Brief Definitive Report

Targeted Disruption of the Leukotriene B_4 Receptor in Mice Reveals Its Role in Inflammation and Platelet-activating Factor–induced Anaphylaxis

By Bodduluri Haribabu,* Margrith W. Verghese,* Douglas A. Steeber,‡ Dwight D. Sellars,* Cheryl B. Bock,§ and Ralph Snyderman*‡

*From the *Department of Medicine, ‡Department of Immunology, and §Department of Genetics, Duke University Medical Center, Durham, North Carolina 27710*

Abstract

Leukotrienes are derived from arachidonic acid and serve as mediators of inflammation and immediate hypersensitivity. Leukotriene B_4 (LTB_4) and leukotriene C_4 (LTC_4) act through G protein–coupled receptors LTB_4 receptor (BLTR) and Cys-LTR, respectively. To investigate the physiological role of BLTR, we produced mice with a targeted disruption of the BLTR gene. Mice deficient for BLTR ($BLTR^{-/-}$) developed normally and had no apparent hematopoietic abnormalities. Peritoneal neutrophils from $BLTR^{-/-}$ mice displayed normal responses to the inflammatory mediators C5a and platelet-activating factor (PAF) but did not respond to LTB_4 for calcium mobilization or chemotaxis. Additionally, LTB_4 elicited peritoneal neutrophil influx in control but not in $BLTR^{-/-}$ mice. Thus, BLTR is the sole receptor for LTB_4-induced inflammation in mice. Neutrophil influx in a peritonitis model and acute ear inflammation in response to arachidonic acid was significantly reduced in $BLTR^{-/-}$ mice. In mice, intravenous administration of PAF induces immediate lethal anaphylaxis. Surprisingly, female $BLTR^{-/-}$ mice displayed selective survival (6 of 9; $P = 0.002$) relative to male (1 of 11) mice of PAF-induced anaphylaxis. These results demonstrate the role of BLTR in leukotriene-mediated acute inflammation and an unexpected sex-related involvement in PAF-induced anaphylaxis.

Key words: arachidonic acid • neutrophil influx • knock-out • sex-related • chemotaxis

Introduction

Leukotriene B_4 (LTB_4) is a potent chemoattractant for neutrophils, eosinophils, and macrophages and also activates the respiratory burst and granule release from neutrophils (1). Leukotriene C_4 (LTC_4) activates smooth muscles and is a potent bronchoconstrictor (2, 3). G protein–coupled receptors LTB_4 receptor (BLTR) and Cys-LTR mediate the activity of LTB_4 and LTC_4, respectively (4, 5). BLTR is expressed in spleen and on leukocytes, whereas Cys-LTR is expressed mainly in smooth muscle and spleen and on leukocytes and pulmonary macrophages. BLTRs activate the G_i family of G proteins to mediate chemotaxis but use other G proteins as well to mediate cytotoxic activities (4, 6). Leukotrienes play a major role in the pathophysiology of asthma and other pulmonary diseases (7, 8). Several inhibitors of leukotriene biosynthesis and activity have been developed for clinical use (9). Studies on mouse models and antagonists of LTB_4 suggested a role for BLTR in rheumatoid arthritis, skin inflammation, and acute septic peritonitis (10–12). Mice with targeted disruption of 5-lipoxygenase (5-LO), 5-LO activating protein (FLAP), and, more recently, LTA_4 hydrolase allowed the determination of the role of leukotrienes in inflammation and hypersensitivity (13–16). Nonenzymatic formation of LTB_4 receptor agonists have been demonstrated (17), and the nuclear receptor peroxisome proliferator-activated receptor α was shown to mediate several of the effects of LTB_4 (18). Therefore, to determine the in vivo biological functions of BLTR and the potential consequences of chronic BLTR deficiency, we used gene targeting in embryonic stem (ES) cells to disrupt the mouse *BLTR* gene. The results demonstrate that BLTR deficiency blocks cellular responses to LTB_4 and diminishes acute inflammatory responses. The data also disclosed an unexpected role for BLTR in sex-dependent leukotriene effects in mediating hypersensitivity to platelet-activating factor (PAF).

Address correspondence to Bodduluri Haribabu, Dept. of Medicine, Duke University Medical Center, Box 3680, Durham, NC 27710. Phone: 919-684-2280; Fax: 919-684-4390; E-mail: Boddu001@mc.duke.edu

