Critical role for inflammasome-independent IL-1β production in osteomyelitis

John R. Lukens[a], Jordan M. Gross[a], Christopher Calabrese[b], Yoichiro Iwakura[c], Mohamed Lamkanfi[d,e], Peter Vogel[f], and Thirumala-Devi Kanneganti[a,1]

[a]Department of Immunology, [b]Small Animal Imaging Core, and [f]Animal Resources Center and the Veterinary Pathology Core, St. Jude Children's Research Hospital, Memphis, TN 38105; [c]Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan; and [d]Department of Biochemistry and [e]Department of Medical Protein Research, Ghent University, B-9000 Ghent, Belgium

Edited by Ruslan Medzhitov, Yale University School of Medicine, New Haven, CT, and approved December 4, 2013 (received for review October 3, 2013)

The immune system plays an important role in the pathophysiology of many acute and chronic bone disorders, but the specific inflammatory networks that regulate individual bone disorders remain to be elucidated. Here, we characterized the osteoimmunological underpinnings of osteolytic bone disease in *Pstpip2*^{cmo} mice. These mice carry a homozygous L98P missense mutation in the Pombe Cdc15 homology family phosphatase PSTPIP2 that is responsible for the development of a persistent autoinflammatory disease resembling chronic recurrent multifocal osteomyelitis in humans. We found that improper regulation of IL-1β production resulted in secondary induction of inflammatory cytokines, inflammatory cell infiltration in the bone, and unremitting bone inflammation. Aberrant *Il1β* expression precedes the development of osteolytic damage in young *Pstpip2*^{cmo} mice, and genetic deletion of *Il1r* and *Il1β*, but not *Il1α*, rescued osteolytic bone disease in mutant mice. Intriguingly, caspase-1 and nucleotide-binding oligomerization domain (NOD)-like receptor family, pyrin domain containing 3 activation in the inflammasome complex were dispensable for *Pstpip2*^{cmo}-mediated bone disease. Thus, our findings establish a critical role for inflammasome-independent production of IL-1β in osteolytic bone disease and identify PSTPIP2 as a negative regulator of caspase-1–autonomous IL-1β production.

osteoimmunology | interleukin-1 | autoinflammation

Bone diseases including osteoporosis, chronic recurrent multifocal osteomyelitis (CRMO), Paget's disease, arthritis, and periodontal disease are a major burden to human health and can cause debilitating pain, physical impairments, and morbidity. Regulated crosstalk between cells of the skeletal and immune systems is required to maintain normal bone integrity and homeostasis (1). It is now widely accepted that inflammatory immune cells contribute centrally to the induction and perpetuation of various inherited and induced bone disorders (2, 3). However, the roles of specific inflammatory pathways and immune cell networks in disease pathogenesis remain to be fully characterized for most bone disorders. Recently, missense mutations in the proline–serine–threonine phosphatase interacting protein 2 (PSTPIP2) were shown to cause a bone disease in mice that is characterized by osteomyelitis and bone deformity (4–6). In particular, the *chronic multifocal osteomyelitis* (*cmo*) mouse that carries a homozygous L98P missense mutation in PSTPIP2 (referred to as *Pstpip2*^{cmo} mice) develops a chronic autoinflammatory disease that resembles CRMO in humans (7). CRMO is a painful genetic inflammatory disorder that primarily affects children and is characterized by bone inflammation, destruction, and deformity (8). The etiology of CRMO remains unknown, and treatment is currently limited to the prescription of nonsteroidal anti-inflammatory drugs (NSAIDs) and bisphosphonates.

Excessive IL-1 production and signaling has recently been identified to centrally contribute to a spectrum of autoinflammatory diseases (9). Recent data suggest that IL-1 can directly influence bone homeostasis, and dysregulation of IL-1 has been found to contribute to bone disorders such as osteoarthritis (2). IL-1 exists in two distinct forms, IL-1α and IL-1β, both of which signal through the IL-1 receptor (IL-1R) to elicit potent proinflammatory responses (10). IL-1β is generated in a biologically inactive proform that requires protease-mediated cleavage to be secreted and elicit its proinflammatory functions. Caspase-1–mediated cleavage of IL-1β following inflammasome complex formation is the major mechanism responsible for secretion of bioactive IL-1β in many disease models (11). Inflammasome-independent sources of IL-1β have also been suggested to contribute to inflammatory disease pathogenesis; however, very little is known about the molecular regulation of these pathogenic pathways (12, 13). IL-1α, on the other hand, does not require cleavage to induce its biological activity and is passively released following inflammatory forms of cell death (14, 15).

In this study, we sought to investigate the immunological factors that contribute to bone disease in *Pstpip2*^{cmo} mice. We show that osteomyelitic disease in these mice is characterized by uncontrolled and unremitting inflammatory cytokine production and immune cell infiltration. Furthermore, we report a critical role for IL-1β that is derived independently of inflammasome activation in osteolytic disease progression.

Results

Osteomyelitis Development in Pstpip2^{cmo} Mice Is Associated with Altered Immune Cell Composition. *Pstpip2*^{cmo} mice develop bone nodules on their tails and paw inflammation that is characterized by severe erythema and hind paw deformities by 7–14 wk of age (Fig. 1*A*). Microcomputed tomography (micro-CT) scans of the inflamed areas reveal extensive loss of bone density and structural malformation in the feet and tails of *Pstpip2*^{cmo} mice (Fig. 1

Significance

The IL-1 cytokines, IL-1α and IL-1β, are proinflammatory cytokines that are implicated in numerous inflammatory and autoimmune diseases. This study demonstrates that dysregulated immune responses centrally contribute to the pathogenesis of osteomyelitis and identifies a critical role for IL-1β in driving the inflammatory cascade that provokes bone destruction. Caspase-1 activation in the inflammasome complex is the most well established mechanism for IL-1β secretion. Interestingly, inflammasome-independent sources of IL-1β were found to provoke inflammation and osteolytic bone disease. Our findings establish a unique role for inflammasome-independent IL-1β in autoinflammatory bone disease and osteomyelitis, and identify proline–serine–threonine phosphatase interacting protein 2 as a negative regulator of inflammasome-autonomous IL-1β.

