Nonredundancy of IL-1α and IL-1β is defined by distinct regulation of tissues orchestrating resistance versus tolerance to infection

Kevin Eislmayr¹, Annika Bestehorn¹, Luisa Morelli¹, Martina Borroni¹, Lieselotte Vande Walle², Mohamed Lamkanfi², Pavel Kovarik¹*

Interleukin-1α (IL-1α) and IL-1β are inflammatory cytokines with important roles in health and disease. They trigger the same receptor and elicit comparable cellular responses but, for poorly understood reasons, are not redundant in vivo. Here, we decoupled IL-1α and IL-1β functions that drive protective responses against invasive infection with group A Streptococcus. IL-1β was essential for pathogen clearance, hence resistance to infection, by inducing granulocyte colony-stimulating factor at the infection site and establishing emergency granulopoiesis. In contrast, IL-1α governed reprogramming of liver metabolic pathways associated with tolerance to infection. The IL-1α–dominated hepatic regulation corresponded to high IL-1α levels in the liver during infection. Conversely, IL-1β was critical for the regulation of the spleen transcriptome, which correlated with ample IL-1β expression in this tissue. The results identify distinct and organ-specific roles of IL-1α versus IL-1β and implicate spatial restriction of their expression and bioavailability during infection as the underlying mechanism.

INTRODUCTION

Interleukin-1 (IL-1) signaling is one of the most potent triggers and amplifiers of inflammatory responses; it regulates basic physiological processes including defense against infections, metabolism, and tissue homeostasis (1, 2). Conversely, dysregulated IL-1 signaling is involved in pathologies ranging from infectious disease to autoimmune disorders and cancer. It is well established that the activators of IL-1 signaling are the cytokines IL-1α and IL-1β. Given the broad importance of these cytokines, it is unexpected that their distinct functions and the mechanisms underlying their nonredundancy remain poorly defined (1, 2).

IL-1α and IL-1β are encoded by the Il1a and Il1b genes, respectively, located on chromosome 2 in both humans and mice (3). The evolutionarily conserved occurrence of Il1a and Il1b genes in mammals suggests that these cytokines accomplish nonredundant functions in the mammalian immune system. IL-1α and IL-1β display a limited amino acid sequence similarity but adopt a conserved structure consisting of 12 antiparallel β strands that are arranged in a β barrel core motif (4). This structural similarity enables both IL-1α and IL-1β to bind and activate the same receptor, i.e., the IL-1 receptor composed of IL-1R1 and IL-1R3; IL-1α and IL-1β are the only known IL-1R1 agonists. IL-1α and IL-1β appear to generate the same response in the target cell: The ligated IL-1 receptor triggers a myeloid differentiation primary response 88 (Myd88)-dependent signaling cascade that culminates in the activation of mitogen-activated protein (MAP) kinases and several transcription factors, nuclear factor κB (NF-κB), and subsequent gene expression changes (3, 5).

The production of IL-1α and IL-1β is precisely regulated at multiple levels, and dysregulation of IL-1α and IL-1β has been associated with human diseases (1). Both Il1a and Il1b genes are transcriptionally activated by stress and inflammatory stimuli (6, 7). The transcription factors NF-κB, Sp1, and activating protein 1 were shown to activate the Il1a gene (7–10). The transcriptional regulation of Il1b appears to be more complex as both activators such as NF-κB and hypoxia-inducible factor 1α as well as repressors including signal transducers and activators of transcription 1 and natural antisense Il1b RNA (Il1bas) are involved (11–14). Another important layer of control of IL-1 production is by means of changes in Il1a and Il1b mRNA stability (15). The bioavailability of IL-1β is critically regulated by posttranslational processing and release from cells (16, 17). The importance of posttranslational regulation is less well explored for IL-1α, which is known to be biologically active both in unprocessed and processed forms (18).

Common versus unique effects of Il1a and Il1b genes in physiological and pathological settings have been addressed in a direct comparison only in few studies. The establishment of gene-targeted mice revealed that Il1a-deficient and wild-type (WT) mice develop fever and induce acute phase proteins upon subcutaneous turpentine administration, while Il1b-deficient mice lack such responses (19). This study suggested that Il1b plays a more important role in this sterile inflammation model. In contrast, lipopolysaccharide (LPS)/d-galactosamine–induced fulminant hepatitis failure (FHF) was similarly reduced in Il1a- and Il1b-deficient mice indicating important functions of both cytokines in FHF (20). Opposing effects of Il1a and Il1b on disease progression were reported in a dextran sulfate–induced colon inflammation model: Il1a deficiency resulted in a milder disease, while Il1b deficiency correlated with exacerbated conditions (21). Both Il1a and Il1b were found to nonredundantly drive host defense by limiting the growth of Mycobacterium tuberculosis in a lung infection model (22). In a model of lung infection with Legionella pneumophila, Il1a was the major player in neutrophil recruitment early in the infection, while Il1b was important in the later phase of the infection (23). Together, these studies indicate that Il1a and Il1b have nonredundant functions in sterile and bacterial-mediated inflammation; the mechanisms underlying these distinct effects remain poorly defined. It is also unclear how Il1a and Il1b

¹Max Perutz Labs, University of Vienna, Vienna Biocenter (VBC), Dr. Bohr-Gasse 9, A-1030 Vienna, Austria. ²Laboratory of Medical Immunology, Department of Internal Medicine and Pediatrics, Ghent University, C. Heymanslaan 10, 9000 Ghent, Belgium.
*Corresponding author. Email: pavel.kovarik@univie.ac.at
Fig. 1. *Il1a* and *Il1b* are both critical for host protection against GAS. (A) Kaplan-Meier survival curves of *Il1a*<sup>−/−</sup>, *Il1b*<sup>−/−</sup>, *Il1r1*<sup>−/−</sup>, and WT mice (*n* = 10 per genotype) subcutaneously infected with GAS (2 × 10<sup>8</sup> colony-forming units (CFU)). *Il1a*<sup>−/−</sup>, *Il1b*<sup>−/−</sup>, and *Il1r1*<sup>−/−</sup> mice were significantly more susceptible than WT mice. The difference between *Il1a*<sup>−/−</sup> and *Il1b*<sup>−/−</sup> was not significant. Log-rank Mantel-Cox test with Bonferroni correction for multiple comparison. **P** < 0.01; ***P** < 0.001. ns, not significant.

(B) Size of necrotic area at the site of infection in *Il1a*<sup>−/−</sup>, *Il1b*<sup>−/−</sup>, *Il1r1*<sup>−/−</sup>, and WT mice 48 hours p.i. (*n* = 35 for each WT, *Il1a*<sup>−/−</sup>, and *Il1b*<sup>−/−</sup> and *n* = 9 for *Il1r1*<sup>−/−</sup>). Data represent pools of three independent experiments. Box-and-whisker plots with the median value and 10th to 90th percentile. Kruskal-Wallis with Dunn’s multiple comparisons test.

**P** < 0.01; ****P** < 0.0001.

(C) Hematoxylin and eosin (H&E), Gram, CD45, and Ly6G staining of skin sections showing epidermis (E), dermis (D), and muscle (M) from infected *Il1a*<sup>−/−</sup>, *Il1b*<sup>−/−</sup>, *Il1r1*<sup>−/−</sup>, and WT mice 24 hours p.i. H&E staining indicates severe acute necrotizing cellulitis and panniculitis in all sections. Necrotic area (n) is more pronounced in *Il1b*<sup>−/−</sup> as well as in *Il1r1*<sup>−/−</sup> mice and range from the dermis into the muscular layer. Gram staining visualizes invasion of tissue with GAS (blue). Note that bacteria show invasion (arrows) of the muscular layer in *Il1b*<sup>−/−</sup> and *Il1r1*<sup>−/−</sup> mice but not in *Il1a*<sup>−/−</sup> and WT mice. Bacteria (Gram) and neutrophils (Ly6G) often do not colocalize in *Il1b*<sup>−/−</sup> and *Il1r1*<sup>−/−</sup> samples. Scale bars, 500 µm.

(D) Quantification of tissue destruction based on staining shown in (C). Parameters used for grading were necrosis, bacterial invasion into the muscular layer, missing colocalization of polymorphonuclear cells (PMNs as Ly6G<sup>+</sup>) with bacteria, edema, and the overall score [in arbitrary units (AU)] as the sum of all categories. Kruskal-Wallis with Dunn’s multiple comparisons test, *n* = 7 per genotype. *P* < 0.05.
accomplish their nonoverlapping functions despite eliciting comparable responses in cells.

Here, we report that Il1a and Il1b drive host defense against severe subcutaneous infection with group A Streptococcus (GAS) in nonredundant and mechanistically distinct ways. Using Il1a−/− and Il1b−/− mice, we show that both Il1a and Il1b are indispensable for a protective defense. Il1b restricted the dissemination of bacteria by promoting bone marrow (BM) emergency granulopoiesis and, consistently, the induction of the neutrophil differentiation factor granulocyte colony-stimulating factor (G-CSF) upon infection. In contrast, Il1a was dispensable for the containment of bacteria and neutrophilia so that its essential role in protection against GAS infection was in line with enhancing host resistance. The distinct host protecting functions of Il1a and Il1b were reflected by markedly different effects of Il1a and Il1b on the tissue transcriptome: Il1a was the key driver of infection-induced transcriptional reprogramming of multiple metabolic pathways in the liver, whereas Il1b was required for the regulation of the spleen transcriptome. This distinct and organ-specific transcriptome regulation by Il1a and Il1b was not caused by tissue-dependent differences in responses to these cytokines; rather they correlated with different expression levels of each of the two cytokines in a given tissue. The differences between Il1a and Il1b during GAS infection were similar in a model of lung infection with Streptococcus pneumoniae, suggesting a broader relevance of our findings.

RESULTS
Il1a and Il1b are each required for host defense against GAS

In previous studies, we and others demonstrated the requirement for IL-1 receptor (Il1r1) signaling in defense of mice against invasive subcutaneous infection with GAS, i.e., a model of necrotizing fasciitis in humans (12, 24). It remained open whether Il1a and Il1b were redundant in triggering the Il1r1-dependent protective response.

Both Il1a and Il1b mRNAs were induced in the lesion, i.e., in the site of pathogen administration, 24 hours postinfection (p.i.), but Il1b induction was by two orders of magnitude higher than that of Il1a (17-fold versus 5641-fold, respectively) (fig. S1A). This implied that Il1r1-dependent host responses could be elicited by either of the two cytokines. To assess the requirement for Il1a or Il1b or both in host protection, we used Il1a−/− and Il1b−/− mice. First, we tested whether the absence of Il1a affected the expression of Il1b or vice versa. The expression levels of Il1a in the infection site were similar in Il1b−/− and WT mice 24 hours p.i. (fig. S1B). As expected, Il1a was not detected in Il1a−/− mice (fig. S1B). Similarly, Il1b expression was comparable in Il1a−/− and WT mice and was undetectable in Il1b−/− mice (fig. S1B). These results demonstrated that the absence of one of the two IL-1 cytokines did not lead to compensatory or impaired expression of the other one.

Survival experiments revealed that Il1a−/− and Il1b−/− mice were significantly more susceptible than WT mice (P < 0.0001), establishing that Il1a and Il1b are nonredundant and essential cytokines in defense against GAS infection (fig. 1A). The susceptibility of Il1a−/− and Il1b−/− mice was comparable (P > 0.05). Il1r1−/− mice were more vulnerable than WT mice (P < 0.001) (Fig. 1A), as reported previously (12). Il1r1−/− mice were more susceptible than Il1b−/− mice (P < 0.01), indicating that a complete lack of IL-1 signaling was more detrimental than the deficiency in one of the two IL-1 receptor agonists (Fig. 1A). Tissue damage markers were not consistently different in the sera of Il1a−/−, Il1b−/−, or Il1r1−/− mice as compared to WT controls 24 hours p.i. The liver damage marker bilirubin and the aspartate transaminase–to–alanine transaminase (AST/ALT) ratio were not increased in Il1a−/−, Il1b−/−, and Il1r1−/−, while glutamate dehydrogenase (GLDH) levels were elevated in Il1r1−/− and Il1a−/−, but not Il1b−/− mice compared to WT controls (fig. S1C).

The muscle damage marker creatinine was higher in Il1r1−/− mice, but creatine kinase was not increased (fig. S1C). Urea, the marker of prerenal failure, was increased in Il1a−/−, but not in Il1r1−/− and Il1b−/− mice, compared to WT mice (fig. S1C).

