IMMUNOLOGY

Pathogen blockade of TAK1 triggers caspase-8–dependent cleavage of gasdermin D and cell death

Pontus Orning[1,2], Dan Weng[1,3]*, Kristian Starheim[1,2]*, Dmitry Ratner[1]*, Zachary Best[1], Bettina Lee[4], Alexandria Brooks[1], Shiyu Xia[5], Hao Wu[5], Michelle A. Kelliher[6], Scott B. Berger[7], Peter J. Gough[7], John Bertin[7], Megan M. Proulx[8], Jon D. Goguen[8], Nobuhiko Kayagaki[4], Katherine A. Fitzgerald[1,2], Egil Lien[1,2]†

Limited proteolysis of gasdermin D (GSDMD) generates an N-terminal pore-forming fragment that controls pyroptosis in macrophages. GSDMD is processed via inflammasome-activated caspase-1 or -11. It is currently unknown whether macrophage GSDMD can be processed by other mechanisms. Here, we describe an additional pathway controlling GSDMD processing. The inhibition of TAK1 or IκB kinase (IKK) by the *Yersinia* effector protein YopJ elicits RIPK1- and caspase-8–dependent cleavage of GSDMD, which subsequently results in cell death. GSDMD processing also contributes to the NLRP3 inflammasome–dependent release of interleukin-1β (IL-1β). Thus, caspase-8 acts as a regulator of GSDMD-driven cell death. Furthermore, this study establishes the importance of TAK1 and IKK activity in the control of GSDMD cleavage and cytotoxicity.

The robust and rapid induction of innate immune signaling is a hallmark of the host response to microbial infection. Successful pathogens subvert, thwart, or dismantle these defensive measures. Growing evidence suggests that the host recognizes these disruptive efforts, eliciting effective backup measures. Cell death processes, including apoptosis and pyroptosis, are integral components of the host response to infection. Multiprotein inflammasome complexes sense the presence of pathogens and activate inflammatory caspases, typically caspase-1 or caspase-11, leading to pyroptotic cell death and maturation of the inflammatory cytokines interleukin-1β (IL-1β) and IL-18. Pyroptosis is an inflammasome-driven cytotoxic process that occurs in macrophages after limited proteolysis of gasdermin D (GSDMD). The generation of an N-terminally cleaved fragment then creates large oligomeric membrane pores and causes lytic cell death (*1*–*7*). At present, caspase-1 and caspase-11 are the only known regulators of GSDMD in macrophages (*5*, *7*), although neutrophil elastase controls GSDMD cleavage in neutrophils (*8*).

Caspase-8 is an upstream activator of caspase-3 and controls apoptotic cell death. In addition, caspase-8 prevents RIPK3–MLKL (mixed lineage kinase domain–like protein)–dependent necroptosis. Increasing evidence indicates important roles for caspase-8 in inflammatory responses in macrophages infected with diverse pathogens. Caspase-8 activation can trigger NLRP3 inflammasomes (*9*) and may also serve as a backup measure when the caspase-1 pathway is blocked or deleted (*10*, *11*). Pathogenic bacteria of the genus *Yersinia* include *Y. pseudotuberculosis* and *Y. enterocolitica*, which cause gastrointestinal disease, and *Y. pestis*, the etiologic agent of plague. *Yersinia* bacteria, through the action of their type III secretion systems, release effectors that manipulate host cells. One of these, YopJ, is a strong activator of caspase-8 via RIPK1 (*12*, *13*). YopJ is an acetyl transferase that blocks the phosphorylation and activation of kinases TAK1, IκB kinase β (IKKβ), and mitogen-activated protein kinase (MAPK) kinases (*14*–*17*). The inhibition of TAK1 is associated with cell death and inflammation (*18*–*20*) and is not limited to *Yersinia*. Several additional pathogens, including *Pseudomonas*, *Vibrio*, and enteroviruses, also target TAK1 (*21*–*23*).

Yersinia bacteria induce cell death, caspase-1 cleavage, and IL-1β release, whereas mutants lacking YopJ do not (*12*, *13*) (fig. S1A). By comparing these responses in wild-type macrophages to responses in macrophages lacking RIPK3 or RIPK3–caspase-8, we previously found that caspase-8 is important for all of these effects (*12*). To investigate the pathways involved, we examined the acute inhibition of TAK1 kinase activity with TAK1 inhibitor (TAK1-i) 5z-7-oxozeaneol (5z7), a specific small-molecule inhibitor, as genetic deletion of TAK1 in macrophages leads to spontaneous death (*20*). TAK1-i induced cell death and IL-1β release in lipopolysaccharide (LPS)–stimulated macrophages (Fig. 1, A to C, and fig. S1, B to D). Similar findings were obtained with NG25, a second inhibitor of TAK1 (fig. S1C). TAK1-i also restored caspase-8–dependent death and IL-1β release in cells infected with YopJ mutant *Yersinia* (Fig. 1, A and B). We found that tumor necrosis factor (TNF) together with TAK1-i could induce similar responses (Fig. 1, D and E). RIPK1 can control caspase-8 activation and is necessary for cell death induced by *Yersinia* and TAK1 inhibition (*12*) (fig. S1, E to I). One function of TAK1 is to activate IKK, which also controls RIPK1 activity. Thus, IKK blockade may effectively trigger a pathway similar to that triggered by TAK1 inhibition (Fig. 1F and fig. S1I). Furthermore, TAK1's effects on IKK are likely key early events in this pathway, rather than effects on MAPK kinase and MAPKs such as p38 (fig. S1J). These responses may serve as a host mechanism to detect the pathological disturbance of TAK1 kinase activity.

