3-Hydroxyl-3-methylglutaryl Coenzyme A (HMG-CoA) Reductase Inhibitor (Statin)-induced 28-kDa Interleukin-1β Interferes with Mature IL-1β Signaling*

Received for publication, April 4, 2014, and in revised form, April 24, 2014. Published, JBC Papers in Press, April 30, 2014, DOI 10.1074/jbc.M114.571505

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Background: HMG-CoA reductase inhibitors (statins) have anti-inflammatory effects, the molecular mechanisms of which remain unclear.
Results: Statin treatment of macrophages induces secretion of a 28-kDa form of IL-1β that interferes with mature IL-1β signaling.
Conclusion: Statins may dampen inflammation through induction of an anti-inflammatory form of IL-1β.
Significance: These observations may provide clues toward elucidating the in vivo anti-inflammatory effects of statins.

Multiple clinical trials have shown that the 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors known as statins have anti-inflammatory effects. However, the underlying molecular mechanism remains unclear. The proinflammatory cytokine interleukin-1β (IL-1β) is synthesized as a non-active precursor. The 31-kDa pro-IL-1β is processed into the 17-kDa active form by caspase-1-activating inflammasomes. Here, we report a novel signaling pathway induced by statins, which leads to processing of pro-IL-1β into an intermediate 28-kDa form. This statin-induced IL-1β processing is independent of caspase-1 activating inflammasomes. The 28-kDa form of IL-1β cannot activate interleukin-1 receptor-1 (IL1R1) to signal inflammatory responses. Instead, it interferes with mature IL-1β signaling through IL1R1 and therefore may dampen inflammatory responses initiated by mature IL-1β. These results may provide new clues to explain the anti-inflammatory effects of statins.

Statins are competitive inhibitors of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the mevalonate pathway for cholesterol biosynthesis (Fig. 1) (1). Large scale randomized clinical trials have shown that statins provide benefits in prevention of both primary and secondary cardiovascular diseases (2–6). However, the striking benefit observed with statin treatment in patients with a dynamic range of cholesterol levels suggested that effects other than lipid-lowering may also contribute to these beneficial effects observed clinically. It has been shown in human patients that statin treatment reduces circulating C-reactive proteins, a biomarker of inflammation, and proinflammatory cytokine levels (7–9). These observations instigated enthusiasm to extend the application of statins to treat inflammatory conditions beyond atherosclerosis, such as sepsis (10), inflammatory arthritis (11, 12), diabetes (13), asthma (14) as well as neural degenerative diseases such as Alzheimer disease (15), Parkinson disease (16), and multiple sclerosis (17, 18).

The interleukin-1 family of proinflammatory cytokines plays an important role in the orchestration of innate immune responses (19, 20). Of note are the prototypical IL-1 family members, IL-1β and IL-18. IL-1β and IL-18 are atypical cytokines that are synthesized as cytoplasmic inactive forms. It is well established that maturation and secretion of these cytokines depend on inflammasomes (21). Recent studies have demonstrated that IL-1β and inflammasomes may contribute to the pathogenesis of atherosclerosis because cholesterol crystals can activate NLRP3 inflammasomes leading to production of mature IL-1β, which promotes inflammation and potentially aggravates the disease (22).

It is known that statins have anti-inflammatory functions (23, 24). It is also reported that statins can induce secretion of proinflammatory cytokine IL-1β (25–28). The latter observation is in direct contradiction to the overall anti-inflammatory effects of statins observed in both clinical practice and in experimental animal studies. The relationship of IL-1β to the anti-inflammatory traits of statins remains enigmatic and paradoxical. Understanding the role of IL-1β in the anti-inflammatory effects of statins may help design novel interventions for cardiovascular diseases. It may also provide mechanistic rationale for expanding the use of statins to treat inflammatory diseases beyond cardiovascular disease.

