The Journal of Immunology

Deficiency of the Leukotriene B_4 Receptor, BLT-1, Protects against Systemic Insulin Resistance in Diet-Induced Obesity

Matthew Spite, Jason Hellmann, Yunan Tang, Steven P. Mathis, Madhavi Kosuri, Aruni Bhatnagar, Venkatakrishna R. Jala and Bodduluri Haribabu

J Immunol 2011; 187:1942-1949; Prepublished online 8 July 2011;
doi: 10.4049/jimmunol.1100196
http://www.jimmunol.org/content/187/4/1942

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Deficiency of the Leukotriene B_4 Receptor, BLT-1, Protects against Systemic Insulin Resistance in Diet-Induced Obesity

Matthew Spite,*,† Jason Hellmann,* Yunan Tang,* Steven P. Mathis,‡ Madhavi Kosuri,* Aruni Bhatnagar,* Venkatakrishna R. Jala,†,‡ and Bodduluri Haribabu†,‡

Chronic inflammation is an underlying factor linking obesity with insulin resistance. Diet-induced obesity promotes an increase in circulating levels of inflammatory monocytes and their infiltration into expanding adipose tissue. Nevertheless, the endogenous pathways that trigger and sustain chronic low-grade inflammation in obesity are incompletely understood. In this study, we report that a high-fat diet selectively increases the circulating levels of $CD11b^+$ monocytes in wild-type mice that express leukotriene B_4 receptor, BLT-1, and that this increase is abolished in *BLT-1*–null mice. The accumulation of classically activated (M1) adipose tissue macrophages (ATMs) and the expression of proinflammatory cytokines and chemokines (i.e., *IL-6* and *Ccl2*) was largely blunted in adipose tissue of obese *BLT-1*$^{-/-}$ mice, whereas the ratio of alternatively activated (M2) ATMs to M1 ATMs was increased. Obese *BLT-1*$^{-/-}$ mice were protected from systemic glucose and insulin intolerance and this was associated with a decrease in inflammation in adipose tissue and liver and a decrease in hepatic triglyceride accumulation. Deletion of *BLT-1* prevented high fat-induced loss of insulin signaling in liver and skeletal muscle. These observations elucidate a novel role of chemoattractant receptor, BLT-1, in promoting monocyte trafficking to adipose tissue and promoting chronic inflammation in obesity and could lead to the identification of new therapeutic targets for treating insulin resistance in obesity. *The Journal of Immunology*, **2011, 187: 1942–1949.**

Obesity is an emerging global epidemic and is one of the most prominent risk factors for the development of type 2 diabetes (1, 2). Extensive studies show that insulin resistance in obesity is induced and sustained by chronic low-grade inflammation (3, 4). Indeed, type 2 diabetes is associated with increased levels of inflammatory mediators that induce insulin resistance, and adipose tissue in obese and diabetic individuals remains in a state of chronic, unresolved inflammation (4, 5). More than 40% of the total adipose tissue cell content from obese rodents and humans is composed of macrophages, compared with 10% in lean counterparts, and inflammatory adipose tissue macrophages (ATMs) directly promote systemic insulin resistance (4, 6, 7). Nevertheless, the mechanisms that contribute and sustain chronic inflammation in obesity and diabetes remain poorly understood.

Leukotriene B_4 (LTB_4) is a proinflammatory lipid mediator generated from arachidonic acid through the sequential activities of 5-lipoxygenase, 5-lipoxygenase–activating protein, and leukotriene A_4 hydrolase (8, 9). LTB_4 is rapidly generated by activated leukocytes and has well-characterized biological actions, including the promotion of leukocyte chemotaxis and the regulation of proinflammatory cytokines (8, 10). The potent biological actions of LTB_4 are mediated primarily through a high-affinity interaction with a G protein-coupled receptor termed BLT-1 (10). Although the LTB_4/BLT-1 axis plays an important role in host defense during acute infection, chronic activation of this pathway contributes to persistent inflammation characteristic of inflammatory pathologies, including atherosclerosis and arthritis (11–16).

Recent studies show that the expression and activity of enzymes required for leukotriene biosynthesis, including 5-lipoxygenase and 5-lipoxygenase–activating protein, are increased in both the liver and adipose tissue in murine models of experimental obesity (16–18). Moreover, LTB_4 levels increase in adipose tissue of both mice and rats consuming a high-fat diet (16, 17, 19). However, a causal role for LTB_4 in promoting or sustaining chronic inflammation and insulin resistance in obesity has not been established. In this study, we report that deficiency of *BLT-1* protects against the development of insulin resistance in diet-induced obesity (DIO) by regulating ATM accumulation and inflammation in insulin-sensitive tissues.

Materials and Methods

Animals and treatment

Male C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and *BLT-1*$^{-/-}$ mice ($Ltb4r1^{tm1Bodd}$) were generated as described (12) and backcrossed for nine generations on a C57BL/6J background as reported before (13). At 8–11 wk of age, mice were placed on either a 10% (kilocalories from fat) low-fat diet or 60% high-fat diet (Research Diets) and maintained for 12 additional weeks. Body weight was recorded weekly. Glucose and insulin tolerance tests were performed during the 12th wk of feeding, as outlined in Fig. 1*A*, whereas all other parameters were evaluated upon euthanasia. All procedures were approved by the University of Louisville Institutional Animal Care and Use Committee.

*Division of Cardiovascular Medicine, Diabetes and Obesity Center, University of Louisville School of Medicine, Louisville, KY 40202; †Department of Microbiology and Immunology, University of Louisville School of Medicine, Louisville, KY 40202; and ‡James Graham Brown Cancer Center, University of Louisville School of Medicine, Louisville, KY 40202

Received for publication January 20, 2011. Accepted for publication June 5, 2011.

This work was supported by the National Institutes of Health-sponsored Diabetes and Obesity Center (Grant P20RR024489 to M.S. and A.B.) and by National Institutes of Health Grant CA138623 (to H.B.).

Address correspondence and reprint requests to Dr. Matthew Spite, University of Louisville, Delia Baxter Building, 580 S. Preston Street, Room 404F, Louisville, KY 40202 and address requests for material and mice to Dr. Bodduluri Haribabu, 505 S. Hancock Street, Room 324 CTR Building, Louisville, KY 40202. E-mail addresses: Matthew.spite@louisville.edu (M.S.) and H0bodd01@louisville.edu (B.H.)

The online version of this article contains supplemental material.

Abbreviations used in this article: ALT, alanine aminotransferase; ATM, adipose tissue macrophage; CLS, crown-like structures; DIO, diet-induced obesity; HOMA-IR, homeostatic model assessment of insulin resistance; LTB_4, leukotriene B_4; MGL-1, macrophage galactose-type C-type lectin 1; WT, wild-type.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1100196

Glucose/insulin tolerance tests

Glucose tolerance tests were performed following a 6 h fast by i.p. injection of D-glucose (1 mg/g) in sterile saline. Insulin tolerance tests were performed on nonfasted animals by i.p. injection of 1.5 U/kg Humulin R (Eli Lilly, Indianapolis, IN). Blood samples were obtained from the tail and glucose levels were measured at indicated time points using an Aviva Accu-Chek glucometer. The homeostatic model assessment of insulin resistance (HOMA-IR) score was calculated based on the formula: glucose (mmol) × insulin (mU/ml)/22.5.

Immunoblotting and PCR

Tissue lysates were prepared as described in Horrillo et al. (17). Equal amounts of protein were separated by SDS-PAGE, transblotted, and probed for phospho-Akt (Ser^{473}), total Akt, phospho-JNK (Thr^{183}/Tyr^{185}), total JNK, phospho-IκBα, and total IκBα (Cell Signaling Technology). Western blots were developed using ECL Plus followed by luminescence detection using a Typhoon 9400 variable mode imager (Amersham Biosciences). Quantification of band intensities was performed using ImageQuant TL software.

