Targeted Disruption of Leukotriene B$_4$ Receptors BLT1 and BLT2: A Critical Role for BLT1 in Collagen-Induced Arthritis in Mice

Wen-Hai Shao, Annalisa Del Prete, Cheryl B. Bock and Bodduluri Haribabu

*J Immunol* 2006; 176:6254-6261; doi: 10.4049/jimmunol.176.10.6254
http://www.jimmunol.org/content/176/10/6254

References

This article cites 50 articles, 20 of which you can access for free at: http://www.jimmunol.org/content/176/10/6254.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Targeted Disruption of Leukotriene B₄ Receptors BLT1 and BLT2: A Critical Role for BLT1 in Collagen-Induced Arthritis in Mice

Wen-Hai Shao,*† Annalisa Del Prete,*‡ Cheryl B. Bock,§ and Bodduluri Haribabu2*†

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.
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 provide 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-HindIII 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 pWSGB 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 (129Sv/EvSor) ES cells (107) were electroporated with 25 μg of NotI-linearized WSGB DNA. The ES cells were grown in DMEM with 200 μg/ml G418 and 2 × 10−3 M ganclovin for 10 days. Surviving clones were tested for recombination using a neomycin-encoding sequence primer—tgcagctcgaccaggatggg. All mice were housed in a specific pathogen-free barrier facility. Mice were 8–12 wk old at the time of use.

Calcium mobilization
For calcium mobilization, 3 × 106 cells were 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-elicted 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, electrophoresed on 1.2% formaldehyde-agarose gel, and transferred to nylon membranes (Hybond-N+; Amersham Biosciences). The membranes were hybridized 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 tuberculosis (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. CIA 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 measured 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, modest infiltration and weak erosion; 3, severe infiltration and invasion of bones; 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 Arthrogen-CIA kit manual (Arthrogen-CIA kit; Chondrex). In brief, precoated plates were washed and incubated with blocking buffer for 2 h at room temperature. Serum at a 1/37,500 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 washed six times. Peroxidase activity was then determined following the addition of ortho-phenylenediamine 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 bloomed 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 demonstrated. To determine whether the mBLT2 is a functional LTB4,

The Journal of Immunology 6255

by guest on April 13, 2017 http://www.jimmunol.org/ Downloaded from
mBLT1 showing at least 10-fold more sensitivity to LTB4 than BLT2, these cells activated dose-dependent calcium release consistent with the results from cell lines expressing human BLT1 and calcium mobilization in cells expressing mBLT1 or mBLT2. Consistently, the same in all cases tested.

FIGURE 1. mBLT2 is a functional LTB$_4$ receptor. The 300.19 cells expressing the mBLT1 or mBLT2 were loaded with Indo-1 and release of intracellular calcium was measured as described in Materials and Methods. Response to increasing concentrations of LTB$_4$ was monitored in real time. Dose-response curves of percent calcium release for mBLT1 and mBLT2 were shown. Data represent average measurements from three different calcium traces for each concentration.

receptor, we generated stable 300.19 cell lines expressing this receptor. Fig. 1 shows dose-response profiles of LTB$_4$-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 LTB$_4$ than mBLT2.

FIGURE 2. Targeted disruption of mouse BLT1/BLT2. a, Genomic locus of BLT1 and BLT2, targeting vector, and the recombinant mutant genomic locus. Coding region of the BLT1 and BLT2 gene is indicated as solid boxes. Six kilobases of the coding region and the untranslated region between BLT1 and BLT2 ORF was replaced with PGK-neo cassette in the targeting vector. An enhanced GFP (EGFP) expression cassette was inserted in-frame 30 bp downstream of BLT2 ORF and upstream of the neo gene. The final construct contained homology arms of 4.5 and 1.5 kb. A BgII-BamHI fragment served as an external probe for Southern blot analysis of genomic DNA from ES cells and mouse tails. b, Southern blotting showing correct targeting and germline transmission of the mutated BLT1/BLT2 gene. Genomic DNA samples prepared from F$_2$ offspring were digested with BamHI and XhoI, separated on 0.75% agarose gels, blotted onto nylon membranes, and hybridized with the $^{32}$P-labeled, 0.9-kb BgII-BamHI fragment. The genotypes of the mice are indicated above the lanes. c, A three-primer PCR was designed to identify the WT, heterozygous, and homozygous mutant alleles at the BLT2/GFP junction (left panel). The previously described three-primer PCR methods used for screening BLT1-deficient mice was also run occasionally to confirm the genotype (right panel). The PCR and Southern blotting methods gave the same in all cases tested.