The assessment of the necrotic area at the site of infection revealed unexpected differences between Il1a−/− and Il1b−/− mice: The lesion size in Il1a−/− mice was comparable to that in WT mice, while Il1b−/− and Il1r1−/− exhibited larger necrotic areas (Fig. 1B). The largest lesion size was detected in Il1r1−/− mice consistent with the most severely impaired defense. Further differences between Il1a−/− and Il1b−/− mice were observed in tissue destruction in the lesion area: Tissue destruction was more pronounced in Il1b−/− and Il1r1−/− mice as compared to Il1a−/− and WT mice (Fig. 1C and D, and fig. S1D). Moreover, Gram staining indicated that GAS was invading deeper tissues in Il1b−/− and Il1r1−/− mice as compared to WT controls.

In conclusion, Il1a and Il1b are each required for host defense against GAS infection. However, Il1b-deficient mice display more severe impact at the site of infection than Il1a-deficient and WT mice. These results imply different modes of action of Il1a and Il1b in response to GAS infection. Both of these modes appear to be required for maximum host protection since defense is most impaired in Il1r1−/− mice.

Il1b promotes bacterial clearance and enhances the neutrophilic barrier, while Il1a is dispensable for these processes

The deeper GAS invasion at the site of infection in Il1b−/− mice as compared to Il1a−/− and WT mice (Fig. 1, B and C) suggested that Il1b, not Il1a, was required for the local containment of bacteria. In agreement, Il1b−/− mice displayed a higher bacterial burden in distant tissues (liver, spleen, kidney, lung, and blood) 24 hours p.i. than Il1a−/− and WT mice (Fig. 2A). Survival analysis did not differ in Il1a−/− versus WT mice, indicating that GAS dissemination was not controlled by Il1a (Fig. 2A). Il1r1−/− mice showed similar bacterial burdens as Il1b−/− mice in all organs except for the spleen in which they were slightly higher in Il1r1−/− mice (Fig. 2A). Since Il1a was not contributing to the containment of bacteria, the higher colony-forming units (CFU) in the spleen of Il1r1−/− mice as compared to Il1b−/− mice were probably caused by the overall poorer defense of the Il1r1-deficient animals (Fig. 1A).

An important consequence of the GAS infection is hemolysis, which is caused by the activity of the cytolysins streptolysin O and streptolysin S (25). The higher bacterial counts in the blood of Il1b−/− mice suggested that hemolysis was likely to be more pronounced in these mice. In agreement, we detected increased hemolysis and hemoglobin levels in the blood of Il1b−/− mice as compared to Il1a−/− and WT mice (fig. S2, A and B).

The increased dissemination of GAS in the absence of Il1b indicated a defect in mechanisms critically important for the containment of...
**Fig. 2. Bacterial clearance and neutrophil infiltration are dependent on Il1b but not Il1a.** (A) Il1a−/−, Il1b−/−, Il1r1−/−, and WT mice were infected, and GAS loads in lesions, livers, spleens, kidneys, and blood were determined 24 hours p.i. as CFU per milliliter of blood or per gram of tissue per animal. For the lesion, liver, spleen, kidney, and blood, n = 13 (WT), n = 24 (Il1α−/−), and n = 24 (Il1b−/−). For the kidney, n = 6 to 9 per genotype; for the blood, n = 5 to 6 per genotype. Kruskal-Wallis with Dunn’s multiple comparisons with median and 10th to 90th percentile. *P < 0.05; **P < 0.01; ***P < 0.001. (B) Ptprc and Elane mRNA in lesions 24 hours p.i. (GAS) and in the skin and underlying muscular tissue from uninfected controls (phosphate-buffered saline (PBS)). Box-and-whisker plot with median value and 10th to 90th percentile. Kruskal-Wallis with Dunn’s multiple comparisons with median and 10th to 90th percentile. *P < 0.05; **P < 0.01. (C) Neutrophil counts (CD45+CD11b+Ly6CmedLy6Ghi) in lesions 24 and 48 hours p.i. (GAS, Il1a−/−, Il1b−/−, and WT mice were infected, and GAS loads in lesions, livers, spleens, kidneys, and blood were determined 24 hours p.i. as CFU per milliliter of blood or per gram of tissue per animal. For the lesion, liver, spleen, kidney, and blood, n = 13 (WT), n = 24 (Il1α−/−), and n = 24 (Il1b−/−). For the kidney, n = 6 to 9 per genotype; for the blood, n = 5 to 6 per genotype. Kruskal-Wallis with Dunn’s multiple comparisons with median and 10th to 90th percentile. *P < 0.05; **P < 0.01. (D) Neutrophil and monocyte recruitment in peritonitis induced by heat-killed (HK) GAS. Numbers of neutrophils (CD45+CD11b+Ly6GmedLy6Ghi) and monocytes (CD45+CD11b+Ly6GmedLy6Cmed) per mouse (right) were determined 24 hours after induction. Student’s t test, n = 8 to 15 per genotype. **P < 0.01. (E) In vivo phagocytosis by peritoneal neutrophils. Mice were primed by injection of heat killed GAS, followed by injection of pHrodo-stained HK GAS 18 hours later. Numbers of pHrodo+ neutrophils were normalized to total neutrophil numbers. One-way analysis of variance (ANOVA), n = 9 (WT), n = 15 (Il1α−/−), and n = 17 (Il1b−/−).
the pathogen. We decided to initially examine neutrophil accumulation as this leukocyte subset is an essential component of the barrier against GAS dissemination (12, 26–28). The mRNA levels of the general leukocyte marker Ptprc (encoding the CD45 protein) and the neutrophil elastase gene Elane at the site of infection were lower in Il1b−/− mice than in Il1a−/− and WT mice in the lesion 24 hours p.i. (Fig. 2B). In agreement, flow cytometry analysis of the lesion revealed lower numbers of infiltrating neutrophils (CD45+CD11b+Ly6CmedLy6G+) in Il1b−/− mice when compared to Il1a−/− and WT mice 24 and 48 hours p.i. (Fig. 2C and fig. S2C). Similar effects of Il1b deficiency were observed in a model of GAS-induced peritonitis: Neutrophils (total numbers as well as percentage in leukocytes) in the peritoneal cavity were lower in Il1b−/− mice when compared to Il1a−/− and WT mice (Fig. 2D and fig. S2D). Notably, phagocytosis, which is an important neutrophilic mechanism of GAS clearance (26), was not impaired in the absence of either Il1a or Il1b (Fig. 2E and fig. S2E).

In summary, these data demonstrate that Il1b, not Il1a, is vital for congestion of neutrophils at the infection site and pathogen clearance. Thus, Il1b drives resistance against GAS as it activates mechanisms targeting the pathogen. In contrast, the critical role of Il1a in host protection is consistent with an augmentation of tolerance rather than resistance.

Il1b promotes G-CSF production and emergency granulopoiesis

Il1r1 is known to be required for alum-induced emergency granulopoiesis (29). We thus asked whether a deficient GAS-induced emergency granulopoiesis is the cause of lower neutrophil numbers at the site of infection observed in Il1b−/− mice (Fig. 2C). Blood counts measured using a hematology analyzer revealed lower granulocyte numbers in Il1b−/− mice when compared to Il1a−/− and WT mice 24 hours p.i. (Fig. 3A). This difference was not seen in naïve [phosphate-buffered saline (PBS)–treated] mice (Fig. 3A). As expected, infected mice displayed higher granulocyte numbers when compared to naïve mice owing to infection–induced emergency granulopoiesis. The monocyte and lymphocyte populations were not affected by the lack of Il1b (Fig. 3A). The specific impact of Il1b deficiency on blood neutrophils was confirmed using flow cytometry (neutrophils, CD45+CD11b+Ly6CmedLy6G+; monocytes, CD45+CD11b+Ly6G−Ly6Cbd; T cells, CD45+CD3+)(fig. S3A).

The lower numbers of blood neutrophils in infected Il1b−/− mice could be caused by a reduction in the BM neutrophil pool. However, BM neutrophils (CD45+CD11b+Ly6CmedLy6G+) were not decreased in Il1b−/− mice as compared to Il1a−/− and WT mice 24 hours p.i. (Fig. 3B and fig. S3B). The neutrophil counts were also similar in uninfected mice in all genotypes (Fig. 3B and fig. S3B). Predictably, BM neutrophil numbers were diminished during infection owing to neutrophil mobilization (Fig. 3B and fig. S3B), which coincided with neutrophilia in the blood (Fig. 3A). BM monocytes (CD45+CD11b+Ly6G−Ly6Cbd) were comparable in all genotypes (Fig. 3B and fig. S3B). Together, the lower number of blood neutrophils in Il1b−/− mice 24 hours p.i. did not mirror changes in the BM compartment.

Mature neutrophils are known to rapidly egress from BM upon GAS infection (30). This inflammatory mobilization leads to low numbers of BM neutrophils. We hypothesized that the rapid mobilization of BM neutrophils upon infection might prevent robust detection of differences in neutrophil production. To directly address the generation of mature neutrophils during reactive granulopoiesis in BM, we analyzed progenitor cells developing from self-renewing pluripotent hematopoietic stem cells (HSCs). The numbers of Lin− cells (CD5+CD45R−CD11b+Gr-1+7-4+Ter-119+) and early lymphoid precursors (Lin−Sca-1+ c-Kit+) (31) were comparable in Il1a−/−, Il1b−/−, and WT mice both 24 hours p.i. and in PBS-treated controls (Fig. 3C). Similarly, the numbers of common myeloid progenitors (CMPs) (Lin−Sca-1−c-Kit+CD16/1632CD434) did not display differences between the genotypes (Fig. 3D and fig. S3, C and D). However, granulocyte-macrophage progenitors (GMPs) (Lin−Sca-1−c-Kit+CD16/1632CD434), which develop from CMPs (31), were reduced in Il1b−/− mice when compared to Il1a−/− and WT mice 24 hours p.i. (Fig. 3D and fig. S3D). Thus, GMPs, unlike other hematopoietic progenitors, were dependent on Il1b.

GMPs give rise to monocytes and granulocytes (including neutrophils) (31). We observed lower numbers of neutrophils but not monocytes in the blood of infected Il1b−/− mice (Fig. 3A). This phenotype resembled hematopoietic impediments observed in mice lacking the Csf3 gene that codes for the G-CSF (32). Csf3−/− mice show lower numbers of GMPs and decreased neutrophil numbers particularly during emergency granulopoiesis (32). Monocytes are barely impaired in young Csf3−/− mice (32). The hematopoietic similarities between Csf3−/− and Il1b−/− mice prompted us to assess Csf3 mRNA expression and G-CSF protein levels in our system. The analysis revealed that the serum G-CSF was lower in Il1b−/− mice as compared to Il1a−/− and WT mice 24 hours p.i. (Fig. 3E). Infection did not increase Csf3 expression in BM, suggesting that this tissue was not the source of the infection–induced serum G-CSF (fig. S3E). In contrast, Csf3 mRNA was induced in the site of infection 24 hours p.i., and the induction was markedly lower in Il1b−/− mice as compared to Il1a−/− and WT mice (Fig. 3F). These results indicated that Il1b is a critical activator of Csf3 expression in the lesion. The requirement for Il1b, not Il1a, in the induction of Csf3 mRNA is in line with high Il1b and low Il1a expression in the lesion (Fig. 1B). Since muscle cells are functional producers of G-CSF (33, 34), the Il1b–induced serum G-CSF is likely to originate from muscle tissue that is associated with the lesion samples (Fig. 1C).

In summary, the results describe a specific requirement for Il1b in Csf3 induction in the lesion and in emergency granulopoieses during infection with GAS. The findings suggest that the high susceptibility of Il1b−/− mice to GAS infection is caused by deficient emergency granulopoiesis, which results from impaired Csf3 induction.

Il1a and Il1b deficiencies affect tissue transcriptomes in distinct ways during GAS infection

The finding that GAS-induced Csf3 expression is largely dependent on Il1b and not on Il1a (Fig. 3E) indicated that these cytokines have distinct impacts on gene expression. To investigate the effects of Il1a and Il1b on gene expression in detail, we analyzed the spleen and liver transcriptomes of Il1a−/−, Il1b−/−, and WT mice 24 hours p.i. using RNA sequencing (RNA-seq). These organs harbor cells known to require IL-1R signaling for physiological responses: Hepatocyte-specific Il1r1 deletion ameliorates acute liver injury (35), and dendritic cells found, among other tissues, in the spleen need Il1r1 for mounting adaptive immunity against influenza virus (36).