TAK1 inhibition by *Yersinia* or by TAK1-i was associated with oligomerization of ASC (apoptosis-associated specklike protein containing a caspase activation and recruitment domain) (Fig. 1, G and H). This oligomerization was dependent on RIPK1, caspase-8, and NLRP3 but not caspase-1 or -11 (Fig. 1, H to J, and fig. S1K). Notably, pan-caspase inhibition blocked ASC oligomerization and IL-1β release but not cytotoxicity. This was likely because of RIPK3-dependent necroptosis triggered by caspase-8 inhibition (Fig. 1J and fig. S1, L to N). Macrophage death was independent of the NLRP3 inflammasome, and IL-1β release was partially dependent on NLRP3 (*24*) (fig. S2). The loss of Toll-like receptor 4 (TLR4), TRIF, or TNF receptor 1 (TNFR1) caused a significant reduction in cell death after bacterial infection or treatment with LPS and TAK1-i (fig. S3, A to C). This suggests activation of the RIPK1–caspase-8 death pathway downstream of TLR4-TRIF activation, perhaps with other contributing bacterial ligands, with concurrent TNF production and TNFR1 autocrine signaling (*25*). TAK1-i combined with additional stimulations, such as TLR2 and TLR7/TLR8 ligands, could also trigger TNFR1-, MyD88-, and caspase-8–dependent cell death and IL-1β release at later time points (fig. S3, D to H). Cell death induced by *Yersinia* has been proposed to be caspase-8–dependent apoptosis (*12*). Although caspase-3 and caspase-7 are activated by *Yersinia* (*12*, *13*) (fig. S4A), deficiency of these caspases did not affect cell death (*13*) (fig. S4B and table S1), and the combined action of multiple components may be necessary. Many *Yersinia*-infected cells display features of apoptosis, including membrane blebs, nuclear condensation, and DNA laddering (*12*). However, we observed significant cleavage of GSDMD after infection, indicating the simultaneous presence of pyroptotic processes (Fig. 1K). The detection antibody we used recognizes the N-terminal 30-kDa pore-forming pyroptotic fragment of GSDMD. *Yersinia* also induced a smaller, 20-kDa fragment and a

[1]Program in Innate Immunity, Department of Medicine, Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, MA 01605, USA. [2]Centre of Molecular Inflammation Research, Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology, 7491 Trondheim, Norway. [3]Center for Molecular Metabolism, Nanjing University of Science and Technology, Nanjing 210094, China. [4]Department of Physiological Chemistry, Genentech, South San Francisco, CA 94080, USA. [5]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, and Program in Cellular and Molecular Medicine, Boston Children's Hospital, Boston, MA 02115, USA. [6]Department of Cancer Biology, University of Massachusetts Medical School, Worcester, MA 01605, USA. [7]Pattern Recognition Receptor Discovery Performance Unit, Immuno-inflammation Therapeutic Area, GlaxoSmithKline, Collegeville, PA 19426, USA. [8]Department of Microbiology and Physiology, University of Massachusetts Medical School, Worcester, MA 01655, USA.
*These authors contributed equally to this work.
†Corresponding author. Email: egil.lien@umassmed.edu