In the present study, we characterized the biochemical nature and function of IL-1β induced by statins.
that stimulation of mouse macrophage or human PBMCs with statins after LPS priming led to processing of IL-1β into a novel 28-kDa form. This process is independent of caspase-1 activating conventional inflammasomes but can be inhibited by pan-caspase inhibitors. Interestingly, this 28-kDa form of IL-1β interferes with mature IL-1β function and potentially contributes to the anti-inflammatory effects of statins in vivo.

EXPERIMENTAL PROCEDURES

Bone Marrow-derived Macrophages and Cell Culture—Mouse bone marrow-derived macrophages (BMDMs) were prepared as described previously (29). Wild type C57BL/6 mice were purchased from The Jackson Laboratory. Caspase-1 KO mice were from Dr. Richard Flavell (30). Caspase-8/Rip3 DKO as well as Rip3 KO BMDMs were derived from bones of the respective genotypes that were kind gifts from Dr. Edward Mocarski (31). Rac1, Rac2 KO, as well as Rac1 and -2 DKO BMDMs were derived from bones of respective genotypes that were kind gifts from Dr. Michael Glogauer (32). Trefoil family factor-2 (TFF2) KO mice were originally from Dr. Timothy Wang (33) and were bred in-house. All of the animals were housed in the specific pathogen-free (SPF) animal facility at the Lazare Research Building under an animal protocol approved by the Institutional Animal Use and Care Committee (IACUC) of the University of Massachusetts Medical School. Human PBMCs were isolated from peripheral blood according to a protocol approved by the institutional Review Board committee of the University of Massachusetts Medical School. Cells were cultured in DMEM with 10% fetal bovine serum in 5% CO₂ in a humidified incubator.

ELISA for Measuring IL-1β and TNFα, Rantes, and Western Blot for Determining IL-1β Secretion in Cell Culture Supernatants—BMDMs were prepared by culturing bone marrow cells in the presence of supernatants from L929 cells for 8 days. On day 8, BMDMs were harvested and plated at 2 × 10⁵ cells/well in 96-well plates for ELISA or 2 × 10⁶ cells/well in 12-well plates for immunoblotting. Cells were primed with ultrapure LPS (from Escherichia coli O111:B4, Invivogen) for 2 h, followed by stimulation with simvastatin or cerivastatin (Sigma) for 6 h, or nigericin for 1 h. IL-1β p17 and caspase-1 p20 immunoblots were conducted as described (34) with antibodies from Adipogen (caspase-1 p20) and R&D Systems (IL-1β). The antibodies against β-actin were from Sigma. ELISAs were performed with commercial kits from R&D Systems and according to the manufacturer’s instructions. Western blot analysis of IL-1β in culture supernatants was described previously (29).

Edman Amino-terminal Amino Acid Sequencing—Wild type BMDMs were primed with LPS (200 ng/ml) for 2 h followed by simvastatin (10μM) treatment for 6 h in OptiMEM medium. Culture supernatants were harvested, passed through a 0.45-μm filter, and IL-1β enriched by affinity chromatography using anti-IL-1β antibody (gift of Abbvie, Worester, MA) immobilized onto Sepharose-4 Fast Flow beads (GE Healthcare). IL-1β was eluted from the antibody affinity column, concentrated, resolved on 4—20% gradient gel (Bio-Rad), and transferred onto PVDF (Immun-Blot® PVDF, Bio-Rad) membrane. The 28-kDa IL-1β band was visualized by Coomassie Blue staining and excised and analyzed by Edman amino-terminal sequencing.