Materials and Methods

Targeting Construct and Generation of BLTR-deficient Mice. The mouse *BLTR* gene was isolated as BAC clones from a genomic library from 129/SvJ strain from Research Genetics using an EST clone for mouse BLTR (19, 20). The BAC clones were mapped by restriction analysis, and a 10-kb BamHI fragment (see Fig. 1 A) was subcloned as two BamHI–SacII fragments into pBluescript. The knockout construct was made in a vector (*pPNT*) containing both *PGK-neo* and *PGK-tk* cassettes (21). A SpeI linker was added to the SacII end of the 3′ BamHI–SacII clone. A 1.5-kb SpeI–KpnI fragment from this clone was subcloned into XbaI–KpnI sites in pPNT vector in between the neo and *tk* cassettes. The 5′ BamHI–XhoI fragment was excised from the pBluescript clone as a NotI–XhoI fragment and cloned into the same sites of the *pPNT* already containing the 3′ SacII (SpeI)–KpnI fragment. The resulting final knockout construct pHB–BLTR was 13 kb in length. A mock construct prepared by inserting the 3′ 4.5-kb SacII–BamHI fragment into the same sites of *pPNT* served as a positive control for PCR screening of the ES cell clones. AK7 (129S4/SvJaeSor) ES cells (10^7) were electroporated with 25 μg of NotI-linearized pHB–BLTR DNA. The transfected cells were grown in DMEM media with 200 μg/ml G418 and 2×10^{-6} M gancyclovir for 10 d. Surviving clones (95) were tested for recombination using a neomycin-coding sequence primer, tcgcagcgcatcgccttctatcg, and a primer from the 3′ end of *BLTR* gene external to the knockout construct, gctgggagtcatcaacaagcactc. Of the 25 positive clones, 15 were expanded, and the genotypes were confirmed by Southern blot analysis with 10–20 μg of DNA using the 0.9-kb probe (BglII–BamHI fragment) external to the 3′ end of the knockout construct (see Fig. 1 A). Six undifferentiated clones were individually microinjected into C57BL/6J 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 blot analysis with the same probe indicated above or more routinely using a three-primer PCR reaction with the primers (i) tcacttcgaagactcaggaatgg, (ii) tgcggggcagcgtctgaggctgg, and (iii) ttccatcagaagctgactctac. 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 Duke University Medical Center.

Zymosan-induced Peritoneal Inflammation. Zymosan (Sigma-Aldrich) was prepared in PBS to a final concentration of 1 mg/ml as described (15), and 1.0 ml was injected intraperitoneally. Mice were killed by CO_2 asphyxiation, and a peritoneal lavage was performed 0, 4, 18, or 72 h after injection, with 9 ml of ice cold RPMI medium containing 2% FCS and 2 mM EDTA. Total cell counts were determined with a hemocytometer, 50 μl of lavage fluid was cytospun and stained with Diff-Quik, and the percentages of macrophages, neutrophils, eosinophils, and lymphocytes were determined from a count of at least 200 cells. These percentages were multiplied by the total cell number to obtain the number of peritoneal neutrophils and macrophages. In some experiments, levels of LTB_4 in peritoneal lavage fluids were measured using an EIA kit from Cayman Chemicals following the manufacturer's protocol.

Neutrophil Calcium Mobilization and Chemotaxis. The 4-h zymosan-elicited peritoneal lavage cells contained >80% neutrophils and were used in functional assays. For calcium mobilization, 3×10^6 cells were washed and loaded with 1.0 μM INDO-1 AM for 30 min at room temperature as previously described (6). Calcium traces were recorded in a Perkin-Elmer fluorescence spectrometer (model 650-19) with an excitation wavelength of 355 nm and an emission wavelength of 405 nm. Chemotaxis of peritoneal exudate cells was measured by a 48-well microchemotaxis chamber technique as described (6).

Arachidonic Acid–induced Ear Inflammation. Mice were intravenously injected with PBS containing 0.5% Evans blue and indomethacin (Sigma-Aldrich) at final amounts of 10 mg/kg body weight to minimize the contribution from cyclooxygenase products (14). The inside of the right ear of each mouse was painted with 20 μl of arachidonic acid (AA; 100 mg/ml in acetone; Sigma-Aldrich), and the left ear was painted with acetone alone. Ear thickness was measured at 0 and 90 min after AA application using a calibrated thickness gauge (Mitutoyo). Mice were killed by CO_2 asphyxiation, a 5-mm-diameter disc of tissue was punched from the center of each ear, and edema was measured by determining the wet weight of the ear punches. The punches were then extracted in 0.5 ml of formamide at 55°C for 48 h. Evans blue extravasation was determined by measuring the absorbance at 610 nm in a spectrophotometer. Histological sections of the ears were stained with hematoxylin and eosin.

PAF-induced Anaphylaxis. Mice were intravenously injected with PAF-16 (Calbiochem) at a dose of 200 μg/kg body weight in saline containing 0.25% BSA. Animals were observed over a 24-h period. All animals alive after 1 h made a full recovery.