For quantitative RT-PCR, RNA was extracted from tissues using the RNeasy lipid tissue kit (Qiagen), followed by cDNA synthesis. Real-time PCR amplification was performed with SYBR Green qPCR Master Mix (SABiosciences) using a 7900HT Fast Real-Time PCR System (Applied Biosystems) and commercially available primers for *Emr-1, IL-10, IL-6*, *PPAR-γ*, and *PPAR-α* (Super Array Biosciences). The following primers were also used (Integrated DNA Technologies): *Ccl2*, forward, 5′-ATGCAGGTCCCTGTCATG-3′, reverse, 5′-GCTTGAGGTGGTTGTGGA-3′; *Blt-1*, forward, 5′-TCC CTT TTT CCT CCA CTT TC-3′, reverse, 5′-GAA AAG ACA CCA CCC AGA TG-3′; *Ym-1*, forward, 5′-GGGCATACCTTTATCCTGAG-3′, reverse, 5′-CCACTGAAGTCATCCATGTC-3′; *Arg-1*, forward, 5′-ATGGAAGAGACCTTCAGCTAC-3′, reverse, 5′-GCTGTCTTCCCAAGAGTTGGG-3′ (20). Relative expression was determined by the $2^{-\Delta\Delta CT}$ method after internal normalization to *hprt* or β-actin.

Immunohistochemistry and quantification of crown-like structures

H&E staining was performed following standard procedures on formalin-fixed paraffin-embedded epididymal adipose tissue and liver concurrent sections. Crown-like structures (CLS) were identified as adipocytes completely surrounded by infiltrating cells and were quantified in five random fields per animal. Oil Red O staining was performed on OCT-embedded liver sections as described in Horrillo et al. (17).

Biochemical analyses

Total plasma cholesterol, high-density lipoproteins, low-density lipoproteins, triglycerides, total protein, albumin (cholesterol CII enzymatic kit; L-type TG-H kit; Bradford reagent, bromocresol green; Wako, Richmond, VA), alanine aminotransferase (ALT), and aspartate aminotransferase (Infinity, ThermoElectron) levels were measured using commercially available assay reagents as indicated. The total hepatic triglyceride content was determined after chloroform/methanol extraction of frozen liver samples. Assays were performed using a Cobas Mira Plus 5600 Autoanalyzer (Roche, Indianapolis, IN). Plasma insulin was measured by ELISA (Mercodia).

Flow cytometry analysis

Analysis of cells in spleen and peripheral blood was carried out as described in Mathis et al. (21) with minor modifications. Cells from spleen were isolated and filtered, while 30 μl peripheral blood was used for analysis. Cells were stained with anti-mouse CD16/32 (BD Pharmingen, San Diego, CA) in 1% FBS in PBS. Biotinylated anti-mouse BLT-1 (3D7) was then used for assessment of BLT-1 expression. Cells were subsequently washed and stained. Abs used include PE-conjugated anti-B220, PerCP-Cy5.5–conjugated anti-CD8, PE-conjugated anti-NK1.1, FITC-conjugated anti-CD3 molecular complex, PE-conjugated anti–Siglec-F, PerCP-Cy5.5–conjugated anti-CD11b (BD Pharmingen, San Diego, CA), allophycocyanin-conjugated anti-CD4, FITC-conjugated anti-GR (eBioscience, San Diego, CA), allophycocyanin-conjugated streptavidin, and PE-conjugated anti-gdTCR (BioLegend, San Diego, CA). Flow cytometry was carried out using a FACSCalibur flow cytometer equipped with CellQuest Pro software (BD Biosciences) and analyzed with FlowJo v.4.3 software.

Adipose tissue stromal vascular cells were isolated as in Lumeng et al. (22). Stromal vascular cell pellets were incubated with Fc Block prior to staining with fluorescent-conjugated primary Abs or isotype controls. Abs used included Alexa Fluor 647 anti–Mgl-1 (CD301a; AbD Serotec), FITC anti-CD11c (BD Biosciences), and PE anti-F4/80 (BioLegend). Flow cytometry was carried out using a BD LSRII flow cytometer equipped with FACSDiva v.6.0. Analysis was performed with FlowJo v.7.6 software.

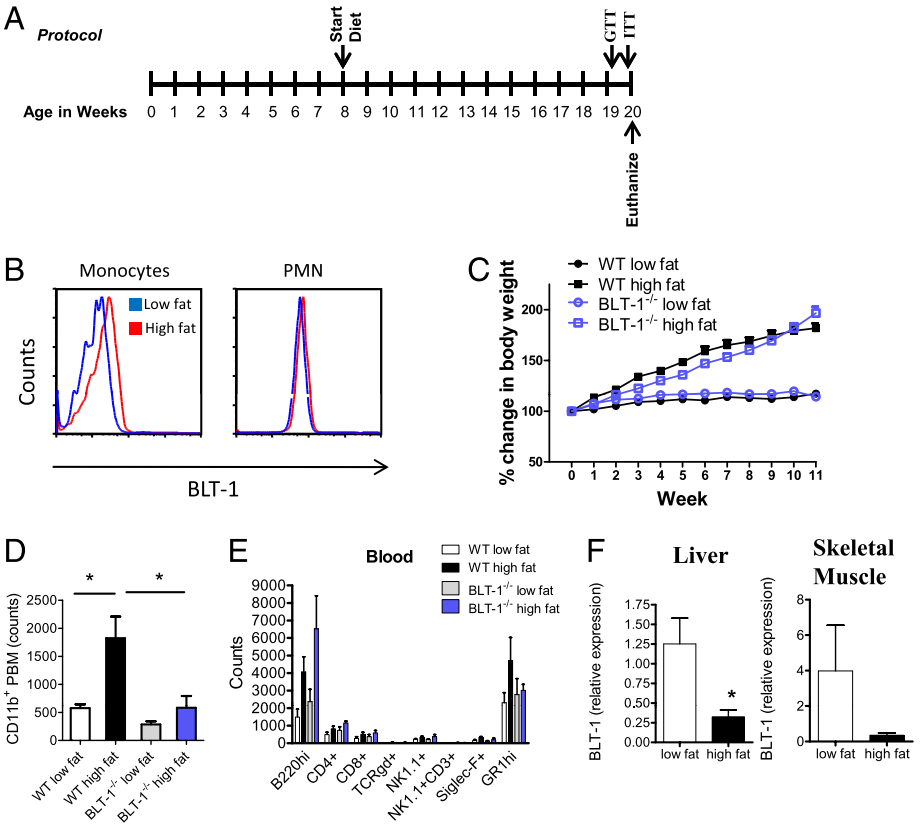

FIGURE 1. Expression of BLT-1 regulates peripheral blood monocyte expansion in DIO. *A*, Feeding protocol. Starting at 8 wk age, WT and *BLT-1*–deficient mice were fed a low-fat (10%) or high-fat (60%) diet for 12 wk. Glucose tolerance tests (GTT) and insulin tolerance tests (ITT) were performed during the 12th week of the feeding protocol. *B*, Surface expression of BLT-1 on peripheral blood monocytes and neutrophils (PMN) in WT mice fed a low-fat or high-fat diet (n = 5/group). *C*, Weight gain of WT and *BLT-1*–deficient mice on a high-fat diet for 12 wk (n = 8–12/group). *D*, Peripheral blood monocytes in WT and *BLT-1*–deficient mice (n = 5/group). *E*, Whole blood leukocyte differentials from WT and *BLT-1*–deficient mice (n = 5/group). *F*, *BLT-1* mRNA expression in liver and skeletal muscle (n = 6/group). Data are presented as means ± SEM. $*p < 0.05$ by Student t test (*F*), one-way ANOVA (*D*), or two-way ANOVA (*C*).

Statistical analysis

Data are expressed as means ± SEM. Multiple group comparisons were made using one-way or two-way ANOVA, followed by Bonferroni post-tests. Direct comparisons were made using an unpaired Student *t* test. A p value <0.05 was considered significant.

