nature medicine

LETTERS

Identification of a key pathway required for the sterile inflammatory response triggered by dying cells

Chun-Jen Chen[1,2], Hajime Kono[1], Douglas Golenbock[3], George Reed[3], Shizuo Akira[4] & Kenneth L Rock[1]

Dying cells stimulate inflammation, and this response is thought to contribute to the pathogenesis of many diseases. Very little has been known, however, about how cell death triggers inflammation. We found here that the acute neutrophilic inflammatory response to cell injury requires the signaling protein myeloid differentiation primary response gene 88 (Myd88). Analysis of the contribution of Myd88-dependent receptors to this response revealed only a minor reduction in mice doubly deficient in Toll-like receptor 2 (Tlr2) and Tlr4 and normal responses in mice lacking Tlr1, Tlr3, Tlr6, Tlr7, Tlr9, Tlr11 or the interleukin-18 receptor (IL-18R). However, mice lacking IL-1R showed a markedly reduced neutrophilic inflammatory response to dead cells and tissue injury *in vivo* as well as greatly decreased collateral damage from inflammation. This inflammatory response required IL-1α, and IL-1R function was required on non–bone-marrow-derived cells. Notably, the acute monocyte response to cell death, which is thought to be important for tissue repair, was much less dependent on the IL-1R–Myd88 pathway. Also, this pathway was not required for the neutrophil response to a microbial stimulus. These findings suggest that inhibiting the IL-1R–Myd88 pathway *in vivo* could block the damage from acute inflammation that occurs in response to sterile cell death, and do so in a way that might not compromise tissue repair or host defense against pathogens.

When cells die *in vivo*, they stimulate a potent inflammatory response[1], including a rapid influx of neutrophils, and later monocytes, into injured tissues. This response is elicited by most types of injured cells and is so stereotypical that it can be used to date the time of injury in, for example, a myocardial infarction[2]. In the absence of infection, this 'sterile inflammation' may cause dysfunction and disease[3–5]. It is therefore important to understand the basis of the sterile inflammatory response to tissue injury.

The two principal stimuli of inflammation are injury and infection. Although the mechanisms that stimulate the inflammatory response to injured cells are poorly understood, those driving inflammation in response to infection are much better known. In infections, extracellular microbial components are recognized by Toll-like receptors (TLRs) that stimulate inflammation. There is evidence that some mammalian cellular components can stimulate TLRs[6–9], raising the possibility that these receptors also have a role in stimulating inflammation in response to dying cells. However, it is presently unknown how important TLRs are in the inflammatory response provoked by dying cells.

All TLRs signal through the TIR adaptor protein Myd88 except for Tlr3, which uses the Trif molecule. To examine whether TLRs were involved in inflammation triggered by cell injury, we injected necrotic EL4 cells (a murine T lymphoma cell line) intraperitoneally (i.p.) into mice deficient in Myd88 or Trif (encoded, respectively, by *Myd88* and by *Trif*, also known as *Ticam2*). After 16 h, wild-type and $Trif^{-/-}$ mice had abundant neutrophils in their abdominal cavities, but this response was markedly less in $Myd88^{-/-}$ mice (**Fig. 1**). In contrast, $Myd88^{-/-}$ mice responded normally to zymosan (yeast cell wall; **Fig. 1d**), indicating that the recruitment of neutrophils to the site of inflammation is not inherently impaired in these mice.

The requirement for Myd88 suggested that TLRs might be involved in necrosis-induced inflammation. Therefore, we injected necrotic EL4 cells into mice deficient in various TLRs. Inflammation was not significantly reduced in any of the mice deficient in single TLRs examined (Tlr1, Tlr2, Tlr3, Tlr4, Tlr6, Tlr7, Tlr9 and Tlr11) (**Fig. 2**). Because there was a trend toward lower responses in $Tlr2^{-/-}$ and $Tlr4^{-/-}$ mice, we also examined Tlr2/Tlr4 double-deficient mice and found a small but significant reduction in neutrophil infiltration (**Fig. 3a**). We do not know, in regard to these responses, whether Tlr5 and Tlr8 (which were not available to us) might be involved or whether multiple TLRs might be participating in a functionally redundant fashion; however, as discussed later, results from analyzing IL-1R mutant mice indicate that these possibilities are unlikely.