Preparation of Recombinant IL-1β and Measurement of IL-1β-induced IL-6 Production in TTF2 KO BMDMs—Mammalian expression vector pMSCVpuro (Clontech) with a phosphoglycerate kinase (PGK) promoter-driven puromycin-resistant cassette were engineered to express mouse pro-IL-1β, a 28-kDa IL-1β-derived from the procytokine, and the mature 17-kDa IL-1β with a signal peptide. These plasmids were used to transfect HEK 293T cells; cell lines stably secreting various forms of IL-1β were established by puromycin selection after transfection. The concentration of IL-1β in the culture supernatants from these stable HEK 293T cells was determined by ELISA using a mouse IL-1β ELISA kit (R&D Systems). For measuring the biological effects of the different forms of IL-1β, we used macrophages deficient in TFF2. TFF2 knock-out macrophages are hyper-responsive to IL-1β (33), and hence can be used as a very useful bioassay to detect the activity of IL-1β. TFF2 KO BMDMs were seeded 2 × 10⁵/well in a 96-well plate. Culture supernatants containing different forms of recombinant IL-1β were added to a final concentration of 10 ng/ml in DMEM with 10% FBS and antibiotics. Cells were incubated at
37 °C, 5% CO₂ overnight. The concentration of IL-6 that was induced was determined by assaying culture supernatants by ELISA (R&D Systems).

RESULTS

Statin Treatment of Macrophages Induces Processing of pro-IL-1β into a 28-kDa Form—To study the effect of statins on IL-1β processing, we exposed LPS-primed BMDMs to simvastatin. Simvastatin alone did not stimulate IL-1β secretion. However, when treated with simvastatin after LPS priming, BMDMs secreted robust amounts of IL-1β (Fig. 2A). Secretion of TNFα was also determined as a control (Fig. 2B). Because ELISA measurement of IL-1β cannot differentiate between mature and pro-IL-1β, cell culture supernatants of simvastatin-stimulated LPS-primed BMDMs were concentrated and subjected to Western blotting analysis with anti-mouse IL-1β antibody. The major species of IL-1β is a protein with an approximate molecular mass of 28 kDa in the LPS and simvastatin-stimulated culture supernatant (Fig. 2C), whereas LPS plus nigericin-stimulated BMDMs secreted the 17-kDa mature IL-1β (Fig. 2C). The processing of IL-1β into the 28-kDa form after simvastatin stimulation began at 1 h after the addition of simvastatin and was sustained for 7 h (Fig. 2D). In addition to simvastatin, cerivastatin (Fig. 2E) as well as lovastatin (data not shown) also induced processing of pro-IL-1β into the 28-kDa form. To further confirm whether statin treatment can cause similar effect in human cells, human PBMCs were stimulated with simvastatin following LPS priming. IL-1β was processed into a 28-kDa form in statin-treated LPS-primed human PBMCs as well (Fig. 2F).

The Caspase-1-activating Conventional Inflammasome Is Not Essential for Simvastatin-induced IL-1β Processing—Caspase-1-activating conventional inflammasomes are the most extensively studied pathway by which pro-IL-1β is processed into its
mature form. The formation of the inflammasome complex brings the proximity of procaspase-1 molecules that promote autocatalysis of procaspase-1 into its p20 and p10 subunits. Those subunits subsequently form tetramers to catalyze the cleavage of pro-IL-1\(\beta\) (35). Therefore, the presence of a p20 or p10 subunit on Western blots has often been used as an indicator of caspase-1 activation.

When BMDMs were stimulated with LPS and simvastatin, there was robust processing of IL-1\(\beta\) into the 28-kDa form; however, there were no detectable levels of p20 subunits present in the Western blot (Fig. 3A). LPS- and nigericin-stimulated BMDMs produced mature IL-1\(\beta\) as well as the p20 subunit of caspase-1 in the culture supernatant, indicating that caspase-1 had been activated (Fig. 3A). To further rule out an essential role of caspase-1 in statin-induced IL-1\(\beta\) processing, BMDMs were pretreated with the pan-caspase inhibitor Z-VAD. This treatment completely abrogated IL-1\(\beta\) processing into the 28-kDa form upon simvastatin stimulation. Similarly, IL-1\(\beta\) processing into its mature form was also abolished when cells were stimulated with LPS and nigericin in the presence of Z-VAD (Fig. 4A). Those results indicate that one or more caspases may contribute to statin-induced IL-1\(\beta\) processing. However, experiments with BMDMs deficient for caspase-6 (Fig. 5A) or caspase-8 (Fig. 5B) demonstrated that those two caspases are not essential for statin-induced IL-1\(\beta\) processing to a 28-kDa intermediate.

