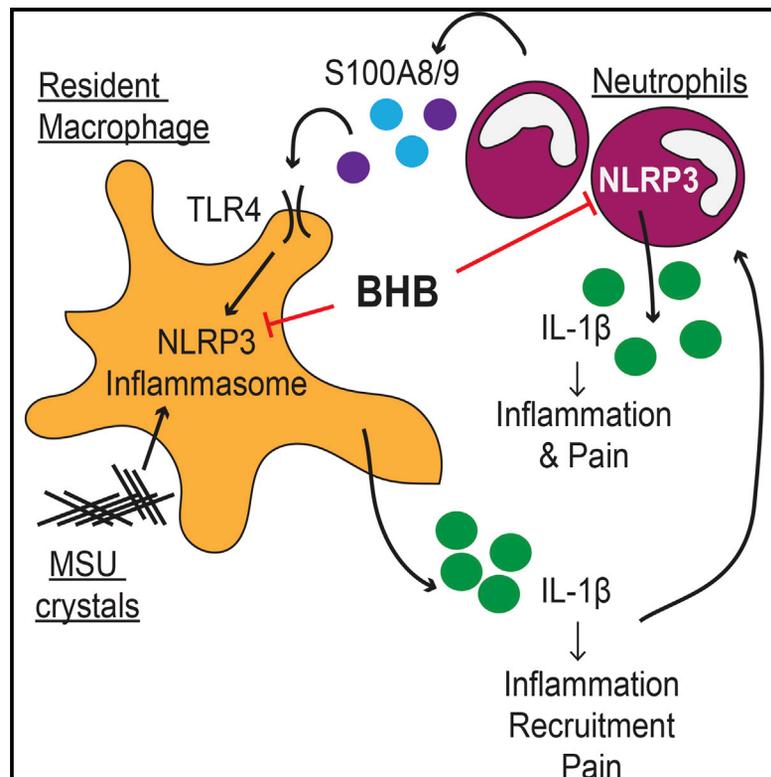


β -Hydroxybutyrate Deactivates Neutrophil NLRP3 Inflammasome to Relieve Gout Flares

Graphical Abstract



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In Brief

NLRP3 inflammasome activation in macrophages and neutrophils drives painful inflammation during gout. Goldberg et al. report that ketogenic diet prevents systemic inflammation and joint damage in a rat model of gouty flare. Mechanistically, the ketone body β -hydroxybutyrate, the most abundant ketone in vivo, inhibits NLRP3/caspase-1-dependent IL-1 β secretion from neutrophils.

Highlights

- Inflammation and joint pathology during gout flare is prevented by ketogenic diet
- BHB inhibits IL-1 β secretion from neutrophils
- Ketogenic diet and BHB inhibit NLRP3 activation in aged neutrophils
- BHB inhibits both priming and assembly steps of NLRP3 activation in neutrophils



β -Hydroxybutyrate Deactivates Neutrophil NLRP3 Inflammasome to Relieve Gout Flares

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SUMMARY

Aging and lipotoxicity are two major risk factors for gout that are linked by the activation of the NLRP3 inflammasome. Neutrophil-mediated production of interleukin-1 β (IL-1 β) drives gouty flares that cause joint destruction, intense pain, and fever. However, metabolites that impact neutrophil inflammasome remain unknown. Here, we identified that ketogenic diet (KD) increases β -hydroxybutyrate (BHB) and alleviates urate crystal-induced gout without impairing immune defense against bacterial infection. BHB inhibited NLRP3 inflammasome in S100A9 fibril-primed and urate crystal-activated macrophages, which serve to recruit inflammatory neutrophils in joints. Consistent with reduced gouty flares in rats fed a ketogenic diet, BHB blocked IL-1 β in neutrophils in a NLRP3-dependent manner in mice and humans irrespective of age. Mechanistically, BHB inhibited the NLRP3 inflammasome in neutrophils by reducing priming and assembly steps. Collectively, our studies show that BHB, a known alternate metabolic fuel, is also an anti-inflammatory molecule that may serve as a treatment for gout.

INTRODUCTION

Gout is a debilitating chronic inflammatory arthritis that afflicts 4% of adults in the United States and is caused by the deposition of monosodium urate (MSU) crystals in the joints (Garrod, 1876; Seegmiller and Howell, 1962). It is widely known that aging and diet-induced lipotoxicity predispose for the development of gout, as the prevalence of gout in elderly persons above the age of 60 years is \sim 10% (Joosten et al., 2010; Li et al., 2013; Roddy and Choi, 2014). MSU crystal-induced gouty flares are characterized by interleukin-1 β (IL-1 β)-driven acute inflammation, fever, and intense pain caused by monocyte-mediated

neutrophil accumulation and activation in joints (Duff et al., 1983). Mechanistically, the inflammatory gouty flares caused by MSU crystals are mediated via the activation of the NLRP3 inflammasome in myeloid cells that causes the release of bioactive IL-1 β and IL-18 (Martinon et al., 2006).

The NLRP3 inflammasome is a carefully regulated inflammatory complex that responds to both endogenous cellular abnormalities and microbial components. Activation of the complex requires two signals: (1) a priming signal (signal 1) that licenses nuclear factor κ B (NF- κ B)-dependent expression of inflammasome complex proteins and (2) a secondary signal (signal 2) that promotes assembly of the complex leading to caspase-1 activation. MSU crystals provide signal 2 of NLRP3 activation, and NLRP3-deficient mice are protected from MSU-induced inflammation (Martinon et al., 2006). Therefore, gout patients face a vicious feed-forward loop in which the chronic deposition and presence of MSU crystals enables constant reactivation of acute inflammatory responses, known as gouty flares, which are associated with intense pain and fever due to high systemic IL-1 β levels (Dalbeth et al., 2016; Duff et al., 1983).

