h zymosan A-elicited peritoneal lavage cells containing over 80% neutrophils was also measured essentially by the same procedure.

Northern blot analysis
Mouse neutrophils and macrophages were isolated from the peritoneal lavage after 4 and 72 h of zymosan A treatment, respectively. Total RNA isolation was performed as described according to the manufacturer’s directions (RNeasy; Qiagen). Twelve micrograms of total RNA from mouse spleen, liver, neutrophils, and macrophages were denatured, electrophore-
sed on 1.2% formaldehyde-agarose gel, and transferred to nylon membranes (Hybond-N+; Amersham Biosciences). The membranes were hyb-
dridized with 32P-labeled open reading frame (ORF) of mouse BLT1, BLT2, cell death-inducing DFF45-like effector B (CIDE-B), and β-actin at 42°C overnight in an ULTRAhyb hybridization buffer (Ambion). The membranes were washed in 2× standard saline citrate phosphate/EDTA, 0.1% SDS, followed by washing in 0.1× standard saline citrate phosphate/ EDTA, 0.1% SDS at 55°C for 1 h, and subjected to autoradiography.

Mice used in CIA experiments
The previously described BLT1−/− mice (27) have been backcrossed onto the B6 background for seven generations and the experimental control mice for this group were purchased from The Jackson Laboratory. The BLT1/BLT2−/− mice were backcrossed onto B6 for nine generations. The BLT1/BLT2−/− mice were then intercrossed to generate three groups of BLT1/BLT2−/−, BLT1/BLT2+/−, and BLT1/BLT2+/+ littermate mice. All mice were ≥8 wk of age at the time of experimentation, and were age matched.

Induction and assessment of arthritis
CFA was prepared by mixing 100 mg of heat-killed Mycobacterium tu-
berculosis (H37Ra; Difco Laboratories) in 20 ml of IFA (Sigma-Aldrich) (6). An emulsion was formed by dissolving 2.0 mg/ml chick CII (CII; Sigma-Aldrich) overnight at 4°C in 10 mM acetic acid and combining it with an equal volume of CFA. CII solution and the emulsion with CFA were always freshly prepared. Mice were injected i.d. at the base of the tail with a total of 100 µl of emulsion containing 100 µg of CII and 250 µg of M. tuberculosis. The same injection was repeated at day 21; however, due to toughening of the skin at the base of the tail, booster injections were distal to the primary injection site.

Clinical and histological assessment of arthritis
All mice were examined two to three times per week for the initial visual appearance of arthritis after immunization. Arthritis of each individual limb was graded using the following scoring system: 0, normal; 1, apparent swelling and redness limited to individual digits; 2, swelling in more than one joint; 3, severe redness and swelling of the entire paw including digits; and 4, maximally inflamed limb with involvement of multiple joints. The maximum score per mouse was 16. Mice were scored as arthritic if more than one paw had a score >2. The thickness of the hind paws was mea-
sured using a dial gauge caliper (Mitutoyo). At the end of the experiment, the rear paws and joints were removed, fixed, decalcified, and paraffin embedded. Joint sections (5 µm) were stained with H&E and examined for the histological changes of inflammation, pannus formation, cartilage, and bone damage. Arthritic changes in the ankle were scored as previously described: 0, normal; 1, weak leukocyte infiltration but no erosion; 2, mod-
est infiltration and weak erosion; 3, severe infiltration and invasion of bone; and 4, loss of bone integrity (30).

Determination of serum anti-collagen Ab levels by ELISA
Blood was collected by cardiac puncture. After clotting at room temperature for 1 h, the samples were kept overnight at 4°C and the serum was collected by centrifuging at 13,000 rpm for 5 min. ELISAs for Abs to CII were performed as described in the Arthritis-CIA kit (Chondrex). In brief, precoated plates were washed and incubated with blocking buffer for 2 h at room temperature. Serum at a 1/50,000 dilution was added to each well. Plates were incubated at 4°C overnight, washed six times and incubated with peroxidase-conjugated goat anti-
mouse IgG for 2 h at room temperature, and then washed six times. Per-
oxidase activity was then determined following the addition of ortho-
pheynediamidine chromagen in urea H2O2 buffer for 30 min by determining the OD at 490 nm. Each sample was tested in duplicate and the mean value was recorded.

Immunohistochemistry
Immunohistochemistry was performed on deparaffinized slides using the traditional primary/secondary Ab-peroxidase technique. Briefly, paraffin-
embedded sections were dewaxed and hydrated. Ag was retrieved with Tris/EDTA buffer, and endogenous peroxidase was blocked by incubating with 0.3% H2O2 for 10 min. Sections were then block with 3% BSA and following with primary Ab and corresponding HRP-secondary Ab staining. Color was developed by treatment with 3,3′-diaminobenzidine (Sigma-Aldrich) and sections were counterstained with hematoxylin.