Principal components analysis (PCA) of liver and spleen RNA-seq derived from infected Il1a−/−, Il1b−/−, and WT mice showed high
intrareplicate reproducibility and sample clustering according to the genotype in each organ (Fig. 4A and data S1 and S2). The analysis indicated that Il1a and Il1b have different effects on the transcriptome in both the liver and the spleen. Clusters of Il1b−/− and WT replicates were closer to each other than to the Il1a−/− cluster in the liver. In contrast, the Il1b−/− replicates formed a cluster that was separated from the Il1a−/− and WT clusters in the spleen (Fig. 4A). This suggested that Il1a was more important for the liver transcriptome, whereas Il1b was played a major in the spleen transcriptome during infection. The distinct effects of Il1a and Il1b deficiencies were confirmed by differential expression analysis (Fig. 4B and data S3). Il1a−/− and WT (Il1a−/−_inf versus WT_inf) liver transcriptomes differed in the expression of 281 genes [adjusted P value (Padj) < 0.001, |log2 fold change| (lfc) > 1], whereas the difference between Il1b−/− and WT samples (Il1b−/−_inf versus WT_inf) comprised only 53 differently expressed genes (Fig. 4B and data S3). In contrast, Il1a deletion had only a minor impact in the spleen (20 differently expressed genes in Il1a−/−_inf versus WT_inf comparison), while Il1b deletion resulted

Fig. 3. Il1b is driver of emergency hematopoiesis. (A) Granulocyte, monocyte, and lymphocyte numbers in the blood of Il1a−/−, Il1b−/−, and WT mice 24 hours p.i. (GAS) and in uninfected controls (PBS), as determined using a hematology analyzer. ANOVA with Dunnett’s correction for multiple comparisons, n = 8 to 14 per genotype. **P < 0.01. (B) Neutrophil (CD45+CD11b+Ly6CmedLy6G−) and monocyte (CD45+CD11b+Ly6CmedLy6G−) percentages (of living cells) in BM from Il1a−/−, Il1b−/−, and WT mice 24 hours p.i. (GAS) and from uninfected controls (PBS), as determined flow cytometry. One-way ANOVA, 10 to 16 per genotype. ****P < 0.0001. (C) Lin− progenitor cells (CD5−CD45R−CD11b−Gr-1−7-4−Ter-119−) and early lymphoid precursors (LSK) (Lin−Sca-1−c-Kit+) in BM from Il1a−/−, Il1b−/−, and WT mice 24 hours p.i. (GAS) and from uninfected mice (PBS), as determined by flow cytometry. ANOVA with Dunnett’s correction for multiple comparisons, n = 7 to 10 per genotype. (D) Common myeloid progenitors (CMPs) (Lin−Sca-1−c-Kit+CD16/32lo,CD34+) and granulocyte-macrophage progenitors (GMPs) (Lin−Sca-1−c-KitloCD16/32hi,CD34hi) in BM from Il1a−/−, Il1b−/−, and WT mice 24 hours p.i. (GAS) and from uninfected controls (PBS), as determined by flow cytometry. ANOVA with Dunnett’s correction for multiple comparisons, n = 7 to 10 per genotype. **P < 0.01. (E and F) G-CSF protein (encoded by the Csf3 gene) (E) and Csf3 mRNA levels (F) at the site of infection from Il1a−/−, Il1b−/−, and WT mice 24 hours p.i. (GAS) and (for Csf3 mRNA) from uninfected controls (PBS), as determined by enzyme-linked immunosorbent assay (ELISA) and reverse transcription quantitative polymerase chain reaction (RT-qPCR), respectively. Kruskal-Wallis with Dunn’s multiple comparisons (E), n = 22 to 24 per genotype. ***P < 0.001; ****P < 0.0001.
Fig. 4. *Il1a* deficiency during infection strongly affects the liver transcriptome, whereas *Il1b* deficiency results in major changes in the spleen transcriptome. (A) PCA plots of liver and spleen RNA-seq derived from *Il1a*−/−, *Il1b*−/−, and WT mice 24 hours p.i. *Il1a*−/− replicates (blue ellipse) are separated from WT and *Il1b*−/− replicates (black ellipse) in the liver (left). *Il1b*−/− replicates (green ellipse) are separated from WT and *Il1a*−/− replicates (black ellipse) in the spleen (right). (B and C) Differential expression analysis of liver (B) and spleen (C) RNA-seq from infected *Il1a*−/− versus infected WT mice (*Il1a*−/−_inf versus WT_inf) (left) and infected *Il1b*−/− versus infected WT mice (*Il1b*−/−_inf versus WT_inf) (right). Genes with differential expression values $P_{adj} < 0.001$ and lfc > 1 are highlighted in red, and their number is indicated within the volcano plots. (D) Visualization of genes regulated by both *Il1a* and *Il1b* in the liver (left, Venn diagram) and the spleen (right, Venn diagram). Genes regulated by *Il1a* ($P_{adj} < 0.001$, lfc > 1) were identified in differential expression analyses shown in (B) and (C) in the comparison *Il1a*−/−_inf versus WT_inf, while genes regulated by *Il1b* were identified in the comparison *Il1b*−/−_inf versus WT_inf. The number of genes regulated by both *Il1a* and *Il1b* is displayed in the overlaps.
in robust gene expression changes (119 differently expressed in Il1b−/−_inf versus WT_inf comparison) (Fig. 4C and data S2). The regulation of Csf3 by Il1b, as shown in lesion (Fig. 3F), could not be assessed in the liver and spleen because of low expression: Csf3 counts were below the detection limit in the liver and low in the spleen (mean normalized counts = 2) from infected WT mice (data S1 and S2). Hence, these organs were not relevant sources of G-CSF.

Visualization of genes regulated by both Il1a and Il1b in the liver (Padj < 0.001, lfc > 1) revealed that Il1a-dependent genes comprised most of the Il1b-dependent genes (41 of 53 genes) (Fig. 4D). Thus, Il1a and Il1b regulated a different number but not a different spectrum of genes in the liver. Similarly, the overlap of Il1a-dependent versus Il1b-dependent genes in the spleen comprised five genes, which is a substantial proportion of Il1a-dependent genes in this organ (Fig. 4D). In contrast, the spectrum of genes regulated by either of the two cytokines in both the liver and the spleen was different, consistent with diverse gene expression profiles in these organs (e.g., one gene was regulated by Il1a in both the liver and spleen) (fig. S4).

Together, two important conclusions can be drawn from the transcriptome analysis in infected mice. First, Il1a and Il1b deficiencies result in distinct impacts on the transcriptome in a given tissue, as evident from liver and spleen analyses. Second, the effects of Il1a and Il1b deficiencies differ in different tissues: The liver transcriptome is largely Il1a dependent, while the spleen transcriptome is more strongly regulated by Il1b during infection.

Il1a is driver of liver transcriptome reprogramming in response to infection

The stark difference between the liver transcriptomes from Il1a−/− versus WT mice 24 hours p.i. (Fig. 4, A and B) prompted us to investigate whether Il1a regulates the liver gene expression in response to infection or already in naïve mice. To this end, we analyzed the liver transcriptomes of uninfected PBS-treated Il1a−/−, Il1b−/−, and WT mice with the aim to compare them to those of infected mice. PCA of samples from uninfected mice confirmed good reproducibility of replicates (Fig. 5A and data S1). Moreover, all uninfected samples clustered close to each other indicating similar variance of both principal components regardless of the genotype. PCA revealed a strong effect of infection on the liver transcriptome: All samples from infected mice were well separated from the uninfected samples (Fig. 5A). The analysis confirmed the more important role of Il1a as compared to Il1b in regulation of the liver transcriptome in infected mice (Fig. 4A). Differential expression analysis did not reveal significant differences between Il1a−/− versus WT livers before infection (Il1a−/−_PBS versus WT_PBS) and only a few differences between Il1b−/− versus WT livers (Il1b−/−_PBS versus WT_PBS) (Fig. 5B, Fig. S5A, and data S4). This indicated the effect Il1a deficiency on the transcriptome observed in infected livers (Fig. 4) developed upon infection.

Comparison of liver samples from infected versus uninfected WT mice (WT_inf versus WT_PBS) confirmed that infection resulted in a deep reprogramming of the liver transcriptome (Fig. 5C and data S5). Comparisons of samples from infected versus uninfected Il1a−/− mice (Il1a−/−_inf versus Il1a−/−_PBS) showed that infection remodeled the liver transcriptome also in the absence of Il1a (Fig. 5C and data S5). Similarly, infection-induced transcriptome changes occurred also in the absence of Il1b (Il1b−/−_inf versus Il1b−/−_PBS) (Fig. S5B and data S5). Moreover, comparison of Il1a−/− samples from infected mice to WT samples from uninfected mice (Il1a−/−_inf versus WT_PBS) showed that the infection-associated transcriptome in Il1a−/− mice was different from the uninfected WT transcriptome (Fig. 5C and data S5). These results showed that the liver transcriptome is regulated during infection by IL-1 signaling–dependent and, as expected, IL-1 signaling–independent cues.

Overlap analysis between Il1a−/−_inf versus Il1a−/−_PBS and Il1a−/−_inf versus WT_PBS comparisons revealed that most of the differentially expressed genes (Padj < 0.001, lfc > 1) in these comparisons were the same (Fig. 5D). Likewise, the effects of Il1a across all expressed genes in Il1a−/−_inf versus Il1a−/−_PBS comparison correlated with those in the Il1a−/−_inf versus WT_PBS comparison ($R^2 = 0.98; y = −0.022 + x$) (fig. S5C). These analyses confirmed that the liver transcriptome in infected Il1a−/− mice was defined by the absence of Il1a-dependent infection–regulated genes. In conclusion, Il1a does not substantially regulate the liver transcriptome under steady-state conditions but is an important driver of transcriptome reprogramming in response to infection.

Adaptation of liver metabolic programs to infection is dependent on Il1a

The identification of infection-induced Il1a-dependent changes in the liver transcriptome (Figs. 4, A and B, and 5D) enabled us to analyze the impact of Il1a on specific liver responses during host defense. We performed gene set enrichment analysis (GSEA) for genes differentially expressed in Il1a−/− versus WT mice 24 hours p.i. (Il1a−/−_inf versus WT_inf; lfc > 1, Padj < 0.05; 281 genes) (data S1) using terms defined in the Gene Ontology (GO) database (http://geneontology.org/). The enrichment data (data S6) were visualized using the EnrichmentMap plugin for Cytoscape (Fig. 6A). Similar GO terms (represented by nodes) were clustered, and related clusters were grouped to multiclusters (data S6). Four multiclusters were identified: Apoptosis & Vesicle signaling, Signaling, Metabolism, and Immune regulation (Fig. 6A). The multiclusters Apoptosis & Vesicle signaling and Signaling comprised GO terms that were positively enriched in the Il1a−/− data when compared to the WT dataset. The multiclusters Metabolism and Immune regulation represented GO terms that were negatively enriched in the Il1a−/− data relative to the WT dataset.

The largest number of nodes for GO terms was found in the negatively enriched multicluster Metabolism (42 nodes) (Fig. 6A and data S6). Moreover, the multicluster Metabolism was well defined as it consisted exclusively of clusters and GO terms representing metabolic pathways. This analysis showed that the induction of metabolic genes by infection was broadly impaired in the Il1a-deficient liver. Metabolic adjustments have been identified in several studies to protect the host by promoting tolerance to infection cues: pathways implicated in tolerance include triglyceride, amino acid, and carbohydrate metabolism (37–39). These pathways were found to be negatively enriched in the liver from infected Il1a−/− mice, suggesting a defective tolerance (Fig. 6A and data S6).

The multiclusters Apoptosis & Vesicle signaling, Signaling, and Immune regulation were characterized by the presence of various signaling clusters. The negatively enriched multicluster Immune regulation included a large cluster Immune cell regulation and proliferation implying a defect in induction of immune cell proliferation–associated genes in the absence of Il1a (Fig. 6A). The enriched multicluster Apoptosis & Vesicle signaling was dominated by the cluster Apoptotic signaling indicating uncontrolled induction of apoptosis-promoting...
genes in the Il1a-deficient liver transcriptome during infection. The enriched multicluster Signaling comprised clusters related to innate immune pathways such as Toll-like receptor (TLR) and chemokine signaling, suggesting unrestricted activation of genes involved in innate immune responses within Il1a−/− livers.

To display dysregulation of individual genes associated with each of the four multiclusters (Apoptosis & Vesicle signaling, Signaling, Metabolism, and Immune regulation), we used heatmaps using the same gene subset as for GSEA. These genes were assigned a ranking z score in the comparisons Il1a−/− inf versus WT_PBS, Il1b−/− inf versus WT_PBS, and WT_inf versus WT_PBS to visualize differences in infection-mediated regulation between the genotypes. The comparison of each of the infected genotype (Il1a−/−, Il1b−/−, and WT) to uninfected WT (WT_PBS) was used since the liver transcriptomes from uninfected mice were comparable in all genotypes (Fig. 5B and fig. S5A). The heatmaps highlighted the marked difference of
Il1a multiclusters defined in (A). The heatmaps highlight differences between infection-induced changes in −/− mice (Il1a).