The long-term prophylactic treatment of gout hinges on reducing hyperuricemia. Intriguingly, a major side effect of all current urate-lowering drugs such as xanthine oxidase inhibitors (allopurinol and febuxostat), recombinant uricase (pegloticase), and uricosurics (probenecid and benzbromarone) is the induction of gouty flares (Dalbeth et al., 2016). Despite progress in the field, the relief from inflammation during acute gouty flares currently relies on non-NLRP3-specific therapeutic approaches such as the use of adrenocorticotropic hormone (ACTH), corticosteroids, or nonsteroidal anti-inflammatory drugs (NSAIDs) (Dalbeth et al., 2016 and references therein). Thus, elucidating the endogenous metabolites that regulate NLRP3 inflammasome activation and resolution of inflammation remain a priority for effective management of gout.

Interestingly, metabolic interventions such as caloric restriction (CR) or moderate carbohydrate restriction reduces gout (Dessein et al., 2000). Of note, a classical feature of adaptive



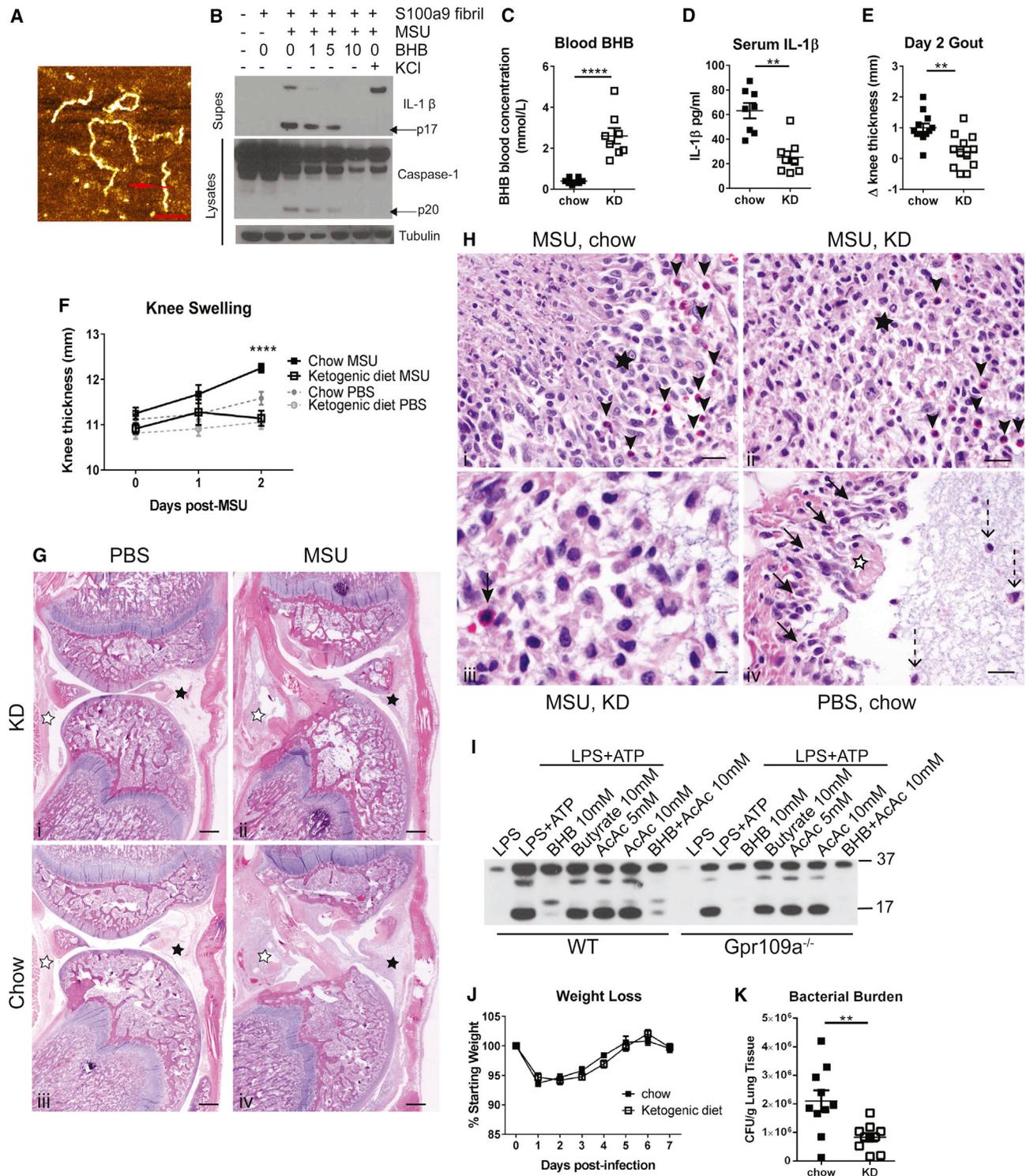


Figure 1. Ketogenic Diet Protects Rats from MSU-Induced Gouty Flare

(A) Atomic force microscopy (AFM) height image of S100A9 amyloid fibrils; scale bar, 120 nm.

(B) Western blot of caspase-1 and IL-1 β activation in BMDMs.

(C) Blood BHB levels after 1 week of ketogenic diet (KD) feeding, prior to injection with MSU.

(D) Serum IL-1 β 48 hr post-MSU injection.

(E) Change in knee thickness 48 hr post-MSU injection.

(F) Knee thickness was measured daily.

(legend continued on next page)

starvation response during negative energy balance is the induction of fatty acid oxidation and production of ketone bodies β -hydroxybutyrate (BHB) and acetoacetate (AcAc) that serve as major substrates for ATP production to support the function of heart and brain (Cahill, 2006). Intriguingly, CR and BHB reduce inflammation and extend lifespan in animals (Edwards et al., 2014; Mitchell et al., 2016), suggesting immune-metabolic interactions driven by BHB may serve as pseudostarvation signals or CR mimetic that could be harnessed against acute inflammatory diseases such as gouty flares.

We made the surprising discovery that myeloid cells also express ketogenic and ketolytic machinery and that BHB blocks the NLRP3 inflammasome in macrophages (Youm et al., 2015). This suggests that ketones may function as regulatory metabolites, serving as endogenous regulators of inflammation (Goldberg and Dixit, 2015). Given that neutrophils are key instigators of inflammation-induced gouty flares, we hypothesized that upregulation of BHB-mediated signals in neutrophils serve as a key immunometabolic checkpoint against gout. Here, we report that BHB prevents NLRP3 inflammasome activation in both mouse and human neutrophils irrespective of the aging process. We found that BHB inhibits the signals that control the priming and assembly of the NLRP3 inflammasome in primary neutrophils and protects against gout.

