nature medicine

Interleukin-33 attenuates sepsis by enhancing neutrophil influx to the site of infection

Jose C Alves-Filho[1,2], Fabiane Sônego[2], Fabricio O Souto[2], Andressa Freitas[2], Waldiceu A Verri Jr[2], Maria Auxiliadora-Martins[3], Anibal Basile-Filho[3], Andrew N McKenzie[4], Damo Xu[1], Fernando Q Cunha[2] & Foo Y Liew[1]

Sepsis is a systemic inflammatory condition following bacterial infection with a high mortality rate and limited therapeutic options[1,2]. Here we show that interleukin-33 (IL-33) reduces mortality in mice with experimental sepsis from cecal ligation and puncture (CLP). IL-33–treated mice developed increased neutrophil influx into the peritoneal cavity and more efficient bacterial clearance than untreated mice. IL-33 reduced the systemic but not the local proinflammatory response, and it did not induce a T helper type 1 (T_H1) to T_H2 shift. The chemokine receptor CXCR2 is crucial for recruitment of neutrophils from the circulation to the site of infection[3]. Activation of Toll-like receptors (TLRs) in neutrophils downregulates CXCR2 expression and impairs neutrophil migration[4]. We show here that IL-33 prevents the downregulation of CXCR2 and inhibition of chemotaxis induced by the activation of TLR4 in mouse and human neutrophils. Furthermore, we show that IL-33 reverses the TLR4-induced reduction of CXCR2 expression in neutrophils via the inhibition of expression of G protein–coupled receptor kinase-2 (GRK2), a serine-threonine protein kinase that induces internalization of chemokine receptors[5,6]. Finally, we find that individuals who did not recover from sepsis had significantly more soluble ST2 (sST2, the decoy receptor of IL-33) than those who did recover. Together, our results indicate a previously undescribed mechanism of action of IL-33 and suggest a therapeutic potential of IL-33 in sepsis.

IL-33 is a recently identified member of the IL-1 family that binds the heterodimeric receptor complex consisting of ST2 (IL-1RL1) and IL-1 receptor accessory protein[7–9]. ST2 is expressed on T_H2 cells[10] and mast cells[11] and has a key role in T_H2 effector functions[12,13]. Moreover, ST2 can negatively regulate TLR activation via sequestration of the TLR signaling components myeloid differentiation factor-88 (MyD88) and Mal[14,15].

TLRs recognize the conserved components of pathogens and endogenous ligands released by injured tissue[16] and are crucial to host defense during infections. However, TLRs may also be detrimental in the pathophysiology of sepsis, as it has previously been shown that deficiency or blockade of TLRs is associated with an enhanced survival rate[4,17–20]. Thus, negative regulation of TLR signaling might be beneficial for treating sepsis, which has a worldwide yearly incidence estimated at 18 million cases, with a mortality rate of up to 30%[21].

Sepsis develops when the initial host response fails to contain the infection, resulting in widespread inflammation and multiple organ failure[1]. Strategies to treat human sepsis, mainly targeting proinflammatory mediators, have only had limited success[2]. We therefore investigated the role of IL-33 in experimental sepsis.

We performed CLP in BALB/c mice, the most relevant animal model for clinical sepsis[22]. We initially injected the mice intravenously (i.v.) twice with recombinant IL-33 (1 μg), 24 h and 1 h before CLP. IL-33 treatment potently protected mice from peritonitis signs (**Fig. 1a**). Moreover, IL-33–treated mice showed significantly reduced mortality compared to PBS-treated controls (**Fig. 1b**). IL-33 given as a single i.v. injection (1 μg) was protective 3 h but not 6 h after CLP (**Fig. 1c**). The protected mice did not show any sign of allergy or asthma (data not shown).

To demonstrate an endogenous role and specificity of IL-33, we performed CLP in *Il1rl1*$^{-/-}$ mice. *Il1rl1*$^{-/-}$ mice had higher mortality rate than that WT mice when tested in a milder form of CLP (**Fig. 1d**). The WT mice after CLP (CLP mice) produced substantial amounts of sST2 and IL-33, whereas the *Il1rl1*$^{-/-}$ CLP mice produced higher concentrations of IL-33 than the WT CLP mice (**Fig. 1e**), consistent with the notion that sST2 can neutralize IL-33. Moreover, mice with severe CLP produced markedly more sST2 than did mice with mild CLP (**Supplementary Fig. 1**). IL-33 reduced the mortality in WT mice but not *Il1rl1*$^{-/-}$ mice (**Fig. 1f**). Thus, IL-33 protects against polymicrobial sepsis via IL-33–ST2 signaling.

Successful clearance of bacterial infection depends on efficient neutrophil migration into the infectious site[23]. IL-33–treated CLP mice contained substantially more neutrophils in the peritoneum than the PBS-treated mice (**Fig. 1g**). Moreover, *Il1rl1*$^{-/-}$ CLP mice had fewer neutrophils in the peritoneum than the WT CLP mice, and the neutrophils from the *Il1rl1*$^{-/-}$ CLP mice showed less chemotaxis to CXCL2 than cells from the WT CLP mice (**Supplementary Fig. 2**).