The finding that Tlr2 and Tlr4 have a small role in sensing and transducing inflammatory signals from injured cells is consistent with reports that they recognize some mammalian molecules potentially released from dying cells[10]. Yet the relatively minor nature of their contribution was surprising because Hmgb1, a nuclear protein released from necrotic cells, has been suggested to be a major trigger of inflammation to necrotic cells[11] and to act through Tlr2 and Tlr4 (refs. 8,12). However, we found that necrotic $Hmgb1^{-/-}$ cells stimulated a robust inflammation in mice that was not significantly different in magnitude from that caused by $Hmgb1^{+/+}$ cells and that this process

[1]Department of Pathology, University of Massachusetts Medical School, 55 Lake Avenue North, Room S2-109, Worcester, Massachusetts 01655, USA. [2]Institute of Microbiology and Biochemistry, National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei 10617, Taiwan. [3]Department of Medicine, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, Massachusetts 01655, USA. [4]Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamada-oka, Suita, Osaka 565-0871, Japan. Correspondence should be addressed to K.L.R. (kenneth.rock@umassmed.edu).

Received 20 November 2006; accepted 3 May 2007; published online 17 June 2007; doi:10.1038/nm1603

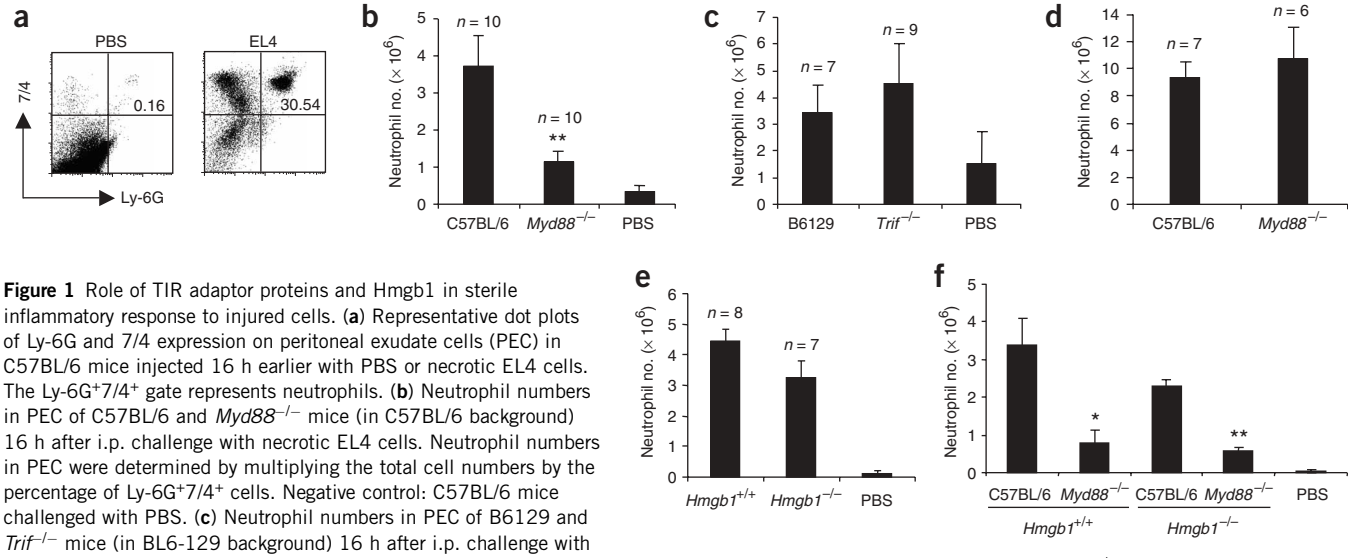

Figure 1 Role of TIR adaptor proteins and Hmgb1 in sterile inflammatory response to injured cells. (**a**) Representative dot plots of Ly-6G and 7/4 expression on peritoneal exudate cells (PEC) in C57BL/6 mice injected 16 h earlier with PBS or necrotic EL4 cells. The Ly-6G$^+$7/4$^+$ gate represents neutrophils. (**b**) Neutrophil numbers in PEC of C57BL/6 and *Myd88*$^{-/-}$ mice (in C57BL/6 background) 16 h after i.p. challenge with necrotic EL4 cells. Neutrophil numbers in PEC were determined by multiplying the total cell numbers by the percentage of Ly-6G$^+$7/4$^+$ cells. Negative control: C57BL/6 mice challenged with PBS. (**c**) Neutrophil numbers in PEC of B6129 and *Trif*$^{-/-}$ mice (in BL6-129 background) 16 h after i.p. challenge with necrotic EL4 cells. Negative control: B6129 mice challenged with PBS. (**d**) Neutrophil numbers in PEC of C57BL/6 or *Myd88*$^{-/-}$ mice 4 h after challenge with 0.5 mg zymosan. (**e**) Neutrophil numbers in PEC of B6129 mice 16 h after i.p. challenge with UV-irradiated *Hmgb1*$^{+/+}$ and *Hmgb1*$^{-/-}$ cells. (**f**) Neutrophil numbers in PEC of C57BL/6 and *Myd88*$^{-/-}$ mice 16 h after i.p. challenge with necrotic *Hmgb1*$^{+/+}$ or *Hmgb1*$^{-/-}$ cells ($n = 4$). Means and s.e.m. values in **b**–**e** are for combined data from two or more independent experiments (n = total number of mice from the multiple experiments for each group). Data shown in **f** are representative of three experiments. *$P < 0.05$, **$P < 0.01$, versus control (C57BL/6 in **b**,**d**,**f** and B6129 in **c**).

was still dependent on Myd88 (**Fig. 1e**,**f**). Therefore, Hmgb1 is at best a minor proinflammatory trigger released by these necrotic cells.

