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SHORT COMMUNICATION

IL-1β and Caspase-1 Drive Autoinflammatory Disease Independently of IL-1α or Caspase-8 in a Mouse Model of Familial Mediterranean Fever

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Mutations in the gene encoding pyrin are associated with autoinflammatory disorder Familial Mediterranean Fever (FMF). A FMF-knock-in mouse strain that expresses chimeric pyrin protein with a V726A mutation (*Mefv*$^{V726A/V726A}$) was generated to model human FMF. This mouse strain shows an autoinflammatory disorder that is prevented by genetic deletion of IL-1 (IL-1) receptor or apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC). ASC-mediated cell death leads to the release of IL-1α and IL-1β, both of which signal through IL-1 receptor. Furthermore, caspase-1 and caspase-8 can interact with ASC to mediate secretion of IL-1 cytokines. The specific IL-1 cytokine instigating development of FMF and the enzymatic caspase involved in its secretion currently are unknown. In this study, we show that the autoinflammation observed in *Mefv*$^{V726A/V726A}$ mice is mediated specifically by IL-1β and not IL-1α. Furthermore, the disorder is dependent on the caspase-1—ASC axis, whereas caspase-8 is dispensable. Concurrently, aberrant IL-1β release by *Mefv*$^{V726A/V726A}$ monocytes in response to stimulation with lipopolysaccharide also is dependent on the caspase-1—ASC axis. In conclusion, our studies have uncovered a specific role for caspase-1—mediated IL-1β release in the manifestation of FMF. *(Am J Pathol 2017, 187: 236—244; http://dx.doi.org/10.1016/j.ajpath.2016.10.015)*

Familial Mediterranean Fever (FMF) is an autosomal-recessive, autoinflammatory disorder characterized by episodic fever and neutrophil-mediated inflammation of serosal tissues. The disorder is highly prevalent in Jewish, Armenian, Arab, and Turkish families geographically located in the Mediterranean basin.[1] Sequence alterations in the gene encoding pyrin (*MEFV*; alias marenostrin), are associated with FMF.[2,3] *MEFV* encodes a protein of 781 amino acids with an N-terminal pyrin, a central boxed-box and coiled coil domain, and a C-terminal PRY/SPRY (B30.2) domain.[4] Nearly one third of mutations found in patients with FMF are located in exon 10 of the *MEFV* gene (*http://fmf.igh.cnrs.fr/ISSAID/infevers/schema.php?n=1*, last accessed October 10, 2016). In human *MEFV*, exon 10 encodes for the B30.2 domain. This domain, however, is absent in the murine ortholog.[5] To study the effect of these mutations, a collection of FMF-knock in (FMF-KI) mouse strains are generated that express a chimeric pyrin protein harboring the human B30.2 domain with FMF-associated mutations.[5] One such FMF-KI strain is represented as *Mefv*$^{V726A/V726A}$ and expresses murine pyrin spliced to the B30.2 domain with a valine to alanine substitution at amino acid position 726. The *Mefv*$^{V726A/V726A}$ strain develops an autoinflammatory disorder characterized by runted growth and neutrophilia, which can be prevented by genetic deletion of apoptosis-associated speck-like protein (ASC) or IL-1—receptor (IL-1R) signaling.[5] A mouse strain with V726A as one allele and wild type as another, represented as *Mefv*$^{V726A/+}$, does not show the signs of autoinflammation observed in FMF-KI strains, highlighting the recessive nature of the disease.[5] FMF-KI mouse strains harboring other FMF-associated pyrin

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mutations within the B30.2 domain such as M680I and M694V develop a less severe form of autoinflammatory disease with similar characteristics.[5]

IL-1α and IL-1β are members of the IL-1 family of cytokines that signal through the same receptor, IL-1R[6]; however, the two cytokines have different modes of release and downstream inflammatory consequences.[7–9] IL-1α is released from necrotic and pyroptotic cells as an alarmin and does not require processing by caspases for its biological activity.[7,10–12] IL-1β, however, requires proteolytic cleavage by an inflammasome complex for its maturation, followed by release from the cell via pyroptosis.[13,14] Both caspase-1 and caspase-8 associate with ASC, and can have either complementary or redundant function in the release of IL-1 cytokines.[15–20] Although both IL-1α and IL-1β are released consequent to lytic cell death and perpetuate inflammatory signal through IL-1R, they mediate distinct neutrophil-driven autoinflammatory diseases. We previously showed that although IL-1α promotes disease in a mouse model of neutrophilic dermatosis,[10] IL-1β drives neutrophil-mediated disease in a mouse model of osteomyelitis.[16,21,22]

Previous studies have shown contradictory roles for wild-type and mutant pyrin (harboring mutations associated with FMF) in IL-1β processing and inflammation.[1,4,23–27] Pyrin recently was identified as an inflammasome-forming sensor that responds to Rho inactivation induced by bacterial infections.[28,29] Loss of pyrin also affects caspase-8 processing and consequent apoptosis.[23] These findings suggest that pyrin may mediate the release of IL-1 cytokines through distinct mechanisms. Therefore, identification of the specific IL-1 cytokine involved in FMF pathogenesis and the upstream regulatory process governing its release would clarify the immunopathogenesis of FMF.