[1]Division of Immunology, Infection and Inflammation, Glasgow Biomedical Research Centre, University of Glasgow, Glasgow, UK. [2]Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil. [3]Division of Intensive Care, Department of Surgery and Anatomy, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil. [4]Medical Research Council Laboratory of Molecular Medicine, Cambridge, UK. Correspondence should be addressed to F.Y.L. (f.y.liew@clinmed.gla.ac.uk) or F.Q.C. (fdqcunha@fmrp.usp.br).

Received 4 March; accepted 16 April; published online 16 May 2010; doi:10.1038/nm.2156

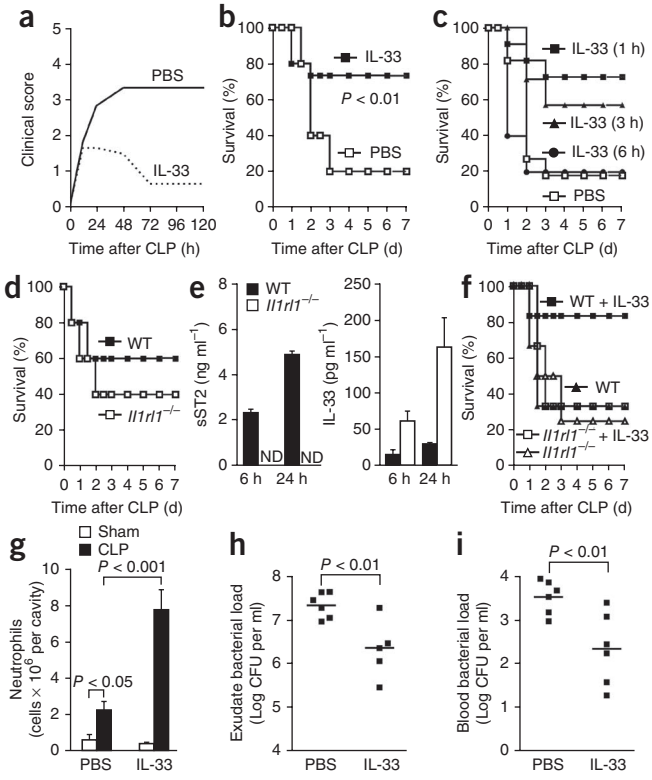

Figure 1 IL-33 attenuates sepsis and increases neutrophil influx to the site of infection and bacteria clearance. (**a**,**b**) Clinical signs (**a**) and mortality (**b**) after IL-33 (1 μg per mouse per injection) was injected i.v. 24 and 1 h before CLP on naive BALB/c mice. Data are pooled from three experiments, $n = 6$ mice per group per experiment. (**c**) Time course of IL-33 treatment. IL-33 (1 μg) was injected i.v. as a single dose 1, 3 or 6 h after CLP in BALB/c mice. (**d**) Survival rate of untreated *Il1rl1*$^{-/-}$ and WT mice given a milder form of CLP. (**e**) sST2 and IL-33 concentration in the peritoneal lavage fluid of WT and *Il1rl1*$^{-/-}$ CLP mice, as determined by ELISA. Similar results were obtained in the serum of the CLP mice (data not shown). (**f**) Survival rate of *Il1rl1*$^{-/-}$ and WT CLP mice treated with IL-33 as in **a**. (**g**) Number of neutrophils in the peritoneum of CLP or sham-operated mice treated with IL-33 or PBS. (**h**,**i**) Bacterial loads in the peritoneum (**h**) and in the blood (**i**) of CLP mice treated with IL-33 or PBS. Data are means ± s.e.m., $n = 5$–10 mice per group and are representative of three experiments (**h**,**i**). *$P < 0.05$.

IL-33 treatment also improved bacterial clearance in the peritoneum (**Fig. 1h**) and in the blood (**Fig. 1i**). Thus, IL-33–mediated protection is associated with augmented neutrophil recruitment and enhanced bacteria clearance.

An increase in systemic proinflammatory cytokines and chemokines (such as IL-6, tumor necrosis factor-α (TNF-α) and CXCL2) and neutrophil sequestration in the lungs are biomarkers and causative agents of poor prognosis in sepsis[24,25]. CLP mice had elevated serum concentrations of TNF-α, IL-6 and CXCL2 (**Fig. 2a**). IL-33 treatment lowered the concentrations of these mediators in the blood (**Fig. 2a**). In contrast, there was no difference in the amounts of TNF-α, IL-6 and CXCL2 in the peritoneal exudates between PBS-treated and IL-33–treated mice after CLP (**Fig. 2b**). IL-33–treated mice had substantially lower myeloperoxidase, IL-6 and CXCL2 abundance in the lungs (**Fig. 2c**). There was no difference in the amounts of IL-4 and IL-13 between the two groups but the IL-33–treated mice had less IL-10 than the untreated mice (**Supplementary Fig. 3**). Thus, the protective effect of IL-33 is associated with a reduction of systemic inflammation but not a T_H1 to T_H2 shift.