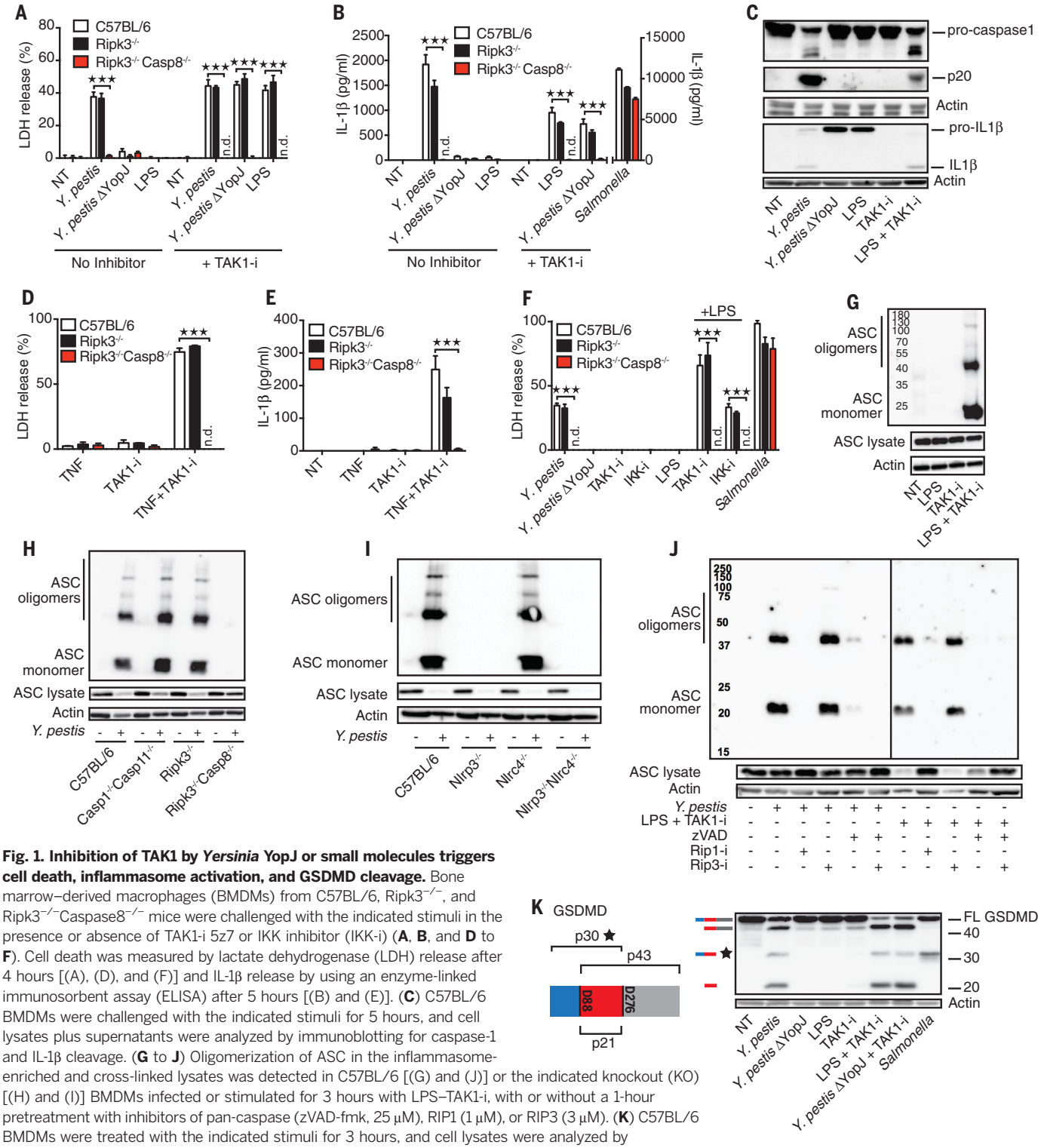

Fig. 1. Inhibition of TAK1 by *Yersinia* YopJ or small molecules triggers cell death, inflammasome activation, and GSDMD cleavage. Bone marrow–derived macrophages (BMDMs) from C57BL/6, Ripk3$^{-/-}$, and Ripk3$^{-/-}$Caspase8$^{-/-}$ mice were challenged with the indicated stimuli in the presence or absence of TAK1-i 5z7 or IKK inhibitor (IKK-i) (**A**, **B**, and **D** to **F**). Cell death was measured by lactate dehydrogenase (LDH) release after 4 hours [(A), (D), and (F)] and IL-1β release by using an enzyme-linked immunosorbent assay (ELISA) after 5 hours [(B) and (E)]. (**C**) C57BL/6 BMDMs were challenged with the indicated stimuli for 5 hours, and cell lysates plus supernatants were analyzed by immunoblotting for caspase-1 and IL-1β cleavage. (**G** to **J**) Oligomerization of ASC in the inflammasome-enriched and cross-linked lysates was detected in C57BL/6 [(G) and (J)] or the indicated knockout (KO) [(H) and (I)] BMDMs infected or stimulated for 3 hours with LPS–TAK1-i, with or without a 1-hour pretreatment with inhibitors of pan-caspase (zVAD-fmk, 25 μM), RIP1 (1 μM), or RIP3 (3 μM). (**K**) C57BL/6 BMDMs were treated with the indicated stimuli for 3 hours, and cell lysates were analyzed by immunoblotting for GSDMD cleavage. In the illustration of GSDMD, predicted cleavage sites and fragment sizes are indicated. Data are presented as the mean ± SD for triplicate wells from three or more independent experiments. For comparisons, Student's *t* test was used. ***$P < 0.001$. NT, nontreated; n.d., not detectable; Rip1-i, RIP1 inhibitor; FL, full-length.

larger p45 fragment, suggesting further cleavage of full-length protein (*26*) or p30 by caspase-3 at GSDMD Asp^{88} (fig. S4C). We found that GSDMD was a central mediator of both cell death and the release of IL-1β and IL-18, which are induced by *Yersinia* infection and treatment with LPS or TNF combined with TAK1-i or IKK inhibition and are controlled by RIPK1–caspase-8 (Fig. 2, A to G, and figs. S1, E to I, and S4, D and E). GSDMD also influenced YopJ- or TAK1-i–induced caspase-1 p20 cleavage and ASC oligomerization (Fig. 2, D, H, and I, and table S2). Excess extracellular potassium completely blocked ASC oligomerization and partially inhibited IL-1β release but did not affect cell death (Fig. 2, J to L, and

