Basophil-derived tumor necrosis factor can enhance survival in a sepsis model in mice

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Basophils are evolutionarily conserved in vertebrates, despite their small numbers and short life span, suggesting that they have beneficial roles in maintaining health. However, these roles are not fully defined. Here we demonstrate that basophil-deficient mice exhibit reduced bacterial clearance and increased morbidity and mortality in the cecal ligation and puncture (CLP) model of sepsis. Among the several proinflammatory mediators that we measured, tumor necrosis factor (TNF) was the only cytokine that was significantly reduced in basophil-deficient mice after CLP. In accordance with that observation, we found that mice with genetic ablation of TNF in basophils exhibited reduced systemic concentrations of TNF during endotoxemia. Moreover, after CLP, mice whose basophils could not produce TNF, exhibited reduced neutrophil and macrophage TNF production and effector functions, reduced bacterial clearance, and increased mortality. Taken together, our results show that basophils can enhance the innate immune response to bacterial infection and help prevent sepsis.

Basophils are the rarest granulocyte (<1% of peripheral blood leukocytes), were named by Paul Ehrlich more than 130 years ago based on the prominent basophilic granules in their cytoplasm. We now know that basophils are evolutionarily conserved in many animal species and that they can help to enhance defenses against certain parasitic infections that induce the adaptive immune responses associated with the production of immunoglobulin E (IgE) isotype antibodies. Basophils activated by IgE-dependent mechanisms also contribute to pathology during allergic disorders.

However, in part because of their rarity and short life span (~2.5 d in mice), it has been challenging to identify important roles for basophils in settings other than those associated with IgE. A few studies have raised the possibility that basophils might participate in host responses to bacteria. For example, basophils can recognize and be activated by staphylococcal enterotoxins via antibody-mediated mechanisms, and basophils can form extracellular traps that can immobilize and kill bacteria. However, no studies have demonstrated that basophils can represent an important host defense component against bacteria in vivo, and so the extent and importance of basophils in contributing to bacterial infection resistance remains undefined.

In this study, we analyzed the potential roles of basophils in the cecal ligation and puncture (CLP) model of polymicrobial bacterial infection and sepsis in mice. We found that basophils are one of the first leukocytes to appear at the infection site. Moreover, by using mice genetically deficient in basophils, we found that basophils reduced the morbidity and mortality associated with CLP, at least in part by enhancing bacterial clearance. Our results with mice genetically deficient in basophil-derived tumor necrosis factor (TNF) also indicate that basophils represent one important source of TNF during lipopolysaccharide (LPS)-induced endotoxemia and that basophil-derived TNF can enhance the ability of other myeloid cells to control bacterial infection and contribute to survival after CLP. These results provide new insights into basophil-dependent mechanisms that can enhance innate immune responses to bacterial infection.

Results

Basophils express active toll-like receptors (TLRs). We first investigated whether mouse basophils express TLRs and can produce proinflammatory cytokines in response to bacterial products. In confirmation of prior work, we found that mouse bone-marrow-derived cultured basophils expressed abundant TLR2 messenger RNA (mRNA) and moderate amounts of TLR1 and TLR4 mRNA (Fig. 1a). Similarly, we found comparable mRNA abundance of TLR2 and TLR4 and very low TLR1 mRNA levels in bone marrow basophils collected from naive or CLP-treated mice (Fig. 1b,c). Moreover, basophils produced TNF and interleukin 6 (IL-6) in response to stimulation with TLR1/2, TLR2, and TLR4 ligands (Fig. 1d,e). These observations show that basophils express TLRs and can respond to TLR ligands by secreting pro-inflammatory cytokines.

Basophils migrate early and enhance survival after CLP. Basophils can migrate into tissues during chronic inflammatory processes, but it is unknown to what extent they can infiltrate into sites of innate immune responses against bacterial infection. After the removal of doublets and dead cells, basophils were gated and defined as FcεRIα+, c-Kit+, and CD49b+ cells to assess their numbers in the peritoneal cavity of naive or CLP-treated mice (Supplementary Fig. 1).
The number of intraperitoneal basophils significantly increased as early as 1 h after induction of CLP of moderate severity (50% cecum ligation and one puncture with a 22 gauge (G) needle, Fig. 2a). Basophils remained elevated throughout the peak of the inflammatory response, which occurred ~24 h after CLP in our model (Fig. 2a and Supplementary Fig. 1). We confirmed that these cells were basophils by detecting increased mast cell protease 8 (Mcpt8)-positive cells in the peritoneal fluid after CLP (Fig. 2b). Despite its name, Mcpt8 is expressed highly by basophils, but in lower amounts by certain mast cells and bone marrow eosinophils and neutrophils. Notably, peritoneal neutrophils and macrophages of naïve mice expressed very low amounts of Mcpt8 mRNA (Supplementary Fig. 2), indicating that basophils, but not other myeloid cells, may be the only substantial cell source of this protease in the peritoneum during CLP. Overall, these observations show that basophils migrate to the site of CLP as part of the early inflammatory response induced by this model, and progressively accumulate at that site as the inflammation reaches its peak.

Next, we studied whether basophils can influence CLP outcomes, using Basoph8 × Rosa-DTα basophil-deficient mice. In Basoph8 mice, the Mcpt8 gene was replaced at the start site with a reporter cassette expressing yellow fluorescent protein (YFP) followed by an internal ribosome entry site and humanized Cre. Then, basophils could be tracked in Basoph8 mice as YFP+ cells or deleted by crossing Basoph8 mice with Rosa-DTα mice (Basoph8 × Rosa-DTα), which contain the diphtheria toxin-α gene inserted into the ubiquitous Rosa26 locus downstream of a loxP-flanked transcriptional site. Our data indicate that basophil-deficient mice exhibit increased mortality