Results

BLT-1 regulates peripheral blood monocyte expansion in obesity

High-fat feeding increases circulating levels of peripheral blood monocytes that infiltrate fat depots and other dysfunctional tissues (6, 23, 24). Because LTB_4 increases in adipose tissue in obesity (16, 17, 19), we asked whether activation of BLT-1 regulates monocyte recruitment in obesity. In mice fed a high-fat diet for 12 wk (see protocol in Fig. 1*A*), BLT-1 expression increased on $CD11b^+$ circulating monocytes (Fig. 1*B*). Importantly, this increase was selective for monocytes, as BLT-1 expression on polymorphonuclear neutrophils (PMNs) did not change with high-fat feeding. To determine whether BLT-1 is required for monocyte expansion in obesity, mice deficient in *BLT-1* were placed on either a low-fat or high-fat diet. Interestingly, *BLT-1*–deficient mice gained weight to a similar extent as did their wild-type (WT) counterparts when fed a high-fat diet (Fig. 1*C*). Note that the *BLT-1*–deficient mice weighed less than their WT counterparts at the initiation of the study (23.59 ± 0.37 versus 26.76 ± 0.41 g, respectively), and thus the *BLT-1*–deficient mice weighed less than the WT mice on a high-fat diet throughout the study protocol (Supplemental Fig. 1*A*). However, at the end of the feeding period, there were no significant differences in body weight between WT and *BLT-1*–deficient mice, indicating that the *BLT-1*–deficient mice had accelerated weight gain toward the end of the study protocol (Supplemental Fig. 1*B*). Consistent with previous reports, high-fat feeding led to an increase in the number of circulating $CD11b^+$ monocytes in WT mice (Fig. 1*D*) (23). Significantly, this increase was completely abolished in *BLT-1*–deficient mice (Fig. 1*D*). Notably, this regulation was specific to monocytes, as other peripheral blood leukocyte populations, including $GR\text{-}1^+$ PMNs, B cells, NK cells, and both $CD4^+$ and $CD8^+$ T cells, were not affected by either high-fat feeding or *BLT-1* deficiency (Fig. 1*E*). Moreover, leukocyte populations in the spleen were also unaffected by high-fat feeding or *BLT-1* deficiency (Supplemental Fig. 1*C*).

We next evaluated changes in the expression of *BLT-1* in liver, skeletal muscle, and adipose tissue of lean and obese mice. As shown in Fig. 1*F*, *BLT-1* was expressed in the liver and skeletal muscle of lean mice, although the expression of *BLT-1* in adipose tissue was not evident. In mice fed a high-fat diet, a decrease in the expression of *BLT-1* was observed in both liver and skeletal muscle.

Deficiency of BLT-1 improves glucose and insulin tolerance in DIO

We next evaluated whether *BLT-1* deficiency modulates metabolic derangements associated with DIO. High-fat feeding markedly increased fasting plasma glucose levels in WT mice (Fig. 2*A*). Similar to the effects of *BLT-1* deficiency on weight gain, no differences in plasma glucose were observed with high-fat feeding compared with WT mice. DIO increased plasma insulin in WT mice, whereas insulin was not significantly elevated in *BLT-1*$^{-/-}$ mice (Fig. 2*B*). Importantly, *BLT-1*$^{-/-}$ mice were less insulin resistant than were obese WT mice, as determined by the homeostasis model assessment of insulin resistance (HOMA-IR; Fig.

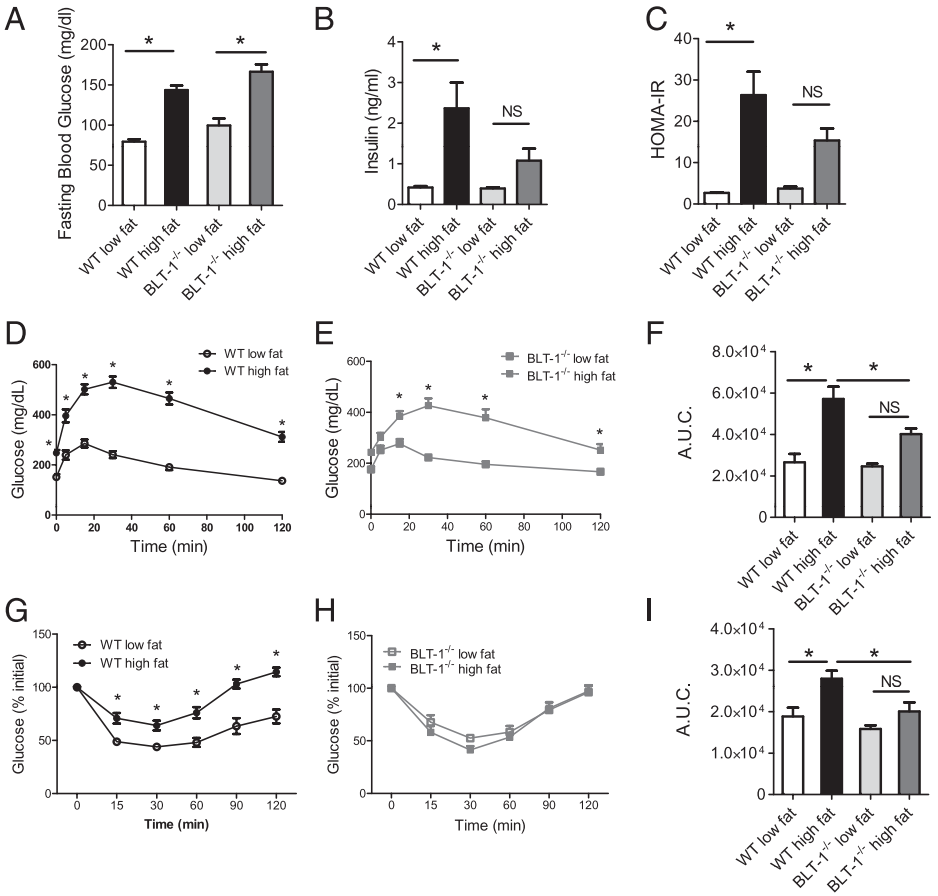

FIGURE 2. Deficiency of *BLT-1* protects against systemic insulin resistance in DIO. *A*, Fasting blood glucose. *B*, Fasting plasma insulin levels. *C*, Calculated HOMA-IR score. *D* and *E*, Glucose tolerance tests. *F*, Area under the curve measurement of glucose tolerance tests in *D* and *E*. *G* and *H*, Insulin tolerance tests. *I*, Area under the curve measurement of insulin tolerances tests in *G* and *H*. Data are presented as means ± SEM. $*p < 0.05$ by one-way (*A*–*C*, *F*, *I*) or two-way (*D*, *E*, *G*, *H*) ANOVA; n = 8–12/group. A.U.C., area under the curve.

2*C*). To evaluate how *BLT-1* deficiency affects glucose homeostasis, we performed glucose and insulin tolerance tests in WT and *BLT-1*$^{-/-}$ mice. DIO induced pronounced glucose intolerance in WT mice (Fig. 2*D*). Similarly, obese *BLT-1*$^{-/-}$ mice were glucose intolerant (Fig. 2*E*), although area under the curve measurements indicated that the magnitude of glucose intolerance was less in the *BLT-1*$^{-/-}$ mice than their WT counterparts (Fig. 2*F*). Importantly, high-fat feeding decreased insulin-stimulated glucose disposal in WT mice, whereas *BLT-1*$^{-/-}$ mice were completely protected from this insulin intolerance (Fig. 2*G–I*). Deficiency of *BLT-1* did not modulate changes in total plasma cholesterol, high-density lipoproteins, low-density lipoproteins, plasma triglycerides, or total protein levels in DIO (Supplemental Fig. 2). Modest changes in the heart/body weight ratios were observed between obese WT and *BLT-1*$^{-/-}$ mice (Supplemental Fig. 2). Serum creatinine and lactate dehydrogenase levels were not significantly increased with high-fat feeding in WT mice (Supplemental Fig. 2). Taken together, these results indicate that deficiency of *BLT-1* protects from glucose and insulin intolerance in obesity.