The marked reduction in inflammation in response to injured cells in Myd88-deficient mice, but not TLR-mutant mice, led us to examine the role of the two other Myd88-dependent receptors, IL-1 receptor I (IL-1R) and the IL-18 receptor (IL-18R), in this process. In IL-1R mutant mice, we observed a reduction of 86.6% in the neutrophil response to necrotic EL4 cells at 16 h (**Fig. 3b**) and similar reductions at later time points (data not shown). In contrast, this inflammatory response was not reduced in IL-18R–deficient mice (**Fig. 3c**). Therefore, the IL-1R has an important role in neutrophil recruitment to injured cells.

The genetic evidence implicating the IL-1R in sterile inflammation in response to dead cells implies that IL-1 is a key mediator driving the acute neutrophilic response. There are two distinct species of IL-1—IL-1α and IL-1β—and both stimulate IL-1R. To test the hypothesis that IL-1 is essential for this response and to identify the active IL-1 species, we measured the neutrophilic inflammatory response to dead cells in mice treated with neutralizing monoclonal antibodies to IL-1α or IL-1β. Notably, antibodies to IL-1α inhibited

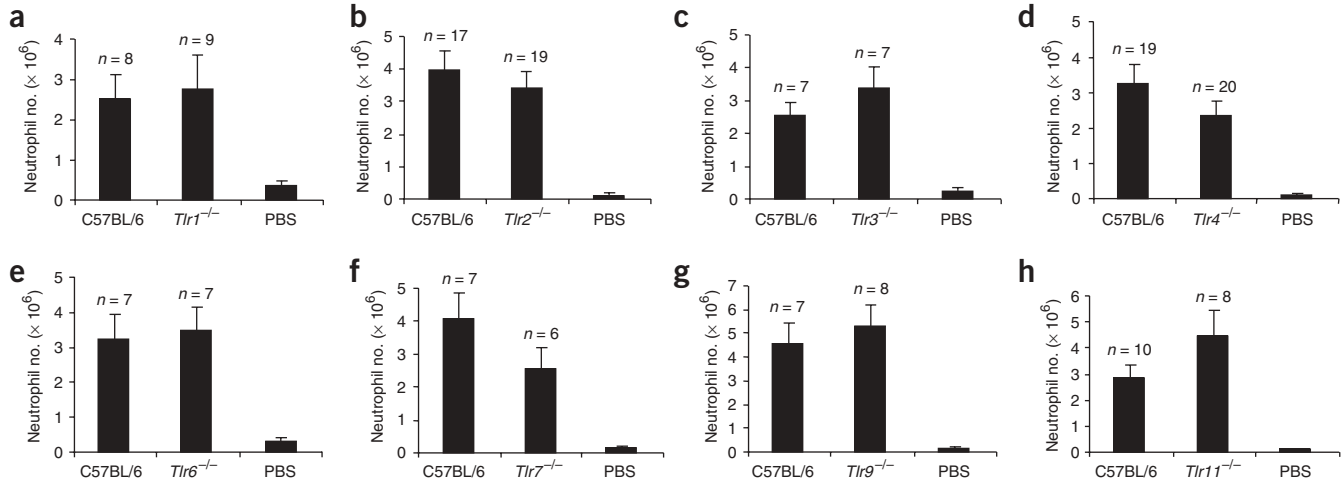

Figure 2 Role of TLRs in sterile inflammatory response to injured cells. (**a**–**h**) Neutrophil numbers in peritoneal exudate cells (PEC) of normal C57BL/6 mice and mice deficient for Tlr1 (**a**), Tlr2 (**b**), Tlr3 (**c**), Tlr4 (**d**), Tlr6 (**e**), Tlr7 (**f**), Tlr9 (**g**) or Tlr11 (**h**) 16 h after i.p. challenge with necrotic EL4 cells. Mice deficient for Tlr1, Tlr3, Tlr6, Tlr7 and Tlr11 were originally of BL6-129 background and were backcrossed to C57BL/6 for at least five generations, and mice deficient for Tlr2, Tlr4 and Tlr9 were fully backcrossed onto the C57BL/6 background. Negative controls in all experiments: C57BL/6 mice challenged with PBS. Means and s.e.m. values are for combined data from two or more independent experiments (n = total number of mice from the multiple experiments for each group).