Fig. 1 | Mouse basophils express TLRs and produce TNF and IL-6 after activation with TLR ligands. a–c. Tlr mRNA expression relative to GAPDH in FceRIα+CD49b-c-Kit+ basophils that were sorted from bone marrow-derived cells cultured in the presence of IL-3 (a) or from bone marrow cells collected from naïve mice (b) or from mice 24 h after CLP (c). The qPCR data are from one representative experiment of the n = 3 independent experiments performed, each of which gave similar results. d. TNF concentrations in the supernatants of CD49b+ cells (1×10^6 cells) following 6 h of TLR ligand stimulation (TLR1/2 (PAM 3CSK4, 250 ng ml⁻¹), TLR2 (HKLM, 1×10^5 ml⁻¹), or TLR4 (LPS, 1 μg ml⁻¹)). e. IL-6 concentrations in the supernatants of CD49b+ cells (1×10^6 cells) following 6 h of TLR ligand stimulation (TLR1/2 (PAM 3CSK4, 250 ng ml⁻¹), TLR2 (HKLM, 1×10^5 ml⁻¹), or TLR4 (LPS, 1 μg ml⁻¹)). Data in d and e are shown as mean ± s.e.m. of the average of duplicate specimens with the circles showing the values obtained from individual experiments (n = 3 for medium, n = 8 for LPS and n = 5 for PAM 3CSK4 or HKLM in d, and n = 3 for medium, PAM 3CSK4 or HKLM, and n = 4 for LPS, in e). P values by Mann-Whitney U test.
and morbidity (drop in body temperature) (Fig. 2c,d), as well as reduced bacterial clearance at the infection site and in the blood (Fig. 2e,f) 24 h after CLP. These findings support the conclusion that a beneficial role of basophils during CLP is to enhance bacterial clearance and that this response is associated with enhanced survival after CLP.

Basophils contribute to intraperitoneal TNF after CLP. To investigate the potential mechanisms by which basophils might contribute to bacterial clearance, we assessed whether the innate immune response following CLP is affected by a deficiency in basophils. Neutrophils and macrophages are essential for bacterial clearance.
Following CLP, we investigated whether basophils might influence neutrophil or macrophage numbers at the infection site. However, we found that the numbers of neutrophils and macrophages in the peritoneal cavity were not significantly different between Basoph8 x Rosa-DTα basophil-deficient mice and their littermate controls 24 h following CLP (Fig. 3a,b).

Among the several pro-inflammatory mediators we tested (IL-6, CXCL1 (KC), MIP-2, and TNF), TNF was the only cytokine whose concentration was significantly reduced in the peritoneum of basophil-deficient mice 24 h after CLP, whereas blood concentrations of TNF and the other mediators were similar in both basophil-deficient mice and wild-type mice in a and b). c-j, Concentrations of TNF, IL-6, CXCL1 (KC), and MIP-2 in the peritoneal cavity (c,e,g,i) and plasma (d,f,h,j) of ROSA-DTα and Basoph8 x Rosa-DTα mice 24 h after CLP. Data are shown as mean ± s.e.m. with the squares representing values from individual mice (n = 15 in c, n = 20 in d, n = 5 in e-g, j, and n = 14 in f for ROSA-DTα; n = 10 in c,f,i, n = 8 in g, and n = 4 in h for Basoph8 x ROSA-DTα mice). Data were pooled from n = 3 independent experiments performed, each of which gave similar results. P values by Mann-Whitney U test.

### Basophils contribute to the increased TNF in the peritoneal cavity following CLP

**Fig. 3** | Basophils contribute to the increased TNF in the peritoneal cavity following CLP. a,b. Number of neutrophils (a) and macrophages (b) in the peritoneal live-cell population analyzed by flow cytometry (Gr-1+ CD11b+ cells for neutrophils and F4/80+ CD11b+ for macrophages). Data in a and b are shown as mean ± s.e.m. with the squares representing values from individual mice (n = 5 or 13 for wild-type mice in a and b, respectively, and n = 10 for basophil-deficient mice in a and b). c–j, Concentrations of TNF, IL-6, CXCL1 (KC), and MIP-2 in the peritoneal cavity (c,e,g,i) and plasma (d,f,h,j) of ROSA-DTα and Basoph8 x Rosa-DTα mice 24 h after CLP. Data are shown as mean ± s.e.m. with the squares representing values from individual mice (n = 15 in c, n = 20 in d, n = 5 in e-g, j, and n = 14 in f for ROSA-DTα; n = 10 in c,f,i, n = 8 in g, and n = 4 in h for Basoph8 x ROSA-DTα mice). Data were pooled from n = 3 independent experiments performed, each of which gave similar results. P values by Mann-Whitney U test.

The data suggest that basophils might be an important local source of TNF, and/or may influence other cells to produce TNF, in the peritoneal cavity during the host's response to bacteria. This observation is relevant because TNF can substantially contribute to the initiation and/or enhancement of the inflammatory response against certain bacterial infections. Notably, we have shown that TNF-deficient mice subjected to CLP with the same severity we used in this study (that is, moderately severe CLP) exhibited similar survival rates to those observed in basophil-deficient mice, as well as significantly increased bacterial colony-forming units (CFUs) in the peritoneum and blood after CLP (Supplementary Fig. 3). From these results, we can conclude that, in this model of CLP, endogenous TNF is required to limit bacterial growth and dissemination and to survive peritonitis.

**Basoph8 x Tnffl/fl** basophils produce less TNF. Next we attempted to produce mice in which basophils were impaired in their ability to produce TNF. We crossed Basoph8 with mice containingloxP-flanked Tnf alleles (Basoph8 x Tnffl/fl mice). We found an approximately 80% reduction in TNF, but not in IL-6, production by Basoph8 x Tnffl/fl mouse bone-marrow-derived CD49b+ cells (which consisted of 60–80% basophils) after activation with 2,4-dinitrophenyl-human serum albumin conjugate (DNP-HSA)-IgE complexes (Fig. 4a,b). Similarly, production of TNF, but not IL-6, in response to TLR ligands was significantly reduced in CD49b+ basophils x Rosa-DTα mice 24 h after CLP. Data are shown as mean ± s.e.m. with the squares representing values from individual mice (n = 15 in c, n = 20 in d, n = 5 in e-g, j, and n = 14 in f for ROSA-DTα; n = 10 in c,f,i, n = 8 in g, and n = 4 in h for Basoph8 x ROSA-DTα mice). Data were pooled from n = 3 independent experiments performed, each of which gave similar results. P values by Mann-Whitney U test.

