Opposing roles of LTB₄ and PGE₂ in regulating the inflammasome-dependent scorpion venom-induced mortality

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Tityus serrulatus sting causes thousands of deaths annually worldwide. T. serrulatus-envenomed victims exhibit local or systemic reaction that culminates in pulmonary oedema, potentially leading to death. However, the molecular mechanisms underlying T. serrulatus venom (TsV) activity remain unknown. Here we show that TsV triggers NLRP3 inflammasome activation via K⁺ efflux. Mechanistically, TsV triggers lung-resident cells to release PGE₂, which induces IL-1β production via E prostanoid receptor 2/4-cAMP-PKA-NFκB-dependent mechanisms. IL-1β/IL-1R actions account for oedema and neutrophil recruitment to the lungs, leading to TsV-induced mortality. Inflammasome activation triggers LTB₄ production and further PGE₂ via IL-1β/IL-1R signalling. Activation of LTB₄-BLT1/2 pathway decreases cAMP generation, controlling TsV-induced inflammation. Exogenous administration confirms LTB₄ anti-inflammatory activity and abrogates TsV-induced mortality. These results suggest that the balance between LTB₄ and PGE₂ determines the amount of IL-1β inflammasome-dependent release and the outcome of envenomation. We suggest COX1/2 inhibition as an effective therapeutic intervention for scorpion envenomation.

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Clinical manifestations of severe scorpion envenomation are local pain and multi-organ failure, including cardiogenic shock and pulmonary oedema\(^1\). Scorpion antiserum is the only available treatment, but it is not always effective and might induce adverse effects\(^1\). To develop a specific and effective treatment, it is necessary to understand the pathogenesis of the reaction induced by a scorpion sting.

Human envenomation by *Tityus serrulatus* venom (TsV) induces a massive and fast release of cytokines such as IL-1\(\beta\), IL-6, IL-8, IL-10 and TNF-\(\alpha\) factor\(^2\). Animal models of envenomation have been instrumental for the characterization of the inflammatory response and cell activation induced by TsV (refs 5,7,8). TsV or venom-associated molecular patterns (VAMPs) act in a way analogous to pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns\(^9,10\) and are also recognized by pattern recognition receptors (PRRs) in leukocytes\(^11\). The recognition of TsV by PRRs induces NFkB and AP-1 or PPAR-\(\gamma\) activation, leading to the production of proinflammatory cytokines and eicosanoids\(^11,12\).

Inflammasome activation induces the production of lipid mediators\(^13\) and contributes to IL-1\(\beta\) secretion induced by bee venom\(^14,15\). Lipid mediators such as PGs and LTs are arachidonate (AA)-derived eicosanoids produced by cyclooxygenases 1/2 (COX1/2) and 5-lipoxygenase (5-LO), respectively, and are produced in abundance by resident and inflammatory cells\(^16\). PGs have important functions in innate and adaptive immune responses through the binding to one of its four different G-protein-coupled E-prostanoid (EP) receptors, known as EP-1-4 (ref. 17). The EP2 and EP4 are Gz-coupled receptors that activate adenylate cyclase and CAMP production, while EP1 receptor increases intracellular calcium, and EP3 is a Gz-coupled receptor that decreases CAMP formation\(^18,19\). PGE\(_2\) is a potent vasodilator and vascular permeability factor\(^20,21\) and induces gene expression of the inflammatory and edematogenic cytokine IL-1\(\beta\) via CAMP-PKA signalling pathway\(^22\). On the other hand, LTB\(_4\) does not induce oedema formation\(^23\), however, via GPCRs, BLT1 (high-affinity receptor) rather than BLT2 (low-affinity receptor)\(^24\) induces recruitment and activation of leukocytes\(^25\) and increases phagocytosis and killing of microorganisms by macrophages\(^26\). Interestingly, low concentrations of LTB\(_4\), but not LTD\(_4\), reduce intracellular cAMP production in macrophages\(^26,27\).

We found that intraperitoneal inoculation of mice with TsV induces the activation of the NLRP3 inflammasome, resulting in the production of IL-1\(\beta\), LTB\(_4\), and PGE\(_2\), lung oedema and neutrophil recruitment and animal mortality. In summary, we identified that, in scorpion envenomation, a complex and intricate CAMP-mediated mechanism is responsible for lung oedema and mortality and unveiled a heretofore unknown cross-talk between IL-1\(\beta\) and eicosanoids, where the balance between 5-LO and COX1/2 products appears to determine the severity of envenomation.

**Results**

**TsV induces the activation of the inflammasome.** Although bee venom activates the inflammasome to produce mature IL-1\(\beta\) (refs 14,15), whether scorpion venom activates inflammasome remains unknown. We initially tested whether TsV induces inflammasome activation and IL-1\(\beta\) release. Bone marrow-derived macrophages (BMDMs) from C57BL/6 wild-type (WT) mice were either pre-treated or not with lipopolysaccharide (LPS) and incubated with a TsV concentration capable of activating macrophages *in vitro*\(^1,12\). TsV alone induces the significant production of IL-1\(\beta\) as early as 1 h after stimulation, demonstrating that the venom, per se, delivery signals are necessary for the activation of the inflammasome in BMDMs (Fig. 1a). Next, we determined which component of the NLRP3 inflammasome accounts for caspase-1/11 activation in response to TsV. Experiments with BMDMs deficient in various inflammasome constituents demonstrated that Nlrp3\(^{−/−}\), Asc\(^{−/−}\) and Casp1/11\(^{−/−}\) cells did not produce IL-1\(\beta\) (Fig. 1b) and did not activate caspase-1 in response to TsV (Fig. 1c). During the TsV challenge, macrophages from Nlrp4\(^{−/−}\) mice produced higher amounts of IL-1\(\beta\) than did macrophages from Nlrp3\(^{−/−}\) mice and lower amounts than those produced by macrophages from WT mice. These data suggest that NLRP3 and the NLRC4 inflammasome, to some extent, contribute to venom-induced IL-1\(\beta\) production (Fig. 1b). Western blotting analysis of the p20 subunit of caspase-1 (ref. 28) confirmed that TsV activated the NLRP3 inflammasome (Fig. 1c) as did nigericin, a positive control\(^28\). As a control, we determined the production of IL-6, an inflammasome-unrelated cytokine. As expected, BMDMs produced IL-6 in response to TsV regardless to the inflammasome components NLRP3, ASC and caspase-1/11 (Fig. 1b). To assess the mechanisms by which inflammasome triggers NLRP3 activation, we investigated the induction of pores in the macrophage membranes and the efflux of potassium, an essential process to enable the activation of the NLRP3 inflammasome\(^29\). We found that elevated concentrations of extracellular K\(^+\) impaired the inflammasome activation in response to TsV (Fig. 1d), suggesting that K\(^+\) efflux is involved in the activation of the inflammasome mediated by TsV. Notably, a higher concentration of NaCl potentiates TsV-induced IL-1\(\beta\) release (Fig. 1d), suggesting that the venom activates the inflammasome by a mechanism that is distinct from that induced by hyperosmotic stress, as reported previously\(^30\). To investigate whether TsV directly triggers cell death, we performed a pore-forming assay using WT-BMDMs treated with TsV in presence or absence of CPS. We verified that, even at 17 h after treatment, the cells did not present significant membrane damage, while cells treated with Triton-X, the positive control, did (Fig. 1e), confirming that TsV-induced cell activation without significant cell death.