In this study, we show that IL-β is the specific IL-1 cytokine that induces the autoinflammatory disorder in a mouse model of FMF. Furthermore, the caspase-1–ASC axis is required to drive the systemic inflammation *in vivo* and for aberrant IL-1β release in monocytes. Caspase-8, however, is dispensable for disease progression in this model of autoinflammatory disorder. Overall, we have shown a pathogenic role of IL-1β and described caspase-1 as a critical upstream inflammatory caspase regulating IL-1β release in a mouse model of FMF.

Materials and Methods

Mice

Mefv$^{V726A/+}$,[5] *Mefv*$^{V726A/V726A}$,[5] *Il1b*$^{-/-}$,[30] *Il1a*$^{-/-}$,[31] *Casp1*$^{-/-}$*Casp11*$^{-/-}$,[12] *Ripk3*$^{-/-}$*Casp8*$^{-/-}$,[32] and *Asc*$^{-/-}$[33] mice have been described previously. *Mefv*$^{V726A/+}$ mice were bred with *Il1b*$^{-/-}$, *Il1a*$^{-/-}$, *Casp1*$^{-/-}$*Casp11*$^{-/-}$, or *Ripk3*$^{-/-}$*Casp8*$^{-/-}$ mice to generate *Mefv*$^{V726A/V726A}$ mice lacking each of these components. Mice were maintained in a specific pathogen-free facility and animal studies were approved by the St. Jude Children's Research Hospital Committee on the Use and Care of Animals.

Cellular Processing and Analysis

Mice were monitored weekly for weight gain starting from 3 to 4 weeks of age and euthanized at 8 to 12 weeks of age for systemic analysis. Peritoneal cells were harvested by peritoneal lavage in RPMI media (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Flowery Branch, GA), nonessential amino acids (Gibco), and penicillin-streptomycin (Sigma, St. Louis, MO). Blood was collected through cardiac puncture, and 50 μL was added to anticoagulant EDTA for cellular analysis. The spleen was homogenized and passed through a 40-μm filter to obtain a single-cell suspension. Both blood and spleen were subjected to red blood cell lysis and washed in complete media. Left lobes of lung were homogenized, filtered, and subjected to a 33% Percoll (GE, Boston, MA) gradient centrifugation to remove debris and obtain a single-cell suspension. Cells obtained from the peritoneal cavity, blood, spleen, and lung were fixed with 2% paraformaldehyde (Affymetrix, Santa Clara, CA) and stained for cellular analysis.

Flow Cytometry

The following antibodies were used for cell-surface staining: CD11b (M1/70), CD19 (6D5), CD3 (17A2), and Gr-1 (RB6-8C5) (eBiosciences, San Diego, CA). Cells were stained, run on a LSRII flow cytometer (BD Biosciences, Franklin Lakes, NJ), and analyzed with FlowJo software version 10.2 (FlowJo LLC, Ashland, OR). After removal of doublets, B cells were gated as $CD19^{+}$ and T cells were gated as $CD3^{+}$; $CD11b^{+}$ and polymorphonuclear leukocytes ($CD11b^{+}Gr1^{+}$) were gated on cells negative for both CD3 and CD19.

Histology and Microscopy Analysis

Liver, kidney, and colon tissues were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. A board-certified pathologist (P.V.) analyzed the hematoxylin and eosin sections for the presence of inflammation and signs of tissue damage in a blinded manner.

Cell Culture and Stimulation

Monocytes were obtained by culturing cells from bone marrow in Dulbecco's modified Eagle's media (Gibco) supplemented with 30% media conditioned by L929 fibroblasts, 10% fetal bovine serum, nonessential amino acids, and penicillin-streptomycin for 3 days. Nonadherent monocytes were collected, counted, and plated at a density of 1×10^6 cells per milliliter for stimulation. Cells were stimulated with lipopolysaccharide (LPS; InvivoGen, San Diego, CA) (200 ng/mL) for 48 hours. At the end of

stimulation, 120 μL of supernatant was removed for enzyme-linked immunosorbent assay. Cells and supernatants then were lysed with RIPA buffer containing protease inhibitors and phosphatase inhibitors (Calbiochem, Billerica, MA), and boiled in SDS sample buffer for Western blot analysis. Caspase-1 activation was assessed by Western blot using the anti−caspase-1 p20 antibody (1:3000) from Adipogen (San Diego, CA) (AG-20B-0042-C100) as previously described.[17]

Enzyme-Linked Immunosorbent Assay

Cytokines in the serum and cell-culture supernatants were measured by enzyme-linked immunosorbent assay, according to the manufacturer's instructions. The IL-1β and multiplex enzyme-linked immunosorbent assay kits were obtained from eBiosciences and Millipore (San Diego, CA), respectively.