Author contributions: J.R.L., C.C., M.L., and T.-D.K. designed research; J.R.L., J.M.G., C.C., and P.V. performed research; Y.I. contributed new reagents/analytic tools; J.R.L., J.M.G., C.C., M.L., P.V., and T.-D.K. analyzed data; and J.R.L. and T.-D.K. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence should be addressed. E-mail: thirumala-devi.kanneganti@stjude.org.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1318688111/-/DCSupplemental.

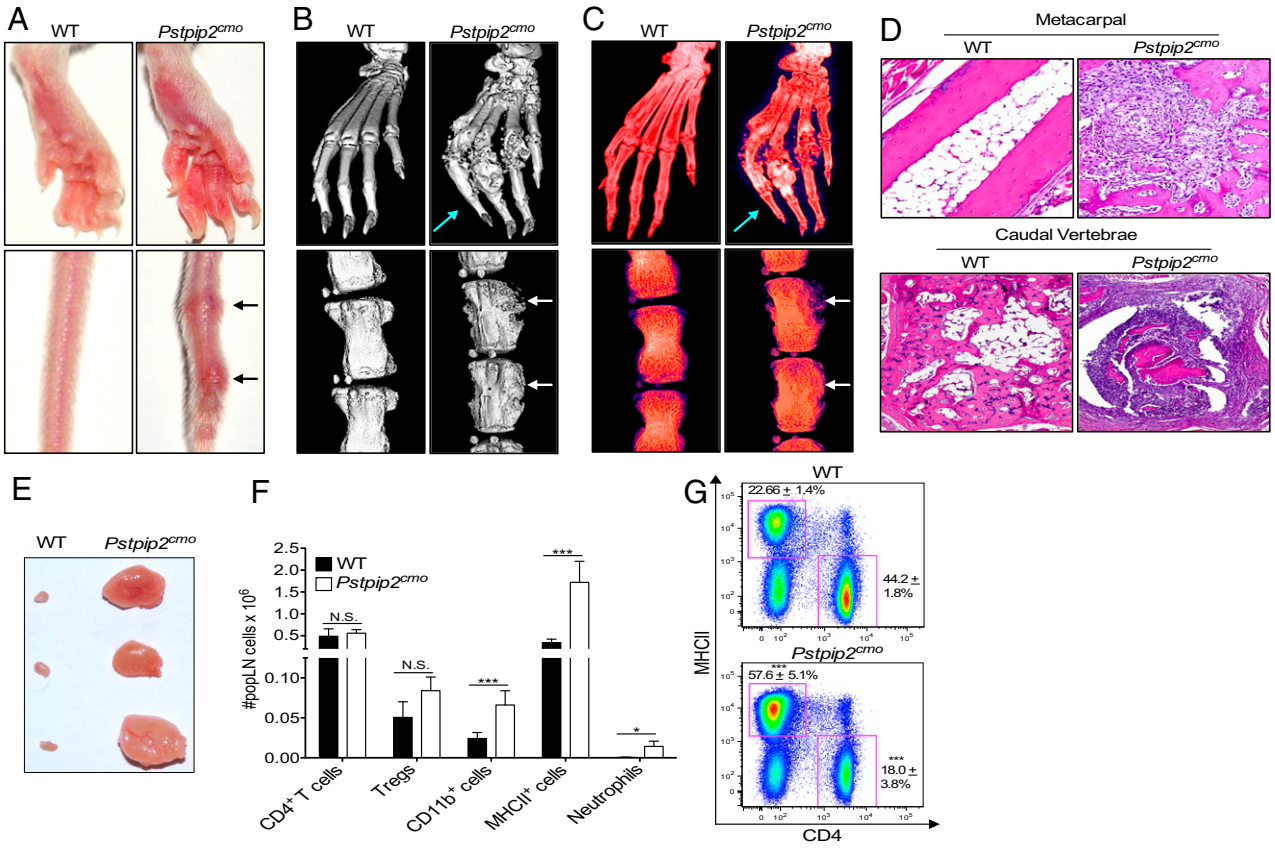

Fig. 1. Mutation in *Pstpip2* results in osteomyelitis and altered immune cell composition. (*A*) Spontaneous induction of paw inflammation and osteoarthropathy (*Top*) and tail kinks (*Bottom*) in *Pstpip2*^{cmo} mice at 7–14 wk of age. Black arrows denote tail kinks. (*B* and *C*) Isosurface microcomputed tomography (micro-CT) scans (*B*) and bone density heat maps (*C*) of WT and *Pstpip2*^{cmo} mice. Blue arrows highlight areas of bone deformity, and white arrows denote sites of bone deposition. (*D*) Hind paw metacarpal bone and caudal vertebrae hematoxylin and eosin (H&E) sections from WT and *Pstpip2*^{cmo} mice. (*E*–*G*) WT and diseased *Pstpip2*^{cmo} mice were harvested at 10–12 wk of age. (*E*) Representative images of popliteal lymph nodes (popLN). (*F*) Number (mean ± SEM) of popLN cells. Data are representative of three independent experiments with at least five to six mice per group. Tregs, Foxp3-expressing regulatory T cells. (*G*) Expression of MHCII and CD4 by popLN cells. Numbers in the FACS plots represent the mean ± SEM. Data are representative of three independent experiments with at least five to six mice per group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

B and *C*). Histological evaluation of the metacarpal bones and the caudal vertebrae further demonstrated osteolytic inflammation that was accompanied by hypertrophic osteoarthropathy and multifocal severe eosinophilic granulomatous inflammation (Fig. 1*D*). Moreover, the inflammatory bone lesions led to secondary s.c. inflammation and fibrosis of the surrounding soft tissue (Fig. 1*D*).