Heatmaps visualizing infection-induced changes within the enriched multicluster Metabolism contained the largest number of GO terms.

Normalized enrichment score (NES) indicates negatively (blue) and positively (red) enriched GO terms in the −/− versus WT livers (Il1a−/−_inf versus WT_inf; lfc > 1, Padj < 0.05) 24 hours p.i. Similar GO terms (represented by nodes) were grouped into clusters, and related clusters were merged into four multiclusters (Metabolism, Immune Regulation, Apoptosis & Vesicle signaling, and Signaling). Normalized enrichment score (NES) indicates negatively (blue) and positively (red) enriched GO terms in the −/− versus WT comparison. Note that the negatively enriched multicluster Metabolism contained the largest number of GO terms (42). cNMP, cyclic nucleotide.

(B) Heatmaps visualizing infection-induced changes within the multiclusters defined in (A). The heatmaps highlight differences between infection-induced changes in Il1a−/− mice (Il1a−/−_inf versus WT_PBS) from those in Il1b−/− mice (Il1b−/−_inf versus WT_PBS) and WT mice (WT_inf versus WT_PBS) in all multiclusters.

Fig. 6. Adjustments in liver metabolic pathways associated with host defense are dependent on Il1a. (A) Cytoscape map of GO terms enriched in a gene set comprising 281 genes differentially expressed in Il1a−/− versus WT livers (Il1a−/−_inf versus WT_inf; lfc > 1, Padj < 0.05) 24 hours p.i. Similar GO terms (represented by nodes) were grouped into clusters, and related clusters were merged into four multiclusters (Metabolism, Immune Regulation, Apoptosis & Vesicle signaling, and Signaling). Normalized enrichment score (NES) indicates negatively (blue) and positively (red) enriched GO terms in the −/− versus WT comparison. Note that the negatively enriched multicluster Metabolism contained the largest number of GO terms (42). cNMP, cyclic nucleotide.
**Distinct effects of IL-1α versus IL-1β in organs are determined by different local expressions of these cytokines during infection**

IL-1α and IL-1β are considered to elicit the same signaling pathways and cellular responses (1, 2). To verify this in our system and to exclude that differences in signaling cause cells from different tissues to activate distinct transcription programs, we investigated responses in relevant primary cells. We used primary hepatocytes as a major cell type present in the liver and BM-derived macrophages (BMDMs), which we considered as a representative of macrophages in the spleen. BMDMs from WT mice were treated with IL-1α or IL-1β, followed by the analysis of signaling events and gene expression changes. IL-1α and IL-1β caused comparable phosphorylation of inhibitor of NF-xB (IκB) and the MAP kinases p38 and extracellular signal–regulated kinase (ERK) (Fig. 7A; replicate in fig. S7A). Similar results were obtained when analyzing responses of hepatocytes to IL-1α and IL-1β (fig. S7B). Moreover, induction of Tnf, Cxcl1, Il6, Ccl2, and Ccl3 in BMDMs and hepatocytes was similar in response to IL-1α and IL-1β (Fig. 7B and fig. S7C). These results confirmed that IL-1α and IL-1β trigger indistinguishable responses in target cells. Together, the data implied that the distinct impacts of IL-1α and IL-1β deficiencies on the liver and spleen transcriptomes during infection (Fig. 4) did not result from different responses to IL-1α and IL-1β by major cell types in these organs.

Thus, we considered the option that the differences between IL-1α−/- and IL-1β−/- transcriptomes in infected mice were caused by an unequal availability of IL-1α and IL-1β in the organ. This model is consistent with the observation that the differences between IL-1α−/- and IL-1β−/- transcriptomes during infection were high with regard to the number but not the identity of the affected genes (Fig. 4D). We then assessed the RNA-seq data (data S1) for IL-1α and IL-1β expression in the liver and spleen. The counts for each of these two genes in the corresponding knockout samples served as a reference baseline. The analysis revealed that IL-1α was more strongly expressed in the liver from infected WT mice than IL-1β: The mean of normalized IL-1α counts was fivefold higher than the IL-1β counts (131.4 versus 26.7 counts) (Fig. 7C). The analysis of the spleen dataset unraveled an opposite pattern of IL-1α and IL-1β expression: The IL-1β counts were 18-fold higher than the IL-1α counts (298.3 versus 16.6 counts) in WT samples (Fig. 7C). Notably, the expression of IL-1α was not impaired in IL-1β−/- samples and vice versa (Fig. 7C). Analysis of cytokine levels in tissues 24 hours p.i. using enzyme-linked immunosorbent assay (ELISA) showed that IL-1α and IL-1β bioavailability reflected the IL-1α and IL-1β mRNA levels: IL-1α levels were high in the liver but low in the spleen, while IL-1β showed the opposite pattern (Fig. 7, D and E). The lesion contained high levels of IL-1β and low amounts of IL-1α (Fig. 7, D and E), which correlated with IL-1α and IL-1β mRNA expression (fig. S1B). These experiments confirmed that the production of either of the two cytokines was not affected by the lack of the other one: IL-1α levels were similar in WT and IL-1β−/- mice, and IL-1β levels were comparable in WT and IL-1α−/- mice (Fig. 7, D and E). The regular production of IL-1α in IL-1β−/- mice and of IL-1β in IL-1α−/- mice persisted at least 48 hours (i.e., until close to death of the knockout mice), indicating that IL-1α and IL-1β were produced independently of each other throughout the infection (fig. S7D). Consistently, maturation of IL-1β proceeded normally in the absence of IL-1α as probed by Western blot analysis of the lesion 24 and 48 hours p.i. (Fig. 7F and fig. S7E). The production of IL-1α and IL-1β was further validated by the analysis of peritoneal macrophages from in IL-1α−/-, IL-1β−/-, and WT mice: LPS stimulation combined with nigerin treatment resulted in comparable levels of IL-1α in the cell supernatants of IL-1β−/- and WT cells and, conversely, IL-1β levels were not affected by IL-1α deficiency (fig. S7F). As expected, tumor necrosis factor (TNf) secretion was similar in all genotypes. Similar results were obtained when using BMDMs (fig. S7G). These data also showed that IL-1α production was not impaired by IL-1β deficiency in our system, in contrast to previously reported reduction in IL-1α production by IL-1β-deficient BMDMs in context of fatty acid or adenosine 5′- triphosphate stimulation (40).

Pores formed by activated gasdermin D (GSDMD) are critical for execution of pyroptosis and release of IL-1β (41, 42). The Western blotting analysis also demonstrated the activation of GSDMD upon GAS infection (Fig. 7F and fig. S7E). To examine whether GSDMD contributes to defense against GAS infection, we analyzed Gsdmd−/- mice. Gsdmd−/- mice were more vulnerable to infection than WT animals, and their susceptibility was similar as that of IL-1β−/- mice (Fig. 7G). Bacterial loads were increased in the spleen of Gsdmd−/- mice as compared to WT controls 24 hours p.i. (fig. S7H). IL-1β−/- mice exhibited higher bacterial loads in all analyzed tissues (fig. S7H), consistent with the experiment shown in Fig. 2A. The counts of blood granulocytes were lower in Gsdmd−/- mice when compared to WT controls, and the reduction of granulocyte numbers was similar to that in IL-1β−/- mice (Fig. 7G). These data reveal a requirement for GSDMD in host defense against GAS. Moreover, the defect in blood granulocyte numbers and the higher bacterial counts in the spleen indicate a role of GSDMD in resistance, similar to the function of IL-1β. However, the phenotype of Gsdmd−/- mice during GAS infection was less severe than that of IL-1β−/- mice since the bacterial loads were increased in the spleen but not in other tissues and the defect in reactive granulopoiesis was less pronounced.

Cumulatively, the data established distinct expression patterns of IL-1α versus IL-1β in organs of infected mice. IL-1α mRNA and protein levels were higher than those of IL-1β in the liver that was consistent with the stronger impact of IL-1α deficiency on the liver transcriptome 24 hours p.i. (Fig. 4). In further support of this, a detailed time course analysis in the liver revealed that the expression of IL-1α was more strongly induced during infection than that of IL-1β throughout the infection (Fig. 7H). Thus, the distinct effects of IL-1α and IL-1β deficiencies on the transcriptome in a given organ are not likely to be caused by different responses of tissues to IL-1α and
**Fig. 7. Different local expressions of Il1a versus Il1b determine their distinct effects in organs during infection.**

(A) Signaling induced by IL-1α and IL-1β in WT BMDMs analyzed by Western blotting using antibodies for phosphorylated ERK (p-ERK), ERK, p38–MAP kinase (p-p38), and IκB-α (p-IκB-α), and for respective total protein as loading control. 

(B) Induction of Tnf, Cxcl1, Il6, Ccl2, and Ccl3 by IL-1α or IL-1β in WT BMDMs analyzed by RT-qPCR. Student’s t test, n = 12. (C) Il1a (left) and Il1b (right) expression (normalized counts) in livers and spleens from Il1a−/−, Il1b−/−, and WT mice 24 hours p.i. determined by RNA-seq. Counts in respective knockouts represent controls. Wald test from DESeq2, means with SDs, ***Padj < 0.001; **Padj < 0.01; *Padj < 0.05, not significant. (D and E) IL-1α (D) and IL-1β (E) protein levels in indicated tissues from Il1a−/−, Il1b−/−, and WT mice 24 hours p.i. determined in homogenates using ELISA, after normalization to total protein amounts. Student’s t test, n = 8 per genotype. ***P < 0.001; **P < 0.001; *P < 0.0001. (F) Lesion homogenates from GAS-infected or PBS-treated WT, Il1a−/−, and Il1b−/− mice analyzed 24 hours p.i. by Western blotting using IL-1α and GSDMD antibodies. Loading control: vinculin. Each replicate represents a different mouse. (G) Il1a−/−, Il1b−/−, Gsdmd−/−, and WT mice were infected with GAS and Kaplan-Meier survival curves (left; n = 9 per genotype, log-rank Mantel-Cox test with Bonferroni correction for multiple comparisons) and blood granulocyte counts 24 hours p.i. (right; n = 6 to 7 per genotype, one-way ANOVA with Tukey’s test for multiple comparisons). ***P < 0.001; **P < 0.01; *P < 0.05. 

(H) Time course of Il1a and Il1b expression in the liver during GAS infection. Means of RT-qPCR data with the SE of median as area, n = 4 mice, each measured in duplicates.
IL-1β. Instead, our data indicate that these effects are a consequence of tissue-specific mRNA expression and, consistently, protein levels and bioavailability of these cytokines.

**Distinct roles and tissue expression of Il1a versus Il1b during lung infection with S. pneumoniae**

To address the distinct functions and expression patterns of Il1a versus Il1b in a different infection model, we used lung infection with S. pneumoniae. Similar to infection with GAS, Il1a and Il1b were each required for mouse protection (Fig. 8A). Moreover, Il1b prevented pathogen dissemination and promoted neutrophilia during lung infection (Fig. 8, B and C). In contrast, Il1a was dispensable for containment of bacteria and for neutrophilia (Fig. 8, B and C). Analysis of Il1a and Il1b expression revealed induction of both cytokines in the lung 48 and 72 hours p.i. (Fig. 8, B and C). However, Il1a was induced also in the spleen, whereas Il1b remained at the basal level in this organ; conversely, Il1b, not Il1a, was induced in the spleen (Fig. 8, B and C). Collectively, the effects of Il1a versus Il1b deficiencies and the differences in Il1a versus Il1b expression in organs during lung infection with S. pneumoniae resemble the findings we obtained for GAS infection. Thus, our model proposing that the nonredundancy of Il1a and Il1b in host defense is defined by distinct induction of these cytokines in tissues is probably relevant in other infections.

**DISCUSSION**

IL-1α and IL-1β induce comparable inflammatory responses in cells but are not redundant at the organismal level. Mechanistic models explaining the nonredundancy of Il1a and Il1b are not well developed. In this study, we used defined genetic models to describe distinct functions of Il1a and Il1b in host defense against GAS and mechanistically explain the nonredundancy of Il1a and Il1b. The vital role of IL-1 signaling in protection against GAS infections is supported by a high incidence of various types of invasive GAS diseases in patients receiving anti-IL-1–directed therapy (43).