Statistical Analysis

All statistical analyses were performed using Prism software version 6.0 (GraphPad Software, San Diego, CA). The *t*-test or one-way analysis of variance was used with the Fisher least significance difference or Dunn's post-test as indicated. Two-way analysis of variance was used to analyze the kinetics of body weight gain and spleen composition. In most analyses, *Mefv*$^{V726A/+}$ mice were used as a control to show the degree of disease or protection. $P < 0.05$ was considered significant.

Results

Runting and Systemic Inflammation in *Mefv*$^{V726A/V726A}$ Mice Are Mediated Specifically by IL-1β and Not IL-1α

We found significantly increased levels of IL-1α and IL-1β in the serum of *Mefv*$^{V726A/V726A}$ mice in comparison with control *Mefv*$^{V726A/+}$ mice (Figure 1A), confirming data from a previous study.[5] To dissect the role of individual IL-1 cytokines in this model of FMF, *Mefv*$^{V726A/V726A}$ mice lacking either IL-1α or IL-1β were generated and tracked for clinical signs of disease, including body weight and an increase in levels of inflammatory mediators.

Although *Mefv*$^{V726A/V726A}$ mice gained significantly less weight than *Mefv*$^{V726A/+}$ mice, genetic deletion of IL-1β completely protected *Mefv*$^{V726A/V726A}$ mice from growth retardation and wasting (Figure 1, B and C). Heterozygosity of *Il1b* in *Mefv*$^{V726A/V726A}$ mice did not prevent runting, showing that a complete loss of IL-1β is required for protection (Supplemental Figure S1A). Genetic loss of IL-1α, on the other hand, did not affect body weight gain in *Mefv*$^{V726A/V726A}$ mice (Figure 1, B and C), suggesting that IL-1α is dispensable for disease progression. A previous study showed that inflammatory mediators that drive clinical manifestations of wasting are increased in the serum of *Mefv*$^{V726A/V726A}$ mice.[5] As expected, the levels of cytokines and chemokines including IL-6, tumor necrosis factor, interferon-γ, IL-17A, IL-10, IP-10, IL-12p70, granulocyte-macrophage colony-stimulating factor, monocyte chemoattractant protein-1, macrophage inflammatory protein-1α, and granulocyte colony-stimulating factor were increased significantly in serum of *Mefv*$^{V726A/V726A}$ mice compared with the levels in *Mefv*$^{V726A/+}$ mice (Figure 1D). Genetic ablation of IL-1β, and not IL-1α, in *Mefv*$^{V726A/V726A}$ mice reduced the level of all cytokines to that observed in *Mefv*$^{V726A/+}$ mice (Figure 1D). Collectively, these data show that IL-1β but not IL-1α is the cytokine that drives hyperinflammation and wasting in a mouse model of FMF.

IL-1β Is a Critical Driver of Neutrophilia and Multiorgan Damage Observed in *Mefv*$^{V726A/V726A}$ Mice

Systemic neutrophilia and inflammation of the serosal tissues is one of the hallmarks of FMF.[1,5] Inflammation in *Mefv*$^{V726A/V726A}$ mice further leads to splenomegaly and a change in cellular composition, such that the number of B and T lymphocytes is reduced drastically, whereas the total number of CD11b^{+} cells and neutrophils (polymorphonuclear leukocytes) is increased.[5] Genetic deletion of IL-1β protected *Mefv*$^{V726A/V726A}$ mice from an increased proportion of neutrophils in blood, an increase in splenic weight, and alterations in the splenic cellular composition (Figure 2, A−C). Furthermore, increased neutrophil infiltration observed in the peritoneal cavity and lungs of *Mefv*$^{V726A/V726A}$ mice also was prevented after genetic loss of IL-1β (Figure 2D). All of these features of neutrophil infiltration in *Mefv*$^{V726A/V726A}$ mice were unaffected by genetic deletion of IL-1α (Figure 2, A−D). The loss of IL-1β further protected *Mefv*$^{V726A/V726A}$ mice against histologic perturbations observed in the liver, kidney, and colon that include moderate and multifocal cellular infiltration (Figure 2E). *Mefv*$^{V726A/V726A}$ mice also showed signs of organ damage such as bile duct hyperplasia in the liver, glomerulopathy, necrosis, and proteinosis in the kidney and mucosal hyperplasia in the colon. These clinical features of organ damage were prevented by genetic ablation of IL-1β (Figure 2E). Altogether, these data show that IL-1β is a critical driver of inflammation and consequent clinical manifestations in a mouse model of FMF.