CXCR2 has a central role in the recruitment of neutrophils[3]. Downregulation of CXCR2 expression on circulating neutrophils is associated with impaired neutrophil migration into infectious sites during sepsis[26,27]. CLP lowered CXCR2 expression on neutrophils (**Fig. 2d**), and the reduction correlated with a lower chemotactic response to CXCL2 (**Fig. 2e**), and was reversed by treatment with IL-33 (**Fig. 2d**,**e**). Neutrophils from *Il1rl1*$^{-/-}$ CLP mice expressed

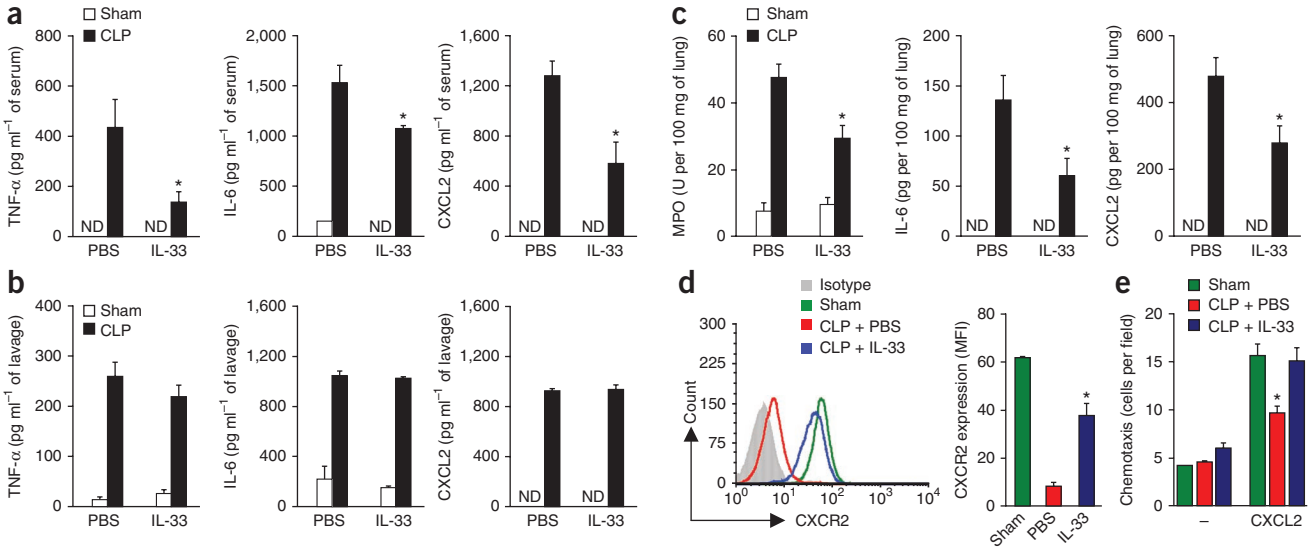

Figure 2 IL-33 treatment reduces systemic proinflammatory cytokine, chemokine and lung myeloperoixdase (MPO) activity but increases CXCR2 expression on, and chemotaxis of, neutrophils. Naive BALB/c mice were treated with IL-33 as in **Figure 1a** and then given CLP or sham operated. Experiments were terminated 6 h after CLP, and blood and lungs were collected for analysis. (**a**,**b**) Serum (**a**) and peritoneal lavage (**b**) TNF-α, IL-6 and CXCL2 concentrations, as determined by ELISA. (**c**) Lung MPO, IL-6 and CXCL2 amounts, as determined by MPO assay and ELISA. ND, not detected. (**d**,**e**) Blood neutrophils were analyzed 4 h after CLP for cell surface CXCR2 expression by FACS (**d**) and chemotaxis toward CXCL2 (**e**). Data are means ± s.e.m., $n = 5$ mice per group, and are representative of three experiments. *$P < 0.05$ compared to PBS-treated group.