Adipose tissue macrophage accumulation and inflammation are decreased in obese BLT-1$^{-/-}$ *mice*

The accumulation of classically activated (M1) macrophages in adipose tissue is a critical underlying component linking adipose tissue expansion with systemic insulin resistance (6, 7, 25). We next asked whether deficiency of *BLT-1* affects the accumulation of M1 ATMs. As shown in Fig. 3*A*, epididymal fat pad weights were increased in both WT and *BLT-1*$^{-/-}$ mice on a high-fat diet, consistent with the observed increase in total body mass (see above). Notably, the magnitude of epididymal fat pad expansion in obese BLT-1$^{-/-}$ mice was significantly higher than in obese WT mice. However, despite this increase, expression of pan-macrophage marker *Emr-1* (F4/80) was significantly decreased in *BLT-1*$^{-/-}$ mice relative to WT mice (Fig. 3*B*). Additionally, the expression of monocyte chemoattractant *Ccl2* was also decreased in *BLT-1*$^{-/-}$ mice (Fig. 3*C*). Interestingly, the expression of *PPARα*, which is a nuclear receptor for LTB_4 that regulates the expression of β-oxidation genes, was significantly elevated in obese *BLT-1*–deficient mice (Fig. 3*D*) (26). Histological analysis of adipose tissue from obese WT mice showed an increase in the formation of CLS compared with their low fat-fed counterparts (Fig. 3*E*) (7). The formation of CLS in obese *BLT-1*$^{-/-}$ mice was not significantly increased compared with their low fat-fed counterparts. Because M1 ATMs contribute to insulin resistance by producing inflammatory cytokines that block insulin action (25, 27), we next evaluated the expression of *IL-6* in adipose tissue. Notably, *IL-6* was drastically reduced in *BLT-1*$^{-/-}$ mice (Fig. 3*F*). To further elucidate how adipose tissue inflammation is affected by *BLT-1* deficiency, we evaluated the activation of JNK, which has been shown to play a causal role in obesity-induced insulin resistance (28). Indeed, phosphorylation of JNK was significantly decreased in adipose tissue of *BLT-1*$^{-/-}$ mice compared with obese WT mice (Fig. 3*G*). Nevertheless, as reported previously (29), high-fat feeding for 12 wk did not induce insulin

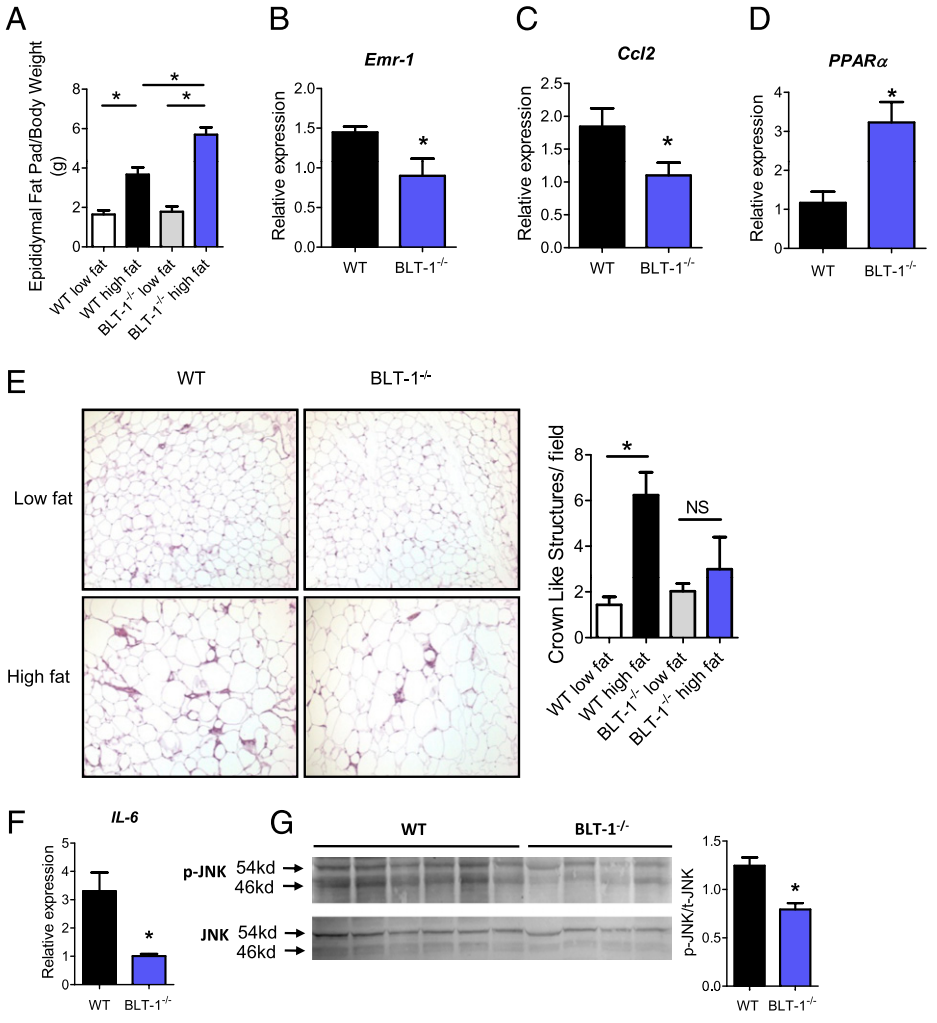

FIGURE 3. Adipose tissue inflammation and macrophage accumulation are decreased in *BLT-1*–deficient mice on a high-fat diet. *A*, Ratio of epididymal fat pad weight to total body weight. *B*, Quantification of mRNA expression of macrophage gene *Emr-1* in adipose tissue isolated from WT or *BLT-1*–deficient mice. *C* and *D*, Quantification of *Ccl2* and *PPARα* in adipose tissue. *E*, Representative histological analysis (H&E stain) of epididymal adipose tissue from WT and *BLT–1* deficient mice (original magnification ×10); *right panel*, quantification of crown-like structures per field. *F*, Adipose tissue mRNA expression of *IL-6* in WT and BLT-1–deficient mice fed a high-fat diet. *G*, Phosphorylation of JNK in adipose tissue of WT and *BLT-1*–deficient mice fed a high-fat diet. Data are presented as means ± SEM. *$p < 0.05$ by one-way ANOVA (*A*, *E*) or Student *t* test (*B–D*, *F*, *G*); n = 3–12/group.

resistance in the adipose tissue, as assessed by insulin-stimulated phosphorylation of Akt (data not shown). These results indicate that *BLT-1*$^{-/-}$ mice are protected from infiltration of inflammatory monocytes into adipose tissue and consequently from adipose tissue inflammation.

Deficiency of BLT-1 alters adipose tissue macrophage phenotypes

Adipose tissue from lean mice contains resident M2 macrophages that serve a homeostatic role. It is now widely accepted that macrophages that infiltrate the expanded adipose tissue in obesity are derived from circulating monocytes and assume an M1 phenotype (22, 30). Because *BLT-1* deficiency prevented the increase in CD11b^{+} monocytes in DIO and also decreased ATM accumulation, we questioned whether the phenotype of ATMs was affected by *BLT-1* deficiency. For this, we isolated stromal vascular cells from adipose tissue obtained from WT or *BLT-1*$^{-/-}$ mice and assessed the surface expression of CD11c and macrophage galactose-type C-type lectin 1 (MGL-1), markers of M1 and M2 macrophages, respectively, on the total macrophage (F4/80^{+}) population (22, 30). High-fat feeding markedly increased the population of ATMs expressing CD11c and lacking MGL-1 (Fig. 4*A*). Importantly, this increase was prevented in *BLT-1*$^{-/-}$ mice. Moreover, the amount of ATMs expressing MGL-1 and lacking CD11c (M2) was significantly increased in *BLT-1*$^{-/-}$ mice compared with WT mice. Consistent with a recent report, we also identified a CD11c^{+}MGL-1^{+} population of ATMs, which was present in both WT and *BLT-1*$^{-/-}$ mice, but this population was significantly increased only in the *BLT-1*$^{-/-}$ mice (30). Representative dot plots of the F4/80^{+} ATMs are shown in Fig. 4*B*. We next measured the expression of characteristic M2 genes to rigorously assess how ATM phenotype is affected by *BLT-1* deficiency. The expression of M2 genes, *Ym-1* and *PPARγ*, was significantly increased in *BLT-1*$^{-/-}$ mice compared with obese WT mice, whereas the expression of other M2 genes, such as *IL-10* and *Arg-1*, was not affected (Fig. 4*C*). Collectively, these results suggest that *BLT-1* deficiency prevents accumulation of M1 ATMs in obesity and thus alters the balance between inflammatory M1 and M2 ATMs.