Autoinflammatory Disease in *Mefv*$^{V726A/V726A}$ Mice Is Dependent on the Caspase-1−ASC Axis

The autoinflammatory disorder observed in *Mefv*$^{V726A/V726A}$ mice is rescued by the loss of inflammasome adaptor ASC, implicating a role for inflammasome in the release of IL-1β in this disorder.[5] After certain infectious and sterile insults, both caspase-1 and caspase-8 associate with ASC to promote IL-1β processing.[15,17,18,20] In addition, caspase-8 can process IL-1β independently of caspase-1.[15,16,18−21,34] To determine

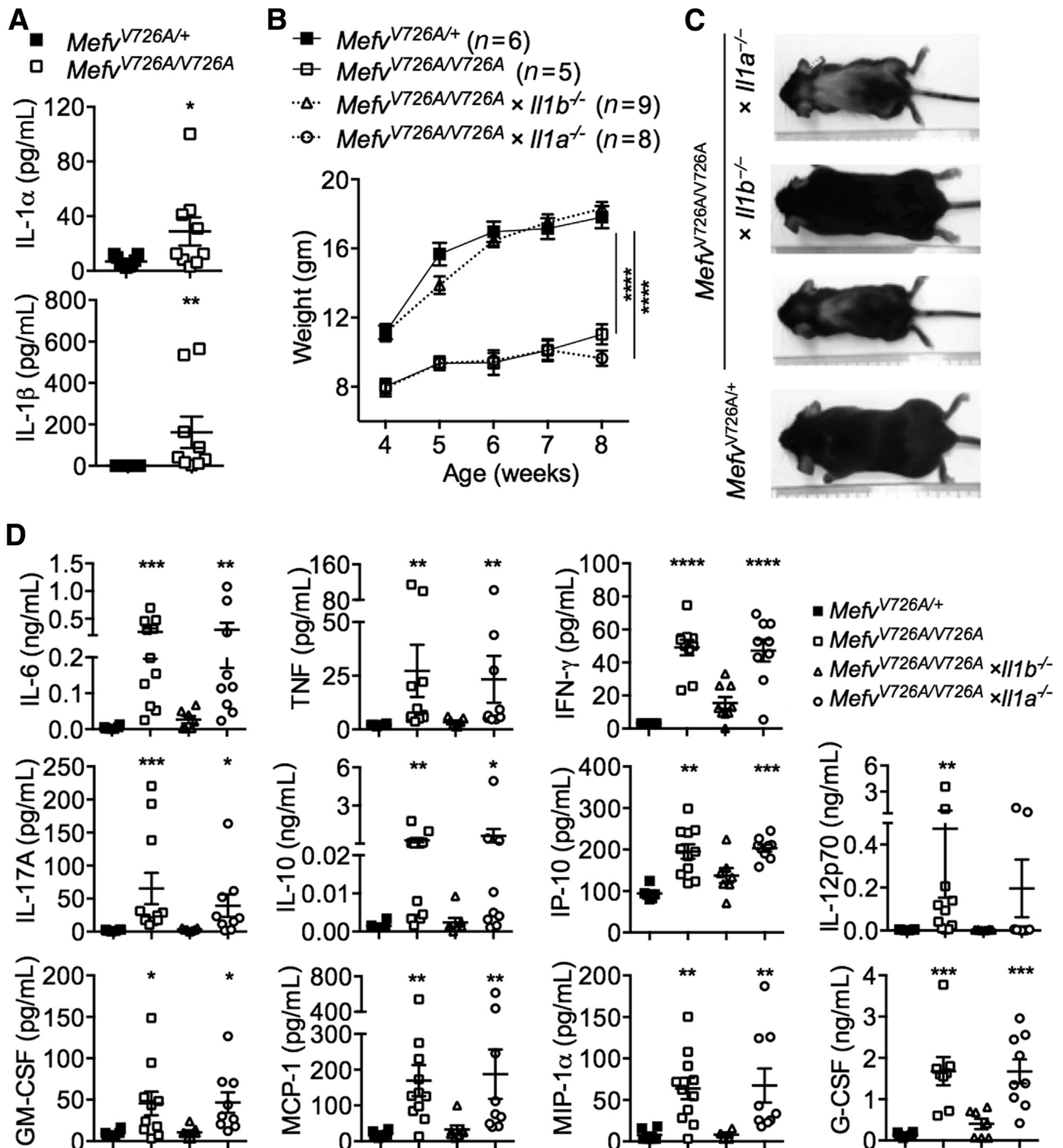

Figure 1 Runting and systemic inflammation in *Mefv*$^{V726A/V726A}$ mice is mediated specifically by IL-1β, but not IL-1α. **A:** Levels of IL-1α and IL-1β in serum samples. **B** and **C:** Body weights of the indicated number of female mice (**B**) and representative whole-body image (**C**) of mice at 8 weeks of age. **D:** Cytokine levels in serum samples. **A** and **D:** The *x* axis in column graphs shows mice strains indicated in separate legend keys. Data are expressed as means ± SEM (**A**, **B**, and **D**). $n = 7$ to 10 for each genotype (**A**, **B**, and **D**). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$ versus *Mefv*$^{V726A/+}$ by [*t*-test (**A**), two-way analysis of variance (**B**), and the Kruskal–Wallis test followed by the Dunn post-test (**D**)]. IFN, interferon; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1α; TNF, tumor necrosis factor.