In recent years, pivotal roles for immune cells in numerous bone disorders have been defined (1, 2). Immune cell-derived cytokines and growth factors are instrumental in the maintenance of bone density and repair, whereas aberrant immune responses contribute to the pathogenesis of multiple bone diseases. To investigate the potential role of immune cells in *Pstpip2*^{cmo}-mediated bone disease, the immune cell composition was characterized. Massive lymphomegaly was observed in the popliteal lymph nodes (popLNs) that drain the inflamed feet (Fig. 1*E*), and enhanced accumulation of MHCII$^+$ cells was found to primarily contribute to the enlarged LNs of *Pstpip2*^{cmo} mice (Fig. 1 *F* and *G*). In addition, neutrophil numbers were significantly higher in *Pstpip2*^{cmo} mice relative to WT mice (Fig. 1*F*). Dysregulated T-cell responses have previously been associated with bone disorders (16), but *Pstpip2*^{cmo} mice did not present with perturbations in peripheral T-cell numbers, activation status, cytokine production, or maintenance of foxhead box P3 (Foxp3)-expressing regulatory T cells (Fig. 1*F* and Fig. S1). Our findings demonstrating normal T-cell responses and activation in PSTPIP2-deficient mice are in agreement with an earlier report showing that osteomyelitic disease ensues in the absence of T cells in a separate *Pstpip2* genetic mouse model (*Pstpip2*^{lupo} mice) (5). Collectively, these results suggest that osteolytic bone disease in *Pstpip2*^{cmo} mice may primarily be linked to dysregulated innate immune signaling.

Pstpip2^{cmo} Mutation Leads to Global Changes in Bone Homeostasis That Are Characterized by Immune Cell Infiltration and Osteoclast/Osteoblast Activation. The most prominent bone lesions in *Pstpip2*^{cmo} mice appeared in the small bones of the hind paws and caudal vertebrae. We were interested in understanding whether this was unique to these anatomical locations or if the osteomyelitc disease was a more general phenomenon that affected bone globally. To investigate this, we performed full body CT scans on WT and *Pstpip2*^{cmo} mutant mice. Full body scans identified aberrations in bone structure and composition in additional areas, including the long bones in the legs (Fig. S2). This indicates that PSTPIP2 deficiency results in global aberrations in bone homeostasis.

To identify the earliest cellular events that trigger osteolytic inflammation and pathology, histological analysis was conducted on young (4- to 5-wk-old) *Pstpip2*^{cmo} mice before the onset of overt bone disease. The initial inflammatory events appear to consist of fibroblastic cells and macrophages plus granulocytes, which are followed by activation of osteoclasts and osteoblasts (Fig. S2). Activation of osteoclasts and osteoblasts in the bone lesions was denoted by evaluating morphological changes that are hallmarks

of activation including the development of ruffled boards in osteoclasts and the identification of prominent Golgi apparatuses in osteoblasts. This ultimately leads to downstream concurrent osteolysis and osteosclerosis in adjacent locations. Furthermore, the areas of necrotic endosteal and periosteal bone, frequently associated with degenerating osteoclasts, appear to induce a severe granulocytic and histiocytic inflammatory response (Fig. S2). Collectively, these histological findings demonstrate extensive cell-to-cell contact and crosstalk between bone cells (osteoclasts and osteoblasts) and immune cells (macrophages and granulocytes) locally in the bone tissue before osteolytic damage ensues.

Defective PSTPIP2 Signaling Promotes Exacerbated Production of Inflammatory Cytokines That Are Associated with Myeloid Cell Recruitment and Activation. To characterize the inflammatory mediators that potentiate osteolytic bone disease in *Pstpip2^{cmo}* mice, the levels of cytokines and chemokines were measured in inflamed paws. Multiple innate immune cell-derived cytokines and chemokines were markedly elevated in the diseased paws of *Pstpip2^{cmo}* mice (Fig. 2*A*). Notably, potent production of IL-1β and TNF-α was observed in the afflicted hind paws. Moreover, the osteolytic inflammatory environment was characterized by enhanced induction of pathogenic factors that are associated with the recruitment and expansion of granulocytes [G-CSF and keratinocyte-derived chemokine (KC)] and macrophages [macrophage inflammatory protein (MIP)-1α and monocyte chemotactic-protein (MCP)-1] (Fig. 2*A*). Defective PSTPIP2 activation was not, however, found to provoke a global induction of proinflammatory cytokines because the levels of several T-cell–generated cytokines (IFN-γ) and other innate cytokines (IL-12) were normal in *Pstpip2^{cmo}* mice (Fig. S3). Collectively, these data suggest that aberrant myeloid cell recruitment and innate immune cell-mediated cytokine production may be associated with autoinflammatory bone disease in *Pstpip2^{cmo}* mice.

IL-1 and TNF-α have both been extensively described to contribute to the pathogenesis and pathology of bone diseases including arthritis and osteoporosis (17, 18). However, the roles of these cytokines in *Pstpip2^{cmo}*-mediated osteolytic disease have not been formally studied. To investigate the involvement of IL-1 and TNF-α in this model of bone disease, we evaluated the regulation of their expression during overt disease and before the onset of clinical symptoms. Consistent with our cytokine ELISA data, expression of *Il1β* (and to a lesser extent *Tnfα*) transcripts was elevated in the inflamed paws of *Pstpip2^{cmo}* mice (Fig. 2*B*). Moreover, enhanced *Il1β* expression was found to precede the development of disease in asymptomatic *Pstpip2^{cmo}* mice (4–6 wk of age), whereas *TNFα* mRNA levels were comparable between WT and young *Pstpip2^{cmo}* mice (Fig. 2*C*). The specific up-regulation of IL-1β in young PSTPIP2-deficient mice suggests IL-1β rather than TNF-α as the apical cytokine responsible for the induction of *Pstpip2^{cmo}*-mediated osteolytic disease.

Osteomyelitis Disease Progression in Pstpip2^{cmo} Mice Is Mediated by IL-1β but Not IL-1α. To ascertain whether IL-1 contributes to osteomyelitis, we first generated *Pstpip2^{cmo}* mice that are deficient in IL-1R. Importantly, genetic ablation of IL-1R rescued osteolytic disease in *Pstpip2^{cmo}* mice (Fig. 2*D*). Deletion of IL-1R prevented bone destruction and deformity in both the tails and feet of PSTPIP2-deficient mice (Fig. 2 *E*–*G*). These results indicate that dysregulated IL-1 signaling is centrally involved in promoting *Pstpip2^{cmo}*-mediated bone disease.

To investigate whether IL-1α– or IL-1β–mediated events were responsible for *Pstpip2^{cmo}*-mediated osteolytic disease, mice were crossed with animals that are deficient in either IL-1α or IL-1β.