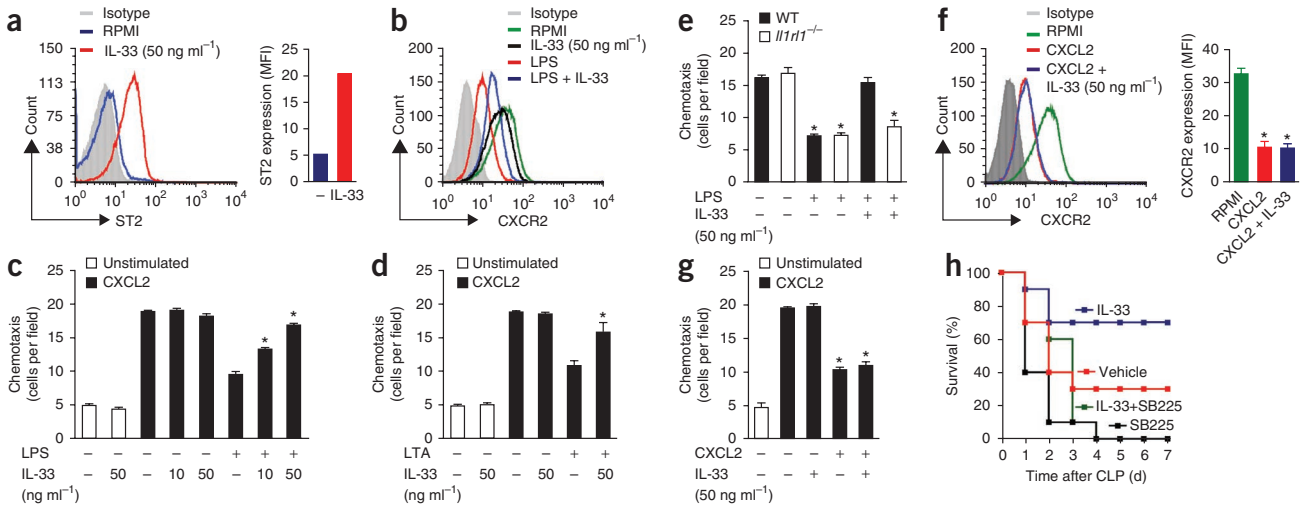

Figure 3 IL-33 blocks the downregulation of CXCR2 and chemotaxis mediated by LPS *in vitro*. (**a**) FACS analysis of naive BALB/c bone marrow neutrophils cultured with IL-33 or medium (RPMI) overnight and stained for ST2. (**b**–**g**) Neutrophils were purified from the bone marrow of naive mice and cultured for 1 h with LPS (1 μg ml^{-1}), LTA (1 μg ml^{-1}), IL-33 (10–50 ng ml^{-1}), CXCL2 (30 ng ml^{-1}) or a combination of these reagents, as indicated. The expression of CXCR2 (**b**,**f**) and chemotaxis to CXCL2 (**c**,**d**,**e**,**g**) were determined. In some experiments (**g**), neutrophils were pretreated with CXCL2 or IL-33 before assaying for chemotaxis toward CXCL2. Data are means ± s.e.m., $n = 5$ replicates per group. *$P < 0.05$ versus cultures without IL-33 (**c**,**d**), untreated neutrophils (**e**), or cells not pretreated with CXCL2 (**g**). (**h**) Survival of CLP mice treated with IL-33 with or without the CXCR2 inhibitor SB225002 (10 mg per kg body weight). Data are representative of two experiments, $n = 10$ mice per group.

less CXCR2 than those from WT CLP mice (**Supplementary Fig. 4**). Therefore, IL-33 may have increased neutrophil migration and thus enhanced bacterial clearance by preventing the downregulation of CXCR2 on circulating neutrophils.

Direct activation of TLRs in neutrophils downregulates the expression of CXCR2 and impairs cellular migration[4]. Resting neutrophils expressed low levels of ST2 mRNA[28]. ST2 expression was markedly upregulated in IL-33–treated neutrophils (**Fig. 3a**). Lipopolysaccharide (LPS) treatment lowered CXCR2 expression on neutrophils and decreased their chemotaxis to CXCL2 (**Fig. 3b**,**c**). IL-33 blocked the LPS-induced downregulation of CXCR2 expression and chemotaxis (**Fig. 3b**,**c**). Treatment with IL-4, IL-5 or IL-13 failed to reverse the LPS-mediated inhibition of neutrophil chemotaxis (**Supplementary Fig. 5**). Thus, IL-33 seems to act directly on neutrophils and not via T_H2 cytokines, which IL-33 might have induced. We confirmed the requirement for TLR4 in the ability of LPS to reduce neutrophil chemotaxis by using neutrophils from *Tlr4*$^{-/-}$ and *Myd88*$^{-/-}$ mice, which were not affected by LPS activation (**Supplementary Fig. 6**). Moreover, neutrophils from *Tlr4*$^{-/-}$ and *Myd88*$^{-/-}$ mice responded to the CXCR2 ligand CXCL2 similarly to WT cells not receiving LPS (**Supplementary Fig. 6**). IL-33 also reversed the inhibition of CXCR2 expression induced by lipoteichoic acid (LTA) (a TLR2 ligand) (**Fig. 3d**). IL-33 failed to prevent LPS-induced inhibition of neutrophil migration in cells from *Il1rl1*$^{-/-}$ mice (**Fig. 3e**). Moreover, IL-33 had no effect on the CXCL2-induced downregulation of CXCR2 (**Fig. 3f**) or chemotaxis (**Fig. 3g**), indicating that the effect of IL-33 was specific for TLR-mediated CXCR2 reduction and did not prevent CXCL2-mediated downregulation of CXCR2 (ref. 29). Notably, the CXCR2 antagonist SB225002 completely abrogated the protective effect of IL-33 in CLP and also increased the mortality of untreated CLP mice (**Fig. 3h**). These results therefore indicate that IL-33 prevents the downregulation of the chemokine receptor CXCR2 by interfering with TLR signaling.

Nitric oxide produced by inducible nitric oxide synthase (iNOS) can downregulate CXCR2 expression[27]. However, it was previously shown that IL-33 had no effect on iNOS expression[30]. GRK2 has a key role in the phosphorylation and downregulation of chemokine receptors[5]. Moreover, the amounts of GRK2 were increased in neutrophils from individuals with sepsis and were associated with a reduction of chemotaxis toward IL-8 (ref. 31). In addition, GRK2 is induced by TLR ligands[4,32] and downregulates CXCR2 expression on TLR2-activated neutrophils[4]. We therefore examined the effect of IL-33 on the expression of GRK2 in neutrophils. LPS-activated neutrophils expressed high amounts of GRK2, a phenotype that was completely inhibited by treatment with IL-33 (**Fig. 4a**). Furthermore, neutrophils from CLP mice expressed high amounts of GRK2 which were also completely suppressed by treatment with IL-33 (**Fig. 4b**). Moreover, neutrophils from *Il1rl1*$^{-/-}$ CLP mice expressed more GRK2 than did cells from WT CLP mice (**Supplementary Fig. 7**). Of note, the inhibition of neutrophil chemotaxis by LPS or LTA could be reversed by a GRK2 inhibitor (methyl 5-[2-(5-nitro-2-furyl)vinyl]-2-furoate) (**Fig. 4c**). Thus, treatment with IL-33 can reverse the CLP-induced reduction of CXCR2 via the inhibition of GRK2.