**TsV induces lung inflammation and mortality via IL-1R/IL-1\(\beta\).** Because TsV activates the inflammasome *in vitro* and since in humans and animals, scorpion envenomation induces IL-1\(\beta\) (refs 5,6), lung oedema\(^1\-3\) and mortality\(^1\-5\), we performed an *in vivo* dose–response experiment to identify the inflammatory, lethal and excessive doses of TsV for C57BL/6 and 129sv mice, which are the WT control mice for the inflammasome, and for Alxox5\(^{−/−}\) mice, respectively (Supplementary Fig. 1). We used a sublethal dose of TsV (120 \(\mu\)g kg\(^{-1}\) intraperitoneally) to study inflammasome activation, IL-1\(\beta\) release and IL-1R signalling-induced lung inflammation *in vivo*. We collected bronchoalveolar lavage fluid (BAL) or whole lungs 4 h after envenomation from C57BL/6 (WT), IL-1r\(^{−/−}\) and Casp1/11\(^{−/−}\) mice. TsV activated inflammasome in lungs, inducing a significant increase in IL-1\(\beta\) release and protein extravasation (Fig. 2a), PGE\(_2\) and LTB\(_4\) production (Fig. 2b), and neutrophil recruitment to lung tissue and bronchoalveolar space in C57BL/6 mice (Fig. 2c). To evaluate if TsV induces the expression of enzymes involved in eicosanoid metabolism, peritoneal macrophages from C57BL/6 mice were incubated *in vitro* with TsV for 2 and 6 h and Ptgs2, Ptges2, Alox5 and AloxSap mRNA expression was determined. Interestingly, we observed that the mRNA expression of these enzymes was increased in a time-dependent manner, suggesting that TsV activates the inflammasome and triggers the expression of enzymes involved in eicosanoid metabolism.
expression of enzymes involved in the PGE₂ biosynthetic pathway were increased earlier than mRNA for enzymes involved in LTs production (Supplementary Fig. 2). Activation of the IL-1β–IL-1R axis during the TsV-induced mediator release and lung inflammation was confirmed in IL-1R⁻/⁻ mice. After TsV inoculation, we detected lower levels of IL-1β and protein extravasation in IL-1R⁻/⁻ mice (Fig. 2a). Moreover, PGE₂ and LTβ₄ production and neutrophil accumulation in lungs were lower in the absence of IL-1R than in WT mice (Fig. 2b,c). These results indicated that IL-1β/IL-1R signalling is essential for TsV-induced lung inflammation. Next, we determined whether the absence of IL-1β/IL-1R signalling protects mice from a lethal
dose of TsV (180 μg kg−1). We found that 100% of IL-1r−/− mice survived the lethal dose, while only 35% of the C57BL/6 mice survived (Fig. 2d). Oedema and cell accumulation induced by the lethal dose of TsV were also assessed macroscopically using Evans blue leakage assay and microscopically by haematoxylin and eosin (H&E) staining of lung tissue. Whole-lung staining in C57BL/6 mice showed intense Evans blue colouring and cell infiltration (Supplementary Fig. 3a,c), whereas the lungs from IL-1r−/− mice did not stain positive for Evans blue and showed milder oedema and weaker infiltration by leukocytes (Supplementary Fig. 3d,f). As expected, TsV intraperitoneal inoculation induced neutrophil recruitment to the peritoneal cavity and increased the number of circulating neutrophils. However, these were lower in IL-1r−/− mice (Supplementary Fig. 3g,h). To confirm the role of caspase-1/11 in the TsV-induced IL-1β secretion in vivo, we performed experiments using the Casp1/11−/− mice. We found that caspase-1/11-deficient animals showed a reduction in protein extravasation (Fig. 2a), in PGE2 and LTB4 release (Fig. 2b), and in neutrophil recruitment to the lung and BAL (Fig. 2c) 4 h after administration of 120 μg kg−1 TsV. The impact of caspase-1/11 deficiency on mortality among the mice after lethal dose of TsV (180 μg kg−1) was examined and, up to 24 h, 100% of Casp1/11−/− survived, while only 25% of WT mice survived (Fig. 2d). These data confirmed that caspase-1/11 and IL-1β are essential participants in lung inflammation and mediated animal death. Because we observed that IL-1r−/− and Casp1/11−/− mice still produced significant amounts of PGE2 after TsV administration, we speculated that PGE2 might be necessary for the release and the amplification loop for IL-1β production.