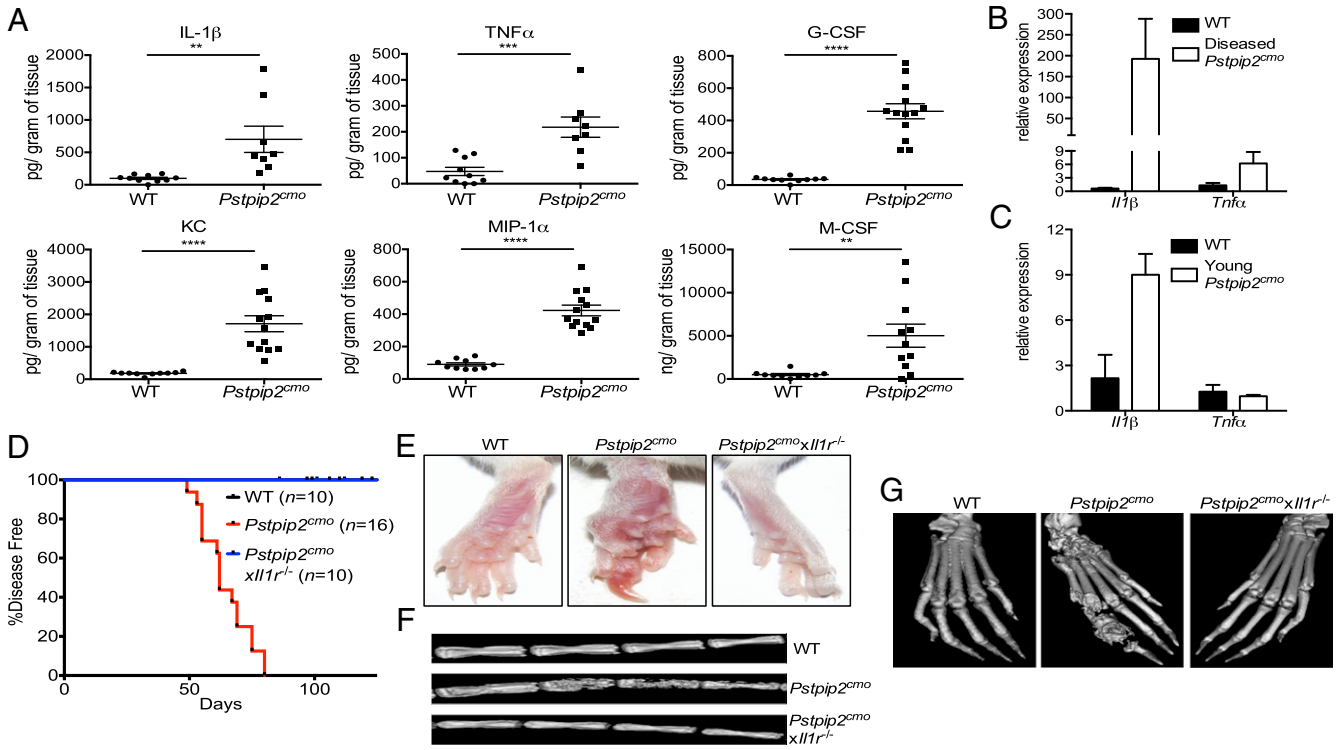

Fig. 2. Dysregulated IL-1 signaling centrally contributes to inflammatory bone disease in *Pstpip2^{cmo}* mice. (*A*) Levels of cytokines in the hind paws of WT and diseased *Pstpip2^{cmo}* mice. Each point represents an individual mouse, and the line represents the mean ± SEM. Data are representative of three independent experiments with at least eight mice per group. (*B* and *C*) *Il1β* and *Tnfα* mRNA expression in the hind paws of WT, diseased *Pstpip2^{cmo}* (*B*), and asymptomatic, young *Pstpip2^{cmo}* (4–6 wk of age) mice (*C*). Data are representative of three independent experiments with at least four mice per group. (*D*) Incidence of disease in WT, *Pstpip2^{cmo}*, and *Pstpip2^{cmo}xIl1r$^{-/-}$* mice over time. (*E*–*G*) Representative paw images (*E*) and isosurface micro-CT tail (*F*) and paw (*G*) scans from WT, *Pstpip2^{cmo}*, and *Pstpip2^{cmo}xIl1r$^{-/-}$* mice. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

Homozygous deletion of IL-1α failed to influence the progression or severity of bone disease in *Pstpip2*^{cmo} mice (Fig. 3 *A*–*C*). In contrast, genetic ablation of IL-1β provided protection against loss of bone density and skeletal deformities in *Pstpip2*^{cmo} mice (Fig. 3 *A*–*C*). Absence of IL-1β also rescued *Pstpip2*^{cmo} mice from lymphomegaly (Fig. 3*D*) and resulted in normal numbers of popLN MHCII^{+} cells and neutrophils (Fig. 3*E*). Moreover, histological evaluation of the bones of *Pstpip2*^{cmo} mice that lack IL-1β confirmed a normal bone architecture and provided further evidence for a marked protection from osteolytic disease pathology and exorbitant inflammatory cell infiltration (Fig. 3*F*).

Dysregulated Control of IL-1β Production Instigates the Production of Secondary Inflammatory Cytokines in PSTPIP2-Deficient Mice. IL-1β is a potent proinflammatory factor that can stimulate the downstream production of multiple pathogenic cytokines that are known to promulgate disease progression. To investigate whether dysregulated IL-1β production is responsible for the secondary induction of proinflammatory factors that we previously found to be up-regulated in diseased *Pstpip2*^{cmo} mice (Fig. 2*A*), we reexamined the production of these cytokines in the absence of IL-1β. Genetic deletion of IL-1β in *Pstpip2*^{cmo} mice completely suppressed the production of key proinflammatory cytokines such as TNF-α and chemokines including KC, G-CSF, M-CSF, MCP-1, MIP-1α, MIP-1β and MIP-2 that typically accompanied osteolytic disease progression in *Pstpip2*^{cmo} mice (Fig. 4 and data not shown). These findings highlight a critical role for IL-1β as the apical cytokine that is responsible for inducing *Pstpip2*^{cmo}-mediated disease progression.

IL-1β–Mediated Osteomyelitis Proceeds Independently of Inflammasomes. To elucidate whether PSTPIP2 regulates osteomyelitic disease progression through its function in hematopoietic or radioresistant cells, bone marrow chimera mice were generated. Expression of the hypomorphic *Pstpip2*^{cmo} allele in the hematopoietic compartment alone promoted the development of tail kinks, bones deformities, and arthritic paw inflammation (Fig. 5*A*). In contrast, chimera mice bearing the *Pstpip2*^{cmo} mutation only in radioresistant cells failed to develop osteolytic disease, suggesting that *Pstpip2* expression in bone marrow-derived immune cells rather than in nonhematopoietic cells is critical for the induction of this autoinflammatory disease.