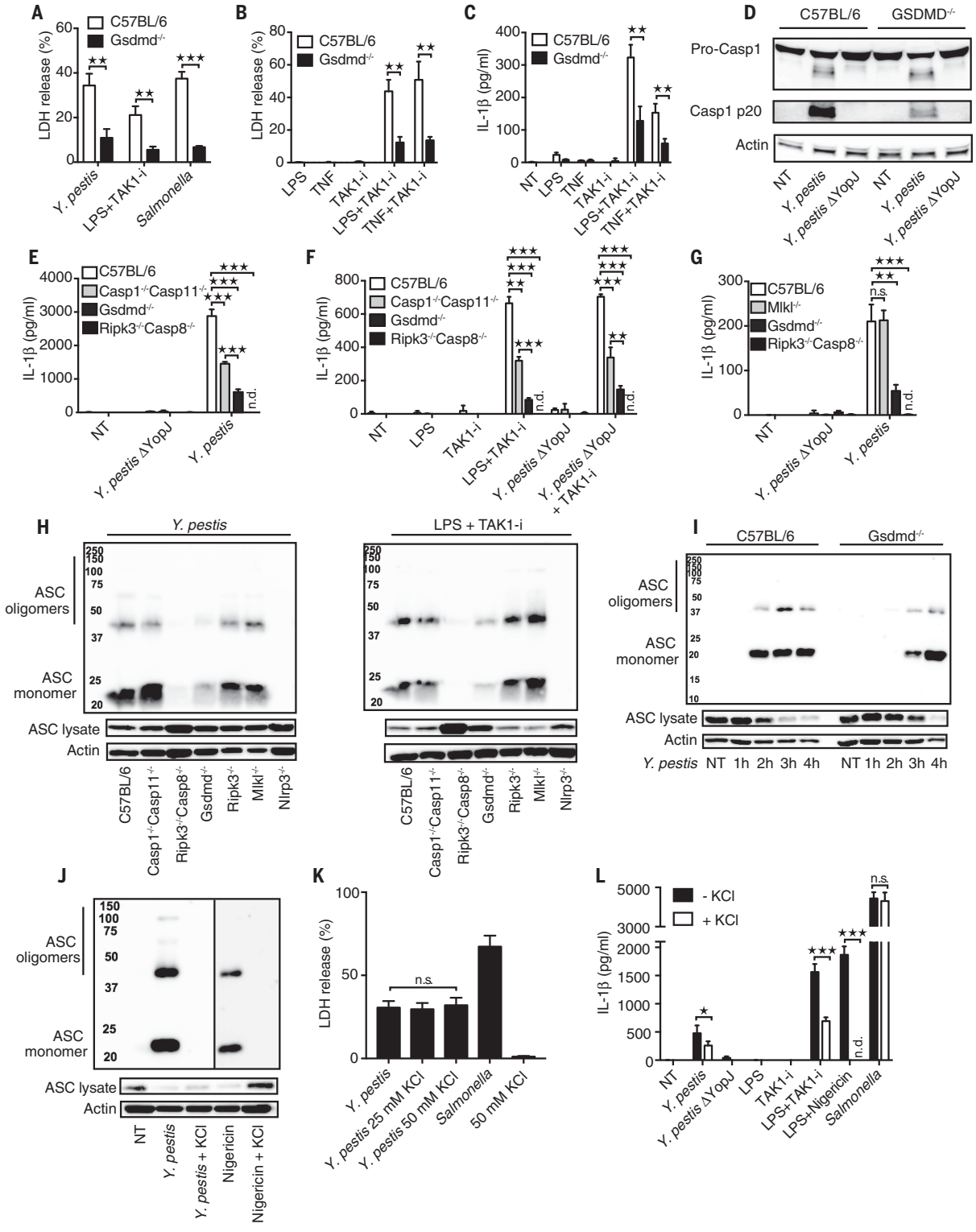

Fig. 2. GSDMD regulates cell death and inflammasome activation in response to TAK1 inhibition. (**A** to **G**) The indicated BMDMs were challenged with *Y. pestis*, a *Y. pestis* mutant lacking YopJ, or *Salmonella enterica* serovar Typhimurium or stimulated with the indicated ligands, TAK1-i, or ligands and TAK1-i. Cell death was measured by LDH release after 3 hours [(A) and (B)] and IL-1β release after 5 hours [(C) and (E) to (G)] or caspase-1 cleavage after 5 hours (D). (**H** to **J**) The oligomerization of ASC in the inflammasome-enriched and cross-linked lysates was detected in BMDMs after *Y. pestis* or LPS–TAK1-i challenge at the indicated time points (I) or after 3 hours. (**J** to **L**) BMDMs were treated with *Y. pestis*, *S.* Typhimurium, or nigericin and LPS in the presence or absence of 50 mM [(J) and (L)] or 25 mM (K) KCl, and the oligomerization of ASC was detected by immunoblotting. Cell death was measured by LDH release or IL-1β release by using ELISA. Data are presented as the mean ± SD for triplicate wells from three or more independent experiments. For comparisons between two groups, Student's *t* test was used; for more than two groups, analysis of variance (ANOVA) was used. n.s., not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