FIGURE 2. a, Genomic locus of BLT1 and BLT2, targeting vector, and the recombinant mutant genomic locus. Coding region of the BLT1 and BLT2 gene is indicated as solid boxes. Six kilobases of the coding region and the untranslated region between BLT1 and BLT2 ORF was replaced with PGK-neo cassette in the targeting vector. An enhanced GFP (EGFP) expression cassette was inserted in-frame 30 bp downstream of BLT2 ORF and upstream of the neo gene. The final construct contained homology arms of 4.5 and 1.5 kb. A BgII-BamHI fragment served as an external probe for Southern blot analysis of genomic DNA from ES cells and mouse tails. b, Southern blotting showing correct targeting and germline transmission of the mutated BLT1/BLT2 gene. Genomic DNA samples prepared from F$_2$ offspring were digested with BamHI and XhoI, separated on 0.75% agarose gels, blotted onto nylon membranes, and hybridized with the $^{32}$P-labeled, 0.9-kb BgII-BamHI fragment. The genotypes of the mice are indicated above the lanes. c, A three-primer PCR was designed to identify the WT, heterozygous, and homozygous mutant alleles at the BLT2/GFP junction (left panel). The previously described three-primer PCR methods used for screening BLT1-deficient mice was also run occasionally to confirm the genotype (right panel). The PCR and Southern blotting methods gave the same in all cases tested.

mBLT2. 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 LTB$_4$ receptor expression and not other chemoattractant receptors, zymosan-elicited peritoneal exudate cells (over 80% neutrophils) were analyzed for calcium mobilization. Both LTB$_4$ 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 LTB$_4$ but equivalent responses to PAF compared with cells from BLT1/BLT2$^{+/+}$ mice.
FIGURE 3. Expression analysis of mBLT2. a, Calcium mobilization of peritoneal neutrophils from BLT1/BLT2+/+ and BLT1/BLT2−/− mice. Calcium flux was monitored in Indo-1-loaded, zymosan-elicited peritoneal neutrophils stimulated with 100 nM LTβ and 100 nM PAF as indicated. Each tracing represents an analysis of 3 × 10^6 cells from a single mouse with the indicated genotype, and the data shown is representative of at least three each of BLT1/BLT2+/+ or BLT1/BLT2−/− animals. b, RNA blot analysis of mBLT1 and mBLT2. Fifteen micrograms of total RNA was isolated from spleen, liver, peritoneal neutrophils, or macrophages were separated on 1.0% agarose gels and transferred to nylon membranes. The RNA blot was sequentially hybridized with BLT1, BLT2, β-actin, and CIDE-B cDNA probes, and exposed to x-ray film for 3 h to overnight after washing. Bottom, Ethidium bromide-stained agarose gel shows comparable RNA loading in each lane.

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 LTβR 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/BLT2−/−, and BLT1/BLT2+/− mice were studied after immunization with CIA on day 0 and a boost with CIA 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. 4, a and c). 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 affect 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−/− or BLT1/BLT2−/− mice had any evidence of arthritis upon histological examination (Figs. 5, c–f, and 6). Pannus formation, fibillation of the articular surface, and eventual ankylosis are hallmarks of RA. Mild to moderate pannus and fibillation 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 fibillation 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/H11002/H11002/H11002 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→→ 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→→ mice. The Southern and PCR analysis of mice and the demonstration that these mice lacked the expression of a functional LTB4 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 LTB4, suggesting that mBLT2 is indeed an LTB4 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 animal 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 injections 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−/− 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+ T cells and CD8+ T cells expressing BLT1 (24, 39) may play an essential role in the B6-CIA model, because substantial numbers of CD3+ cells were detected in the synovial tissue of the BLT1/BLT2+/- arthritic mice but not in the BLT1−/− mice.

Synovial fluid rich in inflammatory cells, in particular neutrophils, characterizes human RA (40). Neutrophils have been considered a key player in the articular and extra-articular manifestations of the disease. This appears to be the case for the CIA model in B6 mice as well. A variety of cells have been suggested as contributing to the initiation and progression of the immune response in rheumatoid synovium, including neutrophils, T cells, 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 articular 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, 34–45). 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 LTβγ biosynthesis 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 LTβγ 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
leukotriene 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 used 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 (S). 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 (N, 25). The BLT1/BLT2−/− mice will be a valuable resource in further studies in these and other models of inflammatory diseases.

Acknowledgments

We thank Dr. Douglas A. Steeber for advice and critical reading of the manuscript.

References


Disclosures

The authors have no financial conflict of interest.

FIGURE 7. Inflammatory cell infiltration in the joint sections. a, Inflammatory cells infiltration in the synovial cavity in the joints of the WT mice but not the BLT1−/− mouse. b, Higher magnification (×1000 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-human CD4 (green) and rat anti-rat 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.

FIGURE 6260 LEUKOTRIENE RECEPTORS IN CIA