PSTPIP1, which shares marked sequence and structural homology with PSTPIP2 (19–21), has recently been identified as a negative regulator of inflammasome activation (22–24). Furthermore, inflammasome-mediated production of IL-1β by macrophages has been found to play instrumental roles in the pathogenesis of numerous autoinflammatory diseases. In particular, nucleotide-binding oligomerization domain (NOD)-like receptor family, pyrin domain containing 3 (NLRP3)-driven activation of caspase-1 has been suggested to instigate multiple sterile inflammatory diseases (10). For all of these reasons we were interested in investigating whether PSTPIP2 also functions as a negative regulator of inflammasome activation and if altered inflammasome-induced IL-1β production contributes to disease in PSTPIP2-deficient mice. To test this, WT and *Pstpip2*^{cmo} bone marrow derived macrophages were stimulated with LPS+ATP and *Salmonella* infection to trigger inflammasome activation. However, deficiency in PSTPIP2 was not observed to influence the secretion of IL-1β by macrophages in response to these inflammasome agonists (Fig. 5*B*).

In addition to macrophages, other cell lineages have also been reported to be important contributors to inflammasome-mediated diseases. To ascertain the overall role of inflammasome activation in *Pstpip2*^{cmo}-induced bone disease, *Pstpip2*^{cmo} mice were crossed with either *Nlrp3*- or *Casp1*-deficient mice. Intriguingly, genetic abrogation of neither NLRP3 nor caspase-1 rescued osteolytic disease in *Pstpip2*^{cmo} mice (Fig. 5 *C* and *E* and Fig. S4), suggesting that IL-1β was produced independently of caspase-1 (and hence inflammasomes) to promote osteomyelitis in *Pstpip2*^{cmo} mice. In line with this, deletion of caspase-1 in *Pstpip2*^{cmo} mutant mice still resulted in elevated IL-1β levels in vivo (Fig. 5*D*).

To further characterize IL-1β maturation in *Pstpip2*^{cmo}-associated bone disease, we evaluated in vivo processing of IL-1β by Western blot analysis of bone lesions. Consistent with our ELISA data, we also detected greater amounts of processed IL-1β in the footpads of *Pstpip2*^{cmo} mice (Fig. 5*F*). Intriguingly, the majority of the processed IL-1β that was generated in *Pstpip2*^{cmo} mice was larger in molecular mass than the typical 17 kDa IL-1β product that is produced following inflammasome activation in macrophages. Detectable levels of 17 kDa IL-1β were still generated in *Pstpip2*^{cmo} mice; however, the alternative cleavage bands constituted the majority of the processed IL-1β in vivo. Importantly, we also observed marked generation of

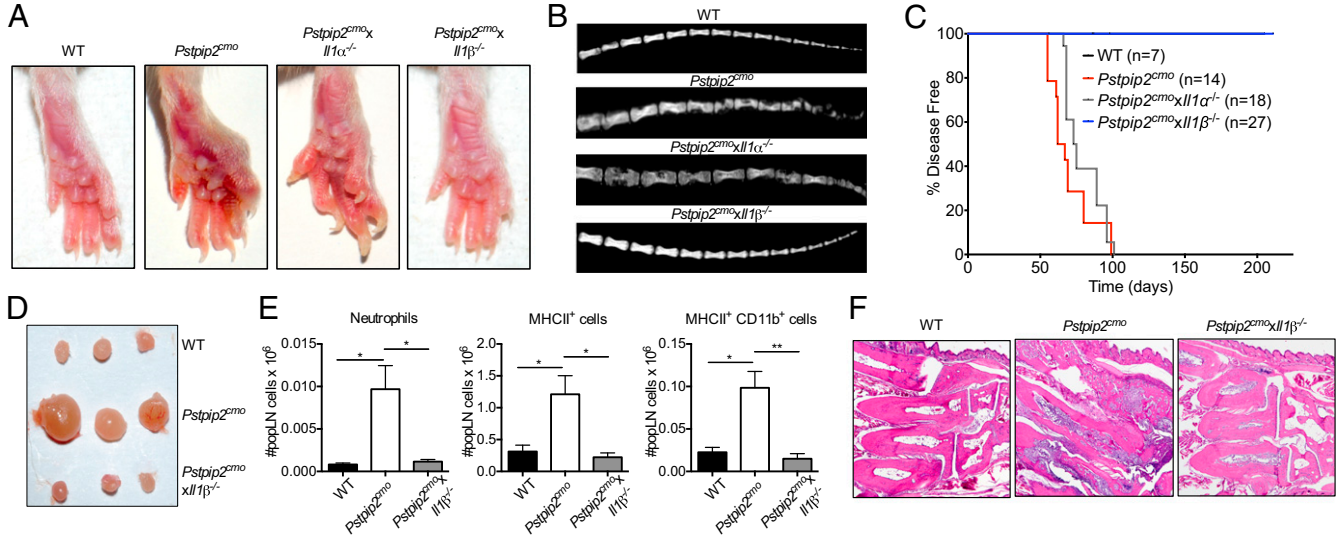

Fig. 3. Deletion of IL-1β prevents *Pstpip2*^{cmo}-mediated bone disease. (*A* and *B*) Representative paw images (*A*) and tail CT scans (*B*) from WT and *Pstpip2*^{cmo} mice that were crossed with mice that are deficient in either IL-1α or IL-1β. (*C*) Incidence of disease over time. Combined data from two independent experiments. (*D* and *E*) PopLNs were harvested from 12- to 16-wk-old WT, *Pstpip2*^{cmo}, and *Pstpip2*^{cmo}x*Il1β*$^{-/-}$ mice. Representative images of the PopLNs (*D*) and numbers (mean ± SEM) of popLN cells (*E*). Data are representative of three independent experiments with at least six mice per group. (*F*) Hind paw metacarpal bone H&E sections from WT, *Pstpip2*^{cmo}, and *Pstpip2*^{cmo}x*Il1β*$^{-/-}$ mice. *$P < 0.05$, **$P < 0.01$.