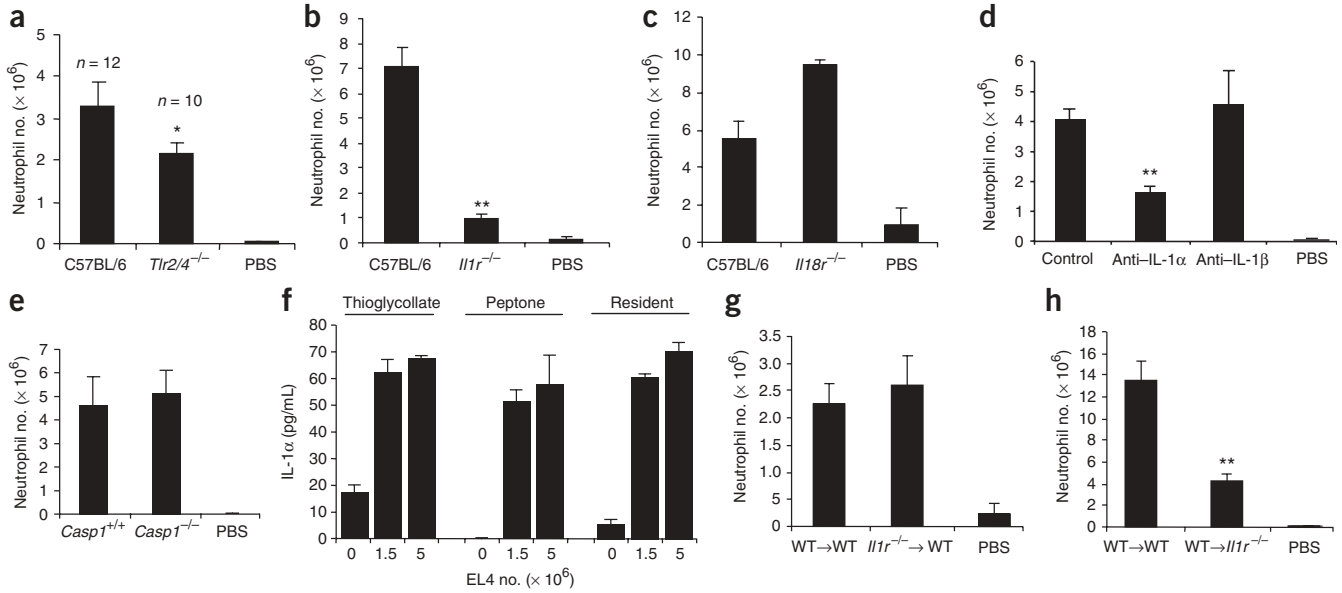

Figure 3 Inflammation induced by injured cells is dependent on IL-1α and on non–bone marrow IL-1R, and independent of IL-18R. (**a**) Neutrophil numbers in peritoneal exudate cells (PEC) 16 h after i.p. challenge with necrotic EL4 cells, for normal and Tlr2/Tlr4-deficient C57BL/6 mice. Mean and s.e.m. are for combined data from three independent experiments (n = total number of mice from the multiple experiments for each group). $^*P < 0.05$ versus control (C57BL/6). Negative controls (in **a**–**d**,**g**,**h**): C57BL/6 mice challenged with PBS. Neutrophil numbers in PEC of normal, IL-1R–deficient (**b**; $n = 3$) or IL-18R–deficient (**c**; $n = 4$) C57BL/6 mice 16 h after i.p. challenge with necrotic EL4 cells. Data are representative of three independent experiments. $^{**}P < 0.01$ versus control (C57BL/6). (**d**) Neutrophil numbers, 16 h after challenge, in PEC of C57BL/6 mice that were treated i.v. with control antibody or antibodies to IL-1α or IL-1β, and 2 h later challenged i.p. with necrotic EL4 cells. Data are representative of two independent experiments analyzing a total of 11 mice per condition. $^{**}P < 0.01$ versus mice treated with control antibody. (**e**) Neutrophil numbers in PEC of *Casp1*$^{+/+}$ or *Casp1*$^{-/-}$ mice 16 h after i.p. challenge with necrotic EL4 cell ($n = 5$). Negative controls: *Casp1*$^{+/+}$ mice challenged with PBS. Data are representative of three independent experiments. (**f**) IL-1α concentrations in the supernatants of resident or thioglycollate- or peptone-elicited peritoneal macrophages stimulated with necrotic EL4 cells, quantified by ELISA ($n = 3$). (**g**,**h**) Neutrophil numbers in PEC of bone marrow–chimeric mice 16 h after i.p. challenge with necrotic EL4 cells ($n = 4$). Chimeric mice were generated using C57BL/6 (WT) mice as hosts and B6.SJL (WT) or *Il1r*$^{-/-}$ as bone marrow donors (**g**), and C57BL/6 (WT) and *Il1r*$^{-/-}$ mice as hosts and B6.SJL (WT) mice as donors (**h**). Data are representative of four independent experiments. $^{**}P < 0.01$ versus control (WT→WT).