5-LO metabolites control lung inflammation and mortality. Our data show that the earlier release of LTB4, while PGE2 production was only partially dependent on the inflammasome (Fig. 2b). Therefore, we hypothesized that Alox5−/− mice would develop a weaker lung inflammation with reduction in mortality after envenomation. Unexpectedly, when we performed a TsV dose–response curve (60, 120, 180 or 360 μg kg−1) and evaluated the survival up to 24 h, we observed that Alox5−/− mice were more susceptible than 129sv and C57BL/6 mice (Supplementary Figs 1,4). We next investigated lung inflammation in Alox5−/− and in WT 129sv mice, 4 h after injection of 120 μg kg−1 TsV. According to the increased susceptibility of these mice to TsV challenge, inflammation was stronger in the lung parenchyma of Alox5−/− mice than that in the lung parenchyma of 129sv mice, as evidenced by an increase in IL-1β and protein concentration (Fig. 3a), PGE2 release (Fig. 3b) and leukocyte accumulation in the lung tissues (Fig. 3c). However, the number of neutrophils in BAL of Alox5−/− mice was lower than that of BAL from 129sv WT mice (Fig. 3c). As expected, LTB4 amount in the lungs of...
129sv mice increased after TsV injection (Fig. 3b). Because we observed a higher production of IL-1β in the lungs of Alox5−/− mice (Fig. 3a), we next assessed the effects of exogenous IL-1 receptor antagonist (IL-1Ra) on TsV-induced lung inflammation and mortality. We found that, regardless of the mouse strain, IL-1Ra treatment decreased TsV-induced lung inflammation (Fig. 3a,b) and BAL neutrophil infiltration (Fig. 3c), confirming the crucial role of IL-1β for oedema and cell recruitment. Furthermore, treatment with exogenous IL-1Ra increased the rate of TsV-induced mortality among Alox5−/− mice closer to that of 129sv IL-1Ra-treated mice. About 60% of the mice in both strains survived up to 24 h (Fig. 3d). To confirm that the high mortality of Alox5−/− mice was not related to a nonspecific compensatory mechanism, we inoculated C57BL/6 mice with a lethal dose of TsV and either treated them or not with MK886, a leukotriene synthesis inhibitor. As expected, MK886 increased mortality, decreased LTB4 and IL-1β and increases PGE2 (Supplementary Fig. 5a–d). Taken together, these results indicate that mediators downstream of 5-LO activation protected the host from excessive inflammasome activation and consequently mortality after scorpion envenomation.

**Figure 3** | Downstream mediators of 5-LO protect mice from excessive lung inflammation and early mortality mediated by IL-1R signalling. (a–c) 129sv WT or Alox5−/− mice were inoculated intraperitoneally with PBS or sublethal (120 μg kg−1) dose of TsV and lung tissues were recovered 4 h later for analysis of (a) IL-1β production and protein content; (b) PGE2 and LTB4 release; and (c) histological features of infiltration by inflammatory cells, as determined by staining with H&E, and the number of neutrophils was determined in BAL within the same period in another group of mice. (d) For survival analysis, 129sv WT or Alox5−/− mice that received PBS or a lethal (180 μg kg−1) dose of TsV were monitored for 24 h. (a–c) A group (n = 6) of 129sv WT or Alox5−/− mice was treated with vehicle or IL-1Ra (10 mg kg−1 intraperitoneally) 1 h before and again 1 h after TsV injection and was subjected to the above analysis. (a–c) The experiment with a sublethal dose of TsV was performed once on six mice and the error bars denote s.d. (d) Data on the lethal dose of TsV are shown as a percentage of surviving animals among six mice. *PBS versus vehicle + TsV, $vehicle + TsV in 129sv versus vehicle + TsV in Alox5−/−, $129sv or Alox5−/− inoculated with vehicle + TsV versus 129sv WT or Alox5−/− inoculated with IL-1Ra + TsV; ±PBS in 129sv WT versus PBS in Alox5−/−. These differences were considered significant with P<0.05 according to one-way ANOVA with Bonferroni’s post-test (a–c) or the log-rank test (d). The histological analyses were single-blinded. Scale bars, 100 μm.

**COX1/2-EP2 axis promotes IL-1β-induced lung inflammation.** As described above, 100% of Alox5−/− mice died after a lethal dose of TsV and produced 100% more PGE2 than 129sv mice did. Given that PGE2 induces IL-1β gene expression and oedema, we next tested whether treatment of 129sv and...
Alox5−/− mice with inhibitors that block both COX1 and COX2 (indomethacin) and COX2 alone (celecoxib) abrogates mortality induced by the lethal dose of TsV. Indomethacin abrogated TsV-induced mortality in both 129sv and Alox5−/− mice and significantly inhibited protein extravasation, PGE2, and IL-1β production, and myeloperoxidase (MPO) expression in the lungs (Fig. 4a). Similar results were observed in C57BL/6 mice injected with a lethal dose of TsV and treated with indomethacin, celecoxib or an EP2 antagonist, AH6809. Although ~50–70% of C57BL/6 mice died after administration of 180 μg kg−1 TsV, indomethacin, celecoxib and EP2 antagonist treatment decreased mortality, PGE2 and IL-1β production and neutrophil infiltration in the lungs (Fig. 4b–d). As expected, COX1/2 inhibition in C57BL/6 mice also resulted in increased LTB4 release (Fig. 4b,c). Inhibition of COX1 by SC-560 partially prevented TsV-induced mortality (Supplementary Fig. 5e). These results indicate that TsV-COX1/2-PGE2-EP2 signalling mediates excessive production of IL-1β, accounted for lung inflammation and is deleterious for the host.

**PGE2 increases IL-1β production via cAMP production.** Previous studies showed that the PGE2-EP2 axis drives cAMP production22,31 and mediates IL-1β production22,31. First, we determined the relative basal expression of all EP receptors in resting macrophages and examined whether TsV upregulates the expression of EP1-EP4 receptors. We observed that

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**Figure 4 | Inhibition of COX1/2, COX2 and PGE2-EP2 signalling decreases lung inflammation and abrogates mortality induced by a lethal dose of TsV.**