Results

Generation of BLTR-deficient Mice. To selectively ablate BLTR, a targeting vector that deleted the coding region of BLTR between amino acids 81 and 316 and replaced it with the PGK–neomycin (*PGK-Neo*) cassette was constructed (Fig. 1 A). After electroporation into ES cells, screening of 95 individual neomycin- and gancyclovir-resistant colonies by PCR yielded 25 positive recombinant targeted clones. A 3′ probe was selected for Southern blot screening of 15 of these clones, and the results indicated that they were all correctly targeted (data not shown). Of the six clones injected into C57BL/6 blastocysts, all yielded chimeric mice; four of the males succeeded in efficient germline transmission. All of the animals in this study consisted of offspring derived from the crossings of F1 heterozygous mice. Fig. 1 B shows a Southern blot of BamHI- and XhoI-digested DNA from the three genotypes. The wild-type littermate lanes (+/+) show the expected 5.2-kb band, and the homozygous lanes (−/−) show the 6.5-kb mutant band. The heterozygous (+/−) lanes show both the wild-type and mutant bands. Mice deficient for BLTR ($BLTR^{-/-}$) were born at the expected Mendelian ratios, showed no overt developmental or morphological abnormalities, and were fertile. A three-primer PCR (Fig. 1 C) was routinely used to determine the genotypes from the DNA isolated from tail biopsies.

To confirm that the mutation disrupted BLTR expression and not other chemoattractant receptors, zymosan-elicited peritoneal exudate cells (>80% neutrophils) were analyzed for calcium mobilization and chemotaxis. LTB_4, C5a, and PAF all induced calcium mobilization in cells

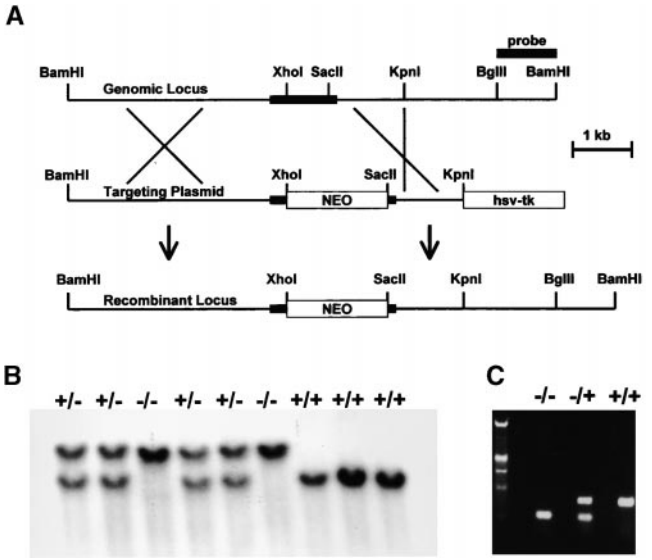

Figure 1. Targeted disruption of mouse BLTR. (A) Wild-type genomic locus of BLTR, targeting vector, and the recombinant mutant genomic locus. Coding region of the *BLTR* gene is indicated as a solid box. 706 bp of the coding region between the XhoI and SacII sites was replaced with *PGK-Neo* in the targeting vector. The final construct contained homology arms of 4.0 and 1.5 kb. A BglII–BamHI fragment served as an external probe for Southern blot analysis of genomic DNA from ES cells and mouse tails. (B) Southern blot showing correct targeting and germline transmission of the mutated BLTR gene. Genomic DNA samples prepared from F2 offspring were digested with BamHI and XhoI, separated on 0.6% agarose gels, blotted onto nylon membranes, and hybridized with the ^{32}P-labeled, 0.9-kb BglII–BamHI fragment. The genotypes of the mice are indicated above the lanes. (C) A three-primer PCR reaction was designed to identify the wild-type, heterozygous, and homozygous mutant alleles. The genotypes of the mice identified by this method confirmed the identifications by the Southern blot method in all cases tested.