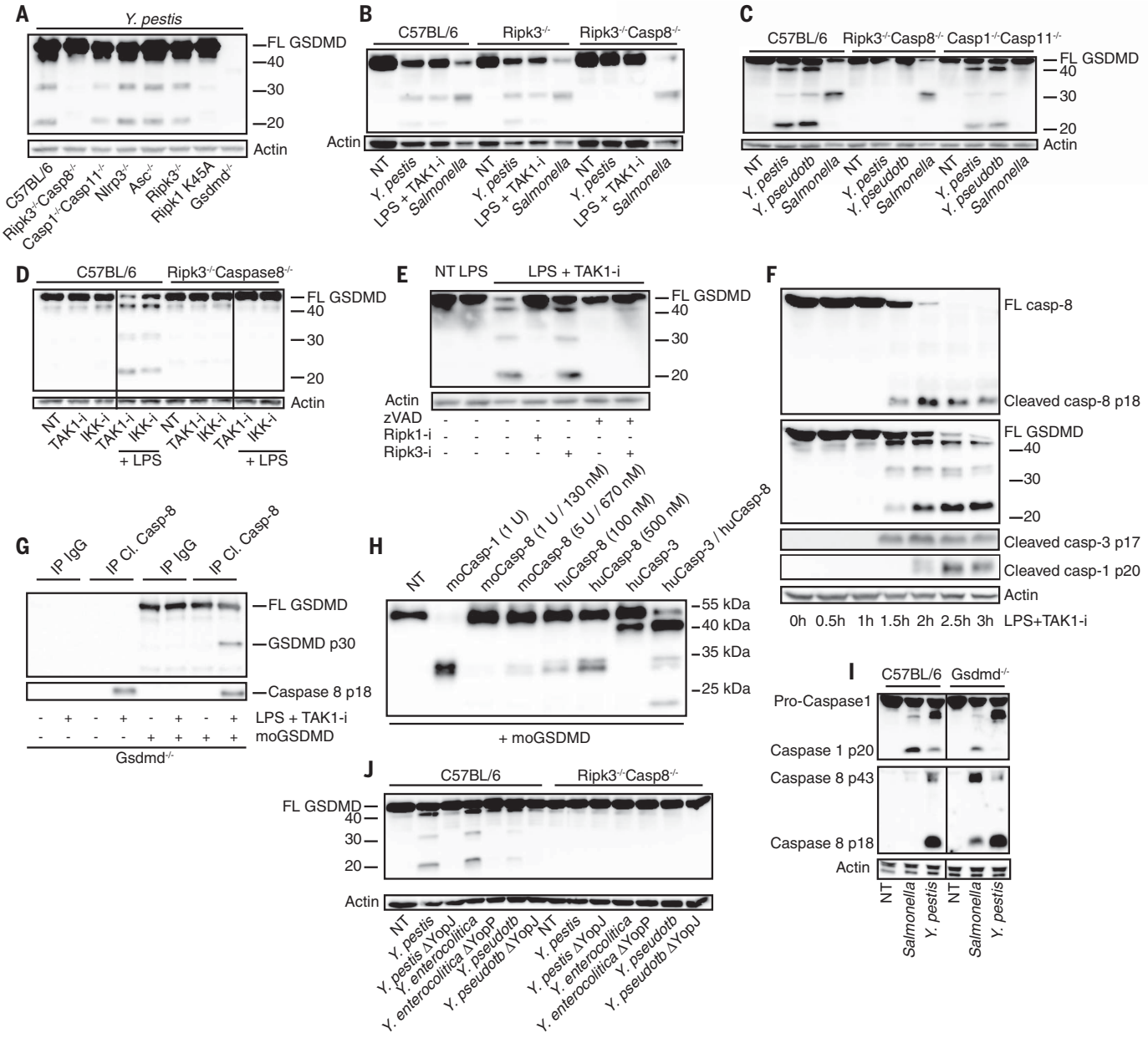

Fig. 3. Caspase-8–dependent GSDMD processing with little contribution by caspase-1 and -11. (**A** to **F**, **I**, and **J**) BMDMs from the indicated mouse strains were challenged with *Y. pestis*, *Y. pseudotuberculosis*, *Y. enterocolitica*, their YopJ (YopP in *Y. enterocolitica*) mutants, or *S.* Typhimurium or treated with LPS–TAK1-i. Cell lysates were harvested after 3 to 4 hours unless otherwise stated and analyzed by immunoblotting for GSDMD cleavage [(A) to (F) and (J)] and/or caspase-1, caspase-3, and caspase-8 cleavage [(F) and (I)]. K45A, Lys^{45}→Ala. (D) BMDMs from C57BL/6 or $Ripk3^{-/-}Casp8^{-/-}$ mice were treated with IKK-i and TAK1-i in the absence or presence of LPS. (E) BMDMs were pretreated with pan-caspase inhibitor zVAD-fmk, RIPK1 inhibitor (RIPK1-i), or RIPK3-i for 1 hour before being treated with LPS–TAK1-i. (**G**) $Gsdmd^{-/-}$ BMDMs were treated with LPS–TAK1-i for 3 hours and then immunoprecipitated with cleaved (Cl.) caspase-8 antibody. Immunoprecipitates (IPs) (G) or recombinant caspases (**H**) were incubated with recombinant mouse GSDMD at 37°C for 1 hour in a protein cleavage buffer before being analyzed by immunoblotting. Panels are representative of three or more independent experiments. IgG, immunoglobulin G; mo, mouse; hu, human.

table S2). Thus, RIPK1–caspase-8 appears to exert full control of the membrane damage that triggers potassium efflux. GSDMD displays partial control—possibly via GSDMD pore formation—over potassium efflux, and these signals direct NLRP3 activation, ASC oligomerization, and ultimately, IL-1β release. However, NLRP3 and caspase-1 and -11 do not appreciably contribute to cell death in this case (fig. S2, A and B).