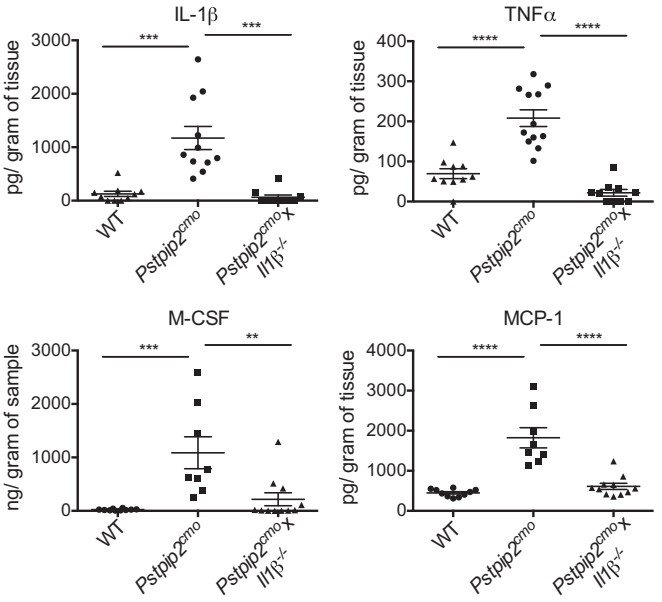

Fig. 4. IL-1β triggers downstream proinflammatory cytokine production. Levels of cytokines in the hind paws of WT, *Pstpip2*^{cmo}, and *Pstpip2*^{cmo}x*Il1β*$^{-/-}$ mice. Each point represents an individual mouse, and the line represents the mean ± SEM. Data are representative of three independent experiments with at least eight mice per group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

alternatively cleaved IL-1β products in *Pstpip2*^{cmo} mice that lack caspase-1 (Fig. 5*F*). Collectively, these findings establish PSTPIP2 as a negative regulator of IL-1β production and suggest an important role for inflammasome-independent IL-1β processing in autoinflammation and osteomyelitis.

Discussion

Our findings define osteomyelitic disease in the *Pstpip2*^{cmo} mouse model as an osteoimmunological disorder that is characterized by dysregulated inflammatory cytokine production and immune cell infiltration. Notably, we demonstrate that IL-1β, but not IL-1α, is required to trigger inflammation, bone deformity, and osteolytic disease. The critical role of IL-1β in driving osteomyelitis in PSTPIP2-deficient mice is consistent with recent clinical studies that show elevated production of IL-1β in CRMO patients (25). Interestingly, bone disease developed in the absence of caspase-1 or NLRP3 expression, which strongly suggests that IL-1β is produced in an inflammasome-independent manner to promote bone disease. IL-1β has been implicated in multiple inflammatory diseases, and its regulation downstream of inflammasome activation has been extensively characterized in recent years. In comparison, the contributions of inflammasome-independent sources of IL-1β to disease pathogenesis are considerably less clear. Our findings presented here establish a unique role for inflammasome-independent IL-1β in autoinflammatory bone disease and osteomyelitis.

Mutation in the highly homologous Pombe Cdc15 homology (PCH) family member, PSTPIP1, causes a rare genetic disorder known as pyogenic arthritis, pyoderma gangrenosum and acne (PAPA) syndrome in humans (22). It was discovered that the PAPA-inducing mutation in PSTPIP1 results in aberrant inflammasome activation and IL-1β–driven pathology in patients, suggesting that PSTPIP1 is a novel negative regulator of inflammasomes (23–26). In line with this, IL-1 blockade therapies including Anakinra treatment have proven effective in controlling inflammatory flares in PAPA syndrome patients (27, 28). Both PSTPIP1 and PSTPIP2 share an N-terminal FerCIP4 homology (FCH) domain and a central coiled coil domain (19). However, PSTPIP2 lacks the terminal *Src* homology 3 (SH3) domain that is present in PSTPIP1 (20). Based upon this high degree of sequence and structural homology it was believed that PSTPIP1 and PSTPIP2 may function in a similar capacity to dampen inflammasome-induced IL-1β. Our findings