whether this autoinflammatory disorder was owing to caspase-1 or caspase-8, we generated *Mefv*$^{V726A/V726A}$ mice lacking either caspase-1 or caspase-8. Genetic deletion of caspase-8 is embryonically lethal, but this lethality is rescued by further deletion of receptor-interacting protein kinase 3 (*Ripk3*$^{-/-}$).[32,35] We therefore generated *Mefv*$^{V726A/V726A}$ × *Ripk3*$^{-/-}$ × *Casp8*$^{-/-}$ mice to assess the role of caspase-8 in this disorder. We tracked *Mefv*$^{V726A/V726A}$ mice lacking

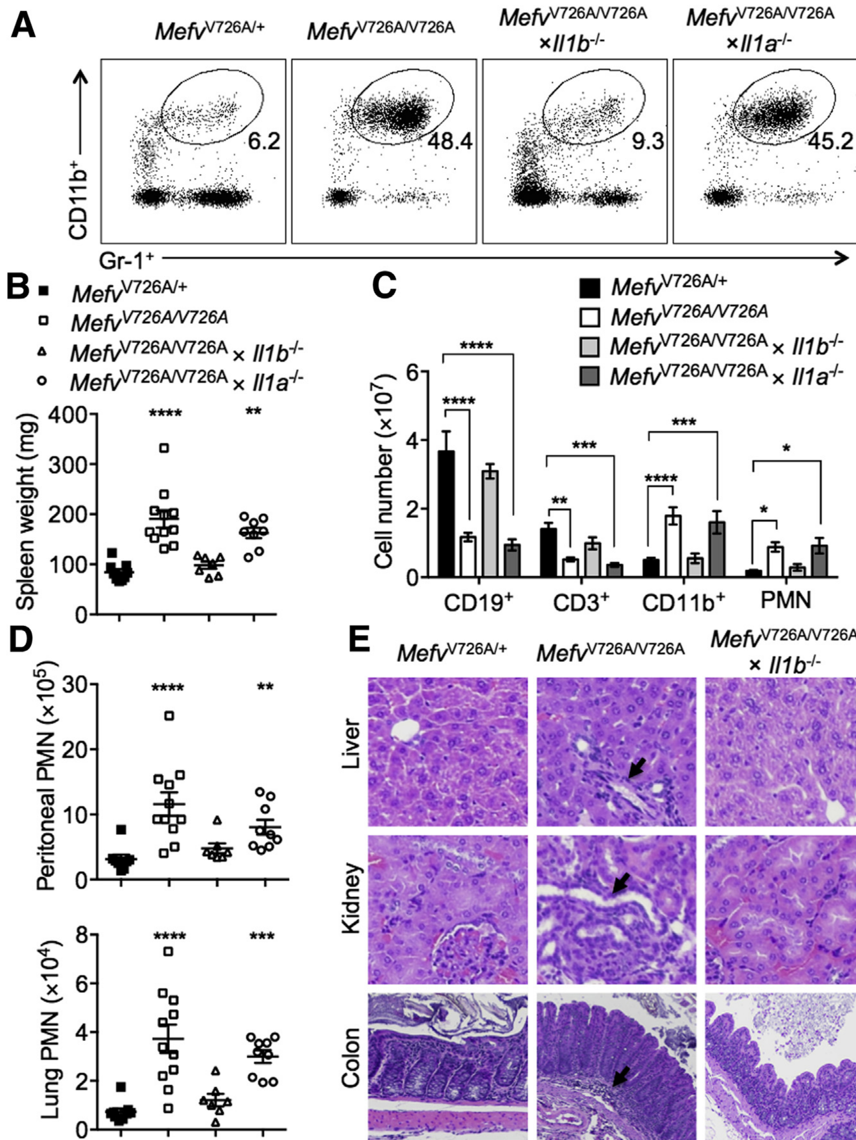

Figure 2 IL-1β is the critical driver of neutrophilia and organ dysfunction observed in *Mefv*$^{V726A/V726A}$ mice. **A:** Representative dot plot showing the proportion of polymorphonuclear leukocytes (PMNs) in the blood of indicated mice. The cells were gated on single cells; the percentage of cells identified as PMN is noted on each plot. **B**–**D:** Spleen weight (**B**), spleen cellular composition (**C**), and PMN infiltration (**D**) in the peritoneal cavity and lung. **E:** Representative hematoxylin and eosin–stained sections of liver, kidney, and colon tissues. **Black arrows** indicate granulocytic infiltration in the tissues. **B** and **D:** The *x* axis in the column graphs shows mice strains indicated in the legend key. Data are expressed as means ± SEM (**B**–**D**). $n = 7$ to 10 for each genotype (**B**–**D**). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$ versus *Mefv*$^{V726A/+}$ by using the Kruskal–Wallis test followed by the Dunn post-test (**B** and **D**) and two-way analysis of variance (**C**). Original magnification, ×20 (**E**).