the inflammatory response, whereas antibodies to IL-1β did not (**Fig. 3d**). To further investigate this point, we examined the sterile inflammatory response to dead cells in mice lacking caspase 1, a protease that is required to generate active IL-1β but not IL-1α (although IL-1α secretion is partially reduced in *Casp1*$^{-/-}$ cells *in vitro*[13]). Consistent with our findings in the mice treated with antibody to IL-1β, the inflammatory response to dead cells was not reduced in the caspase 1–deficient mice (**Fig. 3e**). We also found that dead cells stimulated macrophages to produce IL-1α *in vitro* (**Fig. 3f**). Together these observations demonstrate that the key cytokine in this system is IL-1α rather than IL-1β. These findings (as well as the concordant results in the Myd88-deficient mice) rule out the possibility that the phenotype observed in the IL-1R–mutant mice is due to some defect unrelated to the IL-1–IL-1R pathway.

Although IL-1 had been recognized as a proinflammatory mediator in a number of inflammatory diseases[14–16] and ischemia-reperfusion injury[17–19], its central role in the sterile inflammatory response to cell death was not known. This key role of the IL-1R almost certainly accounts for the much more dramatic phenotype we observed in Myd88-deficient mice as compared to TLR-null mice. If TLRs were important in this response, we would have expected the sterile inflammatory responses to be more impaired in Myd88-deficient mice (which have impaired signaling through both IL-1R and all TLRs except for Tlr3) than in IL-1R–deficient mice (which have intact TLR signaling), and we did not observe this pattern.

IL-1R is broadly expressed on many cell types, and to understand on which of these its presence was required, we analyzed mice chimeric for IL-1R. Wild-type mice that were reconstituted with IL-1R–deficient bone marrow (*Il1r*$^{-/-}$→wt) (**Fig. 3g**) did not show any significant reduction in inflammatory responses to necrotic EL4 cells. In contrast, IL-1R–deficient mice reconstituted with wild-type bone marrow (wt→*Il1r*$^{-/-}$) had markedly reduced inflammatory responses to injured EL4 cells (**Fig. 3h**). Therefore, the IL-1R was required on radioresistant (non–bone-marrow-derived), but not radiosensitive (bone marrow–derived), host cells for them to respond to IL-1.

To determine whether the IL-1R and Myd88 signaling pathways were generally required for the inflammatory response to injured cells, we tested their role in the sterile inflammatory response to additional types of dead cells. UV-irradiated syngeneic mouse melanoma B16 cells also induced a strong influx of neutrophils into the peritoneal cavity, and this response was significantly reduced in *Myd88*$^{-/-}$ (**Fig. 4a**) and *Il1r*$^{-/-}$ (**Fig. 4b**) mice. Similar results were obtained with necrotic primary (non-cultured) liver tissue (**Fig. 4a**,**b**). Therefore, Myd88 and IL-1R have an important and general role in cell- and tissue-injury-induced sterile inflammatory responses.

Monocytes are also recruited to sites of cell injury. When we analyzed the number of monocytes recruited into the peritoneal cavity of *Il1r*$^{-/-}$ (**Fig. 4c**) or *Myd88*$^{-/-}$ (data not shown) mice injected with necrotic EL4, B16 or liver cells, there was no or only a modest reduction in recruitment of monocytes. Therefore, the recruitment of neutrophils to cell injury is more dependent on IL-1 than that of monocytes, indicating that neutrophils and monocytes are recruited through different mechanisms. The relatively intact monocyte