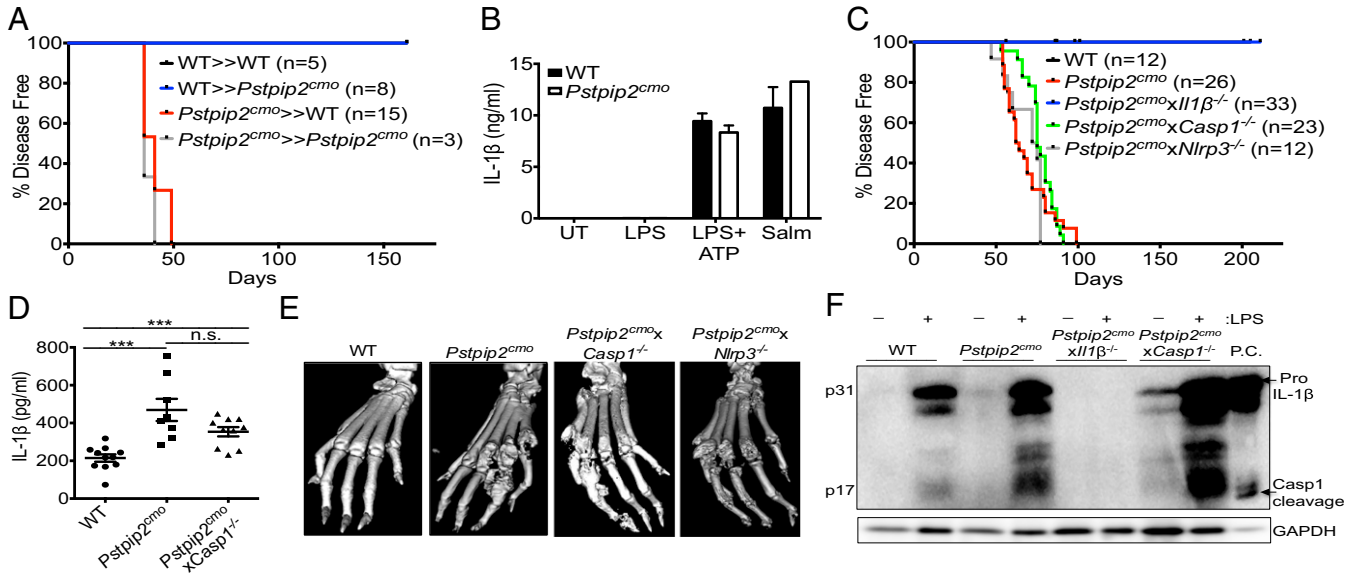

Fig. 5. Inflammasome-independent processing of IL-1β drives osteomyelitis. (*A*) Incidence of disease over time in bone marrow chimeras (donor>>recipient). (*B*) Secretion of IL-1β by WT or *Pstpip2*^{cmo} bone marrow-derived macrophages following stimulation with LPS, LPS+ATP, or *Salmonella* (Salm). (*C*) Incidence of disease over time for WT, *Pstpip2*^{cmo}, *Pstpip2*^{cmo}x*Casp1*$^{-/-}$, and *Pstpip2*^{cmo}x*Nlrp3*$^{-/-}$ mice. (*D*) Levels of IL-1β in the hind paws of diseased *Pstpip2*^{cmo}, *Pstpip2*^{cmo}x*Casp1*$^{-/-}$, and WT control mice. Each point represents an individual mouse, and the line represents the mean ± SEM. Data are combined from two independent experiments. (*E*) Representative micro-CT scans for WT, *Pstpip2*^{cmo}, *Pstpip2*^{cmo}x*Casp1*$^{-/-}$, and *Pstpip2*^{cmo}x*Nlrp3*$^{-/-}$ mice. n.s., not statistically significant. (*F*) Western blot analysis of IL-1β processing in the hind paws of WT, *Pstpip2*^{cmo}, *Pstpip2*^{cmo}x*Casp1*$^{-/-}$, and *Pstpip2*^{cmo}x*Il1β*$^{-/-}$ mice that received footpad injections of PBS or 0.125 mg LPS 2 h before processing. Data are representative of three independent experiments. As a positive control (P.C.) for inflammasome-mediated IL-1β cleavage, WT bone marrow-derived macrophages were treated with LPS+ATP. ***$P < 0.001$.

coupled with older data on PSTPIP1 suggest that both PSTPIP1 and PSTPIP2 are involved in negatively regulating IL-1β production; however, they do so by influencing different pathways. On one hand, PSTPIP1 limits IL-1β production through its regulation of inflammasome activation, whereas PSTPIP2 uniquely dampens caspase-1–independent sources of IL-1β. It is tempting to speculate that PSTPIP2 may have evolved to suppress caspase-1–independent sources of IL-1β.

Our findings demonstrate that unlike most IL-1–dependent diseases, *Pstpip2*^{cmo}-mediated osteomyelitis is not an inflammasomopathy. In contrast, PSTPIP2 negatively regulates IL-1β secretion in caspase-1– and NLRP3-autonomous fashion. Although caspase-1–mediated secretion of IL-1β is the most well characterized mechanism for IL-1β activation, other inflammasome-independent sources of IL-1β have also been reported to contribute to autoinflammatory disease pathogenesis (10, 12). Caspase-1–independent IL-1β has been described to play crucial roles in osteoarthritis (29), particulate-induced lung inflammation (30), host defense against certain pathogens (31, 32), and other inflammatory diseases (33). Our findings suggest that PSTPIP2-targeted therapies may prove helpful in the treatment of such diseases.

Furthermore, genetic deletion of caspase-1 does not lead to complete abrogation of IL-1β production or disease progression in numerous IL-1–dependent disease models (33, 34), suggesting that inflammasome-independent sources can also potentiate disease pathology in some settings. The identification of alternative mechanisms of IL-1β processing and secretion is an emerging area of IL-1 biology and inflammatory disease. Candidate proteases that have been proposed to contribute to caspase-1–independent processing of IL-1β include caspase-8, cathepsin B, PR-3, elastase, chymase, and cathepsin-G (13). The molecular mechanisms that orchestrate the activation of IL-1β by these proteases are just beginning to be elucidated, and not much is known. Our results position PSTPIP2 as a negative regulator of inflammasome-independent IL-1β production.

The rate and severity of bone diseases are expected to rise in the future as a result of increased life expectancies, sedentary lifestyles, and obesity. Continued efforts to define the immunological and molecular underpinnings of bone disorders will offer improved treatments for these debilitating diseases. Our work highlights the central role that inflammasome-independent IL-1β plays in mediating osteolytic bone disease and identifies PSTPIP2 as a negative regulator of such inflammatory pathways. Consequently, our findings suggest that therapeutic neutralization of IL-1β may provide unique approaches to treat CRMO and other osteomyelitic diseases.

Methods

All mice were kept in specific pathogen-free conditions within the Animal Resource Center at St. Jude Children's Research Hospital. Animal studies were conducted under protocols approved by the Institutional Animal Care and Use Committee of St. Jude Children's Research Hospital. Detailed methods are described in *SI Methods*.

ACKNOWLEDGMENTS. We thank Drs. D. Chaplin, J. Bertin, and R. Flavell for the generous supply of mutant mice. We thank J. Kim in the St. Jude Small Animal Imaging Center for helping to acquire and analyze the micro-CT data. We thank M. Barr and G. Johnson for excellent technical assistance and members of the T.-D.K. laboratory for their important suggestions. M.L. is supported by European Union Marie-Curie Grant 256432, European Research Council Grant 281600, and Grants G030212N, 1.2.201.10.N.00, and 1.5.122.11.N.00 from the Fund for Scientific Research-Flanders. This work was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health (NIH), Award AR056296 (to T.-D.K.); the National Cancer Institute, NIH, Award CA163507 (to T.-D.K.); the National Institute of Allergy and Infectious Diseases, NIH, Award AI101935 (to T.-D.K.); and ALSAC.