(a) 129sv WT mice and Alox5−/− mice or (b) C57BL/6 mice were treated with indomethacin (Indo; 2 mg kg−1 intraperitoneally), 4 h and again 30 min before inoculation with a lethal (180 μg kg−1) dose of TsV. Additional administration of vehicle or indomethacin was performed 4 and 8 h later and the survival was monitored for 8–12 h. (c) C57BL/6 mice were treated with celecoxib (5 mg kg−1 intraperitoneally), or with (d) EP2 antagonist (AH6809) (5 mg kg−1 intraperitoneally), 24 h and again 1 h before inoculation of 180 μg kg−1 of TsV. In all experiments, TsV-inoculated mice treated with vehicle were used as controls. The lungs were excised immediately after an animal died or from mice that survived for 8–12 h; then protein content, PGE2 release, IL-1β production and MPO activity were quantified in the lung parenchyma. The experiment was conducted once with six mice and the error bars denote s.d. *PBS versus vehicle + TsV; **vehicle + TsV versus Treatments + TsV; #PBS in 129sv WT mice versus PBS in Alox5−/− mice; $Vehicle + TsV in 129sv WT mice versus Vehicle + TsV in Alox5−/− mice. These differences were considered significant with P < 0.05, according to one-way ANOVA with Bonferroni’s post-test (for soluble mediators and MPO) or the log-rank test (for survival).
non-stimulated macrophages exhibit a higher expression of EP2 and EP4, low expression of EP3 and no expression of EP1. Interestingly, TsV increased the expression levels of Ptg2 and Ptg4 alone (Fig. 5a). Next, we investigated the effects of PGE2 on TsV-induced IL-1β and whether EP2/EP4 mediates cAMP increases, as previously described. We observed that TsV-induced IL-1β expression in C57BL/6-peritoneal or immortalized macrophages (J774.1) was higher in PGE2-pre-treated cells and that indomethacin, the EP2 antagonist AH6809 and the EP4 antagonist AH23848 abrogated IL-1β production (Fig. 5b,c). To further evaluate the participation of PGE2-cAMP in TsV-induced IL-1β expression, we measured cAMP in the supernatant of J774.1 cells pre-treated as indicated. Interestingly, PGE2 alone increases IL-1β and cAMP (Fig. 5b,d). While forskolin, the adenylate cyclase agonist, potentiated TsV-induced IL-1β production, H89, the protein kinase A (PKA) inhibitor, reduced it (Fig. 5e). Moreover, PGE2 increased IL-1β production, while the EP2 antagonist AH6809 and indomethacin inhibited TsV-induced cAMP production (Fig. 5d). Next, we determined whether cAMP and its downstream effector PKA, leading to NFκB activation, are involved in TsV-induced IL-1β. To do so, J774.1 cells were pre-treated with forskolin or with H89, and then challenged with TsV. We discovered that, in the presence of TsV, PGE2 similar to forskolin, amplified the phosphorylation of NFκB p65, while the PKA inhibitor, H89, decreased it (Fig. 5f). The role of NFκB for TsV-induced IL-1β was confirmed by treatment with an NFκB inhibitor (Fig. 5g). Our results suggest that TsV via the PGE2-cAMP-PKA-NFκB pathway increases IL-1β production by inflammasome activation (Fig. 5h).

**LTB4 decreases cAMP generation and rescues mice from death.**

The abovementioned results indicate that PGE2 increases TsV-induced IL-1β by increasing cAMP production. We previously demonstrated that LTB4, but not LTD4, reduces cAMP generation in macrophages. Thus, we hypothesized that LTB4 might play an anti-inflammatory function rather than being an inflammatory mediator during scorpion envenomation, by inhibiting NFκB activation. Our results show that LTB4...
decreased IL-1β in both C57BL/6 peritoneal and immortalized (J774.1) macrophages (Fig. 6a) via BLT1 antagonist (Fig. 6b) or receptor-gene silencing (Fig. 6c). Furthermore, TsV increases Ltb4r1 and Ltb4r2 mRNA expression in macrophages (Fig. 6d). As expected, LTB₄ decreased cAMP-induced by TsV or by PGE₂ (Fig. 6e). To corroborate the anti-inflammatory action of this lipid, lethally envenomed Alox5⁻/⁻ mice were treated with LTBD₄ as indicated. Exogenously administered LTBD₄ rescued Alox5⁻/⁻ mice from mortality (Fig. 7a) and decreased lung inflammation as noticed by the production of IL-1β and PGE₂ and protein extravasation, besides increasing neutrophils recruitment to the lungs (Fig. 7b).

COX1/2 inhibition rescues animals from lethal envenomation. Overproduction of PGE₂-cAMP-IL-1β accounts for uncontrolled lung oedema and mortality in lethal envenomation. Thus, we next investigated whether COX1/2 inhibition could be used as a therapeutic treatment of scorpion stings. For this purpose, we injected C57BL/6 mice with a lethal dose (180 μg kg⁻¹) or superdose (360 μg kg⁻¹) of TsV and then therapeutically treated the animals with indomethacin as indicated. Under both regimens, indomethacin strongly inhibited the envenomation by the superdose: 67% and 50% of the mice survived under the regimens ‘15’ and ‘30 min,’ respectively (Fig. 8a). As our data suggested that the balance between PGE₂ and LTBD₄ regulates the amount of IL-1β produced and lung oedema (Supplementary Table 1), we next calculated the ratio of PGE₂ to LTBD₄ in mice that received TsV at 180 μg kg⁻¹ (Fig. 8b). Our data showed that the PGE₂/LTB₄ ratio affects IL-1β production in vivo and that when LTBD₄ is low, higher levels of PGE₂ and IL-1β are produced and that a positive correlation exists between PGE₂ and IL-1β. As illustrated in Fig. 8c, our data show for the first time that, during scorpion envenomation, an intricate and complex network controls lung oedema, cell recruitment and the stung animal’s fate. Altogether, these results indicate that COX1/2 inhibition might be an effective treatment for patients stung by a scorpion in emergency rooms.

**Figure 6 | LTB₄ decreases cAMP and IL-1β production induced by TsV.** (a) Macrophages were pre-treated with LTBD₄ (0.01-100 nM, 10 min), before TsV (50 μg ml⁻¹) addition for 24 h. Supernatants were collected for IL-1β quantification. (b) J774.1 macrophages were pre-treated or not with BLT1 antagonist (U-75302, 1 μM, 30 min) and with LTBD₄ (100 nM, 10 min) or not before TsV (50 μg ml⁻¹) addition for 24 h. Supernatants were collected for IL-1β quantification. In another set of experiments, (c) J774.1 macrophages were transfected for 48 h to specifically silence Ltb4r1 and Ltb4r2 gene expression. Cells were then pre-treated or not with LTBD₄ (100 nM, 10 min), before TsV (50 μg ml⁻¹) addition for 6 h. In other experiment, (d) Ltb4r1 and Ltb4r2 mRNA expression in C57BL/6 peritoneal macrophages was determined following TsV stimulation (50 μg ml⁻¹) at 2 and 6 h by qRT-PCR. (e) cAMP was determined following stimulation with TsV (50 μg ml⁻¹, 5 min) or PGE₂ (1 μM, 3 min); or LTBD₄ (10 nM, 1 min) followed by TsV or PGE₂. (a-c) Data are representative of two independent experiments performed in quadruplicate. Significant differences P < 0.05 are marked with symbols and the error bars denote s.d. * Median only versus stimulus or treatments, and #TsV versus treatments, according to one-way ANOVA with Bonferroni’s post-test.