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Fig. 4 | TNF production is markedly diminished in CD49b⁺ cells generated from Basoph8 x Tnf⁻/⁻ mice. a, c, TNF concentrations in the supernatants of CD49b⁺ cells (1x10⁶ cells) following overnight sensitization with an IgE mAb to DNP (2 μg ml⁻¹) and 6 h of DNP-HSA (20 ng ml⁻¹) challenge (a, b) or TLR ligand stimulation (TLR1/2 (PAM 3CSK4, 250 ng ml⁻¹), TLR2 (HKLM, 1x10⁴ ml⁻¹), or TLR4 (LPS, 1 μg ml⁻¹)) (c, d). c, d, IL-6 concentrations in the supernatants of CD49b⁺ cells (1x10⁶ cells) following overnight sensitization with an IgE mAb to DNP (2 μg ml⁻¹) and 6 h of DNP-HSA (20 ng ml⁻¹) challenge (a, b) or TLR ligand stimulation (TLR1/2 (PAM 3CSK4, 250 ng ml⁻¹), TLR2 (HKLM, 1x10⁴ ml⁻¹), or TLR4 (LPS, 1 μg ml⁻¹)) (c, d). CD49b⁺ cells enriched from bone-marrow-derived cells of Basoph8 × Tnf⁻/⁻ mice were markedly diminished compared with those observed after challenge of the control Tnf⁻/⁻ mice, whereas IL-6 production was unchanged. Data in a and b are shown as mean ± s.e.m. of the average of duplicate specimens with the squares showing the values obtained from individual experiments (n = 10 and n = 8 for cells from wild-type mice in a and b, respectively, and n = 4 for cells from mice with basophil-derived TNF deficiency). Data in c and d are shown as mean ± s.e.m. of the average of duplicate specimens with the squares showing the values obtained from individual experiments. c, n = 3 for medium, n = 7 for LPS and n = 5 for PAM 3CSK4 or HKLM for cells from wild-type mice, and n = 3 for medium, n = 6 for LPS and n = 4 for PAM 3CSK4 or HKLM for cells from mice with basophil-derived TNF deficiency. d, n = 5 medium, n = 6 for LPS and n = 5 for PAM 3CSK4 or HKLM for cells from wild-type mice; and n = 7 for medium, n = 5 for LPS and n = 7 for PAM 3CSK4 or HKLM for cells from mice with basophil-derived TNF deficiency. Values by Mann-Whitney U test. e, f, g, TNF production by PCMCs (f) and thioglycolate-elicited peritoneal macrophages (g). PCMCs were sensitized overnight with an IgE mAb to DNP (2 μg ml⁻¹) and then challenged with DNP-HSA (20 ng ml⁻¹) for 6 h. Peritoneal macrophages were stimulated with LPS (100 ng ml⁻¹) for 6 h. Data in f and g are shown as mean ± s.e.m. with the squares showing the values from individual experiments (n = 13 and n = 12 for Tnf⁻/⁻ mice in f and g, respectively, and n = 4 for Basoph8 × Tnf⁻/⁻ for f and g) with each condition tested in duplicate. P values by Mann-Whitney U test.

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after activation mediated by IgE/antigen (Fig. 4c), suggesting that the reduced amount of TNF produced by the Basoph8 × Tnffl/fl mouse basophils in vitro cannot be explained by an impairment in the ability of these basophils to undergo activation.

TNF deletion in Basoph8 × Tnffl/fl mice appears to be basophil specific, in that TNF production by peritoneal-cell-derived mast cells (PCMCs) or thioglycolate-elicited peritoneal macrophages obtained from Basoph8 × Tnffl/fl mice was not significantly different from that of the corresponding cells obtained from the control mice (Fig. 4f,g). These findings are in agreement with a previous study that showed that basophils, but not eosinophils and neutrophils, in the bone marrow or mast cells in the peritoneum express mRNA for Mcpt8[24]. Furthermore, our own observations showed that Mcpt8 mRNA abundance in primary neutrophils and macrophages sorted from peritoneal fluids obtained from wild-type and Basoph8 × Tnffl/fl mice was significantly lower than that found in basophils sorted from the bone marrow of wild-type mice (Supplementary Fig. 2).

Overall, this evidence strongly suggests that Cre recombinase expression under the control of the Mcpt8 promoter and the consequent floxed Tnf gene deletion in mice occur primarily in basophils and not in other myeloid cells.

We found no significant differences in the number of basophils or other leukocyte populations, including neutrophils in the spleen, bone marrow, and peritoneal fluid, macrophages in the spleen and peritoneal fluid, monocytes and eosinophils in the blood and mast cells in the peritoneal fluid of Basoph8 × Tnffl/fl mice when compared with littermate controls (Supplementary Fig. 4a–e). In contrast, Basoph8 × Tnffl/fl mice exhibited ~60–66% increase in the number of blood lymphocytes and neutrophils (Supplementary Fig. 4f), but they exhibited a similar reduction in the numbers of blood lymphocytes and neutrophils as their littermate controls 24 h after CLP, as expected in mice subjected to this procedure[1] (Supplementary Fig. 4g). These observations suggest that a modest inhibitory effect of basophil-derived TNF...
Basophil-derived TNF during LPS-induced endotoxemia. TNF production is an important downstream consequence of TLR signaling, in particular through TLR4. Moreover, TNF was reported to influence the results of our CLP studies.

in the development and/or maintenance of normal numbers of blood granulocytes and lymphocytes may not have importantly influenced the results of our CLP studies.
to be a critical mediator of septic shock in humans and of LPS/D-Gal-induced acute systemic toxicity in mice. Notably, we found that Basoph8 × Tnf<sup>fl/fl</sup> mice exhibited a significant reduction in the concentrations of both intraperitoneal (Fig. 5a) and serum (Fig. 5b) TNF 1 h after intraperitoneal administration of LPS, indicating that basophil-derived TNF can contribute to the increased concentrations of TNF observed during LPS-induced endotoxemia. In contrast, Basoph8 × Tnf<sup>fl/fl</sup> mice and their littermate controls exhibited similar intraperitoneal and serum concentrations of IL-6, CXCL1 (KC), and MIP-2 1 h after LPS challenge (Fig. 5c–h), indicating that basophil-derived TNF does not detectably contribute to the increased concentrations of these mediators during LPS-induced endotoxemia.