RESULTS

Elevated BHB Protects against Acute Gout

MSU crystals are the hallmark characteristic of gout (McCarty and Hollander, 1961); the proposed mechanism of a gouty flare is that activation of macrophages by urate crystals recruits neutrophils in an inflammasome-dependent manner, and the accumulation and activation of neutrophils cause substantial pain and swelling (Duff et al., 1983; Martinon et al., 2006). The priming of inflammasome is also a critical step in the process of gouty flare. Interestingly, cytoplasmic S100A8/9 protein heterodimers are highly expressed in neutrophils (Edgeworth et al., 1991) and increase in synovial fluid (Holzinger et al., 2014) and plasma (Ryckman et al., 2003) during a gout flare. Furthermore, S100A8 can activate TLR4 (Vogl et al., 2007), and MSU stimulates the secretion of S100A8/9 from neutrophils (Ryckman et al., 2004). We found that BHB prevented caspase-1 activation and IL-1 β secretion in MSU-treated S100A9-primed bone-marrow-derived macrophages (BMDMs) (Figures 1A and 1B). While S100A9 fibrils provided a priming signal in BMDMs (Figure S1E), they do not function as a danger-associated molecular pattern (DAMP) to provide signal 2 for inflammasome activation in lipopolysaccharide (LPS)-primed BMDM (Figure S1F). Thus, S100A8/9

proteins may represent one of the endogenous sources of priming signal for NLRP3 inflammasome in vivo.

BHB serves as an alternate metabolic fuel during starvation state or in absence of insulin when glucose cannot be utilized. Ketogenic diets (KDs) that are rich in fat and low in carbohydrates are routinely employed to induce BHB or nutritional ketosis, because sustained elevation of BHB through infusion of sodium salts of BHB is contraindicated due to adverse effects on blood acid-base balance. Thus, KD has been safely employed to treat drug-resistant epilepsy (Cahill, 2006). Although IL-1R antagonists have shown benefit in small clinical trials (So et al., 2007, 2010), the high cost of these biologicals and their potential detrimental impact on host defense have limited their use in treatment of gouty flares. Therefore, we next developed a model of gouty arthritis and investigated the induction of nutritional ketosis by feeding KD as a potential therapy against gout. Humans, but not rodents, are susceptible to gout due to loss of the uricase enzyme, which normally functions to prevent high concentrations of uric acid. Therefore, we developed an in vivo gout model in outbred Sprague-Dawley rats by intra-articular injection of MSU crystals in the knee. One week of high-fat, low-carbohydrate KD feeding induced endogenous BHB production (Figure 1C). Rats fed KD were protected from the MSU-induced elevated serum IL-1 β (Figure 1D) and knee swelling observed in the chow-fed rats (Figures 1E and 1F). Pathology analysis of H&E-stained sections of the joints showed that MSU-injected rats displayed combined lesions of intra-articular exudate and synovial soft tissue inflammation (Figure 1G). Intra-articular exudate was characterized by masses of fibrin, amorphous granular foreign material, and clusters of macrophages (Figure 1H). The extent of both intra-articular exudate and synovial inflammation was less severe in KD-fed animals than in those on chow diet. Overall, KD reduced the severity of the inflammatory reactions in MSU-injected knees. Foci of frank necrosis were present in animals on the control diet, but not in those on the ketone-rich diet (Figure 1H). Notably, KD results in elevation of blood ketone bodies BHB as well as AcAc. Our data suggest that the anti-inflammatory effects of ketones are limited to BHB, as neither AcAc nor the microbiota-derived short chain fatty acid butyrate prevented IL-1 β secretion in inflammasome-activated BMDM (Figure 1I). Importantly, reduced inflammatory responses during KD did not increase disease severity in mice infected with *Staphylococcus aureus* (Figures 1J and S1G–S1I) and surprisingly reduced bacterial burdens in the lungs of infected mice (Figure 1K). Taken together, our data show that elevated BHB levels protect against acute gouty flare without compromising the host-defense functions of the immune system.

(G) Representative sections of the femoro-tibial joint showing local tissue reaction (black asterisks) and intrasynovial exudate (white asterisks); scale bar, 500 μ m. (H) Representative images of synovial inflammation. (i and ii) Regions of macrophage infiltration (black asterisk) and neutrophil infiltration (black arrows); scale bar, 20 μ m. (iii) Higher magnification of MSU-induced inflammatory response, where the black arrow points to an infiltrating neutrophil; scale bar, 10 μ m. (iv) Local tissue reaction in a control PBS-injected joint showing focal fibrin exudate (white asterisk) with surrounding macrophages (black arrows) and macrophages within granular amorphous injected material (dashed arrows); scale bar, 20 μ m.

(I) Western blot of IL-1 β secretion from stimulated BMDMs.

(J and K) Body weights (J) and bacterial load (K) after *S. aureus* infection. Data are pooled from at least three independent experiments; in (C)–(F), (J), and (K), data are represented as mean \pm SEM.

Statistical differences calculated by t test (C–E) or two-way ANOVA (F). See also Figure S1.