GSDMD cleavage has previously been associated with caspase-1 and caspase-11 activity (*2*, *4*, *5*, *7*). By contrast, *Yersinia*- and TAK1-i–induced GSDMD p30 was entirely dependent upon RIPK1–caspase-8, with minor contributions from caspase-1 and -11 and RIPK3 (Fig. 3, A to E, and fig. S5, A to D). Caspase-8 and GSDMD were cleaved before the appearance of any detectable cleaved caspase-1 (Fig. 3F and fig. S5E). Thus, we have identified an additional pathway leading to processing of GSDMD. In addition, cleaved caspase-8 immunoprecipitated (by using a p18 antibody) from TAK1-i–LPS–treated GSDMD-deficient cells was capable of cleaving purified mouse GSDMD (Fig. 3G and fig. S5, F to H) and generating the p30 pore-forming fragment. Although recombinant caspase-8 may not process GSDMD as strongly as caspase-1 (*5*, *26*), increased

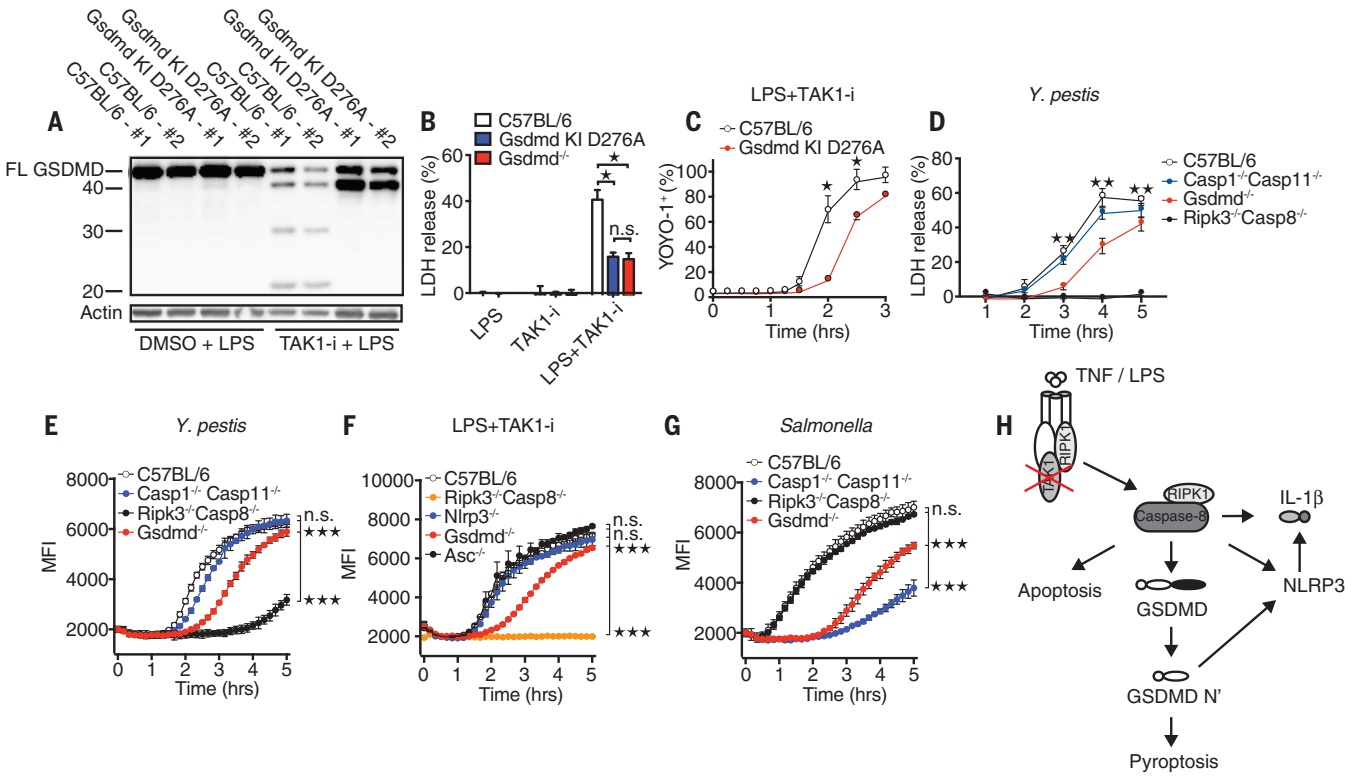

Fig. 4. The GSDMD Asp^{276} residue is critical for GSDMD processing and for regulation of cell death downstream of TAK1 inhibition. (**A**) Immunoblot of GSDMD cleavage in C57BL/6 or GSDMD D276A mutant BMDMs after treatment with LPS and LPS–TAK1-i. Cell death was measured by LDH release (**B** and **D**) or by the percentage of YOYO-1$^+$ cells (**C**) after challenge of the indicated genotypes with LPS–TAK1-i or *Y. pestis*. DMSO, dimethyl sulfoxide. (**E** to **G**) Cell death as measured by entry of EthD-1 into the cell. Samples were read every 10 min at 530-nm excitation and 645-nm emission wavelengths. BMDMs from the indicated genotypes were treated with *Y. pestis* (E), LPS–TAK1-i (F), or *S.* Typhimurium (G). Infections represented in (E) and (G) were without gentamicin. For (E) to (G), statistical analyses were performed on the area under the curve for the whole time course. MFI, mean fluorescense intensity. (**H**) Proposed model of TAK1 inhibition–induced caspase-8 activation and cell death. Data are presented as the mean ± SD for triplicate wells from two [(A) to (C)] or three or more [(D) to (G)] independent experiments. n.d., not detected. For comparisons between two groups, Student's *t* test was used; for more than two groups, ANOVA was used. *P < 0.05; **P < 0.01; ***P < 0.001.