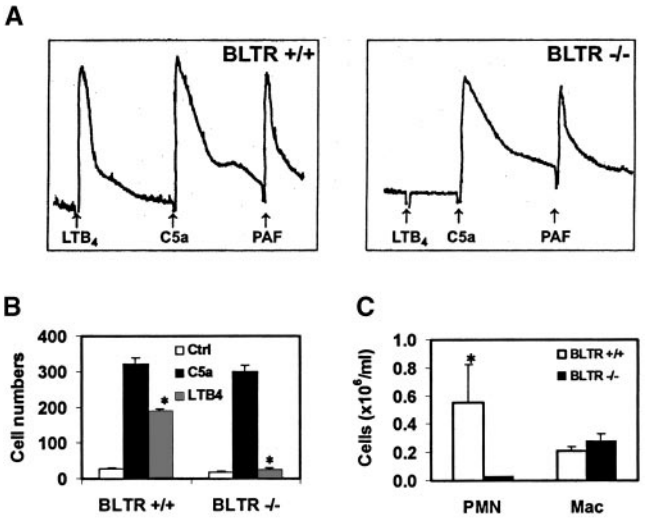

Figure 2. Loss of BLTR function in neutrophils from BLTR$^{-/-}$ mice. (A) Calcium mobilization was monitored in INDO-1–loaded, zymosan-elicited peritoneal neutrophils stimulated with 100 nM LTB_4, 100 nM C5a, or 100 nM PAF as indicated. Each tracing represents an analysis of 3×10^6 cells from a single mouse with the indicated BLTR genotype, and the data shown is representative of at least six each of BLTR$^{+/+}$ and BLTR$^{-/-}$ animals. (B) Neutrophil chemotaxis. The chemotactic response of zymosan-elicited peritoneal neutrophils from a single BLTR$^{+/+}$ or BLTR$^{-/-}$ mouse to LTB_4 (100 nM) and C5a (100 nM) was determined by counting the number of migrated cells within five high-powered fields (400×) representing three individual wells. Results are representative of at least four mice for each genotype. Differences in LTB_4 responses between BLTR$^{+/+}$ and BLTR$^{-/-}$ mice is statistically significant (*$P < 0.05$). (C) LTB_4-induced peritonitis. Six mice of each genotype were injected intraperitoneally with 1.0 ml of saline containing 10 μg of LTB_4, and 4 h later, peritoneal exudate cells were harvested. Values represent the mean ± SEM. The difference in neutrophil accumulation between BLTR$^{+/+}$ and BLTR$^{-/-}$ mice is statistically significant (*$P < 0.05$).

from littermate wild-type animals (Fig. 2 A). In contrast, cells from the BLTR$^{-/-}$ animals showed no calcium mobilization in response to LTB_4 but showed equivalent responses to C5a and PAF compared with cells from BLTR$^{+/+}$ mice. In addition, no calcium response was observed even at 1.0 μM LTB_4 in cells from BLTR$^{-/-}$ animals (data not shown). Chemotaxis assays also showed no response to LTB_4 by BLTR$^{-/-}$ cells, whereas cells from both BLTR$^{-/-}$ and BLTR$^{+/+}$ animals showed similar levels of migration to C5a (Fig. 2 B). Peritoneal injection of LTB_4 led to a rapid neutrophil influx in BLTR$^{+/+}$ mice that was completely abrogated in BLTR$^{-/-}$ mice (Fig. 2 C; $P < 0.05$). No statistically significant differences were observed in macrophage numbers at this time point.

Analysis of lymphoid tissues found no gross alterations in the size of the thymus, spleen, or lymph nodes between BLTR$^{-/-}$ and BLTR$^{+/+}$ 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 BLTR$^{-/-}$ and BLTR$^{+/+}$ 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 BLTR$^{-/-}$ and BLTR$^{+/+}$ mice (data not shown).

Peritoneal Inflammation Induced by Zymosan. To assess the role of BLTR in neutrophil and macrophage recruitment in response to a "nonspecific" inflammatory stimulus, zymosan was injected into the peritoneum of BLTR$^{+/+}$ and BLTR$^{-/-}$ mice. In uninjected mice, there were no significant differences in the resident peritoneal leukocyte populations in the BLTR$^{+/+}$ versus BLTR$^{-/-}$ mice (Fig. 3). In contrast, 4 and 18 h after zymosan injection, significantly fewer neutrophils were recovered from BLTR$^{-/-}$ relative to BLTR$^{+/+}$ mice (Fig. 3 A; $P < 0.05$). Likewise, significantly lower numbers of macrophages were recovered from BLTR$^{-/-}$ relative to BLTR$^{+/+}$ mice at 18 h after injection (Fig. 3 B; $P < 0.01$). However, at 72 h after injection, no differences were seen in the recovery of either cell type from BLTR$^{-/-}$ or BLTR$^{+/+}$ mice. Levels of LTB_4 in peritoneal lavage fluids at 4 h ranged from 1.2 ± 0.3 ng/ml in BLTR$^{+/+}$ animals to 1.0 ± 0.2 ng/ml in BLTR$^{-/-}$ animals.

AA-induced Ear Inflammation. When applied topically, AA induces an acute inflammatory reaction involving both vascular leakage and cellular components that is highly dependent on leukotrienes (13–15). To determine which if

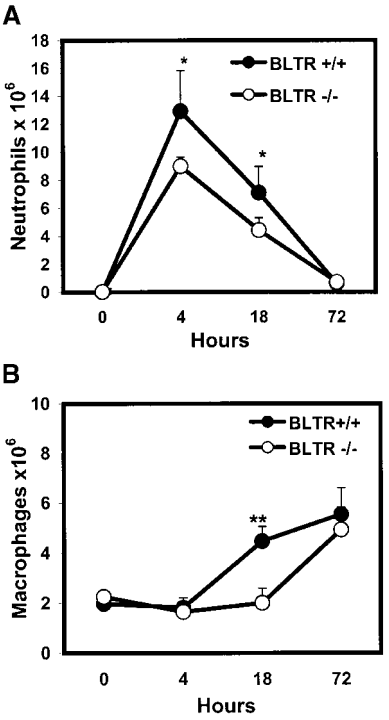

Figure 3. Peritoneal leukocyte accumulation in $BLTR^{+/+}$ and $BLTR^{-/-}$ mice. (A) Neutrophil and (B) macrophage accumulation after zymosan injection. The mean number of each leukocyte type (±SEM) in peritoneal lavage samples isolated at the times indicated after zymosan injection are presented. The number of animals for each time point consisted of five to nine animals. Statistically significant differences are indicated (*$P < 0.05$ and **$P < 0.01$).