**Discussion**

Bee venom activates the inflammasome₁₄,₁₅, resulting in eicosanoid production₁₃,₁₄. However, whether scorpion venom-induced pulmonary oedema and mortality in humans¹⁻³ and animals⁵,⁷ are determined by inflammasome-dependent production of bioactive lipids remains unknown. We hypothesized that TsV activates the inflammasome and induces IL-1β, LTBD₄ and PGE₂ production leading to lung oedema and mortality. In this study, macrophage-derived NLRP3 inflammasome was identified as a platform essential for a systemic response to TsV, resulting in PGE₂, IL-1β and LTBD₄ production, lung inflammation and mortality.

Treatment with IL-1Ra or experiments with Casp1/11⁻/⁻ mice confirmed that the inflammasome is also activated by TsV in vivo, resulting in the production of IL-1β and PGE₂, which are key determinants of the envenomation. The ability of IL-1Ra to control inflammation was reported previously thirty-six and blocking IL-1β along with IL-1Ra has been proposed as an effective treatment of human inflammatory diseases.³⁷ Moreover, PGE₂ upregulates IL-1β (refs 22,31), and both of these mediators promote vascular permeability and oedema, whereas IL-1β recruits neutrophils³⁹,³⁰. To the best of our knowledge, the present report is the first to demonstrate that IL-1Ra protects mice from envenomation, due to the diminished production of PGE₂ and IL-1β and the consequent reduction of lung oedema.

The results demonstrating that the NLRP3 inflammasome is necessary for the LTBD₄ release led us to hypothesize that animals deficient in mediators downstream of 5-LO would be unaffected by scorpion envenomation, because it was previously described that LTs are necessary for the recruitment and activation of polymorphonuclear cells and for pulmonary oedema.⁴⁰,⁴¹ However, Alox5⁻/⁻ animals show overproduced PGE₂ and IL-1β in the lungs leading to strong oedema and leukocyte infiltration in the tissue. Data from the literature demonstrated that PGE₂ causes an array of beneficial and deleterious effects during inflammation.⁴² Among these effects, PGE₂ presents lung protective effects during chronic inflammation.⁴³,⁴⁴ However, induction of oedema by PGE₂ (refs 20,21) (oedema and leukocytes recruitment by IL-1β (refs 38,39)) is very well
Figure 7 | LTB₄ rescues envenomed mice from mortality. (a) For assessment of survival and (b) inflammation in the lung, Alox5⁻/⁻ mice receiving a lethal dose of TsV (180 μg kg⁻¹) were monitored for 8 h with or without exogenous LTB₄ administration (50 ng per mice, intranasal). Lungs were then excised for analysis of total protein content, production of IL-1β, PGE₂ release, and MPO activity. The experiment was conducted once with six mice and the error bars denote s.d. *PBS versus Vehicle + TsV; #Vehicle + TsV versus LTB₄ + TsV. These differences were considered significant with P<0.05, according to one-way ANOVA with Bonferroni’s post-test (for soluble mediators and MPO) or the log-rank test (for survival).

Figure 8 | Treatment with an inhibitor of COX1/2 protects mice from scorpion envenomation. (a) C57BL/6 mice were inoculated with a lethal (180 μg kg⁻¹) or excessive (superdose; 360 μg kg⁻¹) dose of TsV and were treated with vehicle or indomethacin (Indo, 2 mg kg⁻¹) 15 or 30 min after the venom injection, followed by an additional dose of the treatment 4 and 8 h later. Survival was monitored for 12 h. Significant differences (P<0.05) are marked with an asterisk. The experiment was performed once with six mice and the log-rank test was used to compare TsV + vehicle versus TsV + Indo. (b) Using the eicosanoid data obtained from the animals (129sv WT and Alox5⁻/⁻) inoculated with TsV at 180 μg kg⁻¹ for 24 h, we demonstrated the correlation between the PGE₂/LTB₄ balance and the IL-1β production level, in each mouse. Data are representative of one experiment on six mice. (c) Mechanism scheme showing the production of eicosanoids after scorpion envenomation, inflammasome activation and IL-1β release, and indicating that the balance between metabolites of PGE₂ and LTB₄ determines the outcome of inflammasome-mediated envenomation, the severity of lung inflammation and the outcome of envenomation mortality.

Documented the finding that Alox5⁻/⁻ mice are more sensitive to TsV than 129sv and C57BL/6 mice are underscores the protective role of LTB₄ during scorpion envenomation. The recovery of envenomed Alox5⁻/⁻ mice by exogenously administered LTB₄ confirmed its antiedematogenic role, although we cannot disregard a potential role of others eicosanoids such as lipoxins in antiedematogenic effects. In fact, we previously suggested that LTs could present antiedematogenic effects. This was the first demonstration that LTB₄ acts in an autocrine and paracrine manner as an antiedematogenic mediator by controlling PGE₂ and IL-1β production, via cAMP-PKA-NFkB inhibition. Interestingly, our data also indicate that, in scorpion envenomation, IL-1β accounts for neutrophil recruitment to the lungs, but not to the bronchoalveolar space. In fact, decreased IL-1β concentration in IL-1r⁻/⁻, Casp1/11⁻/⁻ and IL-1Ra⁻, indomethacin⁻, celecoxib⁻ and EP2-treated mice correlated with diminished MPO activity in the lung. Although our data indicate that LTB₄ also regulates the intensity of neutrophil recruitment to
the lung parenchyma by controlling IL-1β production, methodologically it is difficult to prove, since as observed in Fig. 7b and as previously described13, LTB4, per se, induces neutrophil recruitment. Altogether, our results show that, during scorpion envenomation, a cross-regulation mechanism exists between PGE2, IL-1β, and LTB4.