**Simvastatin-induced IL-1\(\beta\) Processing Is Independent of its HMG-CoA Reductase Inhibitory Effects**—Statins act on the mevalonate pathway by competitively inhibiting the rate-limiting enzyme HMG-CoA reductase, which converts HMG-CoA into mevalonate. This inhibition leads to the accumulation of mevalonate, which is then converted into farnesylpyrophosphate and geranylgeranylpyrophosphate, the precursors of post-translational modifications that are essential for certain GTPase signaling and cell membrane remodeling. The inhibition of these reactions by statins results in the inhibition of protein prenylation, which is essential for the activation of certain GTPases such as Ras and Rho family members, which are involved in cell proliferation, differentiation, and survival. Therefore, the observation that statins inhibit the activation of caspase-1 and caspase-11 and the processing of IL-1\(\beta\) to its mature form suggests that these effects are due to the inhibition of HMG-CoA reductase, and not due to the inhibition of protein prenylation.
into mevalonate (Fig. 1). We reasoned that statin-induced IL-1β processing may be caused by statin inhibition of the mevalonate pathway and subsequent depletion of the downstream products of the metabolic pathway. To test this idea, we replenished culture medium with mevalonate to offset any deficiency caused by simvastatin treatment and determined whether this treatment would prevent IL-1β processing upon simvastatin treatment. As shown in Fig. 6, supplementation with mevalonate in the culture medium had no effect on statin-induced IL-1β processing into the 28-kDa form, suggesting that this property of statins is independent of their HMG-CoA reductase inhibitory effects.

Simvastatin-induced IL-1β Processing Is Independent of Rac1 or Rac2—It has recently been reported that a signaling cascade involving PI3K, Akt, and Rac1 is involved in statin-induced IL-1β secretion (37). Rac subfamily small GTPases include three members, Rac1, Rac2, and Rac3. Whereas expression of Rac3 is limited to neurological tissues, Rac1 and Rac2 are both expressed in macrophages (38). To elucidate the role of Rac1 and Rac2 in statin-induced IL-1β processing, Rac1 or Rac2 KO or Rac1 and Rac2 DKO BMDMs were stimulated with simvastatin after LPS priming. Expression of Rac1 and Rac2 in different genotypes of BMDMs was determined by Western blot.

FIGURE 4. Statin-induced IL-1β processing depends on caspase activity. A, BMDMs were preincubated with 50 μM pan-caspase inhibitor Z-VAD for 30 min. Cells were then primed with 200 ng/ml LPS followed by stimulation with simvastatin for 6 h or nigericin for 1 h. Processing of IL-1β and caspase-1 were determined by Western blot. B, wild type and ASC−/− BMDMs were primed with 200 ng/ml LPS followed by stimulation with 10 μM simvastatin for 6 h. IL-1β in the culture supernatants was determined by Western blot.

FIGURE 5. Statin-induced IL-1β processing is independent of caspase-6 or -8. Wild type and caspase-6 (A) or caspase-8 (Casp-8)/Rip3 DKO and Rip3 KO BMDMs (B) were primed with 200 ng/ml LPS followed by stimulation with simvastatin for 6 h. IL-1β secretion and proteins in the cell lysate was determined by Western blot.

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production is partially impaired in Rac1 and Rac2 DKO BMDMs, suggesting that Rac proteins are important for TLR induced TNFα production (Fig. 7C).