Treatment with LPS also inhibited human neutrophil chemotaxis toward CXCL8, and this was reversed by treatment with IL-33 (**Fig. 4d**). We further analyzed the peripheral blood neutrophils and serum of 23 humans with sepsis and 12 healthy donors. Neutrophils from individuals who did not survive sepsis had less cell surface CXCR2 expression and chemotaxis compared to survivors and healthy donors (**Fig. 4e**). Whereas healthy donors contained no detectable sST2 and barely detectable IL-33 in their serum, about 50% of subjects with sepsis contained IL-33 (**Fig. 4f**). Notably, those who did not survive had significantly more serum sST2 than did the survivors (**Fig. 4f**). Thus, IL-33 may also play a major part in clinical sepsis by modulating the expression of CXCR2 and chemotaxis of neutrophils.

IL-33 activates T_H2 cells[7], eosinophils[33], basophils[28] and mast cells[34,35]. Here we report that IL-33 activates neutrophils, preventing polymicrobial-mediated sepsis. During acute sepsis, pathogen products such as LPS can trigger the expression of GRK2, which then

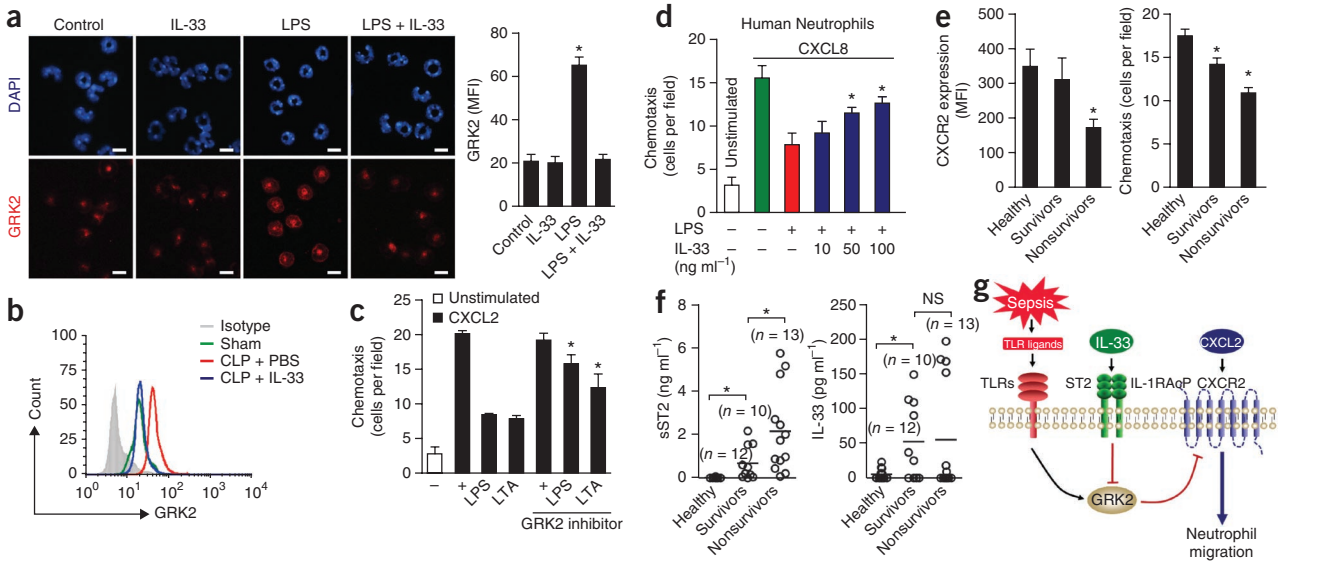

Figure 4 IL-33 blocks the induction of GRK2 by LPS. (**a**) Immunofluorescence staining of GRK2 expression (red) in fixed neutrophils purified from the bone marrow of naive BALB/c mice and cultured for 1 h with LPS (1 μg ml^{-1}), IL-33 (50 ng ml^{-1}) or a combination of LPS and IL-33. Nuclei were counterstained with DAPI (blue). Data show representative individual slide staining of three experiments. Results are shown as the means ± s.d. of the mean fluorescence intensity of each field subtracted from the mean intensity of the area measured as background for each. *$P < 0.05$ compared to all other groups. Scale bar, 10 μm. (**b**) Flow cytometric analysis of GRK2 expression in neutrophils from sham-operated or CLP mice treated with IL-33 or PBS 4 h after CLP ($n = 5$ mice per group). (**c**) Neutrophil chemotaxis (toward CXCL2) in the presence of LPS or LTA in the presence or absence of a GRK2 inhibitor (150 μM). $n = 5$ replicates per group, *$P < 0.05$ versus respective cultures without inhibitor. (**d**) Chemotaxis (toward CXCL8) of purified peripheral blood neutrophils from healthy donors in the presence of LPS and graded concentrations of IL-33. $n = 4$ donors, *$P < 0.05$ versus cultures without IL-33. For **c**–**e**, data are means ± s.e.m. (**e**) CXCR2 expression of peripheral blood neutrophils from individuals with sepsis and healthy donors, as analyzed by FACS. The cells were also examined for chemotaxis (toward CXCL8), $n = 5$–10 donors per group, *$P < 0.05$ compared to healthy donors. (**f**) sST2 and IL-33 concentrations in sera collected from individuals with sepsis and healthy donors, as analyzed by ELISA. (**g**) Schematic representation of the mechanism by which IL-33 facilitates neutrophil migration during sepsis.