According to our data and to one report showing that a high dose of indomethacin decreases the severity of lung oedema induced in rats by one purified scorpion toxin (tityustoxin TsTX)2, we hypothesized that inhibition of COX1/2 or COX2 or blocking EP2/EP4-PGE2 interaction would result in the downregulation of PGE2 and IL-1β and would abrogate the lethality of TsV envenomation. These treatments increased LTB4 production and downregulated IL-1β and PGE2 in the lung and abrogated the mortality induced by a lethal dose of scorpion venom. Moreover, treatment with low dose of indomethacin rescued mice from the death induced by a lethal dose of TsV and significantly increased the survival rate after a high dose of TsV.

In this context, we found that the ratio of 5-LO to COX products determined the amount of IL-1β released and the outcome of the envenomation. Furthermore, exacerbated lung inflammation and greater synthesis of IL-1β (ref. 47) and PGE2 (ref. 48) were previously observed in the presence of reduced level or in the absence of 5-LO products, but the effects of the augmented production of both mediators were not elucidated.

In summary, we can suggest that, in scorpion envenomation, PGE2 is produced in two waves. The first wave comes from peritoneal macrophages and is inflammatory-dependent, but involves the expression of COX2, whereas the second wave of PGE2 (late phase) is generated by structural cells and is dependent on the induction of COX1 expression. The prominent role of PGE2 is also shown by the increase in IL-1β, which is observed after a high dose of TsV and is dependent on the augmented production of PGE2.

Western blot analysis. BMDMs from C57BL/6 (WT), Nlrp3−/−, Nlrp1−/−, Asc−/− and Casp11−/− mice were seeded at 104 per well in 1 ml in a 24-well plate and either pre-treated or not pre-treated with Forskolin from DMSO (1,000 ng ml−1) for 24 h at 37°C in a humidified atmosphere containing 5% CO2. Next, the supernatants were harvested for IL-1β ELISA quantification using an ELISA kit (R&D Systems, Minneapolis, MN, USA). In another set of experiments, resident peritoneal macrophages from naive C57BL/6 mice, harvested by peritoneal washes with RPMI-1640 and the J774.1 cell lineage of peritoneal exudate macrophages, obtained from cell culture were plated at the density of 2 × 105 cells per well in 200 μl of incomplete RPMI-1640 supplemented with 10% FCS and 0.1% L-glutamine (Gibco, Invitrogen, Carlsbad, CA, USA). The cells were cultured at 37°C (5% CO2) for 24 h. Next, the supernatants were removed and the cells were pre-treated or not with either LTβ, for 10 min (0.01, 0.1, 1, 10 or 100 nM), Sigma-Aldrich, St Louis, MO, USA) and then incubated with TsV (50 μg ml−1) for 24 h at 37°C in a humidified atmosphere containing 5% CO2. The supernatants were harvested for IL-1β ELISA quantification.

Activation of BMDMs and peritoneal macrophages by TsV. BMDMs were isolated from C57BL/6 (WT), Nlrp3−/−, Nlrp1−/−, Asc−/− and Casp11−/− mice as described previously28. After differentiation, the culture medium was replaced with 10% (v/v) FCS in RPMI-1640 containing 10 μg ml−1 of LPS (Limulus Amebocyte Lysate Test (LAL; QCL-1000, Bio Whittaker, Cambrex Company, Walkersville, MD, USA) was performed to detect LPS in the TsV samples, according to the manufacturer’s instructions. No LPS was detected in any TsV samples.

**Methods**

**Animals.** Female or male mice (6–8 weeks old) were used for in vivo and in vitro experiments. The mice were matched by sex and age in all procedures. Sample size was determined based on previous studies from our laboratory and literature and considering an alpha and beta errors of 0.05 and 0.20, respectively. Nlrp3−/− (ref. 52), Il-1r−/− (ref. 30), Nkx2.1−/− (ref. 30), Casp11−/− (ref. 53) and Asc−/− (ref. 54) mice were backcrossed with C57BL/6 mice for at least nine generations and the animal facilities of the Faculdade de Ciências Farmacêuticas de Ribeirão Preto, FCFRP/USP. C57BL/6 mice also were obtained from the animal facilities of the Faculdade de Ciências Farmacêuticas de Ribeirão Preto, FCFRP/USP. 5-LO-deficient mice (Alox5−/−) (ref. 55) and 129ov WT strain were obtained from the Jackson Laboratory (Bar Harbor, ME, USA) and raised at FCFRP/USP. Maintenance of and experiments with animals were performed in accordance with the institutional guidelines on ethics in animal experiments approved by the Animal Care Committee of the Prefeitura do Campus de Ribeirão Preto (PCARP) at the University of São Paulo, Ribeirão Preto, Brazil (protocol number 14.1.272.53.7).

**T. serrulatus venom.** The venom was extracted from several T. serrulatus scorpions by electric stimulation, dried at room temperature in a vacuum desicator, and stored at −20°C. Before the experiments, TsV was diluted in PBS and filtered through a 0.22-μm sterilizing membrane (Millipore, Bellerica, MA, USA). Limulus Amebocyte Lysate Test (LAL; QCL-1000, Bio Whittaker, Walkersville, MD, USA) was performed to detect LPS in the TsV samples, according to the manufacturer’s instructions. No LPS was detected in any TsV samples.
In vitro pharmacological treatments. J774.1 macrophages were plated at the density of 2 × 10^5 cells per well in 200 μl of serum-free RPMI supplemented with antibiotics. The cells were then cultured at 37°C in 5% CO_2 for 2 h. Next, the supernatants were removed, and the cells were treated or not with specific inhibitors/antagonists for 30 min: indomethacin (10 μM; Cayman Chemical); AH6809 (1 μM; Cayman Chemical); AH23848 (1 μM; Cayman Chemical); U-73343 (1 μM; Cayman Chemical); and NFkB Activation Inhibitor (20 nM; Calbiochem, Darmstadt, Germany). H89 dihydrochloride hydrate (25 μM; Sigma-Aldrich) was added for 2 h in the cell culture medium before stimulation. AH6809 and U-73343 from ethanol stock solutions were diluted in cell culture medium and the same concentration of ethanol (maximum 0.1%) was added to the medium (Ltb4r1). AH23848 and NFkB inhibitor from DMSO stock solutions were diluted in the cell culture medium and the same concentration of DMSO (maximum 0.1%) was added to the medium only (control). All compounds were diluted in 200 μl of serum-free DMEM, and the same solution with solvent diluents was used as control. After treatment, the cells were stimulated with TsV (50 μg ml^-1) under the same experimental conditions and after 24 h at 37°C in a humidified atmosphere 5% of CO_2, the supernatants were collected for IL-1β quantification.