Il1r1−/− mice exhibited a more severe course of the GAS disease than Il1a−/− and Il1b−/− mice as evident from the survival curves, larger necrotic areas at the site of infection and higher bacterial burden in one of the analyzed organs, i.e., the spleen. It is likely that the deficiency in responses to both IL-1α and IL-1β was causative of the more severe disease observed in Il1r1−/− mice. An involvement

---

**Fig. 8.** Requirement for Il1a and Il1b during lung infection with S. pneumonia. (A) Kaplan-Meier survival curves for of Il1a−−, Il1b−−, and WT mice (n = 7 per genotype) infected intranasally with S. pneumoniae. Log-rank Mantel-Cox test. *P < 0.05; **P < 0.01. (B) Bacterial loads determined as CFUs in the lung, liver, spleen, and blood from Il1a−−, Il1b−−, and WT mice (n = 6 per genotype) 72 hours p.i. with S. pneumonia. The values represent CFUs per milliliter of blood or CFUs per gram total protein concentration of homogenized organ. Kruskal-Wallis with Dunn’s multiple comparisons (all data points, median and 10th to 90th percentile shown). *P < 0.05; **P < 0.01. (C) Numbers of neutrophils (CD45⁺ CD11b⁺ Ly6Cmed Ly6G⁻) and monocytes (CD45⁺ CD11b⁺ Ly6G⁻ Ly6Chigh) in the blood from Il1a−−, Il1b−−, and WT mice 72 hours p.i. ANOVA with Tukey’s multiple comparisons test, n = 7. ***P < 0.0001. (D) Expression of Il1a or Il1b in the lung, spleen, and liver in WT animals 0, 48, and 72 hours p.i. Expression was normalized to the housekeeping gene 36b4. Data are presented with the 10th to 90th percentile, and all data points are shown. ANOVA with Tukey’s multiple comparison test, n = 24. ****P < 0.0001; ***P < 0.001; **P < 0.05.
of other IL-1R ligands such as the IL-1R antagonist IL-1RA cannot be excluded. Thus, the Il1r1−/− data cannot be deconvoluted to visualize the individual functions of Il1a or Il1b; instead, the results highlight the importance of a direct comparison of Il1a−/− mice with Il1b−/− mice for understanding the individual functions of IL-1α and IL-1β.

Protection against infectious diseases is driven by both resistance and tolerance (44, 45). Resistance is caused by mechanisms that eliminate the invading pathogen, e.g., phagocytosis. Tolerance mechanisms do not target the pathogen but rather activate a complex repertoire of host responses that limit tissue damage and restore homeostatic conditions (46). From our infection experiments, it can be inferred that Il1b fosters resistance since the ability of Il1b−/− mice to clear the pathogen is markedly reduced. In contrast, Il1a−/− mice exhibit a regular pathogen clearance implying an essential role of Il1a in tolerance.

The beneficial activity of Il1b in resistance was apparent from higher bacterial loads in organs of Il1b−/− mice when compared to Il1a−/− and WT mice. An exception was the lesion that did not exhibit higher bacterial counts in Il1b−/− mice. This indicated that the pathogen was rapidly spreading from the primary site of infection to distant tissues in these mice, consistent with invasion of deeper tissues compared to Il1a−/− and WT mice. The deficiency in preventing dissemination and clearance of bacteria in Il1b−/− mice was associated with impaired neutrophil infiltration. The numbers of the infiltrating neutrophils are decisive for defeating GAS infection as illustrated in genetic models of enhanced neutrophilia (24, 28). This suggests that the primary cause of the impaired bacterial clearance in Il1b−/− mice is a deficient deployment of neutrophils. In support of this, phagocytosis of GAS by neutrophils was not affected in Il1b−/− mice in vivo. However, selective effects of Il1b on other neutrophil antimicrobial functions cannot be excluded given that in vitro treatment of neutrophils by IL-1β was found to augment antimicrobial peptide expression but not oxidative burst (47).

Infection boosts the neutrophil production several-fold over the steady-state level by activation of emergency granulopoiesis in the BM (48). Emergency granulopoiesis covers the higher demand for neutrophils during infection. Activation of emergency granulopoiesis during sterile immunostimulation with alun or polynosinic-polycytidylic acid and upon infection of the oral cavity with Candida albicans is dependent on Il1r1, but the individual contributions of Il1a and Il1b remained unknown (24, 29, 49). Our finding that Il1b−/−, but not Il1a−/−, mice fail to launch emergency granulopoiesis establishes Il1b as specific driver of GAS-stimulated neutrophilia. Furthermore, our experiments using lung infection with S. pneumoniae imply that the function of Il1b as inducer of neutrophil production is not restricted to GAS infection. IL-1β cytokine was reported to promote HSC differentiation into GMPS in vitro (24, 29, 50). Nevertheless, IL-1β is not likely to directly stimulate granulopoiesis in vivo since Il1r1-dependent reactive neutrophilia requires the expression of Il1r1 on radiation-resistant but not hematopoietic cells (29). A direct driver of emergency granulopoiesis is Csf3, which is known to be produced by various nonhematopoietic cells including endothelial cells during oral infection with C. albicans (32, 34, 49, 51). The induction of endothelial Csf3 in the oral cavity by C. albicans is dependent on Il1r1 (49). Our current study identifies Il1b, not Il1a, as specific inducer of Csf3. The GAS-driven Csf3 expression was observed in the lesion but not in the BM and spleen. This suggested that the Csf3 source were nonhematopoietic cells. We speculate that Csf3 originated from the muscle tissue present in the analyzed lesion since muscle cells were reported as substantial Csf3 producers (34). An increase in levels of Csf3 protein (G-CSF) through exogenous administration phenocopies physiological emergency granulopoiesis (51). Thus, we propose that Il1b induces Csf3 expression, which, in turn, drives emergency granulopoiesis during invasive infection with GAS.

Il1a is the major driver of GAS infection–induced liver adaptation as revealed by our RNA-seq experiments. The liver is the central metabolic hub, and adjustments in liver metabolism are indispensable for infectious disease tolerance, hence host survival (52). Adaptations of the hepatic metabolism to infection cues are readily detected in RNA-seq experiments of the whole liver because hepatocytes are the most abundant liver cells: The ratio of hepatocytes to Kupffer cells, the second most abundant liver cell type, is approximately 4:1 in mice (37, 53). The analysis of the whole liver by RNA-seq is superior to the analysis of hepatocytes since these cells undergo rapid and extensive metabolic changes during isolation and in vitro culture (54). Our RNA-seq analysis unraveled the failure of Il1a−/− mice to induce a number of metabolic pathways including those regulating fatty acid, amino acid, and carbohydrate metabolism; these pathways were previously shown to be involved in context-dependent and mechanistically distinct ways in tolerance to infection cues: (i) Triglyceride synthesis is crucial for tissue protection in models of polybacterial (cecal ligation and puncture) and influenza infections (39), (ii) glucose metabolism exhibits opposing effects on tolerance in models of bacterial versus viral inflammation (38), and (iii) amino acid metabolism prevents liver tissue damage in a model of lymphocytic choriomeningitis virus infection (37). These studies indicate that metabolism is adapted by the host in multiple ways. The complex effects of these adaptations on the physiology and host defense remain to be elucidated. Our data propose the directions of future research that will seek to delineate the contribution of the Il1a-dependent metabolic pathways to host defense. Given the marked effect of Il1a on multiple metabolic pathways, we anticipate that host protection will be influenced by a combined output of these metabolic adaptations. In particular, it will be critical to determine how the Il1a-dependent effects impinge on host tolerance.

Although cell type–specific expression of Il1a and Il1b has been previously demonstrated, its relevance has not been addressed in vivo (55, 56). We propose that the distinct impacts of Il1a and Il1b on the tissue transcriptome are governed by their different expression in a given tissue. This model is supported by several lines of evidence. First, Il1a is more highly expressed in the liver than Il1b, consistent with the profound Il1a-dependent liver transcriptome reprogramming and relatively small effects of Il1b in this organ. Conversely, Il1b, not Il1a, is strongly expressed in the spleen, which is substantially regulated by Il1b but not Il1a. Second, the majority of the small group of Il1b-regulated genes in the liver is also Il1a dependent, implying that Il1a and Il1b trigger qualitatively similar responses. Third, we also noticed higher expression of Il1b than Il1a in the lesion consistent with the dependence of Csf3 induction on Il1b in this tissue. Moreover, responses of hepatocytes and BMDMs to IL-1α and IL-1β treatment are indistinguishable, consistent with the general notion that ligation of the IL-1 receptor with either of the two cytokines triggers the same signaling pathway (1, 2). The possibility that the difference between Il1a−/− and Il1b−/− transcriptomes in a given organ (i.e., liver or spleen) was an indirect consequence of a
different course of infection in \( \text{Il1a}^{-/-} \) and \( \text{Il1b}^{-/-} \) mice is not supported by our data: The liver damage markers bilirubin and AST/ALT ratio were comparable in \( \text{Il1a}^{-/-} \), \( \text{Il1b}^{-/-} \), and WT mice. Moreover, the differences in, e.g., liver transcriptomes from \( \text{Il1a}^{-/-} \) and \( \text{Il1b}^{-/-} \) mice correlate with the expression profile of \( \text{Il1a} \) versus \( \text{Il1b} \) in the liver of WT animals, i.e., animals not perturbed by the lack of either \( \text{Il1a} \) or \( \text{Il1b} \). We observed similar differences in \( \text{Il1a} \) versus \( \text{Il1b} \) expression in a different infection model, i.e., pneumonia caused by \( \text{S. pneumoniae} \). Given that most of the few genes that are \( \text{Il1b} \) dependent in the liver are found in the \( \text{Il1a} \)-dependent group, we argue that the different effects of \( \text{Il1a} \) versus \( \text{Il1b} \) in the liver are a consequence of different levels of these cytokines in this organ rather than an indirect effect of divergent course of the disease in the respective knockout mice. However, a role of a different pathogenesis of infection in \( \text{Il1a}^{-/-} \) and \( \text{Il1b}^{-/-} \) mice in the organ-specific effects of \( \text{Il1a} \) and \( \text{Il1b} \) deficiencies cannot be ruled out.

Our study demonstrates that the biological activity of IL-1\( \alpha \) and IL-1\( \beta \) is largely confined to the tissue of their mRNA expression. IL-1\( \alpha \) activity is determined largely by protein levels since IL-1\( \alpha \) can trigger the IL-1 receptor in the unprocessed form as a secreted or membrane-bound alarmin (18). IL-1\( \beta \) activity is determined by processing of pro-IL-1\( \beta \) into IL-1\( \beta \), which is known to be efficient during GAS infection owing to activation of the NLR family pyrin domain containing 3 (NLRP3) inflammasome (12, 57). In agreement, our study shows efficient maturation of pro-IL-1\( \beta \) to IL-1\( \beta \) in the infected tissue. The release of mature IL-1\( \beta \) during pyroptosis is dependent on GSDMD (41, 42). The involvement of pyroptosis and GSDMD in IL-1\( \beta \) production during GAS infection is supported by our findings showing the requirement for GSDMD for host defense, containment of bacteria and reactive granulopoiesis. The susceptibility of \( \text{Gsdmd}^{-/-} \) mice to GAS infection was less severe as compared to \( \text{Il1b}^{-/-} \) mice, suggesting that IL-1\( \beta \) production was not entirely dependent on GSDMD. IL-1\( \beta \) that is generated independently of GSDMD possibly originates from cleavage of pro-IL-1\( \beta \) by the GAS-secreted protease SpeB, which is known to significantly contribute to IL-1\( \beta \) production during GAS infection (43). Our observation that \( \text{Gsdmd}^{-/-} \) mice show a similar albeit less severe phenotype as compared to \( \text{Il1b}^{-/-} \) mice suggests that GSDMD deficiency affects the function of IL-1\( \beta \) but not IL-1\( \alpha \). Moreover, functional impairment of both \( \text{Il1a} \) and \( \text{Il1b} \) in \( \text{Gsdmd}^{-/-} \) mice would probably result in a more severe susceptibility, i.e., a phenotype resembling \( \text{Il1r1} \) deletion. However, a role of GSDMD in \( \text{Il1a} \) function cannot be excluded. Our data also show that IL-1\( \alpha \) and IL-1\( \beta \) expressions (both mRNA and protein) are not mutually dependent on each other in any of the analyzed tissues. Furthermore, the data imply that IL-1\( \alpha \) and IL-1\( \beta \) are unable to signal over long distances in vivo. This is likely to be caused by efficient sequestering of these cytokines by internalization and degradation of the ligated receptor (58). In the case of IL-1\( \alpha \), the local restriction of signaling can be facilitated by the membrane-bound pool of the incompletely processed cytokine, which is known to signal via direct cell-cell contacts (59).