any of these inflammatory reactions were mediated by BLTR, the $BLTR^{+/+}$ and $BLTR^{-/-}$ animals were treated with AA and edema, and vascular permeability was measured by changes in swelling, ear weight, and Evans blue dye extravasation. $BLTR^{+/+}$ mice showed significant ear inflammation 90 min after AA application (Fig. 4). Specifically, the AA-treated ears showed a 60% increase in weight, 270% increase in leakage of Evans blue, and 63% increase in thickness (data not shown) compared with vehicle only–treated ears. In contrast, AA-treated ears from $BLTR^{-/-}$ animals showed no significant increase in thickness or weight and only a 118% increase in Evans blue leakage compared with control ears. Histological examination of the tissue showed neutrophil infiltration only in $BLTR^{+/+}$ and not in $BLTR^{-/-}$ mice (Fig. 4, C and D).

PAF-induced Anaphylaxis. PAF is an important endogenous mediator of systemic anaphylactic shock in mice (13). Intravenous injection of PAF leads to hypotension, increased vascular permeability, vasodilation, bronchospasm, and endothelial adhesion and activation of neutrophils, macrophages, and eosinophils, resulting in death within an hour. Previous studies indicated that leukotrienes are important if not sole mediators of this response (13–16). PAF, when administered at a dose of 200 μg/kg body weight, resulted in the death of 16 of 20 $BLTR^{+/+}$ animals. This was not significantly different from the death of 13 of 20 $BLTR^{-/-}$ animals. However, a significant difference was observed in the survival of $BLTR^{-/-}$ animals when the results were segregated by sex. As shown in Table I, the female $BLTR^{-/-}$ animals displayed a selective advantage, with 6 of the 9 animals surviving PAF treatment, whereas only 1 of 11 male $BLTR^{-/-}$ animals survived the treatment. By contrast, the littermate $BLTR^{+/+}$ male and female animals did not show any significant differences in their survival.

Discussion

The deletion of the *BLTR* gene reported in this study reveals the critical role this receptor plays in acute inflammation and immediate hypersensitivity. The $BLTR^{-/-}$ mice were viable, developed and reproduced normally, and displayed no overt behavioral or morphological defects. The number and the development of lymphocyte subpopulations was normal in $BLTR^{-/-}$ mice. Although the $BLTR^{-/-}$ mice exhibited no obvious phenotype in a specific pathogen–free environment, exposure to inflammatory challenges revealed significant defects in neutrophil and macrophage recruitment and conferred a survival advantage in females to PAF-induced anaphylaxis.

The lack of developmental defects correlates with the observation that mice deficient for 5-LO, FLAP, or LTA_4 hydrolase all develop normally, indicating that neither enzymatic production of leukotrienes nor their action on specific receptors is essential for growth or development (13–16). Similarly, no developmental defects have been observed in mice deficient for C5a or *N*-formylpeptide receptors, indicating the nonessential nature of individual neutrophil chemoattractant receptors (22, 23). The observation that elicited peritoneal neutrophils did not display any calcium mobilization or chemotaxis to LTB_4, and the complete absence of neutrophil accumulation to peritoneal LTB_4 challenge in the $BLTR^{-/-}$ mice indicates that BLTR is likely the sole LTB_4-responsive G protein–coupled receptor mediating inflammatory responses in mice.

Recent studies demonstrated that transgenic mice overexpressing human BLTR displayed a profound increase in neutrophil recruitment in skin inflammation, peritonitis, and reperfusion-initiated second organ injury (24). These results are in agreement with the current studies that neutrophil influx was reduced at early times (4 and 18 h) in zymosan-induced peritonitis. In addition, a reduction in the 18-h recruitment of macrophages suggests a role for LTB_4 in the early times of monocyte/macrophage migration during peritoneal inflammation. Zymosan activates complement to produce C5a, resulting in neutrophil influx (25). The data from the studies in this paper indicate that LTB_4 and BLTR also play a role in this process. Indeed, previous studies indicated that LTB_4 is the major AA metabolite produced by the entering neutrophils in murine zymosan-induced peritonitis (25). LTB_4 likely produced by the resident macrophages and/or early accumulating neutrophils may be involved in an autocrine loop of neutrophil accumulation during inflammation. The early reduction of neutrophil influx in $BLTR^{-/-}$