As we showed for the CLP model, we found that basophils accumulated at the insult site (peritoneum) 1 h after intraperitoneal injection of a sublethal dose of LPS (Fig. 5i). Moreover, we found that peritoneal macrophages from Basoph8 × Tnf<sup>fl/fl</sup> mice were impaired in their ability to produce TNF during LPS-induced endotoxemia (Supplementary Fig. 5). In contrast, macrophages obtained from Basoph8 × Tnf<sup>fl/fl</sup> mice were able to produce TNF after stimulation with LPS ex vivo (Fig. 4g), indicating that the reduced TNF concentrations observed after LPS administration are not a result of an intrinsic defect in the ability of myeloid cells to produce TNF. These data suggest that the overall reduced intraperitoneal concentrations of TNF detected in Basoph8 × Tnf<sup>fl/fl</sup> mice may be explained by the impaired ability of resident myeloid cells to produce TNF during LPS-induced endotoxemia when not influenced by basophil-derived TNF. Taken together, our findings indicate that basophil-derived TNF can contribute, directly and indirectly, to the elevated concentrations of TNF seen during LPS-induced endotoxemia in mice.

Basophil-derived TNF enhances survival after CLP. Next, we examined whether basophil-derived TNF can influence the outcome of CLP. Basoph8 × Tnf<sup>fl/fl</sup> mice exhibited increased mortality and morbidity (assessed by a drop in body temperature) 24 h after CLP compared with the identically treated Tnf<sup>fl/fl</sup> mice (Fig. 6a,b). Basoph8 × Tnf<sup>fl/fl</sup> mice also had significantly reduced concentrations of intraperitoneal TNF after CLP (Fig. 6c). Basoph8 × Tnf<sup>fl/fl</sup> mice had reduced or absent Mcpt8 (that is, Basoph8) as well as TNF at 24 h of CLP. Basoph8 × Tnf<sup>fl/fl</sup> mice were impaired in their ability to produce TNF during LPS-induced endotoxemia (Supplementary Fig. 5). In contrast, macrophages obtained from Basoph8 × Tnf<sup>fl/fl</sup> mice were able to produce TNF after stimulation with LPS ex vivo (Fig. 4g), indicating that the reduced TNF concentrations observed after LPS administration were not a result of an intrinsic defect in the ability of myeloid cells to produce TNF.

**Fig. 7** | Adoptive transfer of CD49b<sup>+</sup> spleen cells from control littermates enhances bacterial clearance in Basoph8 × Tnf<sup>fl/fl</sup> mice. Tnf<sup>fl/fl</sup> and Basoph8 × Tnf<sup>fl/fl</sup> mice received an adoptive transfer 3 h after CLP with a CD49b<sup>+</sup> basophil-enriched fraction of splenocytes (4 × 10<sup>5</sup> cells) obtained from Tnf<sup>fl/fl</sup> mice or Basoph8 × Tnf<sup>fl/fl</sup> mice. a–d, Concentrations of TNF (a) and IL-6 (c), CFU (b), and number of basophils (FcεR-I<sup>+</sup> CD49b<sup>+</sup> Kit<sup>−</sup> cells) (d) in the peritoneal cavity 24 h after CLP. Data in a–d are shown as mean ± s.e.m. with the squares and circles representing the values for individual mice (n = 4 in a–c and n = 3 in d for Tnf<sup>fl/fl</sup> + CD49b<sup>+</sup> spleen cells from Tnf<sup>fl/fl</sup>; n = 5 in a–c and n = 3 in d for Tnf<sup>fl/fl</sup> + CD49b<sup>+</sup> spleen cells from Basoph8 × Tnf<sup>fl/fl</sup>; n = 7 in a and c, n = 6 in b and n = 5 in d for Basoph8 × Tnf<sup>fl/fl</sup> + CD49b<sup>+</sup> spleen cells from Tnf<sup>fl/fl</sup>; and n = 6 in a, n = 6 in b, n = 7 in c and d for Basoph8 × Tnf<sup>fl/fl</sup> + CD49b<sup>+</sup> spleen cells from Basoph8 × Tnf<sup>fl/fl</sup>). Data were pooled from the n = 3 independent experiments performed, each of which gave similar results. P values by Mann–Whitney U test.
which lacked Mcpt8 but expressed TNF (Supplementary Fig. 6e–j); no alterations in the concentrations of intraperitoneal IL-6 were observed in Basoph8×Tnffl/fl mice 24 h after CLP (Fig. 6d).

There are multiple potential sources of TNF during CLP, including neutrophils and macrophages35,42. Moreover, in vitro studies have shown that exposure to exogenous TNF can enhance the ability of LPS to induce upregulation of TNF mRNA expression in neutrophils and to increase cytotoxic activity in macrophages45. Notably, we found that, when assessed 24 h after CLP, peritoneal neutrophils and macrophages from Basoph8×Tnffl/fl mice exhibited a substantially reduced ability to produce TNF (Fig. 6e,f). To track basophils in Basoph8 mice, we used a YFP reporter as part of the cassette that contained humanized Cre recombinase, resulting in high YFP expression in basophils expressing the Mcpt8 gene (Basophil8+− cells) (Supplementary Fig. 8a). In contrast, peritoneal neutrophils, macrophages, and mast cells obtained from Basoph8+−×Tnffl/fl mice 24 h after CLP induction exhibited low YFP expression, similar to that from cells obtained from Tnffl/fl mice (Supplementary Fig. 8a). Moreover, we did not detect Mcpt8 gene expression by single-cell PCR in neutrophils, macrophages, or mast cells obtained from Basoph8+−×Tnffl/fl mice 24 h after CLP (Supplementary Fig. 8b). These observations indicate that neutrophils, macrophages, and mast cells in the Basoph8+−×Tnffl/fl mice did not upregulate Cre recombinase for TNF deletion after the mice were subjected to CLP, suggesting that genetic deletion of TNF during CLP remained largely, if not entirely, basophil specific. Overall, our findings support the conclusion that basophil-derived TNF can enhance the ability of neutrophils and macrophages to produce TNF during CLP, thus providing an explanation for the low TNF production detected in neutrophils and macrophages after CLP in Basoph8×Tnffl/fl mice.