1. Takayanagi H (2007) Osteoimmunology: Shared mechanisms and crosstalk between the immune and bone systems. *Nat Rev Immunol* 7(4):292–304.
2. Walsh MC, et al. (2006) Osteoimmunology: Interplay between the immune system and bone metabolism. *Annu Rev Immunol* 24:33–63.
3. Schett G (2009) Osteoimmunology in rheumatic diseases. *Arthritis Res Ther* 11(1):210.
4. Ferguson PJ, et al. (2006) A missense mutation in pstpip2 is associated with the murine autoinflammatory disorder chronic multifocal osteomyelitis. *Bone* 38(1):41–47.
5. Grosse J, et al. (2006) Mutation of mouse Mayp/Pstpip2 causes a macrophage autoinflammatory disease. *Blood* 107(8):3350–3358.
6. Chitu V, et al. (2012) PSTPIP2 deficiency in mice causes osteopenia and increased differentiation of multipotent myeloid precursors into osteoclasts. *Blood* 120(15):3126–3135.
7. Chitu V, et al. (2009) Primed innate immunity leads to autoinflammatory disease in PSTPIP2-deficient cmo mice. *Blood* 114(12):2497–2505.
8. El-Shanti HI, Ferguson PJ (2007) Chronic recurrent multifocal osteomyelitis: A concise review and genetic update. *Clin Orthop Relat Res* 462(462):11–19.
9. Lukens JR, Dixit VD, Kanneganti TD (2011) Inflammasome activation in obesity-related inflammatory diseases and autoimmunity. *Discov Med* 12(62):65–74.
10. Lukens JR, Gross JM, Kanneganti TD (2012) IL-1 family cytokines trigger sterile inflammatory disease. *Frontiers Immunol* 3:315.
11. Kanneganti TD (2010) Central roles of NLRs and inflammasomes in viral infection. *Nat Rev Immunol* 10(10):688–698.
12. Netea MG, et al. (2010) IL-1beta processing in host defense: Beyond the inflammasomes. *PLoS Pathog* 6(2):e1000661.
13. Dinarello CA (2011) Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood* 117(14):3720–3732.
14. Lukens JR, et al. (2013) RIP1-driven autoinflammation targets IL-1α independently of inflammasomes and RIP3. *Nature* 498(7453):224–227.
15. Cohen I, et al. (2010) Differential release of chromatin-bound IL-1alpha discriminates between necrotic and apoptotic cell death by the ability to induce sterile inflammation. *Proc Natl Acad Sci USA* 107(6):2574–2579.
16. Pacifici R (2010) T cells: Critical bone regulators in health and disease. *Bone* 47(3):461–471.
17. Yao Z, Xing L, Boyce BF (2009) NF-kappaB p100 limits TNF-induced bone resorption in mice by a TRAF3-dependent mechanism. *J Clin Invest* 119(10):3024–3034.
18. Brennan FM, McInnes IB (2008) Evidence that cytokines play a role in rheumatoid arthritis. *J Clin Invest* 118(11):3537–3545.
19. Chitu V, Stanley ER (2007) Pombe Cdc15 homology (PCH) proteins: Coordinators of membrane-cytoskeletal interactions. *Trends Cell Biol* 17(3):145–156.
20. Wu Y, Dowbenko D, Lasky LA (1998) PSTPIP 2, a second tyrosine phosphorylated, cytoskeletal-associated protein that binds a PEST-type protein-tyrosine phosphatase. *J Biol Chem* 273(46):30487–30496.
21. Yeung YG, Soldera S, Stanley ER (1998) A novel macrophage actin-associated protein (MAYP) is tyrosine-phosphorylated following colony stimulating factor-1 stimulation. *J Biol Chem* 273(46):30638–30642.
22. Wise CA, et al. (2002) Mutations in CD2BP1 disrupt binding to PTP PEST and are responsible for PAPA syndrome, an autoinflammatory disorder. *Hum Mol Genet* 11(8):961–969.
23. Yu JW, et al. (2007) Pyrin activates the ASC pyroptosome in response to engagement by autoinflammatory PSTPIP1 mutants. *Mol Cell* 28(2):214–227.
24. Shoham NG, et al. (2003) Pyrin binds the PSTPIP1/CD2BP1 protein, defining familial Mediterranean fever and PAPA syndrome as disorders in the same pathway. *Proc Natl Acad Sci USA* 100(23):13501–13506.
25. Scianaro RI, et al. (2011) Deregulation of IL-1b axis in peripheral blood mononuclear cells from patients with Chronic Recurrent Multifocal Osteomyelitis. *Pediatric Rheumatology* 9:307.
26. Fernandes-Alnemri T, et al. (2007) The pyroptosome: A supramolecular assembly of ASC dimers mediating inflammatory cell death via caspase-1 activation. *Cell Death Differ* 14(9):1590–1604.
27. Dierselhuis MP, Frenkel J, Wulffraat NM, Boelens JJ (2005) Anakinra for flares of pyogenic arthritis in PAPA syndrome. *Rheumatology* 44(3):406–408.
28. Brenner M, Ruzicka T, Plewig G, Thomas P, Herzer P (2009) Targeted treatment of pyoderma gangrenosum in PAPA (pyogenic arthritis, pyoderma gangrenosum and acne) syndrome with the recombinant human interleukin-1 receptor antagonist anakinra. *Br J Dermatol* 161(5):1199–1201.
29. Joosten LA, et al. (2009) Inflammatory arthritis in caspase 1 gene-deficient mice: contribution of proteinase 3 to caspase 1-independent production of bioactive interleukin-1beta. *Arthritis Rheum* 60(12):3651–3662.
30. Provoost S, et al. (2011) NLRP3/caspase-1-independent IL-1beta production mediates diesel exhaust particle-induced pulmonary inflammation. *J Immunol* 187(6):3331–3337.
31. Mayer-Barber KD, et al. (2010) Caspase-1 independent IL-1beta production is critical for host resistance to mycobacterium tuberculosis and does not require TLR signaling in vivo. *J Immunol* 184(7):3326–3330.
32. Gringhuis SI, et al. (2012) Dectin-1 is an extracellular pathogen sensor for the induction and processing of IL-1β via a noncanonical caspase-8 inflammasome. *Nat Immunol* 13(3):246–254.
33. Kono H, Orlowski GM, Patel Z, Rock KL (2012) The IL-1-dependent sterile inflammatory response has a substantial caspase-1-independent component that requires cathepsin C. *J Immunol* 189(7):3734–3740.
34. Lukens JR, Barr MJ, Chaplin DD, Chi H, Kanneganti TD (2012) Inflammasome-derived IL-1β regulates the production of GM-CSF by CD4(+) T cells and γδ T cells. *J Immunol* 188(7):3107–3115.