Results
mBLT2 is a functional LTB4 receptor
Although the BLT2 gene has been identified from several sources, the functional activity of BLT2 in primary cells is yet to be demon-
strated. To determine whether the mBLT2 is a functional LTB4,
mBLT1 showing at least 10-fold more sensitivity to LTB4 than BLT2, these cells activated dose-dependent calcium release with tent with the results from cell lines expressing human BLT1 and calcium mobilization in cells expressing mBLT1 or mBLT2. Consis-

receptor, we generated stable 300.19 cell lines expressing this receptor. Fig. 1 shows dose-response profiles of LTB4-induced calcium mobilization in cells expressing mBLT1 or mBLT2. Consistent with the results from cell lines expressing human BLT1 and BLT2, these cells activated dose-dependent calcium release with mBLT1 showing at least 10-fold more sensitivity to LTB4 than mBLT2.

Generation of BLT1/BLT2 double-deficient mice

To delete both BLT1 and BLT2 genes simultaneously, we used a modified targeting vector from the one used to generate BLT1-deficient mice (27). In this vector, the entire region between amino acid no. 10 of BLT2 through amino acid 316 of BLT1 was deleted and replaced with the PGK-neomycin (PGK-Neo) cassette (Fig. 2a). In addition, the sequence encoding an in-frame fusion of the GFP coding region was inserted following codon no. 10 of BLT2. BLT1/BLT2−/− mice were generated essentially following the same protocols described for BLT1−/− mice (27). Fig. 2b shows a Southern blot of BamHI- and XhoI-digested DNA from the three genotypes. The BLT1/BLT2−/+ littermate lanes (+/+ ) show the expected 5.3-kb band and the homozygous lanes (−/−) show the 6.5-kb mutant band. The heterozygous (+/−) lanes show both the wild-type (WT) and mutant bands. BLT1/BLT2−/− mice were born at the expected Mendelian ratios and showed no overt developmental or morphological abnormalities. Three primer PCRs to identify the junction of the BLT2 and GFP fusion in the double-deficient mice (Fig. 2c, left) or the neo gene and BLT1 at the 3′ end (Fig. 2c, right) were routinely used to determine the genotypes from the genomic DNA isolated from tail biopsies.

Analysis of lymphoid tissues found no gross alterations in the size of the thymus, spleen, or lymph nodes between BLT1/BLT2−/− and WT littermates. The number and distribution of CD4+ and CD8+ T lymphocytes, or B220+ B lymphocytes found within the spleen, peripheral lymph nodes, mesenteric lymph nodes, or within the blood were similar in BLT1/BLT2−/− and control animals (data not shown). In addition, no significant differences were found in numbers of circulating lymphocytes, monocytes, neutrophils, or eosinophils or in serum IgG and IgM levels between the BLT1/BLT2−/− and BLT1/BLT2+/+ mice (data not shown).

Analysis of mBLT2 gene expression

To confirm that the mutation disrupted the LTB4 receptor expression and not other chemoattractant receptors, zymosan-elicited peritoneal exudate cells (over 80% neutrophils) were analyzed for calcium mobilization. Both LTB4 and platelet activating factor (PAF)-induced calcium mobilization in cells from littermate BLT1/BLT2+/+ animals (Fig. 3a). In contrast, cells from the BLT1/BLT2−/− animals showed no calcium mobilization in response to LTB4 but equivalent responses to PAF compared with cells from BLT1/BLT2+/+ mice.
Northern blot analysis of RNA from spleen, liver, peritoneal neutrophils, and macrophages showed strong expression of mBLT1 in neutrophils, and relatively lower levels of expression in macrophages in the BLT1/BLT2+/+ mice, but no expression in the BLT1/BLT2−/− mice (Fig. 3b). Data also showed weak BLT1 expression in the spleen of BLT1/BLT2+/+ mice (Fig. 3b). Hybridization with the mBLT2 ORF probe (Fig. 3b) or BLT2 5′-UTR or 3′-UTR data (not shown) failed to show any mBLT2 expression in any of the tissues studied. An overlapping divergently transcribed gene, CIDE-B in this locus showed normal liver-specific expression in both the BLT1/BLT2+/+ and BLT1/BLT2−/− mice (Fig. 3b).