concentrations of mouse and human caspase-8 cleaved GSDMD (Fig. 3H and fig. S5I). In addition, a mixture of both caspase-3 and caspase-8 reproduced the cleavage pattern induced by *Yersinia* or TAK1-IKK inhibition (Fig. 3H). The loss of GSDMD had no effect on caspase-8 activation, consistent with the hypothesis that GSDMD acts downstream of caspase-8 (Fig. 3I and fig. S5J). We observed YopJ-dependent caspase-8 cleavage, GSDMD processing, and cell death with *Y. pseudotuberculosis*, *Y. enterocolitica*, and *Y. pestis*, suggesting that the triggering of this pathway is conserved among these *Yersinia* species (Fig. 3J and figs. S1A and S5J). *Salmonella*, normally a strong activator of caspase-1 and GSDMD, induces the processing of caspase-8 when GSDMD is absent (Fig. 3I), as others have suggested (*10*, *11*). We note that *Yersinia* has several effectors inhibiting caspase-1 cleavage (*27*, *28*). This may contribute to the activation of caspase-8 upon infection by this pathogen. Treatment of macrophages from a GSDMD Asp^{276}→Ala (D276A) knock-in (KI) mouse (*29*) containing a mutation in the caspase-1–caspase-11 cleavage motif of GSDMD (^{273}Leu-Leu-Ser-Asp^{276}) with an LPS–TAK1-i combination revealed that the caspase-8–directed cleavage of GSDMD depended on the GSDMD D276 cleavage site (Fig. 4, A to C). These observations expand upon the requirement of caspase-1 or caspase-11 to proteolytically process GSDMD at D276 for cytotoxicity (*5*, *7*). Cell death time-course experiments showed that the absence of GSDMD, but not that of caspase-8, could be overcome by prolonged incubations with bacteria or TAK1-i–LPS (Fig. 4, C to G, and fig. S6). Thus, GSDMD is not the only mediator of cell death downstream of caspase-8 in the TAK1-IKK–regulated pathway. Rather, additional, still undefined components also participate in this process.

The ability of cells to mobilize countermeasures to detect pathogenic inhibition of key signaling pathways is becoming increasingly apparent. One recent example is the pyrin inflammasome pathway, where disturbances in RhoA guanosine triphosphatase by bacterial toxins trigger pyrin-driven caspase-1 activation (*27*, *28*, *30*). On the basis of our current findings, we propose that pathogen blockade of TAK1-IKK triggers a caspase-8–mediated cell death and inflammatory pathway involving GSDMD. GSDMD-mediated cell death has been considered a defining feature of pyroptosis (*4*, *5*, *31*). Our observations suggest that the blockade of the TAK1-IKK pathways leads to cytotoxicity, with features of both apoptosis and pyroptosis (Fig. 4H and fig. S7). Thus, conditions that affect many cellular processes, such as concurrent effects from bacteria and their secretion system components on different signaling proteins (*27*, *28*), can lead to cell death involving multiple pathways. Inhibition of TAK1 represents a strategy that several pathogens may use to their advantage. However, in the arms race between pathogens and their hosts, the host can sense these disturbances as pathogenic and counter these efforts with cytotoxicity and inflammation. This study broadens our understanding of the pathways leading to GSDMD activation and underscores the importance of GSDMD as a key driver of cell death and inflammation induced by a vast array of pathogens.

REFERENCES AND NOTES

1. L. Sborgi *et al.*, *EMBO J.* **35**, 1766–1778 (2016).
2. X. Liu *et al.*, *Nature* **535**, 153–158 (2016).
3. S. B. Kovacs, E. A. Miao, *Trends Cell Biol.* **27**, 673–684 (2017).
4. J. Shi, W. Gao, F. Shao, *Trends Biochem. Sci.* **42**, 245–254 (2017).
5. J. Shi *et al.*, *Nature* **526**, 660–665 (2015).

6. R. A. Aglietti *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **113**, 7858–7863 (2016).
7. N. Kayagaki *et al.*, *Nature* **526**, 666–671 (2015).
8. H. Kambara *et al.*, *Cell Rep.* **22**, 2924–2936 (2018).
9. J. E. Vince, J. Silke, *Cell. Mol. Life Sci.* **73**, 2349–2367 (2016).
10. I. Rauch *et al.*, *Immunity* **46**, 649–659 (2017).
11. D. P. A. Mascarenhas *et al.*, *PLOS Pathog.* **13**, e1006502 (2017).
12. D. Weng *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **111**, 7391–7396 (2014).
13. N. H. Philip *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **111**, 7385–7390 (2014).
14. U. Meinzer *et al.*, *Cell Host Microbe* **11**, 337–351 (2012).
15. R. Mittal, S.-Y. Peak-Chew, H. T. McMahon, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 18574–18579 (2006).
16. S. Mukherjee *et al.*, *Science* **312**, 1211–1214 (2006).
17. N. Paquette *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 12710–12715 (2012).
18. N. Vanlangenakker *et al.*, *Cell Death Differ.* **18**, 656–665 (2011).
19. M. B. Menon *et al.*, *Nat. Cell Biol.* **19**, 1248–1259 (2017).
20. R. K. S. Malireddi *et al.*, *J. Exp. Med.* **215**, 1023–1034 (2018).
21. X. Lei *et al.*, *J. Virol.* **88**, 9830–9841 (2014).
22. X. Zhou *et al.*, *Cell Rep.* **3**, 1690–1702 (2013).
23. C. He *et al.*, *Infect. Immun.* **85**, e00239-17 (2017).
24. Y. Zheng *et al.*, *PLOS Pathog.* **7**, e1002026 (2011).
25. L. W. Peterson *et al.*, *J. Immunol.* **197**, 4110–4117 (2016).
26. C. Y. Taabazuing, M. C. Okondo, D. A. Bachovchin, *Cell Chem. Biol.* **24**, 507–514.e4 (2017).
27. D. Ratner, M. P. A. Orning, E. Lien, *J. Leukocyte Biol.* **101**, 165–181 (2017).
28. D. Ratner *et al.*, *PLOS Pathog.* **12**, e1006035 (2016).
29. B. L. Lee *et al.*, *J. Exp. Med.* **215**, 2279–2288 (2018).
30. H. Xu *et al.*, *Nature* **513**, 237–241 (2014).
31. L. Galluzzi *et al.*, *Cell Death Differ.* **25**, 486–541 (2018).