The 28-kDa Form of IL-1β Interferes with Mature IL-1β Signaling for IL-6 Production—To characterize the biochemical nature of the 28-kDa IL-1β induced by LPS and simvastatin stimulation, we purified the 28-kDa IL-1β from the supernatants of LPS and simvastatin-stimulated BMDMs by antibody affinity chromatography. Purified samples were resolved on a SDS-PAGE and transferred onto a PVDF membrane. The band corresponding to the 28-kDa IL-1β was excised and sent for Edman amino-terminal sequencing. The amino acid sequencing result showed that the 28-kDa IL-1β is a product of cleavage of pro-IL-1β at aspartic acid 26, which corresponds to the first predicted caspase cleavage site (Fig. 8, A and B).

Next, we constructed mammalian expression vectors to express secreted forms of pro-IL-1β, the 28-kDa IL-1β and the mature, 17-kDa IL-1β to further characterize the biological function of the 28-kDa IL-1β. These different forms of IL-1β were expressed in 293T cells and secreted into the culture supernatants. The concentration of each form of IL-1β was determined by ELISA. To evaluate the biological potential of the 28-kDa IL-1β, we used TFF2 KO macrophages, which are hyper-responsive to IL-1β and provide a very sensitive tool for measuring IL-1β biological activity (33). HEK 293T culture supernatants were added to TFF2 KO BMDMs culture medium to achieve a final concentration of 10 ng/ml of IL-1β. The subsequent production of IL-6 was measured by ELISA after 24 h of incubation. Whereas the mature IL-1β stimulated robust IL-6 production, pro-IL-1β, the 28-kDa IL-1β as well as supernatants from vector transfected 293T cells failed to induce any IL-6 production, indicating that the 28-kDa IL-1β does not have any agonist activity by itself (Fig. 8, C and D). However, when TFF2-deficient macrophages were preincubated with the 28-kDa form of IL-1β and then stimulated with the mature, 17-kDa IL-1β, it significantly impaired the mature IL-1β-induced IL-6 production in a dose-dependent fashion, suggesting that the 28-kDa form of IL-1β interferes with mature IL-1β signaling (Fig. 8E).

FIGURE 6. Simvastatin-induced IL-1β processing is independent of its HMG-CoA reductase inhibitory effects. BMDMs were preincubated with mevalonate lactone at different final concentrations for 2 h followed by priming with 200 ng/ml LPS and 10 μM simvastatin stimulation for 6 h. Processing of IL-1β was determined by Western blot.

FIGURE 7. Simvastatin-induced IL-1β is independent of Rac1 or Rac2. Quantitative RT-PCR of Rac1 and Rac2 gene expression in cDNAs prepared from wild type, Rac1−/−, Rac2−/− or Rac1 and Rac2 DKO BMDMs (A). IL-1β (B) and TNFα (C) production upon LPS or LPS and simvastatin stimulation in wild type, Rac1−/−, Rac2−/−, or Rac1 and -2 DKO BMDMs. Western blot analysis of IL-1β processing from wild type or Rac1 and -2 DKO BMDMs after stimulation with LPS, simvastatin or LPS together with simvastatin. GAPDH is used as a loading control.
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DISCUSSION

Statins are the most commonly prescribed drugs for hypercholesterolemia. Multiple clinical studies have shown that in addition to its cholesterol-lowering capacity, statins have a strong anti-inflammatory effect, and this effect is thought to be beneficial in the treatment of atherosclerosis, which is characterized by endovascular inflammation. The anti-inflammatory effect of statins is reflected by the ability of these drugs to reduce the level of inflammation biomarkers such as IL-6 and C-reactive protein (24). Most of those anti-inflammatory effects of statins have been attributed to the inhibition of leukocyte migration into the sub endothelial space as well as class II MHC molecule expression, and the subsequent down-regulation of T-cell activity (39). Inhibition of other immune modulatory molecules such as β2-integrin families has also been proposed (39). However, the exact molecular mechanism by which statins regulate inflammatory processes remains unclear.