Quantitative PCR with reverse transcription. RNA was extracted using a guanidine-based column method, according to the manufacturer protocol (PureLink, Ambion) and the quantity of RNA was determined by means of a fluorometric assay (Qiubit, Invitrogen, Carlsbad, CA, USA). Complementary DNA (cDNA) was synthesized from 1 μg of total RNA (High Quality cDNA Reverse Transcripase Kits, Applied Biosystems, Foster City, CA, USA). Aliquots (2 μl) of the total cDNA were amplified by quantitative reverse transcriptase-polymerase chain reaction (quantitative PCR with reverse transcription (qRT–PCR)) using TaqMan primers for Ptg2, P2ger2, P2ger1, P2ger5, Ptg2r, Alox5, Alox5ap, Ltb4r1, and Ltb4r2 (Applied Biosystems) and Actβ as reference genes. Reactions were performed in duplicate and amplification was performed under the following conditions: denaturation at 95°C for 2 min; followed by 40 cycles of 95°C for 2 s and 60°C for 20 s. The results were normalized to the expression levels of the endogenous internal controls, Actβ and Gapdh and fold change were calculated according to the 2^-ΔΔCt formula. For intracellular cAMP measurement, cells were pre-treated or not with LTB4 (10 nM) for 1 min, and then stimulated with TsV (50 μg ml^-1) for 6 h. Efficiency of transfection was 74% for Libtβ1 and 75% for Libtβ2, as determined by qRT–PCR.

Measurement of intracellular cAMP. For intracellular cAMP measurement, 1 × 10^5 macrophages (peritoneal or J774.1/2) were seeded in 60-mm culture dishes (sc-42587) and Libtβ2 (sc-45323) siRNAs (Santa Cruz Biotechnology, Santa Cruz, CA, USA) using Lipofectamine as the transfection reagent (Life Technologies, Carlsbad, CA, USA) and Opti-MEM reduced serum medium for culture (Life Technologies). Controls included non-targeting siRNA (scrambled and fluorolucifer) and no siRNA (with and without Lipofectamine). After 48 h of transfection, the cells were pre-treated or not with LTB4 (100 nM; Cayman Chemicals) for 10 min and then stimulated with TsV (50 μg ml^-1) for 6 h. Efficiency of transfection was 74% for Libtβ1 and 75% for Libtβ2, as determined by qRT–PCR.

In vivo experiments and drug treatment. IL-1β, Casp1/11 (−/−) and C57BL/6 (WT) mice without treatment (vehicle, 0.9% saline) were inoculated with a sublethal or lethal dose of TsV (or PBS) as described above. Alox5−/− mice and 129sv mice were pre-treated or not with IL-1 receptor antagonist (IL-1ra) (ref. 63) at 63 mg kg^-1, i.p., 1 h before and again 1 h after the sublethal or lethal TsV injection. IL-1Ra was kindly provided by Dr. Stephen Poole, from the National Institute for Biological Standards and Control (South Mimms, Hertfordshire, UK). In a specific experiment, the mice were either treated or not with MK886 (5-LO inhibitor, 5 mg kg^-1, i.p., in 200 μl of 1% alcohol in water)49, indomethacin (COX1/2 inhibitor, 2 mg kg^-1, i.p., in 200 μl of Tris[hydroxymethyl]aminomethane-HCl; TRIS-HCl, pH 8.2)50, SC-560 (selective COX1 inhibitor, 3 mg kg^-1, i.p., in 200 μl of PBS; Sigma-Aldrich)51, celecoxib (COX2 inhibitor, 5 mg kg^-1, i.p., in 200 μl of water)52 or EP2 antagonist (AH6809, 5 mg kg^-1, i.p., in 200 μl of PBS; Cayman Chemical)53. The drugs (MK886 or indomethacin) or vehicles were administered four times, at 4 h and 0.5 h before and again 4 and 8 h after the lethal dose of TsV. The others drugs (SC-560, celecoxib, and EP2 antagonist) or vehicles were administered 1 day and again 1 h before the i.p. injection of lethal dose of TsV (180 μg kg^-1). In other experiments, the Alox5−/− mice were treated or not with LTB4 (50 mg per ng, intranasal (i.n.) administration, in 20 μl of PBS, Cayman Chemical)56. The LTB4 or vehicle (PBS + 0.05% of ethanol) were administered 2 h and 0.5 h before the dose lethal of TsV (180 μg kg^-1). The lungs were excised immediately after death or from mice survivors that were killed 8–12 h after the injection of TsV or vehicle. In some sets of experiments, two groups of mice were inoculated with PBS or a sublethal (120 μg kg^-1) dose of TsV and, in only one, BAL fluids were collected 4 h later, to count the total cell number and neutrophils, as described previously57. In the other group, without BAL, the lungs were excised and weighted and 2 mg of total protein were homogenized in 2% SDS (Complete Protein, Roche), 0.5 h (for 10 min), the supernatants were transferred to new tubes, split into two samples of 1 ml and stored at −80°C until use. One sample was used for IL-1β and protein quantification analysis and the other for PGE2 and LTB4 measurement. For analysis of MPO activity, one lobe of a lung was cut out, immediately frozen in liquid nitrogen, and stored at −80°C until use. Protein concentration in the protocol was the lethal dose (180 μg kg^-1) and superdose (360 μg kg^-1) of TsV were injected and indomethacin (2 mg kg^-1 i.p.) or vehicle were administered either 15 or 30 min after and again 4 and 8 h later. Mice survivors were killed 12 h after the envenomation.