caspase-1 or caspase-8 and RIPK3 for body weight gain, an indicator of wasting and systemic inflammation in this mouse model. Although *Mefv*$^{V726A/V726A}$ mice lacking caspase-1 gained body weight to the same extent as *Mefv*$^{V726A/+}$ mice (Figure 3, A and B), loss of caspase-8 did not protect against growth retardation and runting (Supplemental Figure S1B). Furthermore, the increase in the IL-1β level observed in the serum of *Mefv*$^{V726A/V726A}$ mice was prevented by genetic deletion of ASC or caspase-1 (Figure 3C). Other signs of systemic inflammation such as splenomegaly (Figure 3D), systemic neutrophilia, and multiorgan neutrophil infiltration (Figure 3, E and F) observed in *Mefv*$^{V726A/V726A}$ mice also were prevented by genetic loss of ASC or caspase-1. These data show a critical role for the ASC–caspase-1 axis in driving the IL-1β–mediated autoinflammatory disorder in *Mefv*$^{V726A/V726A}$ mice. The *Casp1*$^{-/-}$ mouse strain additionally lacked caspase-11, but a functional role for caspase-11 in this disease model can be ruled out because caspase-11 is known to engage the NLRP3 inflammasome to process IL-1β,[12] and loss of NLRP3 in *Mefv*$^{V726A/V726A}$ mice did not protect against autoinflammation.[5]

Anemia has been observed in patients with FMF[36] and is a common feature of multiple autoinflammatory disorders.[37] Inflammatory cytokines including IL-1β have been ascribed an inhibitory role in erythropoiesis leading to anemia.[38,39] We analyzed the parameters of anemia such as hematocrit, hemoglobin level, mean corpuscular volume, and red cell distribution width in the blood of FMF-KI mice lacking IL-1α, IL-1β, caspase-1, or caspase-8 (*Ripk3*$^{-/-}$). Anemia was observed in *Mefv*$^{V726A/V726A}$ mice as shown by lower hematocrit and hemoglobin levels (Supplemental Figure S2B). Similar to the inflammatory features of neutrophilia and lymphopenia, anemia observed in *Mefv*$^{V726A/V726A}$ mice also was dependent on the Casp1–IL-1β axis (Supplemental Figure S2, A and B). Lower mean corpuscular volume and higher red cell distribution width is indicative of iron