Obesity-induced hepatic steatosis and inflammation are alleviated by BLT-1 deficiency

Macrophage-mediated adipose tissue inflammation is sufficient to promote insulin resistance in other insulin-sensitive tissues, such as the liver and skeletal muscle (31–33). In particular, insulin resistance in the liver can be driven by fatty acid release from adipose tissue, resulting in hepatic triglyceride accumulation, and it can also arise from increased production of inflammatory cytokines from the adipose tissue (31, 32). Thus, we asked whether a deficiency of BLT-1 would alleviate insulin resistance and hepatic steatosis in DIO. Obesity caused profound liver damage in WT mice, as evidenced by increased plasma ALT levels (Fig. 5*A*). Surprisingly, this increase was completely abolished in *BLT-1*$^{-/-}$ mice. The liver/body weight ratio was significantly decreased in *BLT-1*$^{-/-}$ mice compared with obese WT mice (Supplemental Fig. 3*A*). Moreover, the high-density lipoprotein/low-density lipoprotein ratio was significantly decreased in obese WT mice, but this decrease was prevented in *BLT-1*$^{-/-}$ mice (Supplemental Fig. 3*B*). Histological analysis of the liver showed an apparent decrease in fat accumulation in *BLT-1*$^{-/-}$ mice (Fig. 5*B*). Accordingly, the total triglyceride content of the liver was significantly decreased in *BLT-1*$^{-/-}$ mice (Fig. 5*C*). Fat staining with Oil Red O showed a reduction in total fat content of the liver in *BLT-1*$^{-/-}$ mice compared with obese WT mice (Supplemental Fig. 3*C*). In

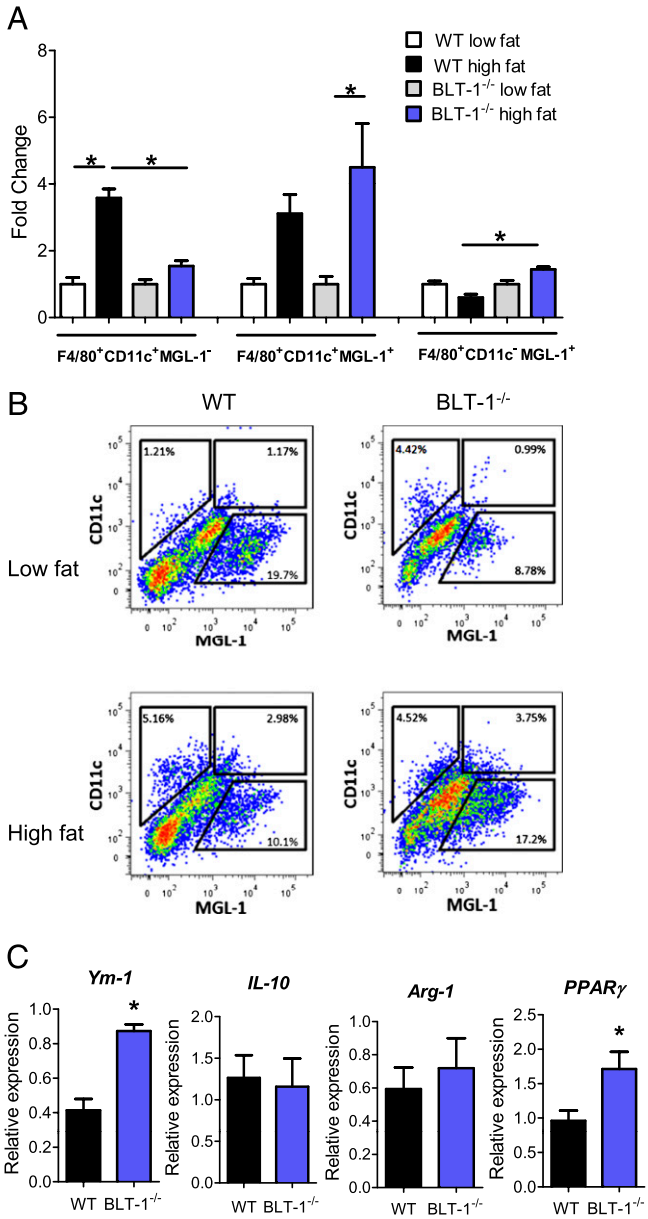

FIGURE 4. *BLT-1* deficiency alters adipose tissue macrophage phenotypes. *A*, Quantification of isolated adipose tissue macrophage populations in WT and *BLT-1*–deficient mice. *B*, Representative flow cytometry dot plots of F4/80^{+} adipose tissue macrophages from WT or *BLT-1*–deficient mice. *C*, Adipose tissue mRNA expression of characteristic alternatively activated adipose tissue macrophage genes. Data are presented as means ± SEM. *$p < 0.05$ by one-way ANOVA (*A*) or Student *t* test (*C*); n = 6–9/group.

contrast to the adipose tissue, *PPARα* transcripts were not modulated by *BLT-1* deficiency in the liver of high fat-fed mice (Supplemental Fig. 3*D*). Because steatosis drives hepatic inflammation, we next evaluated whether the inflammatory NF-κB pathway was affected by *BLT-1* deficiency. Indeed, increased phosphorylation of IκBα, an upstream mediator of NF-κB activation, was observed with DIO in WT mice, whereas this increase was abolished in *BLT-1*$^{-/-}$ mice (Fig. 5*D*). Increased inflammatory signaling through this pathway directly contributes to insulin resistance, so we next evaluated how insulin signaling was affected in the liver of *BLT-1*$^{-/-}$ mice. As expected with this duration of high-fat feeding, the liver was markedly insulin resistant in WT mice, as assessed by reduced insulin-stimulated