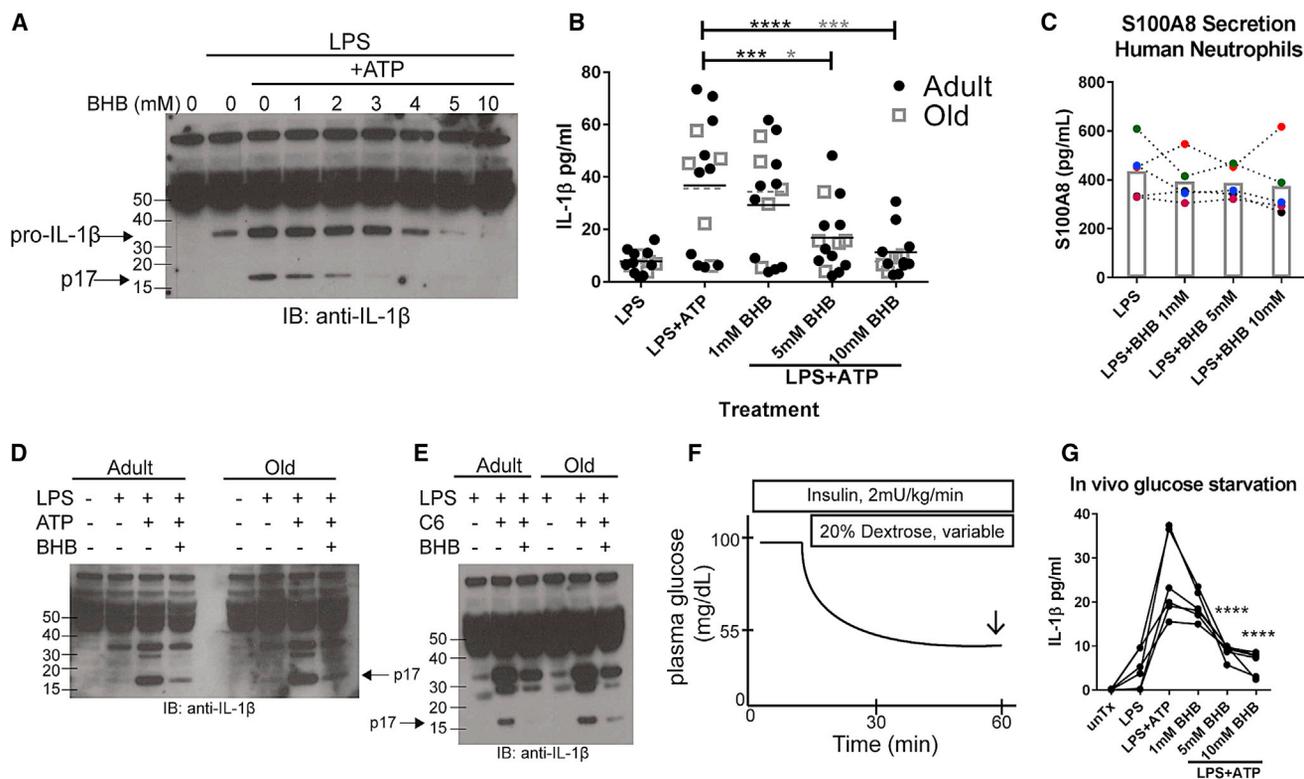


Figure 2. BHB Regulates Neutrophil NLRP3 Inflammasome Activation

(A and B) IL-1 β secretion by stimulated mouse (A) and human (B) neutrophils (lines indicate group mean). (C) Secretion of S100A8 from stimulated human neutrophils. Different-colored symbols connected by dotted line represents a unique individual's response to all treatments. Columns indicate the mean for each treatment. (D and E) Western blot analysis of IL-1 β secretion from LPS-primed adult and old murine neutrophils stimulated with (D) ATP \pm BHB or (E) ceramide \pm BHB. (F) Depiction of insulin-induced hypoglycemia in vivo. Arrow indicates blood collection time point. (G) Neutrophil IL-1 β secretion from hypoglycemic subjects. Lines connect the responses of each individual for each treatment condition. Statistical differences were calculated by paired one-way ANOVA. See also [Figure S2](#).

BHB Acts on Neutrophils to Block IL-1 β Secretion throughout the Lifespan

Gout flares are mediated by both macrophage and neutrophil activation. Because BHB inhibits NLRP3 inflammasome activation in macrophages (Youm et al., 2015) and in an in vitro model of gout (Figure 1B), we next tested whether BHB regulates IL-1 β secretion from neutrophils. Indeed, BHB dose-dependently inhibited IL-1 β secretion in isolated murine neutrophils (Figures 2A and S2). Aging is a major risk factor for gout, and if not properly managed, gouty flares increase in frequency and intensity in the elderly. Importantly, BHB potently inhibited the NLRP3 inflammasome-induced IL-1 β secretion in neutrophils of young and elderly humans (Figure 2B), but not secretion of S100A8 protein implicated in the propagation of gouty flares (Figure 2C). BHB inhibited IL-1 β secretion in response to classical NLRP3 inflammasome activation (Figure 2D) as well as the age-related lipotoxic DAMP ceramide (Figure 2E) in isolated adult and old murine neutrophils. Ketogenesis is typically induced during hypoglycemia or lack of glucose availability, which may alter neutrophil function. Therefore, we next determined whether neutrophils in humans exposed to hypoglycemia in vivo respond to

BHB (Figure 2F). Indeed, IL-1 β secretion is still sensitive to BHB-mediated inhibition regardless of glucose availability in vivo (Figure 2G). These experiments reveal a regulatory role for BHB in neutrophil inflammasome activation regardless of the host's age.

BHB Reduces Urate-Crystal-Induced Inflammation during Aging

Neutrophils are reported to accumulate several defects during aging, including impaired Toll-like receptor (TLR) signaling (Qian et al., 2014) and NETosis (Hazeldine et al., 2014). Neutrophils are also associated with immunopathology during infection in aged hosts (Bou Ghanem et al., 2015; Menter et al., 2014). Bone marrow neutrophils from aged mice exhibited no remarkable differences in abundance or expression of NLRP3 inflammasome proteins compared to young controls (Figure S2). To test neutrophil-intrinsic inflammasome activation defects during aging, isolated neutrophils from adult and old mice were stimulated with LPS+ATP. IL-1 β , but not tumor necrosis factor α (TNF- α) secretion, was NLRP3- and ASC-dependent and no age-related differences were observed (Figures 3A and 3B). Due to the potent inhibitory effects of BHB on aged neutrophils