suppresses the expression of CXCR2 that can otherwise facilitate the migration of neutrophils to the site of infection for the clearance of the pathogens. IL-33 can prevent the induction of GRK2 mediated by TLR signaling and maintain the expression of CXCR2, thus empowering neutrophils to migrate to the site of infection for bacterial clearance (**Fig. 4g**). By doing so, IL-33 also prevents the sequestration of pulmonary neutrophils, a hallmark of severe sepsis[25]. Treatment with IL-33 lowered the concentration of proinflammatory cytokines and chemokines in the blood. Whether this is due to a direct effect of IL-33 or is a consequence of reduced bacterial load is currently unclear.

The effect of IL-33 on mouse neutrophils can be extended to human neutrophils. Individuals who did not recover from sepsis had higher serum concentrations of sST2, and their neutrophils had markedly reduced CXCR2 expression and chemotaxis. Furthermore, in mice, the beneficial effect of IL-33 on sepsis was effective up to 3 h after CLP, demonstrating a therapeutic potential of IL-33 in clinical sepsis. Our finding therefore not only reveals a previously unknown mechanism of action of IL-33 but also suggests a potential new treatment against sepsis.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturemedicine/.

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

ACKNOWLEDGMENTS

This work was supported by the Wellcome Trust, the UK Medical Research Council, the UK and the EU (to F.Y.L.) and the State of São Paulo Research Foundation and National Research Council, Brazil (to F.Q.C.). *Tlr4*$^{-/-}$ and *Myd88*$^{-/-}$ mice were from S. Akira (University of Osaka).

AUTHORS CONTRIBUTIONS

J.C.A.-F., F.Q.C. and F.Y.L. designed the study. J.C.A.-F., F.S. and F.O.S. performed the experiments. A.F. helped with *in vivo* experiments. M.A.-M. and A.B.-F. contributed the clinical data and specimens. D.X., W.A.V. Jr. and A.N.M. contributed key reagents. F.Y.L. and J.C.A.-F. wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Published online at http://www.nature.com/naturemedicine/.
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions/.

1. Hotchkiss, R.S. & Karl, I.E. The pathophysiology and treatment of sepsis. *N. Engl. J. Med.* **348**, 138–150 (2003).
2. Riedemann, N.C., Guo, R.F. & Ward, P.A. Novel strategies for the treatment of sepsis. *Nat. Med.* **9**, 517–524 (2003).
3. Olson, T.S. & Ley, K. Chemokines and chemokine receptors in leukocyte trafficking. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **283**, R7–R28 (2002).
4. Alves-Filho, J.C. *et al.* Regulation of chemokine receptor by Toll-like receptor 2 is critical to neutrophil migration and resistance to polymicrobial sepsis. *Proc. Natl. Acad. Sci. USA* **106**, 4018–4023 (2009).
5. Vroon, A., Heijnen, C.J. & Kavelaars, A. GRKs and arrestins: regulators of migration and inflammation. *J. Leukoc. Biol.* **80**, 1214–1221 (2006).
6. Penela, P., Ribas, C. & Mayor, F. Jr. Mechanisms of regulation of the expression and function of G protein–coupled receptor kinases. *Cell. Signal.* **15**, 973–981 (2003).
7. Schmitz, J. *et al.* IL-33, an interleukin-1–like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2–associated cytokines. *Immunity* **23**, 479–490 (2005).
8. Chackerian, A.A. *et al.* IL-1 receptor accessory protein and ST2 comprise the IL-33 receptor complex. *J. Immunol.* **179**, 2551–2555 (2007).
9. Ali, S. *et al.* IL-1 receptor accessory protein is essential for IL-33–induced activation of T lymphocytes and mast cells. *Proc. Natl. Acad. Sci. USA* **104**, 18660–18665 (2007).
10. Xu, D. *et al.* Selective expression of a stable cell surface molecule on type 2 but not type 1 helper T cells. *J. Exp. Med.* **187**, 787–794 (1998).