ACKNOWLEDGMENTS

We thank G. Germain for help with mice; V. Dixit, N. Kayagaki, and K. Newton (Genentech) for providing GSDMD and RIP3 KO mice; W. Kaiser, E. Mocarski, D. R. Green, and C. Dillon for sending caspase-8 RIP3 double-KO mice; M. O'Riordan for providing *S.* Typhimurium; I. Brodsky and J. Bliska for providing *Y. pseudotuberculosis*; R. Adkins and G. Cornelis for providing *Y. enterocolitica*; G. Salvesen and S. Snipas for providing recombinant caspase-8 and caspase-3; and GlaxoSmithKline and J. Bertin for providing RIPK1 and RIPK3 inhibitors, as well as RIPK1 Lys^{45}→Ala mutant mice. We also thank the A. Poltorak lab for discussions and for sharing data from their unpublished studies and N. Silverman and T. Espevik for critically reading the manuscript. **Funding:** The work was supported by National Institutes of Health grants AI07538 and AI129527 (to E.L.), AI067497 and AI083713 (to K.A.F.), AI095213 (to D.R.), AI139914 (to H.W.), AI075118 (to M.A.K.), and HL092610 (to A.B.); the Norwegian Cancer Society grants 7699133 (to K.S.) and B05035/001 (to E.L.); the UMass Medical School Summer Undergraduate Research Experience Program funding to Z.B.; and the Research Council of Norway Center of Excellence Funding Scheme project 223255/F50 (to P.O., E.L., K.A.F., and K.S.). **Author contributions:** P.O. performed the research with contributions from D.W., K.S., D.R., Z.B., B.L., and A.B. S.X., H.W., M.A.K., S.B.B., P.J.G., J.B., M.M.P., J.D.G., and N.K. provided reagents, mice, and advice. The project was developed by E.L. and supervised by E.L. with input from K.A.F., P.O., K.A.F., and E.L. wrote the paper with help from the other authors. **Competing interests:** B.L. and N.K. are employees of Genentech. S.B.B., P.J.G., and J.B. are employees and shareholders of GlaxoSmithKline. H.W. is a co-founder and consultant for SMOC Therapeutics. K.A.F. is a consultant for Quench Bio. **Data and materials availability:** All data needed to evaluate the conclusions in this paper are present either in the main text or in the supplementary materials. MLKL KO mice were obtained via a material transfer agreement (MTA) with the Walter and Eliza Hall Institute of Medical Research. Caspase 8–deficient mice were obtained via an MTA with the University Health Network, Toronto. RIPK3 KO mice, GSDMD KO mice, GSDMD cleavage-dead KI mice, and mouse GSDMD antibody were obtained via an MTA with Genentech. RIPK1 kinase-dead KI mice and RIPK3 and RIPK1 inhibitors were obtained via an MTA with GlaxoSmithKline.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/362/6418/1064/suppl/DC1
Materials and Methods
Figs. S1 to S7
Tables S1 and S2
References (*32*–*57*)

6 June 2018; accepted 17 October 2018
Published online 25 October 2018
10.1126/science.aau2818

Science

Pathogen blockade of TAK1 triggers caspase-8–dependent cleavage of gasdermin D and cell death

Pontus Orning, Dan Weng, Kristian Starheim, Dmitry Ratner, Zachary Best, Bettina Lee, Alexandria Brooks, Shiyu Xia, Hao Wu, Michelle A. Kelliher, Scott B. Berger, Peter J. Gough, John Bertin, Megan M. Proulx, Jon D. Goguen, Nobuhiko Kayagaki, Katherine A. Fitzgerald and Egil Lien

Science **362** (6418), 1064-1069.
DOI: 10.1126/science.aau2818originally published online October 25, 2018

Caspase-8 is a player in pyroptosis

The activation of certain pattern-recognition receptors by pathogen-associated molecular patterns results in the formation of inflammasome complexes. Inflammasome complexes can initiate both the maturation of inflammatory cytokines and pyroptotic cell death via the caspase-mediated cleavage of gasdermin D (GSDMD). As of now, the only known regulators of GSDMD in macrophages are caspase-1 and caspase-11. Orning *et al.* report an additional pathway controlling GSDMD processing. YopJ, an effector molecule produced by *Yersinia* (the causative agent of plague), inhibits TAK1–IκB kinase signaling. This, in turn, results in caspase-8–directed cleavage of GSDMD, pyroptosis, and the release of interleukin 1 β (IL-1β) and IL-18. Thus, in the arms race between host and pathogen, the host recognizes signaling disturbances as pathogenic and counters with inflammation and cell death.

Science, this issue p. 1064