As observed in basophil-deficient mice, we found that the reduced survival rates and increased morbidity in Basoph8×Tnffl/fl mice were associated with an impairment in the ability of these mice to clear bacteria, both at the infection site (peritoneal cavity) and systemically, as assessed 24 h after CLP (Fig. 6g,h). Moreover, the adoptive transfer of CD49b+ basophil-enriched splenocytes from littermate control mice, but not from basophil-deficient mice, increased intraperitoneal TNF (Fig. 7a) and enhanced the ability of Basoph8×Tnffl/fl mice to clear bacteria at the infection site 24 h after CLP (Fig. 7b). In contrast, we did not observe alterations in intraperitoneal IL-6 concentrations in mice deficient in basophil-derived TNF that underwent adoptive transfer of basophils from littermate controls 24 h after CLP (Fig. 7c).

We also did not observe differences in TNF concentration or bacterial clearance at the infection site 24 h after CLP when we performed an adoptive transfer of basophils obtained from either wild-type littermate controls or basophil-TNF-deficient mice into wild-type mice (Fig. 7a,b), which suggests that the endogenous TNF provided by host basophils (and perhaps other cells) is sufficient to enhance innate immunity during CLP. Notably, basophil numbers in the peritoneal cavity were similar in all mouse groups 24 h after CLP (Fig. 7d). We think that these findings demonstrate a role for endogenous basophil-derived TNF in the enhancement of the innate immune response against bacteria during our model of CLP. Moreover, neutrophil, macrophage, and basophil numbers in the peritoneal cavity were similar in the control and Basoph8×Tnffl/fl mice 24 h after CLP (Fig. 8a–c), providing evidence that the impaired bacterial clearance in these mice cannot be explained by a generalized defect in myeloid cell mobilization to the infection site.

TNF can influence bacterial clearance by increasing neutrophil production of reactive oxygen species (ROS) and macrophage phagocytosis37. We observed that, at 24 h after CLP, Basoph8×Tnffl/fl peritoneal neutrophils produced diminished amounts of ROS (Fig. 8d) and peritoneal macrophages were impaired in their ability to phagocytose bacteria (Fig. 8e). Notably, neutrophils and macrophages of naive Basoph8×Tnffl/fl mice produced ROS or phagocytosed bacteria similar to those of littermate controls to stimuli such as phorbol 12-myristate 13-acetate or opsonized fluorescein isothiocyanate-labeled Escherichia coli (Supplementary Fig. 9a,b), suggesting that basophil-derived TNF can improve mouse survival during CLP in part by enhancing the ability of neutrophils and macrophages to clear bacteria. IL-6 also can improve survival in mouse models of sepsis, and the mortality of IL-6-deficient mice is higher than that of wild-type mice following bacterial infections36,46. We found that peritoneal macrophages from Basoph8×Tnffl/fl mice exhibited diminished production of IL-6 during CLP (Supplementary Fig. 10), suggesting that basophil-derived TNF also induces IL-6 production by myeloid cells that can enhance their activity against bacteria.

To explore a potential contribution of basophils to the outcome of human sepsis, we investigated whether basophil-specific gene expression levels in whole blood are associated with clinical outcomes using a dataset obtained from 35 healthy individuals and 167 severely injured patients, aged 16–55 years (dataset available at http://web.mgh.harvard.edu/TRT/; Supplementary Table 1). In agreement with our observation that circulating basophil numbers increase during infection, we found that expression of FcεRIα positively correlated with the presence of a nosocomial infection (false-discovery-rate-adjusted \( P = 0.0362 \) and \( \rho = 0.27 \). Notably, at day 7 of hospitalization, FcεRIα expression inversely correlated with potential negative outcomes of nosocomial infection, such as multiple organ failure, time to recovery, and hospital length of stay (Supplementary Table 1).

**Discussion**

We used genetically manipulated mice to reveal a potentially important role for basophils in enhancing inflammation at the early stages of the innate immune response to CLP, and in enhancing survival in mice subjected to this long-standing model of polymicrobial infection and sepsis.

Our in vivo studies indicate that the presence of basophils reduces mortality and morbidity after CLP by enhancing bacterial clearance. Notably, we found that TNF was the only cytokine examined that was significantly reduced in the peritoneum of basophil-deficient mice after CLP. There is evidence that TNF can be pathogenic during sepsis, such observations generally having been made in those with severe sepsis or septic shock31. In other settings, death resulting from sepsis occurs in individuals with impaired immunity, including in their ability to produce TNF41. Consistent with such observations, the use of anti-TNF antibodies and genetic approaches in preclinical models of sepsis, including CLP, has consistently shown that the contribution of TNF is critical for an efficient innate antimicrobial defense34,42–44. Despite this evidence, the cellular sources of TNF that can help to initiate inflammatory responses during the early stages of infection have yet to be fully defined.

Having shown that the presence of basophils significantly contributes to TNF concentrations in the peritoneum after CLP and that basophils can produce substantial amounts of TNF in vitro, we then assessed the extent to which basophil-derived TNF might be important in vivo. To do this, we generated mice with basophils lacking TNF (Basoph8×Tnffl/fl mice). Basoph8×Tnffl/fl mice exhibited significantly increased morbidity, reduced concentrations of intraperitoneal TNF and increased CFUs in the peritoneal cavity and blood after moderately severe CLP. It was reported that TNF can prime neutrophils and macrophages to produce ROS and phagocytose bacteria, respectively47. Thus, we hypothesized that the reduced ability of Basoph8×Tnffl/fl mice to clear bacteria may be associated with impaired host response to infection. We observed that, 24 h after CLP, Basoph8×Tnffl/fl mice displayed reduced peritoneal neutrophil ROS production and macrophage phagocytosis. Basoph8×Tnffl/fl mice also exhibited reduced concentrations of TNF in the peritoneal cavity and blood (but not...
In summary, we have demonstrated in mice that basophils are a significant source of the TNF produced in this setting as well.

Considering that basophils constitute < 1% of peripheral blood leukocytes, it was initially surprising to find that peritoneal levels of TNF were almost undetectable after CLP and blood levels of TNF were greatly diminished during LPS-induced endotoxemia in Basoph8 × Tnffl/fl mice. Because there is mounting evidence that basophils are essential initiators of certain inflammatory responses, for example, IgE-dependent chronic allergic inflammation, we considered the possibility that basophil-derived TNF has a major role in the first stages of the host defense against bacteria by priming macrophages and neutrophils to produce large amounts of TNF, that, in an autocrine and/or paracrine fashion, can influence important functions of these myeloid cells, for example, ROS production and phagocytosis. Indeed, we found that neutrophil and macrophage TNF production, and macrophage IL-6 production, were reduced in Basoph8 × Tnffl/fl mice after CLP.