11. Moritz, D.R., Rodewald, H.R., Gheyselinck, J. & Klemenz, R. The IL-1 receptor-related T1 antigen is expressed on immature and mature mast cells and on fetal blood mast cell progenitors. *J. Immunol.* **161**, 4866–4874 (1998).
12. Coyle, A.J. *et al.* Crucial role of the interleukin 1 receptor family member T1/ST2 in T helper cell type 2–mediated lung mucosal immune responses. *J. Exp. Med.* **190**, 895–902 (1999).
13. Löhning, M. *et al.* T1/ST2 is preferentially expressed on murine T_H2 cells, independent of interleukin 4, interleukin 5 and interleukin 10, and important for T_H2 effector function. *Proc. Natl. Acad. Sci. USA* **95**, 6930–6935 (1998).
14. Liew, F.Y., Xu, D., Brint, E.K. & O'Neill, L.A. Negative regulation of Toll-like receptor–mediated immune responses. *Nat. Rev. Immunol.* **5**, 446–458 (2005).
15. Brint, E.K. *et al.* ST2 is an inhibitor of interleukin 1 receptor and Toll-like receptor 4 signaling and maintains endotoxin tolerance. *Nat. Immunol.* **5**, 373–379 (2004).
16. Akira, S. & Takeda, K. Toll-like receptor signalling. *Nat. Rev. Immunol.* **4**, 499–511 (2004).
17. Plitas, G., Burt, B.M., Nguyen, H.M., Bamboat, Z.M. & DeMatteo, R.P. Toll-like receptor 9 inhibition reduces mortality in polymicrobial sepsis. *J. Exp. Med.* **205**, 1277–1283 (2008).
18. Alves-Filho, J.C., de Freitas, A., Russo, M. & Cunha, F.Q. Toll-like receptor 4 signaling leads to neutrophil migration impairment in polymicrobial sepsis. *Crit. Care Med.* **34**, 461–470 (2006).
19. Roger, T. *et al.* Protection from lethal Gram-negative bacterial sepsis by targeting Toll-like receptor 4. *Proc. Natl. Acad. Sci. USA* **106**, 2348–2352 (2009).
20. Spiller, S. *et al.* TLR4-induced IFN-γ production increases TLR2 sensitivity and drives Gram-negative sepsis in mice. *J. Exp. Med.* **205**, 1747–1754 (2008).
21. Angus, D.C. *et al.* Epidemiology of severe sepsis in the United States: analysis of incidence, outcome and associated costs of care. *Crit. Care Med.* **29**, 1303–1310 (2001).
22. Hubbard, W.J. *et al.* Cecal ligation and puncture. *Shock* **24** Suppl 1, 52–57 (2005).
23. Nathan, C. Neutrophils and immunity: challenges and opportunities. *Nat. Rev. Immunol.* **6**, 173–182 (2006).
24. Cavaillon, J.M., Adib-Conquy, M., Fitting, C., Adrie, C. & Payen, D. Cytokine cascade in sepsis. *Scand. J. Infect. Dis.* **35**, 535–544 (2003).
25. Brown, K.A. *et al.* Neutrophils in development of multiple organ failure in sepsis. *Lancet* **368**, 157–169 (2006).
26. Cummings, C.J. *et al.* Expression and function of the chemokine receptors CXCR1 and CXCR2 in sepsis. *J. Immunol.* **162**, 2341–2346 (1999).
27. Rios-Santos, F. *et al.* Down-regulation of CXCR2 on neutrophils in severe sepsis is mediated by inducible nitric oxide synthase–derived nitric oxide. *Am. J. Respir. Crit. Care Med.* **175**, 490–497 (2007).
28. Suzukawa, M. *et al.* An IL-1 cytokine member, IL-33, induces human basophil activation via its ST2 receptor. *J. Immunol.* **181**, 5981–5989 (2008).
29. Mueller, S.G., White, J.R., Schraw, W.P., Lam, V. & Richmond, A. Ligand-induced desensitization of the human CXC chemokine receptor-2 is modulated by multiple serine residues in the carboxyl-terminal domain of the receptor. *J. Biol. Chem.* **272**, 8207–8214 (1997).
30. Kurowska-Stolarska, M. *et al.* IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. *J. Immunol.* **183**, 6469–6477 (2009).
31. Arraes, S.M. *et al.* Impaired neutrophil chemotaxis in sepsis associates with GRK expression and inhibition of actin assembly and tyrosine phosphorylation. *Blood* **108**, 2906–2913 (2006).
32. Loniewski, K., Shi, Y., Pestka, J. & Parameswaran, N. Toll-like receptors differentially regulate GPCR kinases and arrestins in primary macrophages. *Mol. Immunol.* **45**, 2312–2322 (2008).
33. Cherry, W.B., Yoon, J., Bartemes, K.R., Iijima, K. & Kita, H. A novel IL-1 family cytokine, IL-33, potently activates human eosinophils. *J. Allergy Clin. Immunol.* **121**, 1484–1490 (2008).
34. Xu, D. *et al.* IL-33 exacerbates antigen-induced arthritis by activating mast cells. *Proc. Natl. Acad. Sci. USA* **105**, 10913–10918 (2008).
35. Pushparaj, P.N. *et al.* The cytokine interleukin-33 mediates anaphylactic shock. *Proc. Natl. Acad. Sci. USA* **106**, 9773–9778 (2009).

ONLINE METHODS

Chemicals and reagents. We used GRK2 inhibitor (methyl 5-[2-(5-nitro-2-furyl)vinyl]-2-furoate, Calbiochem), CXCR2 antagonist (SB225002, Tocris Bioscience), LPS (from *Escherichia coli* 0111:B4, Sigma) and LTA (from *Staphylococcus aureus*, Sigma). We produced recombinant mouse and human IL-33 as previously described[36] or obtained them from Biolegend, with which we achieved similar results.

Mice. We housed the *Il1rl1*$^{-/-}$ mice on a BALB/c background[37] and *Tlr4*$^{-/-}$ and *Myd88*$^{-/-}$ mice on a C57BL/6 background (from S. Akira) in the School of Medicine of Ribeirão Preto, University of São Paulo. We used the mice at 6–10 week of age. We performed all experiments according to the guidelines of the Animal Welfare Committee of the School of Medicine of Ribeirão Preto, University of São Paulo.