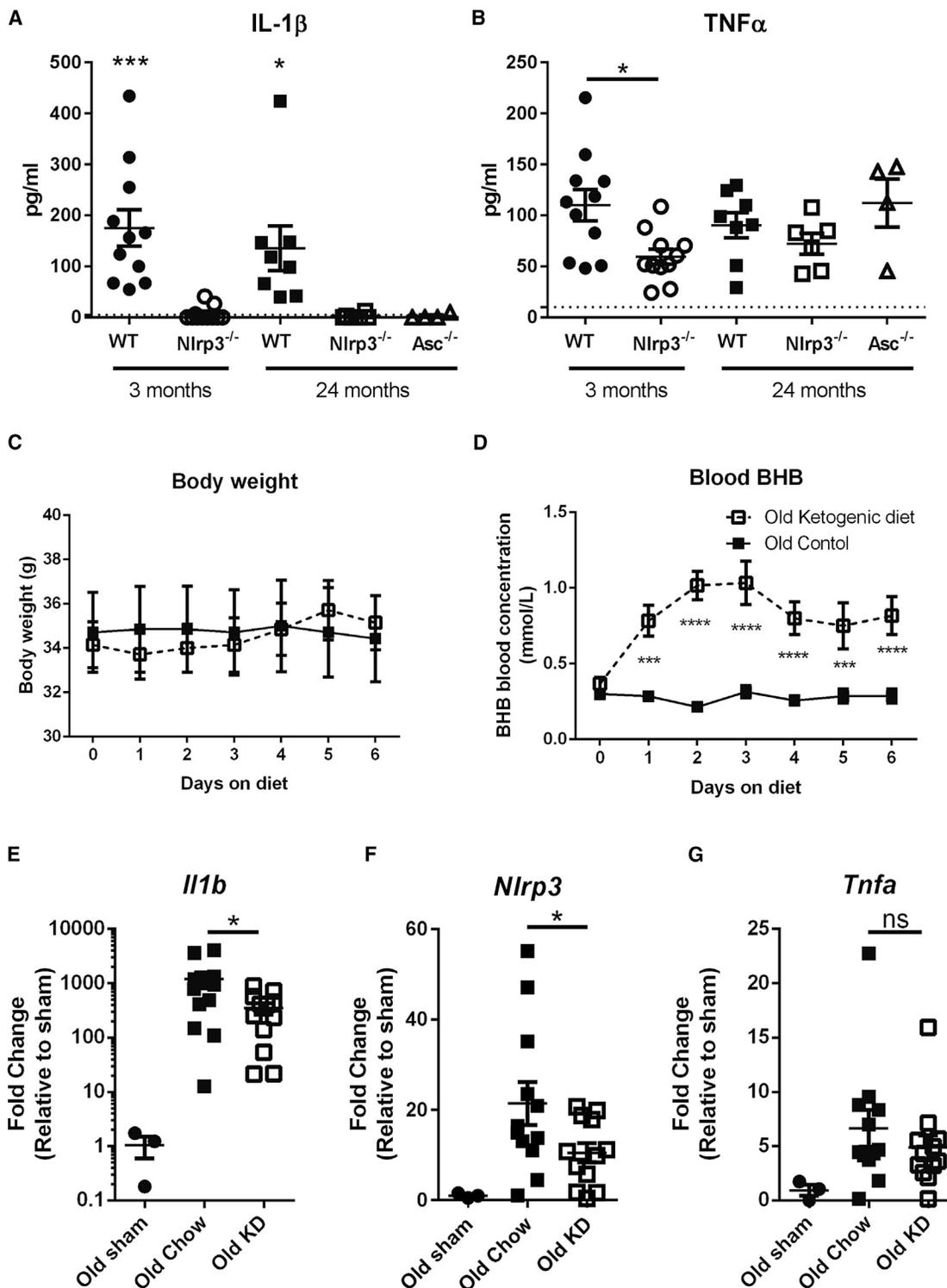


Figure 3. Elevated BHB Protects against MSU-Induced Peritonitis in Aged Mice

(A and B) IL-1 β (A) and TNF- α (B) were measured in culture supernatants after LPS+ATP stimulation of isolated neutrophils from mice of indicated ages and genotypes. Statistical differences were calculated by age-specific t test (3 months) or one-way ANOVA (24 months). Data are pooled from two independent experiments.

(C and D) Body weights (C) and blood BHB concentrations (D) were measured daily in old mice fed KD, prior to MSU injection.

(E–G) Gene expression of (E) *Il1b*, (F) *Nlrp3*, and (G) *Tnfa* within peritoneal cells 4 hr post-MSU. Data are presented as fold change relative to sham and are pooled from three independent experiments.

All data are shown as mean \pm SEM. Statistical differences were calculated by t test between MSU-injected groups. See also Figure S3.

in vitro (Figures 2B, 2D, and 2E), we next sought to determine whether BHB could reduce neutrophilic inflammation in vivo during aging. To induce endogenous ketogenesis, old mice were fed KD for 1 week. During this time, no alterations to body weight (Figure 3C) were observed and blood BHB levels increased rapidly (Figure 3D). KD did not change peritoneal neutrophil infiltration (Figure S3) but prevented upregulation of *Nlrp3* and *Il1b* gene expression, but not the general inflammatory marker *Tnf α* in old mice (Figures 3E–3G) after MSU-induced peritonitis. This suggests that BHB levels can be elevated during aging to reduce acute urate-crystal-induced inflammatory responses.