In conclusion, the results of our study indicate that distinct functions of IL-1\( \alpha \) versus IL-1\( \beta \) in host defense are defined by their different bioavailability relative to each other in a given tissue. The tissue-dependent ratios of IL-1\( \alpha \) versus IL-1\( \beta \) levels result in dominant regulation of organs by one of the two cytokines. Given that organs have different roles in physiology, IL-1\( \alpha \) and IL-1\( \beta \) acquire distinct functions: IL-1\( \beta \) drives resistance by inducing reactive granulopoiesis, while IL-1\( \alpha \) orchestrates tolerance possibly through liver reprogramming. The results have broad physiological relevance, ranging from improved understanding of the nonredundancy of IL-1\( \alpha \) and IL-1\( \beta \) to different regulations of resistance versus tolerance.

**MATERIALS AND METHODS**

**Mice**

\( \text{Il1a}^{-/-} \), \( \text{Il1b}^{-/-} \), \( \text{Il1r1}^{-/-} \), and \( \text{Gsdmd}^{-/-} \) mice have been described (19, 42, 60). All mice including WT mice were on C57BL/6 genetic background and regularly backcrossed. All mice were bred and housed under specific pathogen-free conditions with controlled temperature, humidity, a 12-hour light-dark regimen, as well as water and food ad libitum. Experiments were carried out using 7- to 11-week-old age- and sex-matched mice. This study was performed in accordance with the Austrian law for animal experiments and approved by the Austrian Ministry of Science under the license numbers BMWF-66.006/0019-WF/V/3b/2016 and BMWF-V/3b/2020-0.175.109.

**GAS and \( \text{S. pneumoniae} \) cultures and infection of animals**

GAS infection experiments were performed as described (61). GAS serotype M1 strain ISS3348 was used in all GAS infection experiments (62). Briefly, bacteria were grown at 37°C with 5% CO\(_2\), without agitation in THY medium (Todd Hewitt broth from BD Biosciences, supplemented with 0.2% yeast extract; Sigma-Aldrich). Overnight cultures (stationary phase) were diluted 1:15 in THY medium, and after reaching optical density at 600 nm (OD\(_{600}\)) of 0.5 to 0.6 (mid-log phase), bacteria were harvested by centrifugation at 6000g for 8 min at 4°C and washed twice with cold sterile PBS. Last, bacteria were resuspended in 1:15 of the culture volume with isotonic saline (Sigma-Aldrich), resulting in 2 × 10\(^9\) to 6 × 10\(^9\) CFU/ml. For absolute CFU quantification, 50 μl of 10-fold serial dilutions in PBS were plated onto Columbia blood agar plates (Henry Schein). For infection, mice were anesthetized by intraperitoneal injection of ketamine (100 μg/g weight) (Ketamidor, Richter Pharma) and xylazine (10 μg/g weight) (Sedaxylan, Dechra). The fur of the upper left hind limb from anesthetized animals was shaved, and 50 μl (2 × 10\(^8\) to 3 × 10\(^8\) CFU) of freshly prepared bacterial suspension was injected subcutaneously in the region of the vastus lateralis. The health status of infected animals was monitored regularly and mice were euthanized when reaching predefined humane pathophysiological end points. Survival experiments were monitored for 5 days.

\( \text{S. pneumoniae} \) (American Type Culture Collection, 6303) (gift of S. Knapp) was grown in THY medium [3% (w/v) Todd Hewitt broth, supplemented with 0.2% (w/v) yeast extract] at 37°C with 5% CO\(_2\) until reaching the stationary phase. Subsequently, cultures were diluted and incubated at 37°C with 5% CO\(_2\) until reaching mid-log phase (OD\(_{600}\) = 0.4). Bacteria were pelleted (1000g for 15 min at 4°C), washed twice in PBS, and resuspended in PBS supplemented with 25% glycerol. Bacterial load was determined by plating serial 10-fold dilutions on Columbia agar plates containing 5% sheep blood (Henry Schein), and bacterial stocks were stored at −80°C. For lung infection, aliquots of frozen bacterial stocks were thawed and diluted in sterile PBS to 1 × 10\(^8\) to 1.5 × 10\(^8\) CFU/ml, as determined by plating 10-fold serial dilutions onto blood agar plates. Mice were anesthetized and infected intranasally with 30 μl of bacterial suspension. For survival, disease progression was monitored for 9 days, and mice were euthanized when reaching a humane end point.
Determination of bacterial dissemination and lesion size

At 24 hours p.i., the animals were deeply anesthetized [ketamine (150 μg/g) and xylazine (15 μg/g)], and precisely 500 of blood was withdrawn by vena cava puncture with a 25-gauge heparinized needle connected to a syringe containing 50 μl of heparin (100 U/ml; Sigma-Aldrich) to prevent coagulation and neutrophil activation. After euthanizing, the lesion, liver, spleen, kidney, and lung were isolated and disintegrated in ice-cold PBS with a rotor-stator homogenizer, and 10-fold dilution series were plated onto Columbia blood agar plates. Blood samples were diluted 1:2 in sterile double-distilled H₂O to lyse blood cells before serial dilution. Bacterial load was determined by CFU counting and normalization to volume (blood), tissue weight, or protein concentration (liver, lesion, spleen, and kidney). At 48 hours p.i., the width and length of the necrotic area (dark red– to black-colored skin) were measured and calculated as an elliptical area.

ELISA and hematologic counts and blood laboratory values

For cytokine measurements, tissue was homogenized in PBS containing protease inhibitor cocktail (cComplete Roche), centrifuged for 10 min at 23,000g, and sterile-filtered. ELISAs were performed according to the manufacturer’s protocols, and the results were normalized to total protein amount determined with BCA Pierce assay (Thermo Fisher Scientific). The organ damage markers urea, creatinine, AST, ALT, and GLDH were determined in heparinized plasma samples and analyzed by the veterinary in vitro diagnostics laboratory In Vitro GmbH. Hematologic counts were obtained using the V-Sight automated hematology analyzer (A. Menarini diagnostics). To assess bacteria-induced red blood cell rupture, free heme levels in plasma were measured. Heparinized blood from infected animals was centrifuged at 12,000g for 5 min at ambient temperature and diluted with H₂O in a 96-well plate (total volume, 100 μl), and heme concentration was determined by comparing to a hemin (Sigma-Aldrich) standard curve (0.25 to 16 μM in H₂O). For assessing the percentage of lysis, samples were compared to a standard curve of a dilution series made of the supernatant of completely lysed red blood cells diluted with H₂O. This supernatant was obtained by mixing 400 μl of heparinized blood with 14.5 ml of 0.1% sodium carbonate (Sigma-Aldrich) and incubation for 45 min at 37°C on a turning wheel, followed by centrifugation at 12,000g for 5 min. To each sample and standards, 150 μl of formic acid (Merck) was added, and absorbance was measured at 405 nm using a microplate reader.

Histology and immunohistochemistry

Infected mice were perfused at indicated time points by injecting 15 ml of ice-cold 4% paraformaldehyde (PFA) into the vena cava and at the same time cutting the hepatic portal vein. Tissue was removed, cut once longitudinally through the lesion or the large liver lobe, and fixed overnight in 4% PFA [in PBS (pH 6.9); Sigma-Aldrich]. After dehydration and embedding in paraffin, 3-μm sections were prepared. For hematoxylin and eosin (H&E) staining, a standardized routine protocol was applied. For CD45 and Ly6G staining, the antigen was retrieved by boiling for 10 min at 95°C in citrate buffer (10 mM; pH 6) or 10 min of protease K digestion (20 μg/ml in tris-EDTA; Sigma-Aldrich), respectively, followed by endogenous peroxidase blocking through incubation of defaraffinized and rehydrated slides in 3% H₂O₂ for 10 min. Blocking of unspecified antibody-binding was achieved with 3% mouse serum in tris-buffered saline with Tween 20 (TBS-T) and probed overnight at 4°C with primary rabbit anti-mouse CD45 (Cell Signaling Technology, #70257) or rat anti-mouse Ly6G (eBioscience, #14593182). Biotinylated secondary anti-rabbit or anti-rat antibodies (Vector Laboratories), respectively, were added and incubated at room temperature for 30 min, followed by adding biotin–horseradish peroxidase (HRP) conjugate (ABC-HRP kit, Vector laboratories) for 30 min at room temperature. Subsequently, slides were developed with chromogenic substrate 3,3’-diaminobenzidine (DAKO, Agilent) and counterstained with Harris hematoxylin (Merck). To stain GAS, deparaffinized and rehydrated slides were stained with nuclear fast red-aluminum solution (0.1%; Merck) for 8 min and counterstained with Gentian violet (Merck). To prevent background staining, slides were incubated with Lugol’s solution (Merck) and rinsed with 96% ethanol. Images were dehydrated and in Eukitt (Sigma-Aldrich) mounted specimens were acquired with an Olympus slide scanner and processed with the supporting OlyVIA software (Olympus).

RNA isolation and reverse transcription quantitative polymerase chain reaction

At the indicated time point, tissue was isolated from euthanized mice and homogenized in 1 ml of QIAzol Lysis Reagent (QIAGEN), followed by chloroform extraction. Total RNA was precipitated with a mixture of isopropanol and sodium chloride. cDNA was synthesized using a recombinant M-MuLV reverse transcriptase (RevertAid; Thermo Fisher Scientific) together with oligo(dT)₁₈ primer and used in reverse transcription quantitative polymerase chain reaction (RT-qPCR) with HOT FIREPol EvaGreen qPCR Supermix (Medibena) on CFX Touch qPCR cyclers (Bio-Rad) paired with CFX Maestro Analysis software. Relative expression levels were calculated by applying a twofold standard dilution series from cDNA derived from samples. The following primers were used: 36B4, TCCCTTTC-CAGGCTTGTGGG (forward) and GGACACCCCTCCAGAAACCGCA (reverse); Ccl2, CTTCGTGGCCTCTGTTGTA (forward) and CCAGCCTACTTATTGGAATC (reverse); Cs3f, GCAGGCCCAT-GACCCGAAGAT (forward) and TGAGGAGCATGCTGCTGCT (reverse); Elane, CTTCGAGATAAGTGGTTTGTAC (forward) and CACATTAGCTTTGTGAGCA (forward); Il1b, TCTTTGAGTCTGAGGACCC (forward) and TGATGTACTGCTGCTGCT (reverse); Pptrc, TATATTGCGAAAGATG (forward) and AGCCCGAAGTTGCTCATT (reverse); Tnf, GATCAGTTCTCAAAAGGAGTG (forward) and CACTTGTTGTTTGTACCAG (reverse); Il6, AGTTGGCTTCTTGGGACCTGA (forward) and TTCTGCAAGTGCATCATCGT (reverse).

RNA sequencing

RNA was isolated as described above and DNA digestion was performed using deoxyribonuclease (DNase) (Roche) treatment for...
30 min at 37°C. After RNA recovery with acidic phenol/chloroform/isoamyl alcohol (Ambion) extraction and ethanol precipitation, the quality was assessed using Agilent RNA 6000 Nano Assays (5067-1511) on an Agilent 2100 Bioanalyzer. Automated library preparation and multiplexing with the QuantSeq 3’ mRNA-seq Library Prep Kit (Lexogen), library-quality check, and sequencing on an Illumina HiSeqV4 SR50 platform with a read length of 50 bases were performed in the VBCF (Vienna BioCenter Core Facilities) Next Generation Sequencing (NGS) Facility (www.viennabiocenter.org/facilities). After demultiplexing by the facility, raw data were preprocessed (including quality control as well as barcode, adaptor, and quality trimming) using FastQC (www.bioinformatics.babraham.ac.uk/projects/fastqc/), mapped to the GRCh38/mm10 mouse genome assembly using STAR alignment version 2.5 and counted with HTSeq (version 0.11.6) (63). Overall, each sample contained between 10 million and 15 million reads, of which, on average, 76% were uniquely mapped. Differential expression analysis was performed with obtained read counts using DESeq2 (V1.26.0) (64) with Ifc shrinkage correction in R project version 3.6.1 with RStudio IDE version 1.0.143 (Foundation for Statistical Computing, Vienna, Austria, www.R-project.org/) (65). The raw data are accessible via the National Center for Biotechnology Information (NCBI)’s Gene Expression Omnibus (GEO) database under www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE167874 and the secure token krktocylnoxclv.