Cecal ligation and puncture. We performed CLP as described previously[18]. Briefly, we anesthetized the mice and shaved the abdominal wall. After midline laparotomy, we exposed the cecum, ligated below the ileocecal valve without causing intestinal obstruction and then punctured twice with a 21G needle. In some experiments, we used a 30G needle to induce a mild CLP. We assessed clinical score every 12 h after CLP, as previously described[38]. The symptoms of the maximum score of six are lethargy, piloerection, tremors, periorbital exudates, respiratory distress and diarrhea. Each condition was scored as 1. Mice with a clinical score of >1 were defined as showing signs of sepsis. We determined survival rate daily for 7 d after CLP.

Neutrophil influx. We performed the neutrophil influx experiment as described previously[18]. Briefly, we collected peritoneal lavage fluids 6 h after CLP. Total cell counts were acquired with the Coulter AcT Diff analyzer (Beckman Coulter), and differential cell counts were carried out on Cytospin slides stained with May-Grünwald-Giemsa.

Patients. We enrolled patients (n = 23) admitted for sepsis in the Intensive Care Unit (Department of Surgery and Anatomy, School of Medicine, Ribeirão Preto, University of São Paulo) and compared them with healthy male and female donors. All subjects enrolled in this study fulfilled the criteria defined by the 2001 International Sepsis Definitions Conference[39]. Subjects were excluded if they were older than 75 years or younger than 15 years, if the mean arterial pressure was < 50 mm Hg, if they had bradycardia (heart rate <50 b.p.m.) or tachycardia (heart rate >125 b.p.m.), if they were being treated with high doses of vasopressor agents; if they had oliguria (urine output <50 ml h^{-1}), if they had irreversible circulatory shock and when informed consent could not be obtained. The study was approved by the Human Subjects Institutional Committee of the School of Medicine of Ribeirão Preto, University of São Paulo.

Bacterial counts. We determined bacterial counts as described previously[18]. Briefly, we collected peritoneal lavage fluids and blood 6 h after CLP. We serially diluted the samples, plated them on Mueller-Hinton agar dishes (Difco Laboratories) and incubated the dishes for 24 h at 37 °C. Bacterial counts were determined by counting colony-forming units.

Cytokine, chemokine and myeloperoxidase determination. We assessed cytokine and chemokine concentrations by ELISA using antibody pairs or kits from R&D Systems according to the manufacturer's instructions. Myeloperoxidase activity in lung homogenates (a quantitative measurement of neutrophil sequestration) was assayed as previously described[18].

Neutrophil isolation and chemotaxis assay. We isolated mouse neutrophils from peripheral blood or bone marrow and human neutrophils from peripheral blood by Percoll density gradient, as previously described[4,27]. We assayed chemotaxis in response to CXCL2 or CXCL8 (30 ng ml^{-1}) (R&D Systems) in a 48-well chemotaxis plate (Neuroprobe), as previously described[4].

Flow Cytometry Analysis. We performed surface staining with antibodies to CXCR2 (242216, R&D Systems), ST2L (DJ8, MD Biosciences) and Gr-1 (RB6-8C5, BD Biosciences). We performed intracellular staining with primary rabbit antibody to GRK2 (Y137, Abcam) and secondary FITC-conjugated goat antibody to rabbit IgG (Abcam) according to the manufacturer's protocol. Cells were analyzed by FACSCanto cytometer (BD Biosciences).

Immunofluorescence staining. Cytospin slides were incubated with primary rabbit antibody to GRK2 or isotype control (Santa Cruz Biotechnology) and stained with secondary Alexa-Fluor 594–conjugated goat antibody to rabbit IgG (Invitrogen) and DAPI (Invitrogen). We captured images with an epifluorescence BX-50 microscope (Olympus) equipped with a CoolSnap camera (PhotoMetrics) and analyzed them by Image-Pro Plus 4.0 (Media Cybernetics). The mean fluorescence intensity was determined from a linear measurement of individual cells' fluorescence. All cells of at least five randomly chosen fields of each slide, performed in duplicate, were analyzed from at least two individual experiments.

Statistical analyses. We used Student's t test to compare the differences between groups. Survival studies were analyzed with the log-rank test, and bacterial counts were analyzed by the Mann-Whitney U test. $P < 0.05$ was considered statistically significant.

36. Komai-Koma, M. *et al.* IL-33 is a chemoattractant for human T_H2 cells. *Eur. J. Immunol.* **37**, 2779–2786 (2007).
37. Townsend, M.J., Fallon, P.G., Matthews, D.J., Jolin, H.E. & McKenzie, A.N. T1/ST2-deficient mice demonstrate the importance of T1/ST2 in developing primary T helper cell type 2 responses. *J. Exp. Med.* **191**, 1069–1076 (2000).
38. Manley, M.O., O'Riordan, M.A., Levine, A.D. & Latifi, S.Q. Interleukin 10 extends the effectiveness of standard therapy during late sepsis with serum interleukin 6 levels predicting outcome. *Shock* **23**, 521–526 (2005).
39. Levy, M.M. *et al.* 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit. Care Med.* **31**, 1250–1256 (2003).