β -Hydroxybutyrate Inhibits NLRP3 Inflammasome Priming and Assembly

Neutrophils contain both inflammasome-dependent and inflammasome-independent IL-1 β cleavage processes (Cassel et al., 2014; Joosten et al., 2009; Karmakar et al., 2015; Mankan et al., 2012). In our experimental conditions, neutrophil IL-1 β secretion, in response to several DAMPs, including extracellular ATP, the crystalline silica, or lipotoxic ceramide, was entirely caspase-1/11-dependent (Figure 4A). In cell-free assays, BHB had no effect on enzyme activity of neutrophil serine proteases elastase (Figure 4B) or cathepsin G (Figure 4C), both of which have been implicated in IL-1 β cleavage (Cassel et al., 2014; Guma et al., 2009). We next investigated the mechanism by which BHB inhibits NLRP3 inflammasome activation in neutrophils. GPR109a can bind BHB on the cell surface to inhibit inflammation; however, we found that niacin, a high-affinity ligand for GPR109a, failed to impact neutrophil IL-1 β cleavage, suggesting that in vitro GPR109a signaling is dispensable for neutrophil inflammasome activation (Figure 4D). Ketogenesis occurs during energy restriction, a physiological state in which autophagy is induced. However, BHB's inhibition of IL-1 β secretion does not rely on autophagy, because treatment of neutrophils with the autophagy inhibitor 3-MA did not prevent BHB-mediated inhibition of IL-1 β secretion (Figure 4E). Similarly, treating cells with the tricarboxylic acid (TCA) cycle entry inhibitor (aminoxy)acetic acid hemihydrochloride (AOA) did not prevent BHB's inhibition of IL-1 β secretion, suggesting oxidation of BHB is not required for its effect (Figure 4E). Interestingly, the non-oxidizable chiral enantiomer S-BHB, which cannot enter the TCA cycle, also inhibited IL-1 β secretion from neutrophils (Figure 4F), suggesting that in the presence of glucose in vitro, myeloid cells energetically spare BHB to block inflammasome activation.

Unaltered caspase-11 activation or gasdermin D expression in the presence of BHB (Figure S4A) suggested that BHB also does not impact neutrophil pyroptosis (He et al., 2015; Kayagaki et al., 2015; Shi et al., 2015) and BHB did not alter cell viability (Figure S4B). BHB treatment led to increased histone H3 acetylation (Figure 4G), probably due to its reported histone deacetylase (HDAC) inhibitor activity (Shimazu et al., 2013). Interestingly, BHB inhibited phosphorylation of NF- κ B (Figure 4H), a necessary signaling event for inflammasome activation, confirming previous findings (Fu et al., 2014). Finally, BHB was tested in a mouse model of the severe human disease familial cold autoinflammatory syndrome (FCAS), which contains an L351P nucleo-

tide substitution in *Nlrp3* that facilitates constitutive inflammasome assembly (Brydges et al., 2009; Yu et al., 2006). IL-1 β secretion from neutrophils in this FCAS model was dose-dependently inhibited by treatment with BHB-conjugated nanolipogels (Figure 4I). These data suggest that the inhibitory effect of BHB upon NLRP3 inflammasome activation is two-pronged: (1) it prevents TLR4-mediated priming, and (2) it blocks the physical assembly of the NLRP3 inflammasome complex.

DISCUSSION

Gout is a chronic disease characterized by recurrent painful gouty flares. The incidence of gout has steadily increased, and individuals over age 65 account for the majority of gout-related hospitalizations (Lim et al., 2016). Although a primary treatment strategy for gout is to lower uric acid levels, many of these medications induce gouty flares, presumably due to disruption of tophi, which results in poor adherence by patients. We found that elevated blood BHB levels protected outbred rats from joint swelling and systemic inflammation after intra-articular injection of MSU crystals. Reduced joint swelling was due to a qualitative reduction in the inflammatory response, as pathology analysis revealed reduced tissue damage despite similar neutrophil and macrophage infiltration into the joints.

In vitro, BHB also inhibits neutrophil IL-1 β secretion from adult and elderly individuals. Notably, elevated BHB levels during KD did not increase disease severity during *S. aureus* infection in mice and even reduced bacterial burdens in the lungs of infected mice, suggesting high translational potential of BHB against gouty flares. It was recently reported that glucose metabolism promotes mortality during bacterial infection and LPS sepsis (Wang et al., 2016), which may explain the beneficial effects of KD in *S. aureus*-infected mice, as BHB reduces glucose availability. Notably, other macronutrients in KD can induce hormonal alterations and plasma lipid profile changes in patients. Moreover, at the cellular level, limited glucose availability during KD can induce autophagy, which inhibits the inflammasome. Thus, additional studies are required to rule out the exact contribution of each of these mechanisms in mediating anti-inflammasome effects of KD in vivo in models of gout. Regardless, our findings that BHB can target neutrophil inflammasome have important clinical implications, as an estimated 8 million Americans have gout (Zhu et al., 2011) and the cumulative nature of the disease causes the risk and frequency of gouty flares to increase during aging.

S100A8/9 proteins are also reported to increase during age-related inflammatory diseases, including obesity (Nagareddy et al., 2014), cardiovascular disease (Ma et al., 2012), and Alzheimer's disease (Wang et al., 2014), and in the aged prostate (Yanamandra et al., 2009). This implies that these proteins may provide an endogenous, local signal 1 for NLRP3 inflammasome activation and may be universal biomarkers of inflammation (Vogl et al., 2007). During a gouty flare, neutrophils are recruited to inflamed joints by resident macrophages and secrete a variety of proinflammatory molecules, including S100A8/9 and IL-1 β (Ryckman et al., 2004). BHB inhibited NLRP3 inflammasome activation in S100A9-primed macrophages (Figure 1B). Our data suggest that BHB can break this feed-forward cycle to