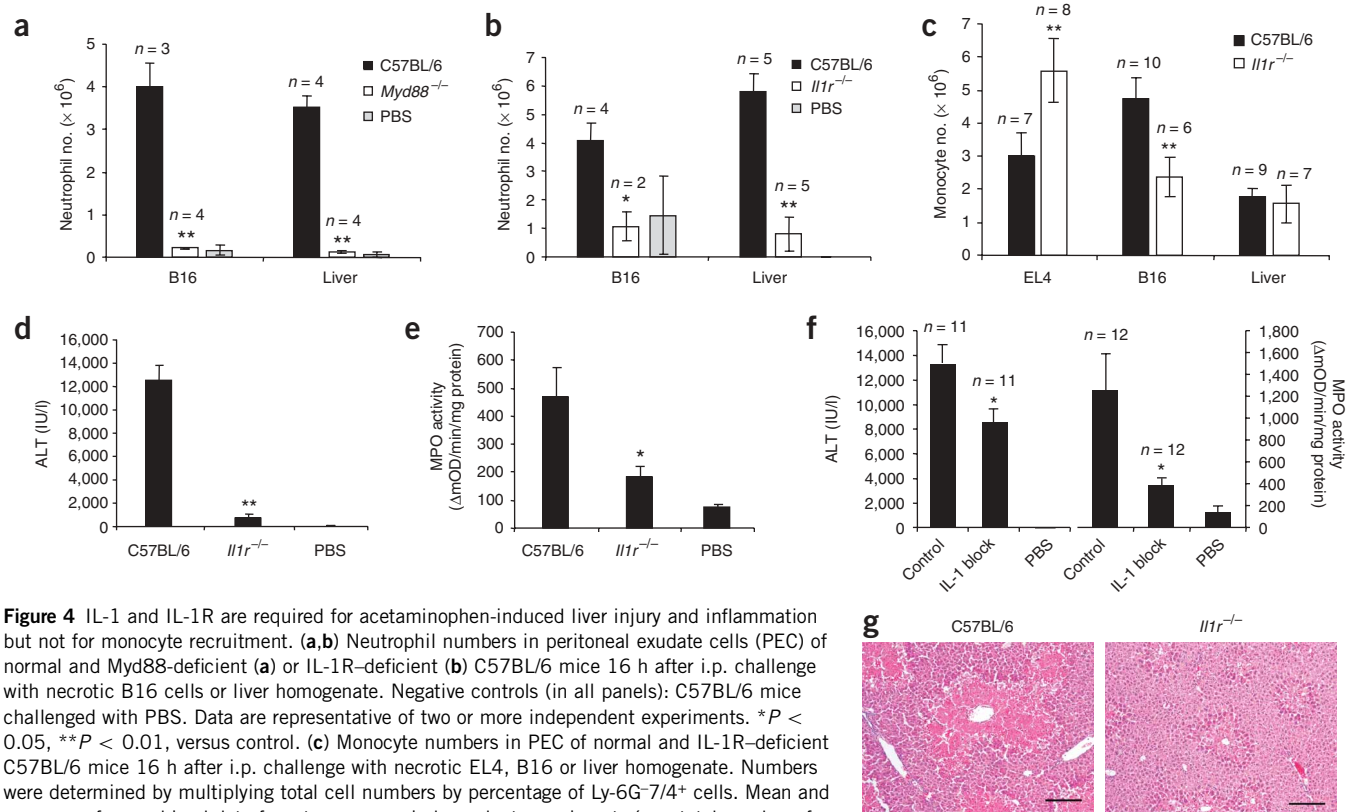

Figure 4 IL-1 and IL-1R are required for acetaminophen-induced liver injury and inflammation but not for monocyte recruitment. (**a**,**b**) Neutrophil numbers in peritoneal exudate cells (PEC) of normal and Myd88-deficient (**a**) or IL-1R–deficient (**b**) C57BL/6 mice 16 h after i.p. challenge with necrotic B16 cells or liver homogenate. Negative controls (in all panels): C57BL/6 mice challenged with PBS. Data are representative of two or more independent experiments. $*P < 0.05$, $**P < 0.01$, versus control. (**c**) Monocyte numbers in PEC of normal and IL-1R–deficient C57BL/6 mice 16 h after i.p. challenge with necrotic EL4, B16 or liver homogenate. Numbers were determined by multiplying total cell numbers by percentage of Ly-6G$^-$7/4$^+$ cells. Mean and s.e.m. are for combined data from two or more independent experiments (n = total number of mice from the multiple experiments for each group). $**P < 0.01$ versus control. (**d**,**e**) Serum ALT activity (**d**) and liver tissue myeloperoxidase activity (**e**) of normal and IL-1R–deficient C57BL/6 mice 18 h after challenge with 300 mg/kg acetaminophen ($n = 6$). Data are representative of three experiments. $*P < 0.05$, $**P < 0.01$, versus control (C57BL/6). (**f**) Serum ALT (left) and liver tissue myeloperoxidase activity (right), 18 h after challenge, of C57BL/6 mice that were first treated with control antibody or a combination of antibodies to IL-1α, IL-1β and IL-1R (0.25 mg each) and then challenged with 300 mg/kg acetaminophen. $*P < 0.05$ versus control antibody group. (**g**) H&E staining of liver tissue from normal and IL-1R–deficient C57BL/6 mice 18 h after challenge with 300 mg/kg acetaminophen (scale bar, 200 μm).

response in the mutant mice indicates that Myd88 is principally required downstream of the initial triggering event, providing further evidence that TLRs (which should trigger the whole inflammatory cascade) are unlikely to be major sensors of cell death in this response.