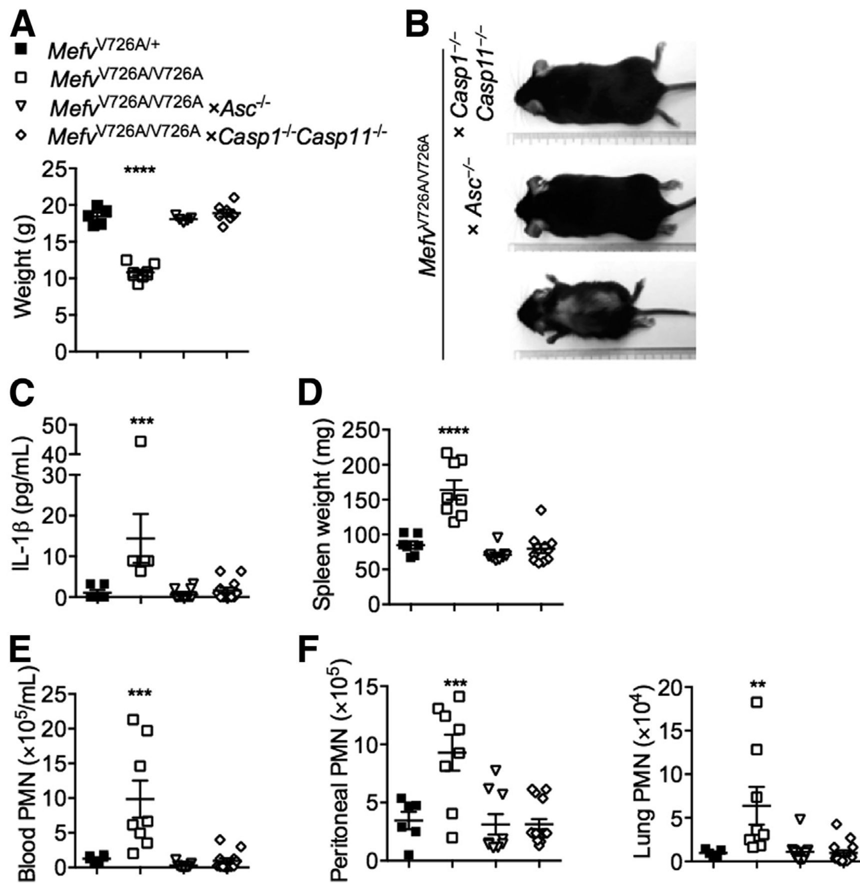

Figure 3 Autoinflammatory disease in *Mefv*$^{V726A/V726A}$ mice is dependent on the caspase-1—ASC axis. **A** and **B:** Body weights of female mice (**A**) and representative whole-body image (**B**) of mice at 8 weeks of age. **C**—**F:** Level of IL-1β in serum samples (**C**), spleen weight (**D**), total number of circulating polymorphonuclear leukocytes (PMNs) per milliliter of blood (**E**), and PMN enumeration (**F**) in the peritoneal cavity and lung of indicated mice. The *x* axis in the column graphs shows mice strains indicated in the legend key (**A** and **C**—**F**). Data are expressed as means ± SEM (**A** and **C**—**F**). $n = 5$ to 11 for each genotype (**A** and **C**—**F**). $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$ versus *Mefv*$^{V726A/+}$ (**A** and **C**—**F**, one-way analysis of variance followed by the Fisher least significance difference test).

deficiency and is consistent with the role of IL-1 in iron homeostasis and anemia.[40,41]

Chronic LPS Exposure Leads to Overt Inflammasome Activation and IL-1β Release in *Mefv*$^{V726A/V726A}$ Monocytes

Immune cells from *Mefv*$^{V726A/V726A}$ mice and peripheral blood mononuclear cells isolated from patients with FMF have shown aberrant IL-1β release in response to LPS stimulation.[5,42] In line with these observations, we observed an increase in IL-1β release and caspase-1 cleavage in *Mefv*$^{V726A/V726A}$ monocytes after 48 hours of LPS stimulation (Figure 4, A and B). Release of IL-1β from *Mefv*$^{V726A/V726A}$ monocytes was abolished in the absence of ASC or caspase-1 (Figure 4C). FMF-associated pyrin mutations also have been ascribed a role in promoting NF-κB signaling.[27] However, production of NF-κB signaling-dependent cytokines IL-6 and tumor necrosis factor was similar between *Mefv*$^{V726A/V726A}$ and *Mefv*$^{V726A/+}$ monocytes (Figure 4A). Therefore, aberrant caspase-1 activation drives the production of IL-1β, which leads to overt inflammation and its deleterious consequences in this mouse model of FMF.

Discussion

FMF is an autoinflammatory disorder marked by increased production of inflammatory mediators and neutrophilia in both humans[42] and mice.[5] Both IL-1α and IL-1β are capable of instigating sterile inflammation and we previously have shown that these cytokines drive distinct neutrophil-mediated autoinflammatory disorders.[10,34] Although a reason for this specificity currently is unknown, a possible reason could be the nature of cell death perturbed in each autoinflammatory disorder.[9] Although necrotic cell death releases bioactive IL-1α, IL-1β requires caspase-1— and/or caspase-8—mediated maturation and release. Thus, depending on the type of cell death that is instigated, either IL-1α or IL-1β could initiate and promote sterile inflammation, driving the disease pathogenesis. In this study, we showed that in a mouse model of FMF, aberrant caspase-1 activation mediated the maturation and release of IL-1β. Overt IL-1β production led to systemic and multiorgan hyperinflammation observed in the FMF-KI mice. Genetic deletion of IL-1β or caspase-1 in the FMF-KI mice provided protection against multiple parameters of the disorder including runting/wasting, neutrophilia, and anemia, suggesting that the clinical basis of these features is aberrant IL-1β production. Furthermore, IL-1α—mediated IL-1R