Although BLT2 was expressed in a wide variety of tissues, including liver and spleen in humans, we did not detect any GFP expression in these tissues either in the BLT1/BLT2+/+ or in BLT1/BLT2−/− mice. Although flow cytometry revealed weak GFP expression in platelets, we could not detect any functional activity of BLT2 in these or other cells (data not shown).

BLT1−/− and BLT1/BLT2−/− mice are completely resistant to CIA

Mice of the H-2b (DBA/1J) background are highly susceptible to CIA whereas mice of the H-2b (B6) are resistant. However, modification of the immunization procedure results in high incidence of CIA in B6 background mice (6). In two separate preliminary experiments, using the same method, we successfully induced arthritis in B6 strain mice (data not shown). To determine the role of LTβ3 receptors in arthritis, we set up two different study groups: the BLT1+/− mice were set up together with control B6 (BLT1/BLT2+/+) mice and the littermate offspring from BLT1/BLT2+/− breeders (BLT1/BLT2+/+, BLT1/BLT2−/−, and BLT1/BLT2−/−) constituted a second group. The arthritis symptoms in BLT1/BLT2+/+, BLT1−/−, BLT1+/−, and BLT1/BLT2−/− mice were studied after immunization with CII on day 0 and a boost with CII on day 21. Mice were examined weekly after the first immunization and every 2–3 days after the boost for signs of developing arthritis. The severity of the arthritis was assessed using a visual scoring system standardized under our laboratory experimental conditions (Fig. 4a). The BLT1/BLT2+/+ animals developed clinical signs of arthritis with an incidence of 37% and 60% by 38 and 25 days, respectively (Fig. 4a). Because male and female mice developed arthritis with comparable incidence, only the total number of mice is shown. The average cumulative clinical score and the swelling measured as Δpaw thickness are shown in Fig. 4, d–g. The clinical appearance of the swollen joints, the range of severity, and the progression to severe swelling was similar to that observed in DBA/1 mice. None of the BLT1−/− or BLT1/BLT2−/− developed any signs of arthritic disease as compared with the BLT1+/+ control mice (Fig. 4). The intermediate incidence and severity of arthritis in the BLT1/BLT2+/− mice in this group indicated a possible gene dosage effect in the CIA model (Fig. 4, c, e, and g).

Histological features of immunized BLT1/BLT2+/+, BLT1−/−, and BLT1/BLT2−/− mice

An observer unaware of the genotype of the animals scored the histopathology of hind limb knee joints. The severity of disease as determined by the histological features correlated with the observed visual scores (Figs. 5, a and b, and 6). None of the BLT1−/− and BLT1/BLT2−/− had any evidence of arthritis upon histological examination (Figs. 5, c–f, and 6). Pannus formation, fibrillation of the articular surface, and eventual ankylosis are hallmarks of RA. Mild to moderate pannus and fibrillation of the articular surface were common in the BLT1/BLT2+/+ mice (Fig. 5, g–i). In contrast, none of the BLT1−/− or BLT1/BLT2−/− mice showed any sign of pannus formation or fibrillation of the cartilage (data not shown).

Immune response against CII in BLT1−/− and BLT1/BLT2−/− mice

A high level of anti-CII Ab generation accompanies the development of disease in the CIA model (3). To investigate whether the disease-free incidence of arthritis in BLT1−/− and BLT1/BLT2−/− mice was due to the lack of an Ab response to type II collagen, the anti-CII-specific levels of IgG in the serum were determined at the termination of the CIA experiment. Anti-CII Ab levels were similar in BLT1−/− mice and in BLT1−/− and BLT1/BLT2−/− mice (Fig. 6).

Inflammatory cell infiltration in B6-CIA synovitis

To determine the type and extent of inflammatory cell infiltration occurring during disease development, histopathological examination and immunohistochemical staining of synovial tissues were performed. Fig. 7 shows the typical inflammatory cell infiltration found in BLT1+/+ animals and complete absence of these cells in the BLT1−/− animals. Examination of arthritic tissue sections at
higher magnification showed a predominant neutrophil-based inflammation (Fig. 7b) but also some lymphocytes (arrows) and macrophages (arrowhead). Immunohistochemical analysis with Gr-1 and CD3 Abs confirmed these observations (Fig. 8), whereas the BLT1<sup>−/−</sup> mice showed no detectable immunostaining with any of these markers.

**Discussion**

The deletion of the BLT1/BLT2 genes reported in this study reveals a potential role for these receptors in inflammatory arthritis. The BLT1/BLT2<sup>−/−</sup> mice were viable, developed normally, and displayed no overt behavioral or morphological defects. The number and development of leukocyte subpopulations were normal in BLT1/BLT2<sup>−/−</sup> mice. The Southern and PCR analysis of mice and the demonstration that these mice lacked the expression of a functional LTB<sub>4</sub> receptor in peritoneal lavage cells clearly establishes the generation of a leukotriene receptor double-deficient mouse line.