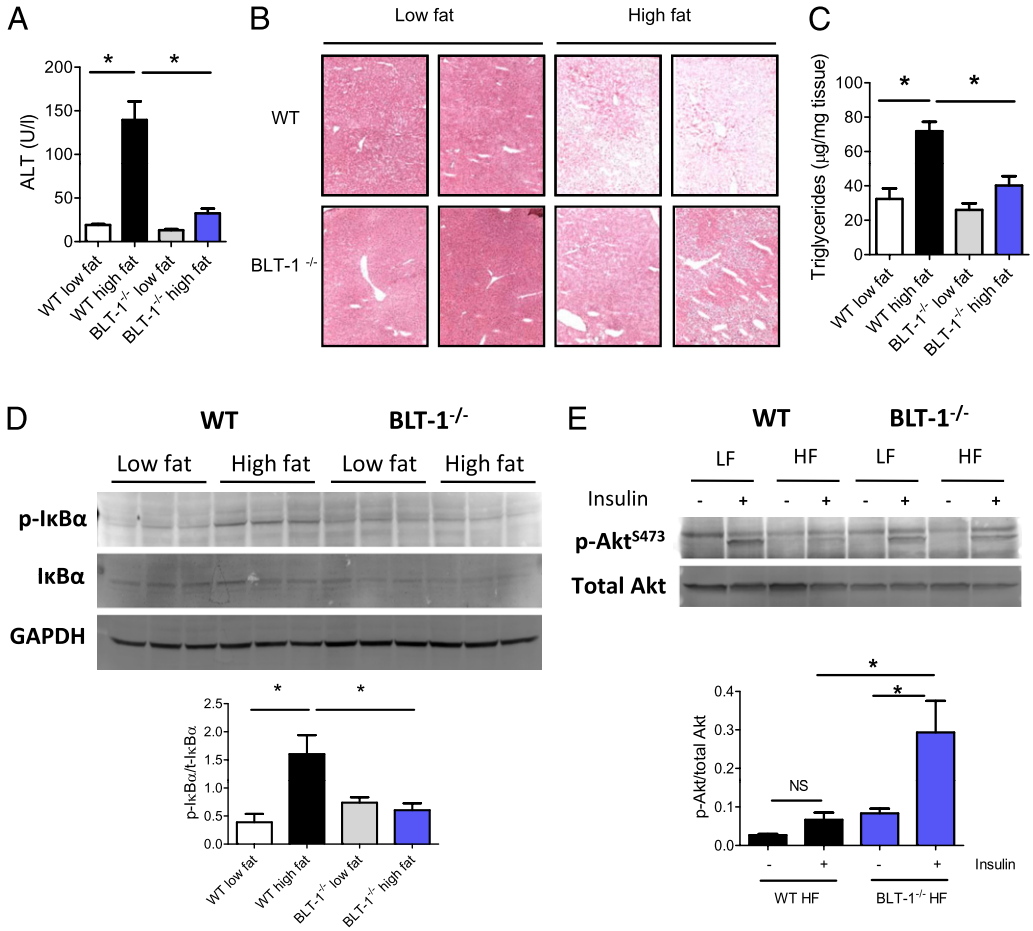

FIGURE 5. Hepatic steatosis, inflammation, and insulin resistance are diminished in *BLT-1*–deficient mice in DIO. *A*, Plasma ALT levels in WT and *BLT-1*–deficient mice. *B*, Histological analysis (H&E staining) of liver tissues from WT or *BLT-1*–deficient mice (original magnification ×10). *C*, Hepatic triglyceride levels in WT or *BLT-1*–deficient mice. *D*, Immunoblot of phospho-IκBα in the liver of WT and *BLT-1*–deficient mice, with quantification shown (*lower panel*). *E*, Insulin-stimulated Akt phosphorylation in liver of WT and *BLT-1*–deficient mice fed a low-fat (LF) or high-fat (HF) diet. *Lower panel*, Quantification of Akt phosphorylation in high fat-fed WT and *BLT-1*–deficient mice. Data are presented as means ± SEM. $*p < 0.05$ by one-way ANOVA; $n = 6$–9/group.

phosphorylation of Akt. Importantly, insulin signaling was largely preserved in the liver of obese *BLT-1*$^{-/-}$ mice (Fig. 5*E*).

Deficiency of BLT-1 preserves insulin signaling in the skeletal muscle in DIO

In addition to the liver, insulin resistance in skeletal muscle is central to altered glucose disposal in DIO. High-fat feeding resulted in pronounced insulin resistance in skeletal muscle, as evidenced by a relative lack of insulin-stimulated Akt phosphorylation in high fat-fed mice (Supplemental Fig. 4). Although the level of insulin-stimulated phosphorylation of Akt in *BLT-1*$^{-/-}$ mice fed a low-fat diet was lower in magnitude than in lean WT mice, obese *BLT-1*–deficient mice were markedly protected from obesity-induced loss in insulin signaling (Supplemental Fig. 4).

Discussion

The LTB_4/BLT-1 axis is an important proinflammatory pathway involved in host defense. During the acute inflammatory response, LTB_4 is one of the first leukocyte chemoattractants generated and it promotes leukocyte migration in response to invading pathogens and tissue injury (8, 12, 14). Indeed, *BLT-1*–deficient mice have defects in leukocyte infiltration in acute peritonitis and show a decrease in leukocyte-mediated bacterial clearance (12, 14, 34). Conversely, increased activation of BLT-1 is associated with chronic inflammatory diseases, and deficiency of *BLT-1* confers protection against the development of arthritis and atherosclerosis. Although BLT-1 contributes to the progression of chronic inflammation and recent studies show that LTB_4 is increased in obesity, a direct causative role of this pathway in DIO and insulin resistance had not been assessed before (16, 17, 19). The results of the current study document a previously unrecognized role of BLT-1 in promoting monocyte recruitment, inflammation, and insulin resistance in obesity.

Obesity promotes the mobilization of monocytes from the bone marrow in part by activating the chemokine receptor CCR2 (23, 24, 35). Global deficiency of *Ccr2* or its ligand, *Ccl2* (MCP-1), in mice results in a failure of monocyte mobilization and is associated with protection from monocyte infiltration into adipose tissue and insulin resistance (24, 35). The current study demonstrates that BLT-1 is also required for obesity-induced increases in peripheral monocytes and subsequent ATM accumulation. Interestingly, BLT-1 deficiency also led to a decrease in the adipose tissue expression of *Ccl2*, suggesting that activation of the BLT-1 pathway may be important for subsequent production of chemokine-driven amplification loops in obesity. Indeed, activation of BLT-1 by LTB_4 induces production of CCL2 in monocytes and, conversely, CCL2 stimulates the production of LTB_4 to establish a positive feed-forward cycle (13, 36). Recently, BLT-1 was shown to be required for the initiation of cytokine and chemokine gradients in K/BxN-induced arthritis (11). Thus, in light

of these previous findings, our current data indicate that activation of BLT-1 may be an important early contributor to the increase in circulating monocytes and monocyte infiltration into tissues in obesity. One potential caveat of our study is that the WT and *BLT-1*–deficient mice used were not littermates. However, the *BLT-1*$^{-/-}$ mice have been backcrossed for more than nine generations onto a C57BL/6 background, and we routinely house the WT mice obtained from the vendor for a 2- to 3-wk period for acclimatization in the same facility before they enter the study. Notably, the high-fat feeding protocol itself extends over 12 wk, during which time the mice were housed under identical conditions, thus minimizing the contribution of differences in housing to the observed phenotype.

Consistent with our findings that BLT-1 expression increases on peripheral blood monocytes in obesity, BLT-1 is also highly expressed on classically activated "inflammatory" human monocytes (CD14^{+}CD16^{-}) (37, 38). Significantly, the expression of BLT-1 is lower on CD14^{+}CD16^{+} "resident" monocytes, and this dynamic expression pattern parallels that of CCR2 (37). Our findings also demonstrate that DIO decreases the expression of *BLT-1* in tissues. Previous studies have documented that BLT-1 expression decreases on leukocytes after they infiltrate arthritic joints and are exposed to ligand LTB_4 (11, 39). Moreover, the expression of both BLT-1 and CCR2 is negatively regulated by proinflammatory cytokines, including IFN-γ and TNF-α (37, 40). This well-documented negative feedback system likely explains why BLT-1 expression decreased in both skeletal muscle and liver of obese mice and was not readily detected in adipose tissue. Although our studies suggest a key role of BLT-1 in mediating tissue recruitment of monocytes in obesity, we cannot rule out that BLT-1 might also be an important factor in perpetuating inflammatory signaling at the tissue level. As noted, recent reports demonstrate that LTB_4 levels are increased in adipose tissue in obesity, and our studies show a profound decrease in the activation of inflammatory signaling in both adipose tissue and liver of *BLT-1*–deficient mice. Thus, it is likely that the LTB_4/BLT-1 pathway plays an important role in both recruitment and local activation of monocytes/macrophages in obesity.