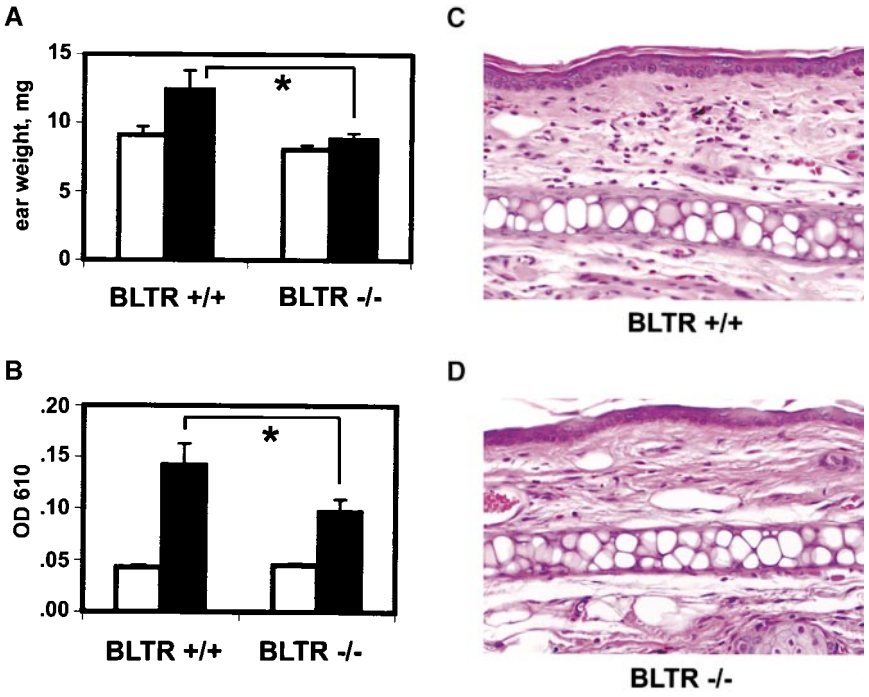

Figure 4. AA-induced ear inflammation. AA (2 mg) was applied to the mouse ears and analyzed for edema and protein extravasation after 90 min as described in Materials and Methods. (A) Edema: wet weight of a 5-mm ear punch of the control (white bars) and AA-treated ears (black bars) was measured. (B) Extravasation: Evans blue dye leakage was measured at a wavelength of 610 nm (OD 610): open bar, control ears; closed bars, AA-treated ears. The decreased inflammatory response in the AA-treated ears of $BLTR^{-/-}$ mice compared with $BLTR^{+/+}$ mice was statistically significant (*$P < 0.05$) for data in A and B. (C and D) Histology: hematoxylin and eosin–stained tissue sections from $BLTR^{+/+}$ (C) and $BLTR^{-/-}$ (D) mice treated with AA for 90 min.

mice may have resulted in reduced synthesis of other mediators for further monocyte influx. However, the similar macrophage levels at 72 h after zymosan treatment in the $BLTR^{-/-}$ and $BLTR^{+/+}$ mice indicate the existence of as yet unknown compensatory mechanisms.

Previous studies with mice deficient in leukotriene synthesis showed decreased inflammatory responses to AA (14–16). Although the relative contributions of LTB_4 and LTC_4 were not clear, both protein extravasation and cellular infiltration were affected. Intermediate responses were observed in LTA_4 hydrolase–deficient mice relative to 5-LO– and FLAP-deficient mice. However, the LTA_4 hydrolase–deficient mice produced larger amounts of LTC_4 relative to control mice. Current studies with $BLTR^{-/-}$ mice indicate that both edema and cellular components of inflammation were decreased, suggesting a role for BLTR in both vascular leakage and neutrophil accumulation.

A surprising observation from the current studies in this paper is the relative resistance of female $BLTR^{-/-}$ mice to PAF-induced immediate hypersensitivity. Previous studies with 5-LO– and FLAP-deficient animals showed a strong protection from PAF-induced lethality (13–15). However, sex differences in survival, if any, were not reported. These studies show clear differences in the survival of female versus male $BLTR^{-/-}$ mice to challenge with PAF. Several autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus (SLE) display sex-dependent predilection, with females being most severely affected (26, 27). In autoimmune disease–prone MRL-*lpr/lpr* mice, females are more susceptible to SLE-like pathology, whereas males appear to express increased inflammatory responsiveness to leukotrienes (28). Studies with 5-LO–deficient mice on the MRL-*lpr/lpr* background showed that males lost their survival advantage and became as susceptible to disease as females, suggesting that leukotrienes protect against SLE-like disease (28). The relevance, if any, of the sex-related difference in PAF anaphylaxis in $BLTR^{-/-}$ mice to autoimmune diseases is unknown at this time. The loss of male advantage against SLE in 5-LO–deficient MRL-*lpr/lpr* mice needs to be placed in context with our results of gain of a female advantage in $BLTR^{-/-}$ mice. We postulate that a threshold level of leukotriene responsiveness provides protection against autoimmune disease but produces susceptibility to PAF-mediated anaphylaxis. If so, males may have greater leukotriene responsiveness than females. The mice with 5-LO or BLTR deficiency cross these thresholds to result in either male susceptibility to SLE or female protection to PAF anaphylaxis, respectively. Clearly, PAF-induced anaphylaxis is a complex process, as all 5-LO–deficient mice are protected but the male $BLTR^{-/-}$ mice are not. Further studies on hormonal effects will likely delineate the sex-dependent leukotriene effects in these mice.