The studies reported here demonstrate that expression of mBLT2 in cell lines results in a low-affinity receptor that responds to LTB<sub>4</sub>, suggesting that mBLT2 is indeed an LTB<sub>4</sub> receptor. Although BLT2 was identified several years ago, its expression pattern in different tissues remains unclear. Although macrophages and to some extent neutrophils were suggested to express BLT2 (22, 31) and BLT2 mRNA was detected in a wide variety of human tissues, the expression of BLT2 in mouse tissues is unknown. Tager and Luster (32) suggested they could not detect BLT2 expression in mice. Our results are consistent with these findings that BLT2 mRNA was not detectable in murine tissues by Northern blot analysis. An examination of relative abundance of human and murine leukotriene receptor cDNA clones in public expressed sequence tag databases is also consistent with this observation. Although human BLT1 and BLT2 are represented at similar abundance (43 and 41 clones, respectively), the mBLT1 is more frequently identified than mBLT2 (22 vs 3 clones). These observations suggest that while the mBLT2 gene is indeed expressed, the level of transcription of mBLT2 gene is very weak relative to human BLT2. Based on the design of the construct, we expected the GFP expression to represent the native BLT2 expression pattern. Although the absence of GFP expression in most mouse tissues in the knockout/knockin mouse generated here may not represent the true expression pattern of BLT2, it is consistent with the failure to detect any BLT2 mRNA. Further biochemical as well as functional
studies with BLT2 single-deficient mice are needed to precisely define the mBLT2 function.

CIA has been the most widely used model for studying the pathogenesis of human RA and for screening novel therapeutic compounds. Although B6 mice are known to be resistant to CIA, it was the secondary but not the primary immune response to collagen that is defective in these mice (33). Campbell et al. (6) first reported that CIA could be induced in B6 at an incidence approaching that of congenic DBA/1 mice using an altered immunization protocol. Following the same procedure, with increasing the CFA concentration and administering both the primary and boost injection i.d., we could induce 40–70% incidence of arthritis in B6 mice. It should be noted that this level of incidence is relatively low compared with 70–100% incidence routinely observed for CIA in the DBA strain. However, the severity scores reported here are comparable to most studies on arthritis in DBA mice (34). These data suggest that the method of immunization but not the MHC haplotype might be a critical determinant of CIA incidence in B6 mice. The precise mechanisms whereby immunization with CII leads to a chronic arthritis are not known; however, data have shown that the CIA model is absolutely dependent on B cells and is significantly dependent on CD4+ T cell involvement (35–38).

Our results of comparable levels of anti-CII Ab indicate the normal functioning of B cells in the BLT1/BLT2+/− and BLT1/BLT2−/− mice. It should also be noted that levels of anti-CII Abs in the WT animals also did not correlate with the disease incidence i.e., most animals had similar levels of anti CII Ab but only some (40–60%) did get the disease. This may be related to an activation event that requires BLT1. In this regard, the function of LTB4 as a chemoattractant for activated CD4+ and CD8+ T cells expressing BLT1 and CD11b+ macrophages, fibroblasts, synoviocytes, and endothelial cells. Although the interplay between these cell types and the specific site of BLT1 activity remains to be established, lack of T lymphocytes in synovial cavity of the immunized BLT1 mice suggests an early role of BLT1 in disease development. Moreover, complete absence of neutrophil recruitment in BLT1−/− mice, and the known activity of BLT1 in neutrophils suggest that BLT1 could also play a direct role in neutrophil recruitment to the arthritic joints.

Multiple studies over the past two decades have suggested a role for arachidonic acid-derived lipid mediators in human RA (41, 42). Moreover, mice deficient in cyclooxygenase 2 (COX2), cytosolic phospholipase A2, and FLAP are all protected from CIA (20, 43–45). Although COX2 inhibitors were in extensive use for human RA treatment until recently, cardiovascular side effects led to a drastic reduction in use of these compounds (46, 47). Leukotrienes were recently demonstrated to be effective promoters of atherosclerosis (14). Inhibition of COX2 could result in increased arachidonic acid production, a substrate for 5-LO pathway leading to increased leukotriene generation that might account for the observed side effects of COX2 treatment. Thus, leukotrienes could offer an alternate target for treating inflammatory arthritis.