IL-1β is an important proinflammatory cytokine that appears to play an important role in the evolution of atherosclerosis (22). IL-1β is atypical in that it is synthesized as an inactive precursor. Caspase-1-activating large protein complexes termed inflammasomes play an important role in processing of pro-IL-1β into its mature, biologically active form (21). There are two caspase-1 cleavage sites in pro-IL-1β that are located at Asp-26 and Asp-116 (Fig. 8A). Processing of pro-IL-1β happens in a sequential manner. Site 1 at Asp-26 is accessible to caspase-1, whereas site 2 at Asp-116 is hidden inside the pro-IL-1β molecule. Cleavage at site 1 and removal of the 3-kDa amino-terminal fragment renders site 2 accessible to caspase-1 following confirmation changes (40). The data reported here strongly suggests that site 1 is also a cleavage site for one or more additional caspasases.

Multiple studies have shown that statin-stimulation of leukocytes may lead to secretion of IL-1β (26–28, 37). However, it was not clear whether such secreted IL-1β is the mature 17-kDa form or proform because the ELISA assay used in those reports cannot differentiate between the various forms of IL-1β. It also is not clear whether those secreted IL-1β are biologically active. In this report, we provide strong evidence that statin-stimulated LPS-primed mouse BMDMs or human PBMCs secret a unique form of IL-1β with a molecular mass close to 28 kDa in addition to trace amount of mature 17-kDa IL-1β (Fig. 2D). It was previously reported that prolonged (longer than 8 h) treatment of leukocytes with statins following LPS priming may lead to production of mature IL-1β that is dependent on caspase-1-activating inflammasomes (41). Our observations showed that processing of IL-1β into the 28-kDa form begins as early as 1 h after statin stimulation (Fig. 1D). More importantly, processing of IL-1β into the 28-kDa form upon statin treatment is not impaired in caspase-1 or Asc KO macrophages, whereas processing of that into mature IL-1β is completely abrogated in those mutant cells (Figs. 3B and 4B). It is suggested that prolonged statin treatment may lead to depletion of down stream intermediates such as geranylgeranyl pyrophosphate, which in turn activates caspase-1 inflammasomes. The underlying mechanisms remain unclear (41). Statins inhibit cholesterol biosynthesis by inhibiting the rate-limiting enzyme HMG-CoA reductase (22). IL-1β appears to play an important role in the evolution of atherosclerosis and has been linked to increased risk of cardiovascular disease. The exact mechanism by which statins inhibit IL-1β production and inhibition of inflammation is still unclear; however, it is clear that statins interfere with the processing of pro-IL-1β into mature IL-1β.

FIGURE 8. The 28-kDa form of IL-1β interferes with mature IL-1β signaling. A, a schematic graph of pro-IL-1β, the putative 28-kDa IL-1β, and the mature, 17-kDa IL-1β. Asp-26 and Asp-116 are the two cleavage sites on pro-IL-1β by caspase-1. B, PVDF membrane showing the band corresponding to the 28-kDa IL-1β purified by affinity chromatography and partial amino acid sequence of the N terminus of pro-IL-1β with the cleavage site at Asp-26. C, Western blot of culture supernatant from 293T cells stably transfected with plasmids expressing pro-IL-1β, the 28-kDa intermediate IL-1β, and the mature IL-1β. D, TFF2-deficient BMDMs were stimulated with control culture supernatant or 293T cell supernatants containing pro-IL-1β, 28-kDa IL-1β, or mature, 17-kDa IL-1β for 24 h; production of IL-6 in the supernatants was determined by ELISA. E, TFF2-deficient BMDMs were stimulated with mature IL-1β in the presence or absence of different concentrations of recombinant 28-kDa IL-1β for 24 h. IL-6 production was determined by ELISA. N.S., not significant.
reductase, which converts HMG-CoA into mevalonate (Fig. 1). However, pretreatment of BMDMs with mevalonate at a concentration as high as 20 mM did not abrogate statin-induced IL-1β processing into the 28-kDa form (Fig. 6), indicating that this process is not caused by depletion of downstream products of the mevalonate pathway upon statin treatment. Together, those results suggest that statin-induced IL-1β-processing can be divided into at least two distinct phases. At the early phase (first 7 h), statin treatment lead to production of the 28-kDa IL-1β, which is independent of caspase-1. Whereas prolonged exposure of macrophages to statins (longer than 8 h) results in depletion of geranylgeranyl pyrophosphate, activation of caspase-1 and subsequent processing of IL-1β into the mature 17-kDa form. Therefore, our studies unraveled a novel pathway of IL-1β processing that is distinct from the conventional inflammasome pathway.