Table I. *Sex-related Differences in PAF-induced Anaphylaxis*

Genotype:	$BLTR^{+/+}$		$BLTR^{-/-}$	
Sex	Male	Female	Male	Female
Survivors/total	3/11	1/9	1/11	6/9

Mice were injected intravenously with PAF at a dose of 200 μg/kg body weight and observed for 24 h. The total number of animals tested for each genotype and sex is shown separately with the number of surviving animals. The difference in mortality between female and male $BLTR^{-/-}$ mice is statistically significant ($P = 0.002$, Fisher's exact test).

In summary, this study has shown that deletion of BLTR reduced the vascular and cellular components of acute inflammatory responses in mice. In addition, these data suggest a role for BLTR in mediating systemic anaphylaxis, with female mice being more dependent on this pathway than males. Inhibition of BLTR function may provide a target for therapeutic intervention in certain human inflammatory conditions. Further studies with expression of the BLTR deficiency on different strain backgrounds will allow investigation of the role of BLTR in asthma, rheumatoid arthritis, lupus, and colitis as well as infectious diseases.

We thank Dr. Thomas F. Tedder for advice and encouragement, Dr. Beverly H. Koller for sharing unpublished results and advice on various methods used in these studies, and Dr. Larry Liao for suggestions on construction of the targeting vector.

This work was supported by National Institutes of Health grants AI-43184, AR-39162, and DE-03738.