The current results of complete inhibition of CIA in BLT1-deficient B6 mice need to be considered in the context of several earlier studies using LTβRI synthesis inhibitors or receptor antagonists in mice and in human RA clinical trials. First, several studies have shown that antagonists of BLT1 offer significant but not complete reduction of CIA development in mice (18, 19). In addition, in a model of IL-18 enhanced CIA in the DBA strain, a role for LTβRI was established and inhibition of CIA induction by MK-886, a FLAP inhibitor, was observed (48). Moreover, in a DBA model of CIA partial protection from arthritis was observed in FLAP-deficient mice (20). The partial protection observed with
receptor antagonists as well other inhibitors and mouse models may be related to the incomplete targeting of the BLT1 and the differences in the DBA vs B6 model.

Several leukotriene antagonists and biosynthesis inhibitors have been approved in clinical trials for human RA, but none have been approved for the treatment of RA (49, 50). Although the elements of inflammatory response are similar, murine CIA has limitations in modeling human arthritis, including the absence of rheumatoid factor as well as lack of clear mechanisms for the initiation of disease in humans (5). There are several potential explanations for the clinical failure of past leukotriene-based drugs for treatment of arthritis. Because all of the leukotriene antagonists are selected based on assays of neutrophil function, they may not have complete blocking activity on other cell types such as T cells. Another reason might be related to genetic variation in human populations. Although most of the inbred mouse models have shown strong leukotriene effects in CIA models, only subsets of human RA patients might benefit from the leukotriene antagonist-based therapies. Although the current study has shown complete protection against CIA in BLT1-deficient mice, the known expression pattern of BLT2 in human synovial tissues (23) suggests another potential difference in leukotriene involvement in human RA vs murine CIA. Despite these limitations, studies on RA patients might benefit from reinvestigation of dose, efficacy, and pharmacokinetics of the previously tested compounds as well as direct examination of the involvement of this pathway in human RA.

In summary, this study has described the generation of BLT1/BLT2−/− mice and defined a critical role for BLT1 in CIA. Because the loss of BLT1 alone is sufficient to offer complete protection against CIA, a role for BLT2 in this model could not be established. However, in a number of other models, including atherosclerosis and asthma, loss of BLT1 only offers partial protection or delays the progression of the disease (11, 25). The BLT1/BLT2−/− mice will be a valuable resource in further studies in these and other models of inflammatory diseases.

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FIGURE 7. Inflammatory cell infiltration in the joint sections. a. Inflammatory cells infiltration in the synovial cavity in the joints of the WT mouse but not the BLT1−/− joint. b. Higher magnification (×100 original) showed the majority of the infiltration is neutrophils (right), with occasional lymphocytes (arrows) and macrophages (arrowhead) among the neutrophils (middle and left).

FIGURE 8. Immunohistochemistry staining of inflammatory cells in joint sections. Sections of CIA synovial tissues were stained with rat anti-mouse neutrophils Ab and rat anti-human CD3, which cross-reacts with mouse CD3. Correspondent isotype control was included as indicated. All sections were counterstained with H&E. All images shown are ×400 original magnification.

Disclosures

The authors have no financial conflict of interest.

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9. Williams, R. O., M. Feldmann, and R. N. Maini. 1992. Anti-tumor necrosis factor factor as well as lack of clear mechanisms for the initiation of disease in humans (5). There are several potential explanations for the clinical failure of past leukotriene-based drugs for treatment of arthritis. Because all of the leukotriene antagonists are selected based on assays of neutrophil function, they may not have complete blocking activity on other cell types such as T cells. Another reason might be related to genetic variation in human populations. Although most of the inbred mouse models have shown strong leukotriene effects in CIA models, only subsets of human RA patients might benefit from the leukotriene antagonist-based therapies. Although the current study has shown complete protection against CIA in BLT1-deficient mice, the known expression pattern of BLT2 in human synovial tissues (23) suggests another potential difference in leukotriene involvement in human RA vs murine CIA. Despite these limitations, studies on RA patients might benefit from reinvestigation of dose, efficacy, and pharmacokinetics of the previously tested compounds as well as direct examination of the involvement of this pathway in human RA.

In summary, this study has described the generation of BLT1/BLT2−/− mice and defined a critical role for BLT1 in CIA. Because the loss of BLT1 alone is sufficient to offer complete protection against CIA, a role for BLT2 in this model could not be established. However, in a number of other models, including atherosclerosis and asthma, loss of BLT1 only offers partial protection or delays the progression of the disease (11, 25). The BLT1/BLT2−/− mice will be a valuable resource in further studies in these and other models of inflammatory diseases.

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