Macrophage polarization toward a classically activated (M1) or alternatively activated (M2) state depends largely on soluble factors, such as cytokines and lipid mediators (38, 41). Adipose tissue expansion in DIO is associated with the infiltration of M1 macrophages that localize to CLS surrounding apoptotic adipocytes (42). A high ratio of inflammatory M1 ATMs (F4/80^{+} CD11c^{+}MGL-1^{-}) to resident M2 ATMs (F4/80^{+}CD11c^{-}MGL-1^{+}) is reflective of both adipose tissue as well as systemic insulin resistance (7, 22, 30). In comparison with WT controls, ATMs were drastically decreased in obese *BLT-1*$^{-/-}$ mice and this was associated with a higher M2/M1 ratio. This observation was confirmed by the identification of higher M2 transcripts, including *Ym-1* and *PPARγ*, in *BLT-1*$^{-/-}$ mice. Because PPARγ controls alternative activation in macrophages and the expression of this nuclear receptor is associated with increased insulin sensitivity (43), it is likely that PPARγ may be in part responsible for the higher proportion of M2 macrophages in adipose tissue of *BLT-1*$^{-/-}$ mice. Additional studies are required to fully elucidate this relationship.

The central role of ATM-mediated inflammation to systemic insulin resistance is evidenced by studies showing that adipocyte-specific expression of *Ccr2* promotes adipose tissue macrophage accumulation and is sufficient to induce insulin resistance in other tissues such as the liver (33). In our studies, *BLT-1*–deficient mice were largely protected from ATM accumulation, and insulin signaling was preserved in the liver as well as in the skeletal muscle. These data suggest that BLT-1 regulates infiltration of macrophages into the adipose tissue and agrees with the paradigm that adipose tissue inflammation drives insulin resistance and inflammation in other insulin-sensitive tissues (31–33). Consistent with this scenario, triglyceride accumulation in the liver was largely blunted in *BLT-1*–deficient mice, which is in agreement with the well-documented phenomenon that lipolysis in adipose tissue promotes hepatic triglyceride accumulation and subsequent insulin resistance (32). Note that we chose a duration of high-fat feeding that is sufficient to drive adipose tissue inflammation with associated insulin resistance in the liver and skeletal muscle, but precedes the development of insulin resistance in the adipose tissue itself, which requires nearly 14 wk of high-fat feeding to develop (29).

Activation of both the JNK and NF-κB pathways directly promotes insulin resistance through serine phosphorylation of the insulin receptor substrate-1 and deficiencies of *jnk1* and *Ikkβ* protect mice from obesity-induced insulin resistance (28, 44). Our study also shows that activation of these inflammatory signaling pathways is decreased in the adipose tissue and the liver of *BLT-1*$^{-/-}$ mice. Importantly, the expression of *IL-6*, which directly promotes insulin resistance in the liver and adipocytes (27), was also decreased in adipose tissue of *BLT-1*$^{-/-}$ mice. These protective effects of *BLT-1* deficiency are in agreement with the known role of BLT-1–dependent signaling in the activation of JNK and NF-κB by LTB_4 in macrophages (15, 16, 45). The activation of these signaling pathways has been shown to underlie the BLT-1–dependent induction of both CCL2 and IL-6 by LTB_4 (46).

Collectively, these studies lend new insight into the mechanisms by which chronic inflammation contributes to insulin resistance and implicate BLT-1 as a key regulator of macrophage accumulation in adipose tissue and systemic insulin signaling. Identification of this pathway points toward promising new avenues for the therapeutic management of obesity and type 2 diabetes.

Acknowledgments

We thank David Young and Laura Wheat for expert technical assistance.

Disclosures

The authors have no financial conflicts of interest.

References

1. Boyle, J. P., T. J. Thompson, E. W. Gregg, L. E. Barker, and D. F. Williamson. 2010. Projection of the year 2050 burden of diabetes in the US adult population: dynamic modeling of incidence, mortality, and prediabetes prevalence. *Popul. Health Metr.* 8: 29.
2. Flegal, K. M., M. D. Carroll, C. L. Ogden, and L. R. Curtin. 2010. Prevalence and trends in obesity among US adults, 1999–2008. *JAMA* 303: 235–241.
3. Hotamisligil, G. S., and E. Erbay. 2008. Nutrient sensing and inflammation in metabolic diseases. *Nat. Rev. Immunol.* 8: 923–934.
4. Olefsky, J. M., and C. K. Glass. 2010. Macrophages, inflammation, and insulin resistance. *Annu. Rev. Physiol.* 72: 219–246.
5. Spiegelman, B. M., and J. S. Flier. 2001. Obesity and the regulation of energy balance. *Cell* 104: 531–543.
6. Weisberg, S. P., D. McCann, M. Desai, M. Rosenbaum, R. L. Leibel, and A. W. Ferrante Jr. 2003. Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* 112: 1796–1808.
7. Lumeng, C. N., J. L. Bodzin, and A. R. Saltiel. 2007. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J. Clin. Invest.* 117: 175–184.
8. Samuelsson, B., S. E. Dahlén, J. A. Lindgren, C. A. Rouzer, and C. N. Serhan. 1987. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science* 237: 1171–1176.
9. Haeggström, J. Z. 2004. Leukotriene A_4 hydrolase/aminopeptidase, the gatekeeper of chemotactic leukotriene B_4 biosynthesis. *J. Biol. Chem.* 279: 50639–50642.
10. 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.