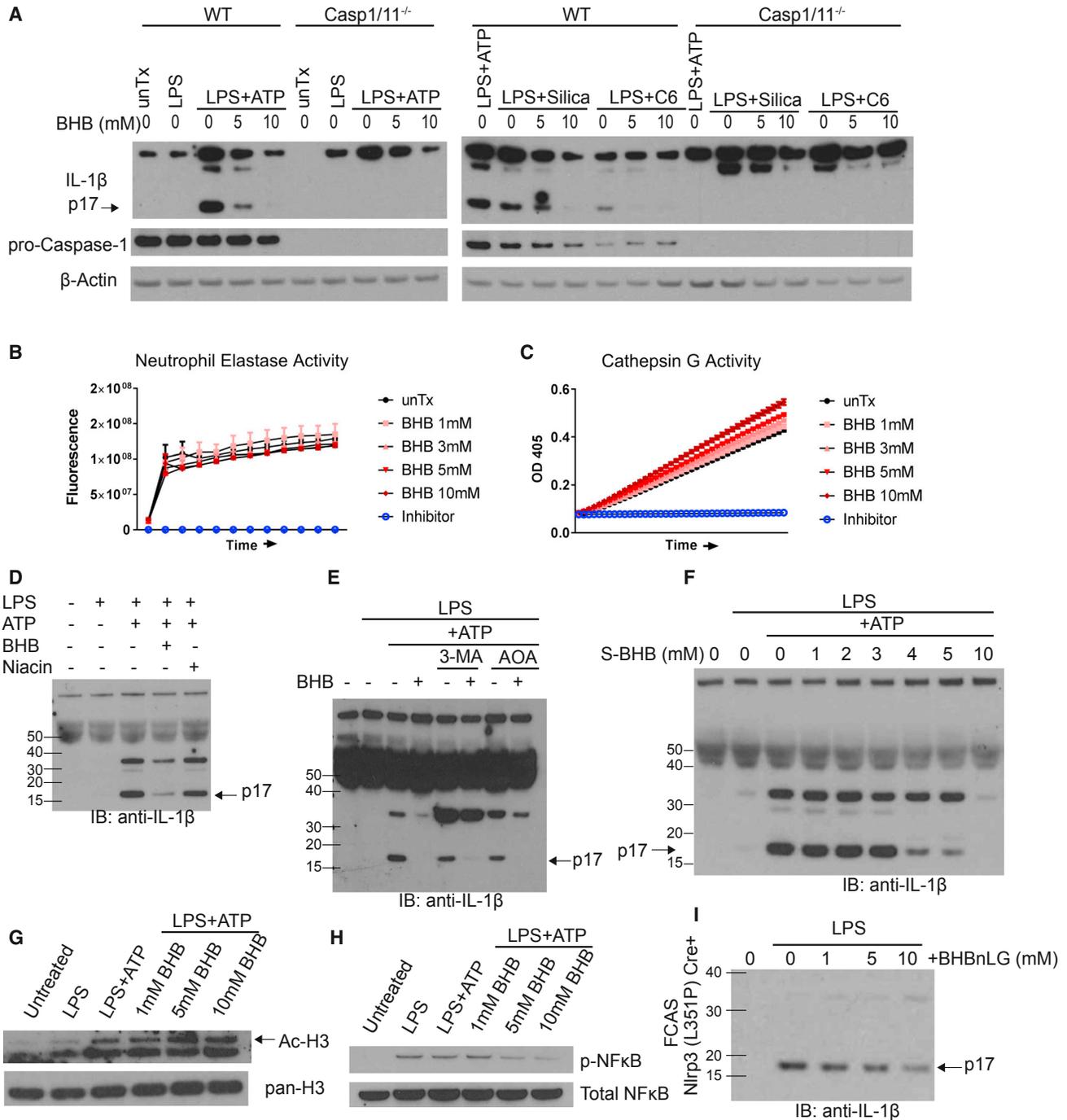


Figure 4. BHB Inhibits Inflammasome Priming and Assembly

(A) Western blot of culture supernatants from LPS-primed wild-type (WT) and caspase-1/11^{-/-} neutrophils after ATP, silica, or ceramide stimulation as indicated. (B and C) Elastase (B) and cathepsin G (C) activity; n = 4, data are presented as mean \pm SEM.

(D-F) Culture supernatants were analyzed for neutrophil IL-1 β secretion by western blot after treatment with BHB \pm (D) niacin, (E) 3-MA or AOA, and (F) S-BHB as indicated.

(G) Total and acetylated H3 expression in cell lysates.

(H) NF- κ B phosphorylation in neutrophil cell lysates.

(I) IL-1 β secretion from neutrophils derived from a mouse model of FCAS bearing activating mutation of Nlrp3.

For all blots, each sample is pooled from at least n = 4 mice per experiment. Each blot is representative of at least two independent experiments. See also Figure S4.

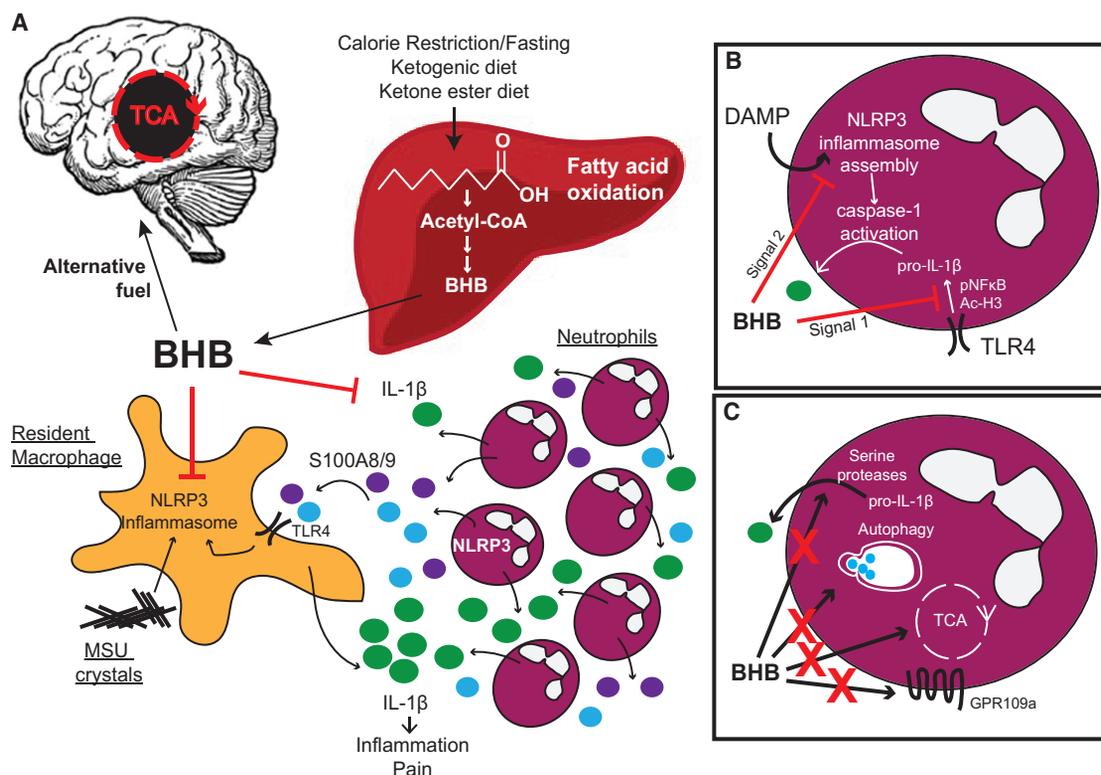


Figure 5. Hypothetical Model for the Dual Role of BHB as a Regulatory Metabolite

(A) The metabolic role of BHB as an alternative energy source during limited energy availability, coordinated with the anti-inflammatory role of BHB on the NLRP3 inflammasome.