Submitted: 29 March 2000
Revised: 1 May 2000
Accepted: 5 May 2000

References

1. Smith, M.J., A.W. Ford-Hutchinson, and M.A. Bray. 1980. Leukotriene B: a potential mediator of inflammation. *J. Pharm. Pharmacol.* 32:517–518.
2. Samuelsson, B., S.E. Dahlen, J.A. Lindgren, C.A. Rouzer, and C.N. Serhan. 1987. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science.* 237:1171–1176.
3. Dahlen, S.E., P. Hedqvist, S. Hammarstrom, and B. Samuelsson. 1980. Leukotrienes are potent constrictors of human bronchi. *Nature.* 288:484–486.
4. Yokomizo, T., T. Izumi, K. Chang, Y. Takuwa, and T. Shimizu. 1997. A G-protein-coupled receptor for leukotriene B-4 that mediates chemotaxis. *Nature.* 387:620–624.
5. Lynch, K.R., G.P. O'Neill, Q.Y. Liu, D.S. Im, N. Sawyer, K.M. Metters, N. Coulombe, M. Abramovitz, D.J. Figueroa, Z.Z. Zeng, et al. 1999. Characterization of the human cysteinyl leukotriene CysLT(1) receptor. *Nature.* 399:789–793.
6. Haribabu, B., D.V. Zhelev, B.C. Pridgen, R.M. Richardson, H. Ali, and R. Snyderman. 1999. Chemoattractant receptors activate distinct pathways for chemotaxis and secretion-role of G-protein usage. *J. Biol. Chem.* 274:37087–37092.
7. Henderson, W.R., Jr., D.B. Lewis, R.K. Albert, Y. Zhang, W.J. Lamm, G.K. Chiang, F. Jones, P. Eriksen, Y.T. Tien, M. Jonas, et al. 1996. The importance of leukotrienes in airway inflammation in a mouse model of asthma. *J. Exp. Med.* 184:1483–1494.
8. Peters-Golden, M. 1999. Pulmonary diseases other than asthma as potential targets for antileukotriene therapy. *Clin. Rev. Allergy. Immunol.* 17:247–260.
9. Showell, H.J., and K. Cooper. 1999. Inhibitors and antagonists of cyclooxygenase, 5-lipoxygenase, and platelet activating factor. *In* Inflammation: Basic Principles and Clinical Correlates. 3rd ed. J.I. Gallin and R. Snyderman, editors. Lippincott, Williams & Wilkins, Philadelphia. 1177–1193.
10. Griffiths, R.J., E.R. Pettipher, K. Koch, C.A. Farrell, R. Breslow, M.J. Conklyn, M.A. Smith, B.C. Hackman, D.J. Wimberly, A.J. Milici, et al. 1995. Leukotriene B4 plays a critical role in the progression of collagen-induced arthritis. *Proc. Natl. Acad. Sci. USA.* 92:517–521.
11. Tsuji, F., Y. Miyake, M. Horiuchi, and S. Mita. 1998. Involvement of leukotriene B4 in murine dermatitis models. *Biochem. Pharmacol.* 55:297–304.
12. Matsukawa, A., C.M. Hogaboam, N.W. Lukacs, P.M. Lincoln, R.M. Strieter, and S.L. Kunkel. 1999. Endogenous monocyte chemoattractant protein-1 (MCP-1) protects mice in a model of acute septic peritonitis: cross-talk between MCP-1 and leukotriene B-4. *J. Immunol.* 163:6148–6154.
13. Chen, X.S., J.R. Sheller, E.N. Johnson, and C.D. Funk. 1994. Role of leukotrienes revealed by targeted disruption of the 5-lipoxygenase gene. *Nature.* 372:179–182.
14. Goulet, J.L., J.N. Snouwaert, A.M. Latour, T.M. Coffman, and B.H. Koller. 1994. Altered inflammatory responses in leukotriene-deficient mice. *Proc. Natl. Acad. Sci. USA.* 91:12852–12856.
15. Byrum, R.S., J.L. Goulet, R.J. Griffiths, and B.H. Koller. 1997. Role of the 5-lipoxygenase-activating protein (FLAP) in murine acute inflammatory responses. *J. Exp. Med.* 185:1065–1075.
16. Byrum, R.S., J.L. Goulet, J.N. Snouwaert, R.J. Griffiths, and B.H. Koller. 1999. Determination of the contribution of cysteinyl leukotrienes and leukotriene B-4 in acute inflammatory responses using 5-lipoxygenase- and leukotriene A(4) hydrolase-deficient mice. *J. Immunol.* 163:6810–6819.
17. Harrison, K.A., and R.C. Murphy. 1995. Isoleukotrienes are biologically active free radical products of lipid peroxidation. *J. Biol. Chem.* 270:17273–17278.
18. Devchand, P.R., H. Keller, J.M. Peters, M. Vazquez, F.J. Gonzalez, and W. Wahli. 1996. The PPARalpha-leukotriene B4 pathway to inflammation control. *Nature.* 384:39–43.
19. Huang, W.W., E.A. Garcia-Zepeda, A. Sauty, H.C. Oettgen, M.E. Rothenberg, and A.D. Luster. 1998. Molecular and biological characterization of the murine leukotriene B4 receptor expressed on eosinophils. *J. Exp. Med.* 188:1063–1074.
20. Martin, V., P. Ronde, D. Unett, A. Wong, T.L. Hoffman, A.L. Edinger, R.W. Doms, and C.D. Funk. 1999. Leukotriene binding, signaling, and analysis of HIV coreceptor function in mouse and human leukotriene B_4 receptor-transfected cells. *J. Biol. Chem.* 274:8597–8603.
21. Tybulewicz, V.L., C.E. Crawford, P.K. Jackson, R.T. Bronson, and R.C. Mulligan. 1991. Neonatal lethality and lymphopenia in mice with a homozygous disruption of the c-abl proto-oncogene. *Cell.* 65:1153–1163.
22. Hopken, U.E., B. Lu, N.P. Gerard, and C. Gerard. 1996. The C5a chemoattractant receptor mediates mucosal defence to infection. *Nature.* 383:86–89.
23. Gao, J.L., E.J. Lee, and P.M. Murphy. 1999. Impaired antibacterial host defense in mice lacking the *N*-formylpeptide receptor. *J. Exp. Med.* 189:657–662.
24. Chiang, N., K. Gronert, C.B. Clish, O.B. JA, M.W. Freeman, and C.N. Serhan. 1999. Leukotriene B-4 receptor transgenic mice reveal novel protective roles for lipoxins and aspirin-triggered lipoxins in reperfusion. *J. Clin. Invest.* 104:309–316.
25. Rao, T.S., J.L. Currie, A.F. Shaffer, and P.C. Isakson. 1994. In vivo characterization of zymosan-induced mouse peritoneal inflammation. *J. Pharmacol. Exp. Ther.* 269:917–925.
26. Ansar Ahmed, S., W.J. Penhale, and N. Talal. 1985. Sex hormones, immune responses, and autoimmune diseases. Mechanisms of sex hormone action. *Am. J. Pathol.* 121:531–551.
27. Pisetsky, D.S. 1991. Systemic lupus erythematosus. *Curr. Opin. Immunol.* 3:917–923.
28. Goulet, J.L., R.C. Griffiths, P. Ruiz, R.F. Spurney, D.S. Pisetsky, B.H. Koller, and T.M. Coffman. 1999. Deficiency of 5-lipoxygenase abolishes sex-related survival differences in MRL-lpr/lpr mice. *J. Immunol.* 163:359–366.