11. Chou, R. C., N. D. Kim, C. D. Sadik, E. Seung, Y. Lan, M. H. Byrne, B. Haribabu, Y. Iwakura, and A. D. Luster. 2010. Lipid-cytokine-chemokine cascade drives neutrophil recruitment in a murine model of inflammatory arthritis. *Immunity* 33: 266–278.
12. Haribabu, B., M. W. Verghese, D. A. Steeber, D. D. Sellars, C. B. Bock, and R. Snyderman. 2000. Targeted disruption of the leukotriene B_4 receptor in mice reveals its role in inflammation and platelet-activating factor-induced anaphylaxis. *J. Exp. Med.* 192: 433–438.
13. Subbarao, K., V. R. Jala, S. Mathis, J. Suttles, W. Zacharias, J. Ahamed, H. Ali, M. T. Tseng, and B. Haribabu. 2004. Role of leukotriene B_4 receptors in the development of atherosclerosis: potential mechanisms. *Arterioscler. Thromb. Vasc. Biol.* 24: 369–375.
14. Tager, A. M., J. H. Dufour, K. Goodarzi, S. D. Bercury, U. H. von Andrian, and A. D. Luster. 2000. BLTR mediates leukotriene B_4-induced chemotaxis and adhesion and plays a dominant role in eosinophil accumulation in a murine model of peritonitis. *J. Exp. Med.* 192: 439–446.
15. Bäck, M., D. X. Bu, R. Bränström, Y. Sheikine, Z. Q. Yan, and G. K. Hansson. 2005. Leukotriene B_4 signaling through NF-κB-dependent BLT1 receptors on vascular smooth muscle cells in atherosclerosis and intimal hyperplasia. *Proc. Natl. Acad. Sci. USA* 102: 17501–17506.
16. Bäck, M., A. Sultan, O. Ovchinnikova, and G. K. Hansson. 2007. 5-Lipoxygenase-activating protein: a potential link between innate and adaptive immunity in atherosclerosis and adipose tissue inflammation. *Circ. Res.* 100: 946–949.
17. Horrillo, R., A. González-Périz, M. Martínez-Clemente, M. López-Parra, N. Ferré, E. Titos, E. Morán-Salvador, R. Deulofeu, V. Arroyo, and J. Clària. 2010. 5-lipoxygenase activating protein signals adipose tissue inflammation and lipid dysfunction in experimental obesity. *J. Immunol.* 184: 3978–3987.
18. López-Parra, M., E. Titos, R. Horrillo, N. Ferré, A. González-Périz, M. Martínez-Clemente, A. Planagumà, J. Masferrer, V. Arroyo, and J. Clària. 2008. Regulatory effects of arachidonate 5-lipoxygenase on hepatic microsomal TG transfer protein activity and VLDL-triglyceride and apoB secretion in obese mice. *J. Lipid Res.* 49: 2513–2523.
19. Chakrabarti, S. K., Y. Wen, A. D. Dobrian, B. K. Cole, Q. Ma, H. Pei, M. D. Williams, M. H. Bevard, G. E. Vandenhoff, S. R. Keller, et al. 2011. Evidence for activation of inflammatory lipoxygenase pathways in visceral adipose tissue of obese Zucker rats. *Am. J. Physiol. Endocrinol. Metab.* 300: E175–E187.
20. Oh, D. Y., S. Talukdar, E. J. Bae, T. Imamura, H. Morinaga, W. Fan, P. Li, W. J. Lu, S. M. Watkins, J. M. Olefsky; Oh da. 2010. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell* 142: 687–698.
21. Mathis, S. P., V. R. Jala, D. M. Lee, and B. Haribabu. 2010. Nonredundant roles for leukotriene B_4 receptors BLT1 and BLT2 in inflammatory arthritis. *J. Immunol.* 185: 3049–3056.
22. Lumeng, C. N., J. B. DelProposto, D. J. Westcott, and A. R. Saltiel. 2008. Phenotypic switching of adipose tissue macrophages with obesity is generated by spatiotemporal differences in macrophage subtypes. *Diabetes* 57: 3239–3246.
23. Takahashi, K., S. Mizuarai, H. Araki, S. Mashiko, A. Ishihara, A. Kanatani, H. Itadani, and H. Kotani. 2003. Adiposity elevates plasma MCP-1 levels leading to the increased CD11b-positive monocytes in mice. *J. Biol. Chem.* 278: 46654–46660.
24. Tsou, C. L., W. Peters, Y. Si, S. Slaymaker, A. M. Aslanian, S. P. Weisberg, M. Mack, and I. F. Charo. 2007. Critical roles for CCR2 and MCP-3 in monocyte mobilization from bone marrow and recruitment to inflammatory sites. *J. Clin. Invest.* 117: 902–909.
25. Lumeng, C. N., S. M. Deyoung, and A. R. Saltiel. 2007. Macrophages block insulin action in adipocytes by altering expression of signaling and glucose transport proteins. *Am. J. Physiol. Endocrinol. Metab.* 292: E166–E174.
26. Narala, V. R., R. K. Adapala, M. V. Suresh, T. G. Brock, M. Peters-Golden, and R. C. Reddy. 2010. Leukotriene B_4 is a physiologically relevant endogenous peroxisome proliferator-activated receptor-α agonist. *J. Biol. Chem.* 285: 22067–22074.
27. Kristiansen, O. P., and T. Mandrup-Poulsen. 2005. Interleukin-6 and diabetes: the good, the bad, or the indifferent? *Diabetes* 54(Suppl. 2): S114–S124.
28. Hirosumi, J., G. Tuncman, L. Chang, C. Z. Görgün, K. T. Uysal, K. Maeda, M. Karin, and G. S. Hotamisligil. 2002. A central role for JNK in obesity and insulin resistance. *Nature* 420: 333–336.
29. Kim, F., M. Pham, E. Maloney, N. O. Rizzo, G. J. Morton, B. E. Wisse, E. A. Kirk, A. Chait, and M. W. Schwartz. 2008. Vascular inflammation, insulin resistance, and reduced nitric oxide production precede the onset of peripheral insulin resistance. *Arterioscler. Thromb. Vasc. Biol.* 28: 1982–1988.
30. Shaul, M. E., G. Bennett, K. J. Strissel, A. S. Greenberg, and M. S. Obin. 2010. Dynamic, M2-like remodeling phenotypes of $CD11c^+$ adipose tissue macrophages during high-fat diet–induced obesity in mice. *Diabetes* 59: 1171–1181.
31. Rosen, E. D., and B. M. Spiegelman. 2006. Adipocytes as regulators of energy balance and glucose homeostasis. *Nature* 444: 847–853.
32. Guilherme, A., J. V. Virbasius, V. Puri, and M. P. Czech. 2008. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat. Rev. Mol. Cell Biol.* 9: 367–377.
33. Kanda, H., S. Tateya, Y. Tamori, K. Kotani, K. Hiasa, R. Kitazawa, S. Kitazawa, H. Miyachi, S. Maeda, K. Egashira, and M. Kasuga. 2006. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J. Clin. Invest.* 116: 1494–1505.
34. Scott, M. J., W. G. Cheadle, J. J. Hoth, J. C. Peyton, K. Subbarao, W. H. Shao, and B. Haribabu. 2004. Leukotriene B_4 receptor (BLT-1) modulates neutrophil influx into the peritoneum but not the lung and liver during surgically induced bacterial peritonitis in mice. *Clin. Diagn. Lab. Immunol.* 11: 936–941.
35. Weisberg, S. P., D. Hunter, R. Huber, J. Lemieux, S. Slaymaker, K. Vaddi, I. Charo, R. L. Leibel, and A. W. Ferrante Jr. 2006. CCR2 modulates inflammatory and metabolic effects of high-fat feeding. *J. Clin. Invest.* 116: 115–124.
36. 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.
37. Pettersson, A., A. Sabirsh, J. Bristulf, K. Kidd-Ljunggren, B. Ljungberg, C. Owman, and U. Karlsson. 2005. Pro- and anti-inflammatory substances modulate expression of the leukotriene B_4 receptor, BLT1, in human monocytes. *J. Leukoc. Biol.* 77: 1018–1025.
38. Gordon, S., and P. R. Taylor. 2005. Monocyte and macrophage heterogeneity. *Nat. Rev. Immunol.* 5: 953–964.
39. Goldman, D. W., and E. J. Goetzl. 1984. Heterogeneity of human polymorphonuclear leukocyte receptors for leukotriene B_4: identification of a subset of high affinity receptors that transduce the chemotactic response. *J. Exp. Med.* 159: 1027–1041.
40. Tangirala, R. K., K. Murao, and O. Quehenberger. 1997. Regulation of expression of the human monocyte chemotactic protein-1 receptor (hCCR2) by cytokines. *J. Biol. Chem.* 272: 8050–8056.
41. Biswas, S. K., and A. Mantovani. 2010. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat. Immunol.* 11: 889–896.
42. Cinti, S., G. Mitchell, G. Barbatelli, I. Murano, E. Ceresi, E. Faloia, S. Wang, M. Fortier, A. S. Greenberg, and M. S. Obin. 2005. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *J. Lipid Res.* 46: 2347–2355.
43. Odegaard, J. I., R. R. Ricardo-Gonzalez, M. H. Goforth, C. R. Morel, V. Subramanian, L. Mukundan, A. Red Eagle, D. Vats, F. Brombacher, A. W. Ferrante, and A. Chawla. 2007. Macrophage-specific PPARγ controls alternative activation and improves insulin resistance. *Nature* 447: 1116–1120.
44. Arkan, M. C., A. L. Hevener, F. R. Greten, S. Maeda, Z. W. Li, J. M. Long, A. Wynshaw-Boris, G. Poli, J. Olefsky, and M. Karin. 2005. IKK-β links inflammation to obesity-induced insulin resistance. *Nat. Med.* 11: 191–198.
45. Rola-Pleszczynski, M., and J. Stanková. 1992. Leukotriene B_4 enhances interleukin-6 (IL-6) production and IL-6 messenger RNA accumulation in human monocytes in vitro: transcriptional and posttranscriptional mechanisms. *Blood* 80: 1004–1011.
46. Sánchez-Galán, E., A. Gómez-Hernández, C. Vidal, J. L. Martín-Ventura, L. M. Blanco-Colio, B. Muñoz-García, L. Ortega, J. Egido, and J. Tuñón. 2009. Leukotriene B_4 enhances the activity of nuclear factor-κB pathway through BLT1 and BLT2 receptors in atherosclerosis. *Cardiovasc. Res.* 81: 216–225.