(B) BHB inhibits both the priming signal and the secondary assembling signal in neutrophils.

(C) Inhibition of IL-1 β secretion by BHB does not involve inhibition of serine protease activity, autophagy, oxidation in the TCA cycle, or the surface receptor GPR109a, although additional experiments will be needed to further clarify the mechanism.

prevent swelling and inflammation (Figure 5), although defining the exact mechanism will require further experimentation. Neutrophils can secrete IL-1 β by caspase-1-independent mechanisms. Although we were not able to detect IL-1 β secretion from caspase-1/11 knockout mice, cell-free assays revealed no effect on enzymatic activity of serine proteases neutrophil elastase or cathepsin G. These data highlight the targeted effects of BHB upon the NLRP3 inflammasome, making it an ideal candidate for preventing NLRP3-driven inflammation.

Together with our prior findings that BHB inhibits NLRP3 inflammasome activation in macrophages (Youm et al., 2015) and our current data that BHB also regulates neutrophil inflammasome activation, we propose that strategies to increase BHB levels are likely to be therapeutically beneficial in gout patients. Furthermore, the NLRP3-targeted effects of BHB make it ideal for reducing or preventing many age-related inflammatory disease that have been shown to be driven by chronic NLRP3 inflammasome activation (Youm et al., 2013). Adherence to a high-fat low-carbohydrate KD is difficult and can promote dyslipidemia in gout patients. However, ketone esters have been delivered orally to humans, which increased circulating BHB levels and enhanced physical performance in athletes (Clarke et al., 2012; Cox et al., 2016), and should be explored for

inducing mild ketosis to prevent inflammation in individuals with gout.

EXPERIMENTAL PROCEDURES

Animals

All animals were housed under specific-pathogen-free conditions under a 12-hr light/dark cycle. All mice used were on the C57BL/6 genetic background. See Supplemental Experimental Procedures for details of mouse and rat strains. KD (Research Diets D12369B) was initiated 1 week prior to experimental manipulation. Blood BHB concentrations were measured with Precision Xtra β -ketone strips. All animal experiments were performed in compliance with the Yale University Institutional Animal Care and Use Committee.

Human Subjects

Healthy adult (18–45 years) and old (>65 years) males and females with no current steroid use were recruited. Individuals were not fasting at time of peripheral blood collection, except for hypoglycemic studies. Insulin was used to induce hypoglycemia, see Supplemental Experimental Procedures for detailed procedure. Informed consent was obtained from all subjects and all studies were approved by the Institutional Review Committee of Yale University.

Cell Isolation and Activation

Neutrophils were isolated from bone marrow (mice) or peripheral blood (humans) by negative magnetic selection (Stem Cell Technology; Figure S2).

BMDM were generated and all cells were stimulated as previously described (Youm et al., 2015), see [Supplemental Experimental Procedures](#) for detailed treatment conditions.

In Vitro Activation Measurements

All biochemical analysis methods are described in detail in [Supplemental Experimental Procedures](#). Cytokine secretion was measured by Multiplex (Figures 3A and 3B; IL-1 β and TNF- α from Life Technologies) or ELISA (human IL-1 β from eBioscience 88-7261-22; S100A8 from Thermo EHS100A8) according to manufacturers' protocols. Elastase (ab118971) and cathepsin G (ab204693) enzymatic activity was assessed using kits (Abcam) according to manufacturer's instructions.

Peritonitis Model

Peritonitis was induced by intraperitoneal (i.p.) injection of MSU crystals (InvivoGen). Mice were injected with 2.5 mg MSU in PBS. 4 hr later, total peritoneal cells were collected by lavage. Cells were counted using a hemacytometer, and phenotype and gene expression were analyzed by flow cytometry and RT-PCR, respectively.

Staphylococcus aureus Infection

Mice were infected intranasally with 10⁸ colony-forming units (CFUs) or 10⁶ CFUs of *S. aureus* (strain 14458). Bacterial burdens were determined by plating serial dilutions 24 hr post-infection. Bronchoalveolar lavage fluid (BALF) was collected by washing lungs three times with 1 mL sterile PBS.

Gout Model

Gout was induced in rats by intra-articular injection of 1.25 mg MSU in the knee. Knee thickness was measured with digital calipers. IL-1 β was measured in serum by ELISA (eBioscience BMS630). Knees were fixed and decalcified in Bouin's solution (Sigma). Tissue sectioning and H&E staining were performed by the Yale Mouse Research Pathology and Histology Core. For pathology analysis, all sections were taken from the mid-sagittal region of the femoro-tibial joint, encompassing cruciate ligaments and menisci. Images are oriented with the patellar ligament on the right. In high-power images, all images were taken from the anterior synovial tissue.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism software (GraphPad) as indicated in the text and figure legends. $p < 0.05$ was considered statistically significant for all tests. All experiments were performed at least twice. All graphs shown are combined from all replicates of each experiment, and each data point represents an individual test subject. All data are expressed as mean \pm SEM unless otherwise specified. For all statistical differences, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. Western blot experiments were repeated at least three times, each time pooling cells from $n = 3$ –5 mice.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2017.02.004>.

AUTHOR CONTRIBUTIONS

E.L.G. performed experiments and data analysis and prepared the manuscript. J.L.A. performed gout experiments and clinical evaluations. R.D.M. performed infection experiments. C.W. and L.A.M.-R. provided S100A9 reagents and expertise. A.C.S. provided human samples. R.I.H. provided expertise and samples from hypoglycemic clamp studies. C.J.Z. evaluated gout pathology. A.I. designed and interpreted the infection experiments. V.D.D. conceived and supervised the project, interpreted the data, and prepared the manuscript.

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