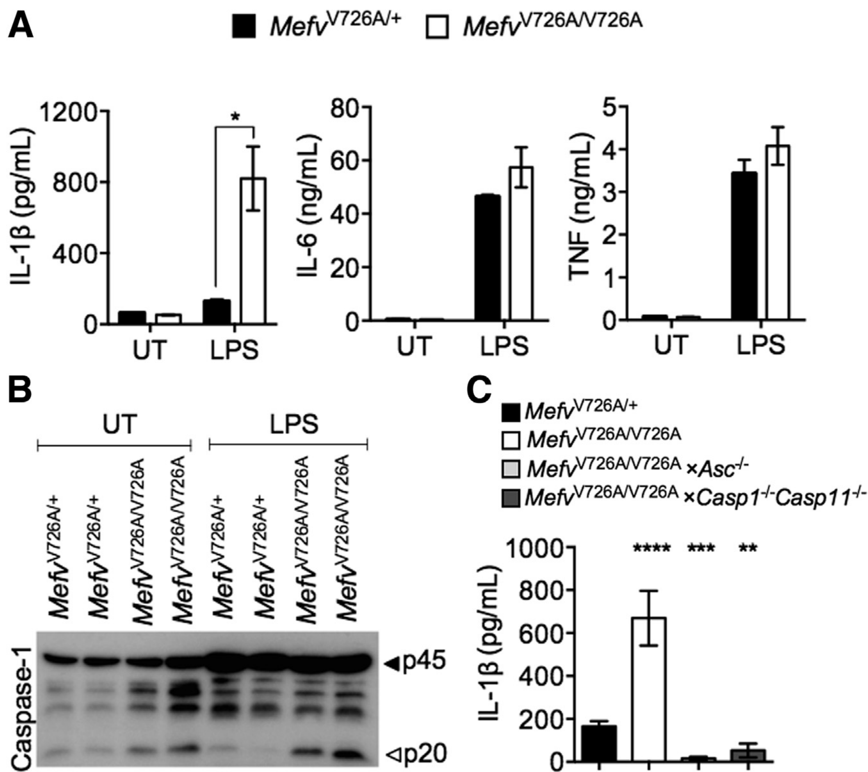

Figure 4 Chronic lipopolysaccharide (LPS) exposure leads to overt inflammasome activation and IL-1β release in $Mefv^{V726A/V726A}$ monocytes. Monocytes stimulated with LPS (200 ng/mL) for 48 hours were analyzed for cytokine release (**A** and **C**) and caspase-1 processing (**B**). Data are expressed as means ± SEM. $n \geq 3$ independent experiments. $^*P < 0.05$, , $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$ versus with $Mefv^{V726A/+}$ [two-way (**A**) or one-way (**C**) analysis of variance followed by the Fisher least significance difference test]. TNF, tumor necrosis factor; UT, untreated.

signaling and caspase-8–mediated IL-1β processing was dispensable for disease pathogenesis.

It has been shown previously that the autoinflammatory disease in FMF-KI mice is instigated by cells of the hematopoietic compartment and is independent of the adaptive immune system.[5] This suggests that the disease is perpetuated through IL-1β production by innate immune cells. FMF-KI cells of either granulocytic or monocytic lineage release higher amounts of IL-1β in response to LPS stimulation *in vitro* (data not shown), suggesting that both of these populations might contribute to this IL-1β–mediated disorder.

Although both anti–IL-1R and anti–IL-1β antibodies are used to treat human patients with FMF,[43–47] our studies provide a mechanistic rationale for specific blockade of IL-1β as the therapy of choice in patients with FMF. Compared with anti–IL-1R, anti–IL-1β therapy has the benefits of a longer biological half-life and an absence of side effects associated with IL-1α blockade.[48] Our studies further indicate that inhibition of caspase-1 activity would provide a therapeutic benefit to patients with FMF.

Recent studies have identified that protein kinase N1–mediated phosphorylation of pyrin (at serine 242) allows its association with 14-3-3 proteins, and this interaction inhibits pyrin inflammasome activation.[49,50] FMF-associated mutations in the B30.2 domain inhibit the interaction between pyrin and protein kinase N1, and reduce the inhibitory control on pyrin activation.[49] Our work, along with the earlier-mentioned findings, define the upstream molecular mechanisms involved in IL-1β release during FMF pathogenesis. How mutations in the C-terminal B30.2 domain affect phosphorylation at an amino acid position within the N-terminus domain is currently unknown. Mutations in the B30.2 domain reduce the threshold for pyrin activation, release pyrin from inhibitory control, and promote inflammasome activation even in the absence of pyrin-specific stimuli. This promotes aberrant IL-1β production, which drives the pathogenesis in this autoinflammatory disorder. Recent studies also have highlighted the role of pyrin inflammasome in another autoinflammatory disorder called hyperimmunoglobulonemia D syndrome.[49,51] Future studies aimed at elucidating the molecular underpinnings of pyrin activation and inflammasome regulation by the disease-associated mutations would help identify molecular targets for therapy in patients with FMF and other pyrin-associated disorders.

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D.S. and T.-D.K. conceptualized the project; D.S. and B.R.S. executed the experiments; P.V. performed the histologic assessment; D.S. conducted the data analysis;

D.S. and T.-D.K. wrote the manuscript; and T.-D.K. acquired funding and provided resources and overall supervision.

Supplemental Data

Supplemental material for this article can be found at *http://dx.doi.org/10.1016/j.ajpath.2016.10.015*.

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