Interestingly, this novel IL-1β processing pathway can be inhibited by pan-caspase inhibitor Z-VAD-FMK, suggesting that processing of pro-IL-1β induced by statins is mediated by caspase(s). When BMDMs deficient for caspase-1/11, caspase-6, or caspase-8 were used, statin-induced IL-1β processing was not impaired. The specific caspase responsible for the processing of pro-IL-1β upon statin treatment remains to be discovered.

It was reported that statin-induced IL-1β processing and secretion depends on the Rac1-P13K-AKT pathway (37). The Rac family of small GTPases includes three members: Rac1, Rac2, and Rac3. Only Rac1 and Rac2 are expressed in hematopoietic cells including macrophages, whereas Rac3 expression is limited to neurological tissues (38). Using Rac1 and Rac2 double knock-out BMDMs, our data showed that deficiency of either Rac1 or Rac2 or simultaneous deficiency of both did not impair statin-induced IL-1β processing (Fig. 7). Those results ruled out the possibility of the involvement of Rac proteins in statin-induced IL-1β production.

Edman amino-terminal sequencing has shown that the 28-kDa IL-1β is the product of pro-IL-1β cleaved at the first caspase cleavage site. Recent studies have shown that caspases other than caspase-1 may also cleave pro-IL-1β (42, 43). It is known that full-length pro-IL-1β is biologically inactive (44). By measuring IL-6 production induced by recombinant forms of pro-IL-1β, the 28-kDa IL-1β and the mature 17-kDa IL-β, we discovered that the 28-kDa IL-1β is not biologically active by itself (Fig. 8D). However, pretreatment of TFF-2 KO BMDMs with the 28-kDa form of IL-1β impaired mature IL-1β-induced IL-6 production indicating that the 28-kDa IL-1β is, in all likelihood, an IL-1 receptor antagonist. This interference of IL-1β signaling may in part explain the anti-inflammatory effects of statins in human patients.

In order to see the effect of statins on IL-1β processing in cells, we required an LPS-priming step. This priming step results in the production of pro-IL-1β, which is not expressed otherwise in resting macrophages. Patients who are treated with statins are not primed with LPS. However, the liver is responsible for the clearance of bacterial products absorbed from the gastrointestinal tract. It is likely that as a result of this constant assault upon the liver by gut-derived bacterial products, the liver always expresses large amounts of pro-IL-1β, and hepatic macrophages (i.e. Kupffer cells) are always in a primed state. Indeed, we (45) have observed that the liver of otherwise untreated mice expresses pro-IL-1β in abundance, and that this pro-IL-1β is processed to the active form upon appropriate stimulation. It is likely that human patients who take oral statins for hypercholesterolemia can generate the 28-kDa IL-1β intermediate without the need for additional priming stimuli on this basis.

Together, our data have unraveled a novel signaling pathway leading to processing of pro-IL-1β into a 28-kDa form upon statin stimulation following LPS priming. The 28-kDa, intermediate form of IL-1β is not biologically active by itself but interferes with mature IL-1β-IL-1RI signaling.

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