Gene set enrichment analysis
For GSEA, normalized read counts of genes with significantly higher expression levels ([|Ifc| > 0.05]) between infected Ifc− and infected WT samples were used. Enrichment of GO biological processes was assessed with the GSEA desktop software (V4.0.3) and the MSigDB (V7.0) gene set database. Significantly enriched GO terms ([normalized enrichment score] (NES) > 1.5, \( P < 0.01 \)) were visualized using the Enrichment Map plug-in (V1.1.0) for Cytoscape (V3.8.2) and grouped using the AutoAnnotate function. Heatmaps of selected AutoAnnotate clusters were generated with a \( z \) score (\( z \) score = sign (Ifc) \cdot \log_{10}(P_{adj}) \) for \(|\text{Ifc}| > 1 \) and \( z = 1 \) for \(|\text{Ifc}| < 1 \) between the indicated genotypes and the color range globally scored.

Preparation of single-cell suspensions for flow cytometry

**Lesion**
The lesion tissue was taken from euthanized mice, minced into small pieces, and incubated for 1 hour at 37°C with agitation in 5 ml of Dulbecco’s modified Eagle’s medium (DMEM) supplemented with collagenase-1 (1.5 mg/ml; Worthington), DNase-1 (0.4 mg/ml; Roche), and 0.5 mM CaCl₂. Digestion was stopped by adding 10 ml of PBS with 5 mM EDTA and 2% bovine serum albumin (BSA), and cell suspension was passed through a 70-µm cell strainer to obtain single cells.

**Bone marrow**
Femur and tibia of the noninfected hind leg were cut open at one end, transferred into a 1-ml tube with a hole at the bottom, and subsequently placed into a 1.5-ml centrifugation tube. Cells were obtained after a brief centrifugation step at 3000g for 30 s.

**Spleen**
Isolated spleens were homogenized through a 45-µm cell strainer with a 2-ml syringe plunger. Independent of the isolation method, red blood cells were lysed using a hypotonic ACK buffer (ammonium-chloride-potassium) and then washed twice with PBS.

Flow cytometry
To exclude dead cells, single-cell suspension was stained with Fixable Viability Dye eFluor 450 or eFluor 780 (eBioscience, Thermo Fisher Scientific), before blocking the FcγII/III receptor with an anti-CD16/anti-CD32 antibody (BioLegend). For staining of hematopoietic progenitor cells, no Fc block was performed; instead, cells were incubated with biotinylated antibody mixture from the lineage cell detection cocktail (Miltenyi), followed by staining with streptavidin–fluorescein isothiocyanate (FITC) conjugate (eBioscience, Thermo Fisher Scientific). For cell surface marker, an appropriate combination (as indicated in the figure legends) of antibodies conjugates was used and acquired with a BD LSR Fortessa flow cytometer. Debris and cell doublets were excluded based on the signal ratio of the forward scatter height/area as well as side scatter height/area and dead cells with the fluorescence of the viability dye. For blood samples, 15 µl of heparinized blood were mixed with 100 µl of fluorescence-activated cell sorting buffer and 25-µl containing beads (CountBright Absolute Counting Beads, Thermo Fisher Scientific). After centrifugation and washing, cells were stained with 25 µl of antibody mix including the viability dye and Fc block. Subsequently, cells were fixed with 4% PFA (Sigma-Aldrich) for 10 min, and red blood cells were lysed with ACK buffer. Antibody conjugates used are as follows: (specificity, conjugate, clone, supplier, and catalog no.): CD11b, BV605; M1/70, BD Biosciences 563015; CD11b, PE-Cy7; M1/70, BD Biosciences 561098; CD16/32, PE93, eBioscience (Thermo Fisher Scientific), 12-0161-82; CD3, PE-CF594, 145-2C11, BD Biosciences 562286; CD34 APC, HM34, BioLegend, 128611; CD45, BV421, 30-F11, eBioscience (Thermo Fisher Scientific), 48-0451.82; c-Kit (CD117), BV711, 2B8, BD Biosciences 563160; Ly6C, PerCP-Cy7, HK14, eBioscience (Thermo Fisher Scientific), 45-5932-82; Ly6G, PE; 1A8, BioLegend, 127608; Ly6G, FITC, 1A8, BD Biosciences 551460, Sca-1 (Ly6A/E), PE-Cy7, D7, BD Biosciences 561021; Fc Block, TrueStain, 93, BioLegend, 101320; Fixable Viability Dye, eFluor 780, eBioscience (Thermo Fisher Scientific), 65-0865-14; Fixable Viability Dye, eFluor 450, eBioscience (Thermo Fisher Scientific), 65-0863-14; Lineage Cocktail, Biotin, Miltenyi, 130-092-613; Streptavidin, FITC, eBioscience (Thermo Fisher Scientific), 11-4317-87.

Peritonitis model and in vivo phagocytosis assay
Mid-log phase grown GAS cells were washed twice with sterile PBS, incubated for 30 min at 70°C to obtain heat-killed (HK) bacteria that were diluted in physiological NaCl (0.9%, B. Braun) and injected (2 × 10⁸ CFU) into the peritoneum. After 24 hours, mice were euthanized, peritoneal cavity was flushed with 8 ml of PBS containing 0.02% EDTA, and the obtained cells in the extrudate were analyzed by flow cytometry. For in vivo phagocytosis assay, mice were first primed with intraperitoneal injection of HK bacteria for 16 hours to facilitate neutrophil recruitment. Subsequently, mice were injected with pHrodo Red, SE (Thermo Fisher Scientific)–stained HK GAS after labelling according to the manufacturer’s protocol [pHrodo (20 ng/ml) for 1 × 10⁸ bacteria/ml]. After 2 hours, the peritoneal extrudate was collected and stained, and phagocytic efficiency was calculated as counts of pHrodo⁺ neutrophils normalized to the total number of counted neutrophils.

Primary cells and in vitro stimulation
**Bone marrow–derived macrophages**
BMMDCs were differentiated from BM isolated from the lower limb bones of 7- to 9-weeks old mice with colony-stimulating factor-1 (derived from L929-cells) in DMEM (Sigma-Aldrich) supplemented with colony-stimulating factor-1 and phagocytic efficiency was calculated as counts of pHrodo⁺ neutrophils normalized to the total number of counted neutrophils.
with 10% fetal bovine serum (FBS) (Sigma-Aldrich), streptomycin (100 µg/ml) (Sigma-Aldrich), and penicillin (100 U/ml) (Sigma-Aldrich) for 8 days.

**Primary murine hepatocytes**

WT mice were deeply anaesthetized [ketamine (150 µg/g) and xylazine (15 µg/g)] and the vena cava inferior cannulated (22G Venflon, BD) in its infrarenal region. After reasurring correct positioning, the vein was fixed with a surgical suture (USP 5-0 SILON, Vitrex), the portal vein was punctured, and a clamp was placed onto the supradiaphragmatic vena cava inferior. Initially, the liver was perfused with 50 ml of Hanks’ balanced salt solution (Gibco) containing 0.5 mM EDTA (Sigma-Aldrich) and heparin (120 U/ml) (Sigma-Aldrich), followed by in situ digestion with 20 ml of DMEM (Sigma-Aldrich) containing Liberase (40 mg/liter) (TM Research Grade, Roche, Sigma-Aldrich), both at a flow rate of 5 ml/min and prewarmed to 37°C. Subsequently, the liver was carefully isolated and placed in a petri dish, the gall bladder was removed, and the liver capsule was thoroughly ruptured with forceps. Obtained cell suspension was passed through a 100-µm cell strainer, filled up to 30 ml with William’s E medium (Gibco, Thermo Fisher Scientific) containing 10% FBS, and centrifuged at 50 g for 5 min at 4°C. Cell pellet was gently resuspended and washed twice with FBS-supplemented medium before plating in William’s E medium (Gibco, Thermo Fisher Scientific) containing 10% FBS, and centrifuged at 50g for 5 min at 4°C. Cell pellet was gently resuspended and washed twice with FBS-supplemented medium before plating in William’s E medium (Gibco, Thermo Fisher Scientific) with 10% FBS, streptomycin (100 µg/ml; Sigma-Aldrich), penicillin (100 U/ml; Sigma-Aldrich), 5 mM 1-glutamine (Sigma-Aldrich), and 1× nonessential amino acids on collagen-coated dishes. [For coating: type I collagen (0.1 mg/ml) in PBS for 24 hours at 4°C]. Hepatocytes were stimulated 24 hours after plating with recombinant mouse IL-1α (10 ng/ml) or mIL-1β (10 ng/ml; PeproTech).

**Peritoneal macrophages**

The peritoneal cavities of CO2-euthanized untreated mice were flushed with 5 to 6 ml of PBS containing 3% FBS. Obtained cell suspension was centrifuged with 500g for 10 min at 12°C, and 1.5 x 10^6 cells per well were seeded into a non-tissue culture treated six-well plates. After 3 hours of incubation, all nonadherent cells were washed away, and cells were stimulated the next day with LPS (10 ng/ml; O55:B5, Sigma-Aldrich) for 9 hours. For inflammasome activation, additional 10 µM nigericin (Sigma-Aldrich) was added 2 hours before taking cell culture supernatant for subsequent ELISA measurements.

**Western blotting**

After stimulation, dishes were placed on ice and washed twice with ice-cold PBS, followed by scraping into cold Fractelon buffer [10 mM tris-HCl, 50 mM NaCl, 50 mM NaF, 30 mM Na2HPO4, 1% Triton X-100, 1 mM dithiothreitol (DTT), 1 mM vanadate, and 1× protease inhibitor cocktail (Complete Roche)]. After five additional minutes on ice, the lysate was centrifuged at 13,000g for 5 min at 4°C, mixed at a ratio of 2:1 with SDS loading dye, and boiled for 5 min at 95°C. For lesion, tissue from infected or untreated mice was homogenized in 1 ml of PBS containing protease inhibitor cocktail and centrifuged with 3000g for 5 min at 4°C, and 400 µl of the obtained supernatant was mixed with 300 µl Fracelton buffer (without DTT), incubated 5 min on ice, and centrifuged with 21,000g for 5 min at 4°C. Protein concentration was adjusted after BCA (Thermo Fisher Scientific) measurement and mixed at a ratio 5:1 with a 6× SDS loading dye before boiling for 5 min at 95°C. SDS-polyacrylamide gel electrophoresis separation was performed with a 12% separation gel, and proteins were subsequently transferred to a nitrocellulose membrane (GE Healthcare) with the semidyrid method (Bio-Rad Trans-Blot Turbo) or for in vivo samples with the wet transfer method (Bio-Rad Trans-Blot cell). Next, the membrane was blocked with 5% BSA in TBS-T before probing overnight with primary antibody. The next day, the membrane was incubated with HRP-linked secondary antibody and SuperSignal West Chemiluminescent Substrate (Thermo Fisher Scientific; according to the manufacturer’s protocol), followed by scanning using the ChemiDoc MP Imaging system (Bio-Rad). Antibodies for Western blot analyses were phosphorylated (p) p38, rabbit, Cell Signaling Technology, catalog no. 9211S; p38, rabbit, Santa Cruz Biotechnology, catalog no. L0707; p-IXKB-α, mouse, Cell Signaling Technology, catalog no. 9246; IXKB-α, rabbit, Cell Signaling Technology, catalog no. 4812; p-ERK, rabbit, Cell Signaling Technology, catalog no. 9101; ERK, rabbit, Cell Signaling Technology, catalog no. 9012; IL-1β, goat, R&D Systems, catalog no. #AF401-NA; GSDMD, mouse, Santa Cruz Biotechnology, catalog no. sc-393581; Vinculin, mouse, Sigma-Aldrich, catalog no. #V9131; α-tubulin, mouse, Sigma-Aldrich, catalog no. T0926; Peroxidase AffiniPure Anti-Rabbit IgG (H + L), goat, Jackson ImmunoResearch, catalog no. 115-035-144; Peroxidase AffiniPure Anti-Mouse IgG (H + L), goat, Jackson ImmunoResearch, catalog no. 115-035-003.

**Statistics**

All visualizations and statistical analysis were performed using GraphPad Prism 9.0.0 or the R project. Statistical significances were calculated using an unpaired two-tailed Student’s t test, one-way analysis of variance (ANOVA) with Bonferroni correction, Mann-Whitney U test, Kruskal-Wallis test, linear regression model, or Pearson’s correlation tests, as indicated in the figure legends. Results were considered to be significant with *P < 0.05, **P < 0.01, and ***P < 0.001.