We next sought to test the generalizability of these findings to an *in vivo* situation where cell death occurred *in situ*. For this purpose, we induced liver injury with acetaminophen (AAP), which, as expected[20], resulted in necrosis, as indicated by elevations of alanine aminotransferase (ALT, a hepatic enzyme) in the blood (**Fig. 4d**), and the recruitment of neutrophils into the liver as assayed by the abundance of the neutrophil enzyme myeloperoxidase (MPO) (**Fig. 4e**). In contrast, liver MPO activities were very significantly reduced in *Il1r*$^{-/-}$ mice (**Fig. 4e**) and in mice treated with antibodies to block IL-1 and IL-1R (**Fig. 4f**), indicating that IL-1 is an important mediator for sterile neutrophilic inflammation to tissue death *in situ*. Notably, serum ALT (**Fig. 4d**) was also significantly reduced in *Il1r*$^{-/-}$ mice, and histological evidence of damage was likewise reduced (**Fig. 4g**). Serum ALT was also reduced in mice treated with antibodies to IL-1 and IL-1R (**Fig. 4f**). These results are consistent with the concept that the sterile acute inflammatory response contributes substantially to the extent of tissue damage, and demonstrate that it is possible to prevent this process by blocking the IL-1R pathway.

Taken together, these results have potentially important medical implications. The sterile inflammatory response to injured cells can cause disease. For example, sterile neutrophilic inflammation is thought to contribute to the pathogenesis of acute ischemia-induced injuries[21], lung injury[22] and liver injury[23] and to impair healing[24]. Chronically, sterile inflammation provoked by ongoing tissue damage is thought to contribute to many chronic diseases, including ones affecting the lung[25], joints[26] and bowel[27]. In many of these settings, neutrophils are thought to be a principal culprit causing tissue damage[23,28].

Blocking sterile neutrophilic inflammation is a potentially attractive strategy to limit the damage of acute sterile inflammation and to stop the ongoing damage in chronic inflammation to tissue injury. Our findings raise the possibility that agents that block the actions of IL-1 might be particularly useful in limiting tissue damage during sterile inflammation. In direct support of this concept, we found that hepatocyte damage induced by a toxic insult is markedly reduced in IL-1R–deficient mice.

A potential problem with therapeutic strategies to potently block the sterile inflammatory response as a means to prevent further tissue injury is that this might decrease the ability of a host to fight infections and interfere with tissue repair. However, we found that neutrophil recruitment to a microbial stimulus was not reduced in mutant mice, raising the possibility that inflammation in response to microbes might be less affected by IL-1 pathway blockade than is sterile inflammation. Moreover, although Myd88- and IL-1R–deficient mice show a marked reduction in the neutrophil response to tissue injury, the recruitment of monocytes is unaffected or is diminished to

a much lesser degree. Therefore, blocking the IL-1 pathway therapeutically might limit the damaging effects of neutrophils while preserving the host defense and tissue repair functions of monocytes. Consistent with this idea, patients treated with IL-1 blocking agents have not shown an increase in opportunistic infections. Accordingly, we speculate that IL-1 pathway blockade might have therapeutic benefit in sterile inflammation without markedly increasing susceptibility to infection or compromising healing.

METHODS

Animals and cell lines. Mice were purchased from commercial sources or obtained from their laboratories of origin and bred as previously described[29]. *Casp1*$^{-/-}$ mice were a gift (see Acknowledgments). Although some TLR mutants were of a mixed BL6-129 genetic background, we found that sterile inflammatory responses were no different in BL6 and BL6×129 F1 mice (**Supplementary Fig. 1** online). Irradiation bone marrow chimeras were prepared as described[29]. All animal protocols were approved by the University of Massachusetts animal care and use committee. EL4 and B16 cells were maintained in hybridoma culture medium as described[29]. *Hmgb1*$^{+/+}$ and *Hmgb1*$^{-/-}$ fibroblast cells were a gift (see Acknowledgments) and were maintained in DMEM plus 10% FBS.

Cell injury induction. EL4 cells were harvested by centrifugation, washed three times with PBS, resuspended in PBS at a density of 13–20 × 10^7 cells/ml and heated at 45 °C for 10 min. Heat-shocked EL4 cells were incubated at 37 °C for 5 h before being used to challenge mice. Adherent cells (B16, *Hmgb1*$^{+/+}$ and *Hmgb1*$^{-/-}$) cultured on 15-cm dishes were rinsed once with 10 ml of PBS and covered with 6 ml of PBS. The cells were then exposed to a UV source for 10 min, trypsinized, washed three times with PBS and resuspended in PBS at a density of 13–20 × 10^7 cells/ml. UV-irradiated B16, *Hmgb1*$^{+/+}$ and *Hmgb1*$^{-/-}$ cells were incubated at 37 °C for 5 h before being used to challenge mice. Before being used for challenge, heat-shocked EL4 and UV-irradiated B16 cells were confirmed to be necrotic by staining and flow cytometry (annexin V$^+$, 7-AAD$^+$). Mouse liver homogenate was prepared through the use of a motor-driven Potter homogenizer followed by five freeze-thaw cycles.