It is unclear to us how basophil-derived TNF influences myeloid function during CLP, but it may do so indirectly by controlling the expression of third-party cytokines. In support of this hypothesis, we found that peritoneal macrophages from Basoph8 × Tnffl/fl mice exhibited a reduced ability to produce IL-6 during CLP. It has been shown that IL-6 administration can improve survival in mouse models of sepsis. Moreover, the mortality of IL-6-deficient mice is high following bacterial infection compared with that of wild-type mice. Accordingly, it is possible that basophil-derived TNF induces IL-6 production by macrophages that, in an autocrine manner, enhances macrophage activity against bacteria.

The contribution of basophils to innate immunity during bacterial infection and sepsis in humans is not yet clear. Humans with a total selective lack of basophils have not been observed. However, it has been suggested that alterations in basophil numbers and/or basophil-associated molecules are consistent with specific roles for basophils in the pathophysiology of human diseases, such as anaphylaxis.

In a dataset generated by the Inflammation and Host Response to Injury Program (Glue Grant)47,48, we found that 39 patients with severe traumatic injuries who participated in this study were evaluated for basophil counts at admission (no infection present) and at a time when the same patients developed a nosocomial infection. We found that the numbers of basophils were significantly increased in patients who acquired a nosocomial infection (median: 0.07 basophils × 10⁹ l⁻¹ of blood (range: 0–0.4) versus 0.04 basophils × 10⁹ l⁻¹ of blood (range: 0–0.11) at baseline, P < 0.001). This finding, as well as the inverse correlation of the whole blood expression of FcεRIα with potential negative outcomes of nosocomial infection at day 7 of hospitalization49, suggests that good outcomes in nosocomial infections may be correlated with a more robust a blood basophil response in humans.

In summary, we have demonstrated in mice that basophils are among the first leukocytes to migrate to the site of infection after induction of CLP and have provided several lines of evidence that basophils, at least in part by releasing TNF, can enhance the inflammatory response against bacteria and promote bacterial clearance via effects on other myeloid cells. These effects of basophils and basophil-derived TNF are not trivial, in that the basophil-TNF axis can significantly diminish the morbidity and mortality associated...
with this model of polymicrobial infection and sepsis. These findings show that basophils, despite their low numbers, can trigger a cascade of events that both helps to initiate an innate immune response against polymicrobial infection and enhances the effectiveness of this response. Together, these findings provide insights into how basophils, and basophil-derived TNF, might have key roles in the early stages following bacterial infection and in resisting the progression of such infection to bacteremia and sepsis.

Online content
Any methods, additional references, Nature Research Reporting Summary, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41590-018-0288-7.

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Author contributions
A.M.P., A.K.L., P.T., M.C., N.J.S., L.L.R., K.N., A.L.T., and K.M. performed the experiments. S.A.N. generated the \textit{Tnf}^{fl/fl} mice. A.M.P. and S.J.G. designed the study. A.M.P., S.A.N., H.K., L.L.R., M.T., K.M., and S.J.G. analyzed the data. A.M.P. and S.J.G. wrote the manuscript. All of the authors contributed to the final editing of the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
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Methods

Mice. C57BL/6 mice were purchased from The Jackson Laboratories. Mice with transgenic Basoph8-Cre expression\(^\text{\textsuperscript{1}}\) (Basoph8 mice) on the C57BL/6 background were kindly provided by R. Locksley (University of California, San Francisco). To track basophils in Basoph8 mice, a reporter gene used for the assay that contained humanized Cre recombinase, resulting in high YFP expression in basophils expressing the Mcp8 gene. Basoph8 mice were crossed with mice containing loxp-Banked Tnf alleles. Basoph8 mice were also crossed with Rosa-DTR mice purchased from The Jackson Laboratories to generate basophil-deficient mice at described previously\(^\text{\textsuperscript{2}}\). A combination of Basoph8\(^+\) and Basoph8\(^-\) mice were used, unless otherwise specified.

All mice were bred and maintained at the Seattle Children’s Research Institute animal facility. Unless specified otherwise, all of the experiments were performed using male mice that were 12 weeks old at the beginning of the experiment. All animal care and experimentation were conducted in accordance with the current National Institutes of Health guidelines and with the approval of the Institutional Animal Care and Use Committee of the Seattle Children’s Research Institute.

LPS-induced endotoxemia. For LPS-induced endotoxemia, mice were injected intraperitoneally with 10 μg of LPS (LPS from E. coli, Serotype O55:B5; catalog number ALX-581-013; Enzo Life Sciences) in sterile saline (pyrogen-free 0.9% NaCl); serum and peritoneal fluids were collected 1 h after challenge.

CLP. CLP was performed as described previously\(^\text{\textsuperscript{3}}\). Briefly, mice were deeply anesthetized with a 1–2 cm midline incision on the anterior abdomen; the distal half of the cecum was ligated (moderate CLP) and punctured once with a 22 G needle in the ligated segment. The cecum was returned to the anterior abdomen; the distal half of the cecum was ligated (moderate CLP) and punctured once with a 22 G needle in the ligated segment. The cecum was then placed back into the abdomen, 1 ml of sterile saline (pyrogen-free 0.9% NaCl) was administered into the peritoneal cavity, and the incision was closed with 9-mm steel wound clips. To assess mouse morbidity, mouse rectal temperatures were measured 24 h after CLP. For survival experiments, mice were observed for mortality at least four times daily for the first 3 d, and then twice a day for up to 7 d. Mice that were clearly moribund were killed by CO\(_2\) inhalation.

Adoptive transfer of basophils. Adoptive transfer of basophils was performed using a protocol described previously\(^\text{\textsuperscript{4}}\). Briefly, spleen cells were isolated from mice and the basophil-enriched CD49b\(^+\) fraction was prepared by using CD49b (Dxs) MicroBeads (catalog number 130-052-501; Miltenyi Biotec). Mice were injected intraperitoneally with the CD49b\(^+\) fraction (4×10\(^8\) cells) 3 h after CLP.