**Ethics statement**

Animal experiments were carried out according the Austrian law for animal experiments (BGBl. I Nr. 114/2012). Experiments were approved and authorized through the licenses BMWF-66.006/0019-WF/V/3b/2016 and BMWF-V/3b/2020-0.175.109 issued by the Austrian Ministry of Science and Research.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at https://science.org/doi/10.1126/sciadv.abj7293

**View/request a protocol for this paper from Bio-protocol.**

**REFERENCES AND NOTES**

1. C. A. Dinarello, The IL-1 family of cytokines and receptors in rheumatic diseases. *Nat. Rev. Rheumatol.* **15**, 612–632 (2019).
2. A. Mantovani, C. A. Dinarello, M. Molgora, C. Garlanda, Interleukin-1 and related cytokines in the regulation of inflammation and immunity. *Immunity* **50**, 778–795 (2019).
3. C. A. Dinarello, Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunol. Rev.* **281**, 8–27 (2018).
4. B. Krumm, Y. Xiang, J. Deng, Structural biology of the IL-1 superfamily: Key cytokines in the regulation of immune and inflammatory responses. *Protein Sci.* **23**, 526–538 (2014).
5. S. Moorlag, R. J. Roring, L. A. B. Joosten, M. G. Netea, The role of the interleukin-1 family in trained immunity. *Immunol. Rev.* **281**, 28–39 (2018).
6. Y. Zhang, S. Saccani, H. Shin, B. S. Nikolajczyk, Dynamic protein associations define two phases of IL-1β transcriptional activation. *J. Immunol.* **181**, 503–512 (2008).
7. N. Mori, D. Prager, Transactivation of the interleukin-1alpha promoter by human T-cell leukemia virus type 1 and type II Tax proteins. *Blood* **87**, 3410–3417 (1996).
8. K. Alheim, T. L. McDowell, J. A. Symons, G. W. Duff, T. Bartfai, An AP-1 site is involved in the NGF induction of IL-1α in PC12 cells. *Neurochem. Int.* **29**, 487–496 (1996).
9. S. Bailly, M. Fay, N. Israel, M. A. Gougerot-Pocidalo, The transcription factor AP-1 binds to the human interleukin 1 alpha promoter. *Eur. Cytokine Netw.* **7**, 125–128 (1996).
12. V. Castiglia, A. Piersigilli, F. Ebner, M. Janos, O. Goldmann, U. Damböck, A. Kröger, Sci. Adv. et al

23. K. C. Barry, M. F. Fontana, J. L. Portman, A. S. Dugan, R. E. Vance, IL-1

25. G. Sierig, C. Cywes, M. R. Wessels, C. D. Ashbaugh, Cytotoxic effects of streptolysin O

27. A. S. Zinkernagel, A. M. Timmer, M. A. Pence, J. B. Locke, J. T. Buchanan, C. E. Turner,

28. F. Ebner, V. Sedlyarov, S. Tasciyan, M. Ivin, F. Kratochvill, N. Gratz, L. Kenner, A. Villunger,

31. H. Iwasaki, K. Akashi, Myeloid lineage commitment from the hematopoietic stem cell.

496. D. Stoiber, S. Lienenklaus, P. Kovarik, Type I interferon signaling prevents IL-1

P. E. Auron, R. J. Xavier, L. A. J. O'Neill, Succinate is an inflammatory signal that induces

V. Nizet, M. Whyte, C. T.aylor, H. Lin, S. L. Masters, E. Gottlieb, V. P. Kelly, C. Clish,

S. C. Corr, M. Haneklaus, B. E. Caffrey, K. Pierce, S. Walmsley, F. C. Beasley, E. Cummins,

Y. Ueda, D. W. Cain, M. Kuraoka, M. Kondo, G. Kelsoe, IL-1R type I-dependent hemopoietic

H. Iwasaki, K. Akashi, Myeloid lineage commitment from the hematopoietic stem cell.

D. Stoiber, S. Lienenklaus, P. Kovarik, Type I interferon signaling prevents IL-1

P. E. Auron, R. J. Xavier, L. A. J. O'Neill, Succinate is an inflammatory signal that induces

V. Nizet, M. Whyte, C. T.aylor, H. Lin, S. L. Masters, E. Gottlieb, V. P. Kelly, C. Clish,

S. C. Corr, M. Haneklaus, B. E. Caffrey, K. Pierce, S. Walmsley, F. C. Beasley, E. Cummins,

Y. Ueda, D. W. Cain, M. Kuraoka, M. Kondo, G. Kelsoe, IL-1R type I-dependent hemopoietic

H. Iwasaki, K. Akashi, Myeloid lineage commitment from the hematopoietic stem cell.

D. Stoiber, S. Lienenklaus, P. Kovarik, Type I interferon signaling prevents IL-1

P. E. Auron, R. J. Xavier, L. A. J. O'Neill, Succinate is an inflammatory signal that induces

V. Nizet, M. Whyte, C. T.aylor, H. Lin, S. L. Masters, E. Gottlieb, V. P. Kelly, C. Clish,

S. C. Corr, M. Haneklaus, B. E. Caffrey, K. Pierce, S. Walmsley, F. C. Beasley, E. Cummins,

Y. Ueda, D. W. Cain, M. Kuraoka, M. Kondo, G. Kelsoe, IL-1R type I-dependent hemopoietic

H. Iwasaki, K. Akashi, Myeloid lineage commitment from the hematopoietic stem cell.

D. Stoiber, S. Lienenklaus, P. Kovarik, Type I interferon signaling prevents IL-1

P. E. Auron, R. J. Xavier, L. A. J. O'Neill, Succinate is an inflammatory signal that induces

V. Nizet, M. Whyte, C. T.aylor, H. Lin, S. L. Masters, E. Gottlieb, V. P. Kelly, C. Clish,

S. C. Corr, M. Haneklaus, B. E. Caffrey, K. Pierce, S. Walmsley, F. C. Beasley, E. Cummins,

Y. Ueda, D. W. Cain, M. Kuraoka, M. Kondo, G. Kelsoe, IL-1R type I-dependent hemopoietic

H. Iwasaki, K. Akashi, Myeloid lineage commitment from the hematopoietic stem cell.

D. Stoiber, S. Lienenklaus, P. Kovarik, Type I interferon signaling prevents IL-1

P. E. Auron, R. J. Xavier, L. A. J. O'Neill, Succinate is an inflammatory signal that induces

V. Nizet, M. Whyte, C. T.aylor, H. Lin, S. L. Masters, E. Gottlieb, V. P. Kelly, C. Clish,

S. C. Corr, M. Haneklaus, B. E. Caffrey, K. Pierce, S. Walmsley, F. C. Beasley, E. Cummins,

Y. Ueda, D. W. Cain, M. Kuraoka, M. Kondo, G. Kelsoe, IL-1R type I-dependent hemopoietic

H. Iwasaki, K. Akashi, Myeloid lineage commitment from the hematopoietic stem cell.

D. Stoiber, S. Lienenklaus, P. Kovarik, Type I interferon signaling prevents IL-1

P. E. Auron, R. J. Xavier, L. A. J. O'Neill, Succinate is an inflammatory signal that induces

V. Nizet, M. Whyte, C. T.aylor, H. Lin, S. L. Masters, E. Gottlieb, V. P. Kelly, C. Clish,

S. C. Corr, M. Haneklaus, B. E. Caffrey, K. Pierce, S. Walmsley, F. C. Beasley, E. Cummins,

Y. Ueda, D. W. Cain, M. Kuraoka, M. Kondo, G. Kelsoe, IL-1R type I-dependent hemopoietic

H. Iwasaki, K. Akashi, Myeloid lineage commitment from the hematopoietic stem cell.
53. B. G. Lopez, M. S. Tsai, J. L. Baratta, K. J. Longmuir, R. T. Robertson, Characterization of Kupffer cells in livers of developing mice. *Comp. Hepatol.* **10**, 2 (2011).

54. S. Cassim, V. A. Raymond, P. Lapierre, M. Biloodeau, From in vivo to in vitro: Major metabolic alterations take place in hepatocytes during and following isolation. *PLoS ONE* **12**, e0190366 (2017).

55. G. Chen, L. Sun, T. Kato, K. Okuda, M. B. Martino, A. Abzhanova, J. M. Lin, R. C. Gilmore, G. Núñez, Activation of the Nlrp3 inflammasome by Streptococcus pyogenes requires streptolysin O and NF-κB activation but proceeds independently of TLR signaling and P2X7 receptor. *J. Immunol.* **194**, 499–503 (2015).

56. J. Lugrin, R. Parapanov, N. Rosenblatt-Velin, M. Kesimer, C. M. P. Ribeiro, B. Waeber, O. Müller, C. Vergely, M. Zeller, A. Tardivel, P. Schneider, P. Pacher, L. Liaudet, Cutting edge: IL-1α is a crucial danger signal triggering acute myocardial inflammation during myocardial infarction. *J. Immunol.* **194**, 499–503 (2015).

57. J. Harder, L. Franchi, R. Muñoz-Planillo, J. H. Park, T. Reimer, G. Nüñez, Activation of the Nlrp3 inflammasome by *Streptococcus pyogenes* requires streptolysin O and NF-κB activation but proceeds independently of TLR signaling and P2X7 receptor. *J. Immunol.* **183**, 5823–5829 (2009).

58. S. B. Mizel, P. L. Kilian, J. C. Lewis, A. Zagon, J. M. Lin, R. C. Gilmore, A. Livraghi-Butrico, M. Kesimer, C. M. P. Ribeiro, W. K. O’Neal, R. C. Boucher, IL-1α dominates the promucin secretory cytokine profile in cystic fibrosis. *J. Clin. Invest.* **129**, 4433–4450 (2019).

59. J. N. E. Chan, M. Humphry, L. Kitt, A. Tardivel, P. Schneider, P. Pacher, L. Liaudet, Cutting edge: IL-1α is a crucial danger signal triggering acute myocardial inflammation during myocardial infarction. *J. Immunol.* **194**, 499–503 (2015).

60. M. Labow, D. Shuster, M. Zetterstrom, P. Nunes, R. Terry, E. B. Cullinan, T. Bartfai, M. C. H. Clarke, Cell surface IL-1α and associates with the membrane via IL-1R2 and GPI anchors. *Eur. J. Immunol.* **50**, 1663–1675 (2020).

61. M. Labow, D. Shuster, M. Zetterstrom, P. Nunes, R. Terry, E. B. Cullinan, T. Bartfai, C. Solorzano, L. L. Moldawer, R. Chizzonite, K. W. M. Intyre, Absence of IL-1α signaling and reduced inflammatory response in IL-1α receptor-deficient mice. *J. Immunol.* **159**, 2452–2461 (1997).

62. N. Gratz, H. Hartweger, U. Matt, F. Kratchovill, M. Janos, S. Sigel, D. Drobis, X. D. Li, S. Knapp, P. Kovarik, Type I interferon production induced by *Streptococcus pyogenes*-derived nucleic acids is required for host protection. *PLoS Pathog.* **7**, e1001345 (2011).

63. S. Anders, V. P. Rygiel, W. Huber, HTSeq—A Python framework to work with high-throughput sequencing data. *Bioinformatics*, 166–Bioinformat169 (2015).

64. M. I. Love, W. Huber, S. Anders, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 550 (2014).

65. R. C. Team, A. R. Language and Environment for Statistical Computing (2017).

**Acknowledgments:** We thank R. Eferl, J. Schmid, and A. Berghaeter for helpful discussions. We are grateful to P. Berthold for animal husbandry. The VBCF NGS Facility (www.viennabiocenter.org) and L. Paul from Lexogen are acknowledged for sequencing advice. We thank A. LeHeron for critically reading the manuscript. *Funding:* Research in the Lamkanfi laboratory is supported by grants from the European Research Council (grant 683144) and the Fund for Scientific Research-Flanders (grants G014221N and G017121N). This work was supported by the Austrian Science Fund (FWF) grants P33000-B, P31848-B, and W1261 to P.K. and the University of Vienna uni:docs fellowship to M.B. *Author contributions:* Conceptualization: K.E. and P.K. Methodology: K.E., L.V.W., and M.L. Investigation: K.E., A.B., L.M., and M.B. Figures: K.E. Supervision: P.K., K.E., and M.L. Writing: K.E. and P.K. *Competing interests:* The authors declare they have no competing interests. *Data and materials availability:* All raw RNA-seq data are accessible via the NCBI's GEO under the accession number GSE167874. Gsdmd−/− mice can be provided by Genentech Inc., Il1a−/− and Il1b−/− mice can be provided by the University of Vienna uni:docs fellowship to M.B. Requests for the Gsdmd−/−, Il1a−/−, or Il1b−/− should be submitted to Y. Iwakura, Tokyo University of Science, pending scientific review and a completed material transfer agreement. Requests for the Gsdmd−/−, Il1a−/−, or Il1b−/− should be submitted to Genentech Inc. and Y. Iwakura, Tokyo University of Science, respectively. All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials.

Submitted 28 May 2021
Accepted 5 January 2022
Published 2 March 2022
10.1126/sciadv.abj7293