AAP treatment. Mice were fasted for 16–18 h and were then injected i.p. with 300 mg/kg AAP or 20 ml/kg PBS. Eighteen hours after AAP administration, blood was drawn for serum collection and ALT assay (Synchron LX Systems, Beckman Coulter), and mice were killed to obtain liver tissues for MPO activity assay and histology. In some experiments mice were injected intravenously (i.v.) either with a cocktail of monoclonal antibodies to IL-1α, IL-1β and IL-1R (250 μg each) or with isotype control antibody to glutathione *S*-transferase (gifts; see Acknowledgments) 2 h before AAP treatment.

Myeloperoxidase (MPO) activity assay. Mouse liver tissues were homogenized in MPO buffer (0.5% hexadecyl trimethyl ammonium bromide, 10 mM EDTA, 50 mM Na_2HPO_4, pH 5.4) using a Polytron homogenizer. Liver homogenates were then subject to three freeze-thaw cycles and cleared by centrifugation. MPO reaction was carried out by first mixing 25 μl liver lysate with 25 μl assay buffer (1.67 mg/ml *o*-dianisidine, 50 mM Na_2HPO_4, pH 5.4) in a 96-well plate and, after adding 200 μl development solution (0.01% H_2O_2, 50 mM Na_2HPO_4, pH 5.4), measuring absorbance at 450 nm every 15 s. MPO activity is expressed as the change in absorbance per min per mg of liver lysate protein (ΔmOD/min/mg protein).

Injured cell– and zymosan-induced inflammation. Mice were injected i.p. with 2–3 × 10^7 necrotic EL4, B16, *Hmgb1*$^{+/+}$ or *Hmgb1*$^{-/-}$ cells in 0.15 ml PBS, 36 mg of mouse liver homogenate in 0.15 ml PBS, or PBS alone. At 16 h after challenge, the numbers of neutrophils (Ly-6G$^+$7/4$^+$) and monocytes (Ly-6G$^-$7/4$^+$) in the peritoneum were evaluated as described[29]. In preliminary experiments examining dose-response characteristics in this system, acute inflammatory responses were still detected in mice injected with the lowest number of EL4 cells tested (7.5 × 10^6) and increased as the number of dead cells injected was increased. Mice were injected with zymosan (0.5 mg) i.p. and, 4 h after challenge, the peritoneal content of neutrophils and monocytes was quantified as above. In experiments where zymosan was injected 16 h before analysis, the acute inflammatory response was more intense but still independent on the IL-1R pathway (data not shown). In some experiments mice were injected i.v. with 250 μg of antibodies to glutathione *S*-transferase (control), to IL-1α or to IL-1β 2 h before the injection of dead cells.

Macrophage stimulation and IL-1α assay. Peritoneal cells (5 × 10^5) from untreated mice or mice injected i.p. with 10% proteose peptone (1 ml) or 4% thioglycollate 4 d earlier were cultured in 24-well plates, and after 2 h nonadherent cells were removed and the indicated number of necrotic EL4 cells were added to the cultures. After 18 h the amount of IL-1α in the culture supernatants was determined by ELISA (Peprotec).

Statistical analyses. Statistical analysis in each independent experiment was performed with an unpaired, two-tailed Student's *t*-test. **Figure 2** shows data from multiple repeats of the experiment that were combined and analyzed using a linear mixed model[30] with experiment as the random effect. Data are reported as mean ± s.e.m. $P < 0.05$ was considered statistically significant.

Note: Supplementary information is available on the Nature Medicine website.

ACKNOWLEDGMENTS

The authors thank R. Schreiber (Washington University) for the generous gift of IL-1α, IL-1β, IL-1R and glutathione *S*-transferase (isotype control) antibodies, R. Flavell (Yale University) for the caspase 1–deficient mice, M. Bianchi (San Raffaele Scientific Institute) for *Hmgb1*$^{+/+}$ and *Hmgb1*$^{-/-}$ fibroblast cells, and K. Dresser and T. Smith (University of Massachusetts Medical School) for help with histology and photomicrographs. H.K. was supported in part by funds from Kanae Foundation. C.J.C. was supported in part by National Science Council, Taiwan (NSC95-2313-B002-120). This work was supported by grants from the US National Institutes of Health to K.L.R., and core resources supported by the Diabetes Endocrinology Research Center grant DK32520 were also used.

AUTHOR CONTRIBUTIONS

C.J.C. and H.K. performed the experiments and contributed to the writing of the manuscript. D.G. provided reagents and advice and contributed conceptually to the project. G.R. performed statistical analyses. S.A. provided mice. K.L.R. initiated and supervised the project and contributed to the writing of the manuscript.

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

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