Cytometric analysis. Peritoneal cells were collected from mice 24 h after CLP. Basophils were then purified using the CD49b\(^+\) magnetic bead (Miltenyi Biotec) separation. Approximately 2×10\(^6\) CD49b\(^+\) peritoneal cells were then allowed to adhere to glass slides with cytofunnel filters and dried for at least 1 h. The cells were then fixed with methanol followed by incubation with 0.3% H\(_2\)O\(_2\) in methanol to inhibit endogenous peroxidase reactions. Then, the cells were blocked with 5% goat serum and 5% bovine serum albumin in PBS, treated with 5 μg/ml of purified rat IgG2a anti-mouse MCP-8 antibody (clone 2B8, catalog number 105825). From BioLegend: Alexa Fluor 700 anti-mouse monoclonal antibody (clone 44.1522, catalog number 51-08421); APC anti-mouse mAb conjugated to F4/80 (clone BM8, catalog number 123115); APC anti-mouse mAb conjugated to CD49b (pan-NK cells, clone DX5, catalog number 150099); PE anti-mouse mAb conjugated to Fc\(\alpha\)RI (clone MAR-1, catalog number 134507); and APC/CY7 anti-mouse mAb conjugated to c-Kit (clone 2B8, catalog number 105825). From Thermo Fisher Scientific: CD11b mAb eFluor 450 EBiocision (clone M1/70, catalog number 48-0112-82) and CD200 Receptor mAb PerCP-eFlour 710 (clone OX110, catalog number 46-5201-82), 4,6-Diamidino-2-phenylinoldi (DAPI) (catalog number D9542; Sigma-Aldrich) was used to detect dead cells. Only cells that were negative for DAPI were used for cell population analysis. To measure intracellular cytokine expression, cells from CLP-treated mice were maintained in media alone in the presence of GolgiPlug Protein Transport Inhibitor containing brefeldin A (catalog number BD Biosciences). Cells were maintained in vitro for 2 h, washed with PBS, and the cells were stained for neutrophil- and macrophage-specific surface markers. The stained cells were incubated with oposizened fluorescein isothiocyanate-labeled E. coli (strain K12) bioparticles (catalog number E2861; Thermo Fisher Scientific) for 10 min at 37 °C; flow cytometry analysis was performed immediately thereafter.

Flow cytometry. Single-cell suspensions were stained with a combination of the following antibodies. From BioLegend: Alexa Fluor 700 anti-mouse monoclonal antibody (clone 44.1522, catalog number 51-08421); APC anti-mouse mAb conjugated to F4/80 (clone BM8, catalog number 123115); APC anti-mouse mAb conjugated to CD49b (pan-NK cells, clone DX5, catalog number 150099); PE anti-mouse mAb conjugated to Fc\(\alpha\)RI (clone MAR-1, catalog number 134507); and APC/CY7 anti-mouse mAb conjugated to c-Kit (clone 2B8, catalog number 105825). From Thermo Fisher Scientific: CD11b mAb eFluor 450 EBiocision (clone M1/70, catalog number 48-0112-82) and CD200 Receptor mAb PerCP-eFlour 710 (clone OX110, catalog number 46-5201-82), 4,6-Diamidino-2-phenylinoldi (DAPI) (catalog number D9542; Sigma-Aldrich) was used to detect dead cells. Only cells that were negative for DAPI were used for cell population analysis. To measure intracellular cytokine expression, cells from CLP-treated mice were maintained in media alone in the presence of GolgiPlug Protein Transport Inhibitor containing brefeldin A (catalog number BD Biosciences). Cells were maintained in vitro for 2 h, washed with PBS, and then stained with either E. coli or heat-killed Listeria monocytogenes (HKLM, TLR2 agonist, catalog number tlr-hklin; InvivoGen) or LPS (TLR4 agonist, Sigma-Aldrich, L4005) for 6 h. Cell supernatants were then collected for cytokine measurements.

Cytokine release assays. For IgE-antigen-mediated activation, basophils and mast cells (1×10\(^6\) per 100 μl) were sensitized with IgE-mAb to DNP (H1-e-26)\(^\text{\textsuperscript{5}}\) (2 μg/ml\(^\text{-}\)1 overnight at 37 °C. The cells were then challenged with DNP-HSA \((10 \text{ng ml}^-1)\) for 18 h at 37 °C. For TLR-mediated activation, basophils were incubated with either synthetic lipopeptide 20:4 (Pam3CysLR, TLR1/2 agonist, catalog number B6C-t026; InvivoGen), heat-killed Listeria monocytogenes (HKLM, TLR2 agonist, catalog number tlr-hklin; InvivoGen) or LPS (TLR4 agonist, Sigma-Aldrich, L4005) for 6 h. Cell supernatants were then collected for cytokine measurements.

Cytokine and chemokine measurements. The cytokine measurements outlined in the figures were performed at different time intervals, which are indicated in the legends for the respective figures.

Concentrations of mouse TNF and IL-6 produced by bone-marrow-derived cultured CD49b\(^+\) cells were measured with the Essential TGF I/II/III, 2 Cyto6-6 Plex Mouse ProcartaPlex Panel (catalog number EPX060-20831-901; Thermo Fisher Scientific) with detection limits of 0.39 pg ml\(^{-}\)1 for TGF\(\beta\)1 and 0.21 pg ml\(^{-}\)1, respectively. Concentrations of TNF produced by basophils isolated from the bone marrow were measured with a high-sensitivity TNF enzyme-linked immunosorbent assay (ELISA) kit (catalog number BM5067HS; Thermo Fisher Scientific) with a detection limit of 0.75 pg ml\(^{-}\)1.

Concentrations of Tnf IL-6, CXCL1 (KC) and MIP-2 (for serum and intraperitoneal lavage fluids) were measured with ELISAs. The TNF and IL-6 ELISAs were obtained from BioLegend (Mouse TNF-α ELISA MAX Deluxe, catalog number 439094; Mouse IL-6 ELISA MAX Deluxe, catalog number 431304); and the CXCL1 (KC) and MIP-2 ELISAs were obtained from PeproTech (Murine KC (CXCL1) Standard AntiT3 ELISA Development Kit, catalog number 500-9127; Mouse MIP-2 CXCL2 STAND AntiT3 ELISA Development Kit, catalog number 500-9125). The detection limits for these assays were 4 pg ml\(^{-}\)1 for TNF, 2 pg ml\(^{-}\)1 for IL-6, 4 pg ml\(^{-}\)1 for CXCL1 (KC) and 16 pg ml\(^{-}\)1 for MIP-2.
qPCR. RNA (1 μg) was isolated from cells with the RNeasy Mini Kit (catalog number 74104; QiAGEN) and converted to first-strand complementary DNA (cDNA) with the iScript cDNA Synthesis Kit (catalog number 1708890; Bio-Rad Laboratories). cDNA was analyzed for gene expression with the aforementioned TaqMan probes and the Gapdh gene probes were used. Cells underwent reverse transcription (Mm00484933_m1) and following by 22 cycles of preamplification. The resulting amplified cDNA was then analyzed for gene expression with the Mann–Whitney U test. All other data were analyzed for statistical significance with the logrank (Mantel–Cox) test. Unless otherwise specified, all data are presented as the mean ± s.e.m.

**Data availability**
The authors declare that the main data supporting the findings of this study are available within the article and its supplementary figures. Additional data are available from the corresponding authors upon request.

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- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
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- Clearly defined error bars
- State explicitly what error bars represent (e.g. SD, SE, CI)

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No software was used.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No sample size calculation was performed. In vitro experiments were repeated a minimum of three independent times with three different batches of primary cells. Based on our many years of experience with the experimental models used in our study, we estimated the use of the following numbers of animals:
- For CLP: a minimum of 5 animals/time point for inflammation and morbidity assessment, and 15 animals for the 7d morbidity/mortality assessment time point.
- For LPS-induced endotoxemia: 5 mice/group were utilized to analyze the inflammatory process after injection of a sub-lethal dose of LPS. |

| Data exclusions | Data were excluded only if a value was determined as an outlier by using the Grubbs’ test. |

| Replication | Attempts at replication were successful once the conditions of the experiment were carefully optimized with pilot experiments. |

| Randomization | Samples or mice were allocated into experimental groups according to treatment (type of stimulation or in vivo model, respectively) and genotype for specific mouse work. |

| Blinding | Investigators were blinded to group allocation during sample processing and data collection, including histology assessments. |

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
|     | Unique biological materials |
|     | Antibodies |
|     | Eukaryotic cell lines |
|     | Palaeontology |
|     | Animals and other organisms |
|     | Human research participants |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
|     | ChIP-seq |
|     | Flow cytometry |
|     | MRI-based neuroimaging |

Antibodies

Antibodies used

The list of the antibodies used is as follows: Histology: HRP-conjugated goat anti–rat IgG (catalog number sc-2065, Santa Cruz Biotechnology, Dallas, TX), rat IgG2a anti-mouse Mcpt8 antibody (clone TUG8, catalog number 647401, BioLegend), rat isotype control antibody of irrelevant antigen specificity (clone RA3-6B2, catalog number 103201, BioLegend). FACS, surface staining: Alexa Fluor 700-conjugated mAb to Ly-6G/Ly-6C (clone RB6-8C5, catalog number 108421), allophycocyanin (APC)-conjugated mAb to FceRIa (clone MAR-1, catalog number 134307), and APC/Cy7-conjugated mAb to c-kit (clone 2B8, catalog number 105825), all from Biolegend, San Diego, CA; and eFluor 450-conjugated mAb to CD11b (clone M1/70, catalog number 48-0112-82), perCP-eFluor 710 (PE-Cy5.5)-conjugated mAb to CD200R (clone DX110, catalog number 46-5201-82), from eBioscience, San Diego, CA). FACS, intracellular staining: PE/Cy7-conjugated mAb to TNF (clone MP6-XT22, catalog number 5063232) and PE-conjugated mAb to IL-6 (clone MP5-20F3, catalog number 504503) from BioLegend.

Validation

We used well-validated commercial antibody reagents for histology and flow cytometry panels. All commercial antibodies have been authenticated by the commercial provider and we have datasheets showing the degree of non-specific binding as measured in cells that do not express the epitope.
Eukaryotic cell lines

Policy information about cell lines

- Cell line source(s): No eukariotic cell lines were used
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Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

- Laboratory animals: C57BL/6 mice were purchase from Jackson Laboratories. Mice with transgenic Basoph8-Cre expression on the C57BL/6 background were kindly provided by Dr. R. Locksley (UCSF). Basoph8-Cre mice were crossed with mice containing loxP-flanked Tnf alleles. Basophil-8-Cre mice were also crossed with Rosa-DTa mice purchased from Jackson Laboratories to generate basophil-deficient mice. All of the mice were bred and maintained in the Seattle Children's Research Institute animal facility. All of the experiments were performed using male mice that were 12 weeks old at the beginning of the experiment.
- Wild animals: Not applicable
- Field-collected samples: Not applicable

Flow Cytometry

Plots

- Confirm that:
  - The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
  - The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
  - All plots are contour plots with outliers or pseudocolor plots.
  - A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

- Sample preparation: Single peritoneal cell suspensions were stained with a combination of antibodies to assess the expression of cell surface markers. For intracellular cytokine expression measurements, peritoneal cells were maintained in media alone in the presence of GolgiPlug (catalog number BDB555029, FisherScientific) (1:1,000) at 37°C for 4h. The cells were surface-stained with a combination of the antibodies listed above, fixed and permeabilized using a commercially available kit (catalog number 554722, BD Biosciences), and then stained with antibodies against TNF or IL-6.
- Instrument: The cells were acquired on a BD LSR II.
- Software: The cells were acquired with the FACSDiva software and analyzed with the FlowJo software (version 8.8.7, Tree Star).

- Cell population abundance: Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.

Gating strategy

- Representative flow cytometry plots and frequencies used to identify basophils amongst peritoneal cells obtained from mice 24 h after induction of moderate CLP. Cell population considered for analysis using FSC and SSC. Doublets were removed using FSC-A and FSC-W gating. Dead cells were removed from the analysis using DAPI dead cell stain. FcERIα+ c-Kit- CD49b+ cells were gated and defined as basophils. The same gating strategy was used to identify basophils amongst peritoneal cells obtained from CLP-treated basophil deficient mice, which shows a significant reduction in the basophil percentage. Peritoneal mast cells from naive mice were used to establish gates for FcERIα+ and c-Kit- cells.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.