Quantification of inflammatory markers. IL-1β present in the supernatant of a lung homogenate or cell culture and IL-6 in cell culture were quantified using IL-1β (BD Biosciences, Franklin Lakes, NJ, USA) or R&D Systems) ELISA kits. Lypids were purified from 1 ml of filtered supernatants from the lung homogenates using Sep-Pak C18 cartridges (Waters Corp). Measurement of LTB4 and PGE2 levels was performed by means of an ELISA assay (Enzo Life Sciences). The supernatants of the lung homogenates were used for the measurement of MPO activity as described previously58 and the quantification of total protein was performed using the Coomassie Protein Assay Reagent (Pierce, Rockford, IL). A set of experiments, peripheral cavity fluid and 1 ml of peripheral blood were also collected to count total cells and neutrophils, using a Neubauer chamber and cytospin preparation. Immediately after an animal died or in mice survivors, the lungs were always weighed before processing and this data were used for the calculation of the lung/body weight index to evaluate oedema. When indicated in the figures, the samples of lung parenchyma were stained with H&E for histological analysis and blind observers performed the analysis. In one set of experiments, three mice received 200 μl of a 1% Evan blue solution according to the manufacturer’s instructions (Cell Signaling). Briefly, the lyses were diluted with sample diluent and 100 μl of the lyses was added to wells that had been pre-coated with the primary antibody. The plate was left overnight at 4°C and then washed. Next, the plate was incubated with the detection antibody and horseradish peroxidase-conjugated secondary antibody, with subsequent washes. The substrate 3,5,5′-tetramethylbenzidine was added to the wells, the reaction was stopped with acid solution, and the absorbance of the samples was read at 450 nm. The results and horizontal bars are presented as relative percentages of the levels of phosphorylated NFκB to total NFκB, normalized by 100 mg of total protein.

Dose-response experiments with TsV in vivo. In all experiments under all conditions, the mice were weighed before an i.p. injection of TsV. To determine the sublethal, lethal and excessive doses in WT mice (C57BL/6 and 129sv) or Alox5−/−, we tested TsV at 60, 120, 180 and 360 μg kg^-1 in 200 μl of PBS and the survival was monitored for 24 h. Death of each animal was recorded immediately and used to calculate the rate of survival (in percentage points). On the basis of these results (Supplementary Fig. 1), the TsV dose of 120 μg kg^-1 was considered sublethal and used to assess lung inflammation 4 h later; 180 μg kg^-1 was designated as lethal, and 360 μg kg^-1 as a superdose. These doses were used in survival experiments (with or without treatment) as indicated below. The animals were monitored for 8, 12 or 24 h. The animals that were inoculated with 200 μl of PBS (i.p.) served as negative controls. The mice were killed with an overdose of a solution of 20% ketamine and 10% xylazine.
(Sigaal–Aldrich) intravenously 60 min before euthanasia and the lungs were excised, weighed and photographed.

Statistical analysis. For comparison of multiple groups, we performed one-way analysis of variance (ANOVA) followed by Bonferroni’s post-test. The differences between any two groups were evaluated using two-tailed Student’s t-test. All calculations were performed in the GraphPad Prism 5.0 software (GraphPad, San Diego, CA, USA). Differences in survival were analysed using the log-rank test. Differences with P < 0.05 were considered statistically significant.

References

1. Chippaux, J. P. & Geryon, M. Epidemiology of scorpionism: a global appraisal. Acta Trop. 107, 71–79 (2008).
2. Amaral, C. F., de Rezende, N. A. & Freire-Maia, L. Acute pulmonary edema after Tityus serrulatus scorpion sting in children. Am. J. Cardiol. 71, 242–245 (1993).
3. Bahloud, M. et al. Scorpion envenomation among children: clinical manifestations and outcome (analysis of 685 cases). Am. J. Trop. Med. Hyg. 83, 1084–1092 (2010).
4. el-Amin, E. O., Sultan, O. M., al-Magами, M. S. & Eldrissi, A. Serotherapy in the management of scorpion sting in children in Saudi Arabia. Ann. Trop. Paediatr. 14, 21–24 (1994).
5. Petricevich, V. L. Scorpion venom and the inflammatory response. Mediators Inflamm. 2010, 903295 (2010).
6. Fukuhara, Y. D., Reis, M. L., Dellalibera-Joviliano, R., Cunha, F. Q. & Donadi, E. A. Increased plasma levels of IL-1beta, IL-6, IL-10 and TNF-alpha in patients moderately or severely envenomed by Tityus serrulatus scorpion sting. Toxicol. 41, 49–55 (2005).
7. Freire-Maia, L., Almeida, H. O., Cunha-Melo, J. R., Azevedo, A. D. & Barroso, J. Mechanism of the pulmonary edema induced by intravenous injection of scorpion toxin in the rat. Agents Actions 8, 113–118 (1978).
8. Pessini, A. C., de Souza, A. M., Faccioli, L. H., Gregorio, Z. M. & Arantes, E. C. Time course of acute-phase response induced by Tityus serrulatus venom and TsTX-I in mice. Int. Immunopharmacol. 3, 765–774 (2003).
9. Kumar, H., Kawai, T. & Akira, S. Pathogen recognition by the innate immune system. Rev. Immunol. 30, 16–34 (2011).
10. Medzhitov, R. Recognition of microorganisms and activation of the immune response. Nature 449, 819–826 (2007).
11. Zoccal, K. F. et al. TLR2, TLR4 and CD14 recognize venom-associated molecular patterns from Tityus serrulatus to induce macrophage-derived inflammatory mediators. PLoS ONE 9, e88174 (2014).
12. Funk, C. D. Prostaglandins and leukotrienes: advances in eicosanoid biology. Eur. J. Pharmacol. 10, 201–215 (2010).
13. Ip, W. K. & Medzhitov, R. Macrophages monitor tissue osmolality and induce inflammatory response through NLRP3 and NLRC4 inflammammasome activation. Nat. Commun. 6, 6931 (2015).
14. Palm, N. W. & Medzhitov, R. Role of the inflammasome in defense against pathogens and in the inflammatory response. J. Immunol. 184, 5030–5037 (2009).
15. Zhou, H., Voll, R. E. & Ghosh, S. Phosphorylation of NF-kappa B by P65 by PKA stimulates transcriptional activity by promoting a novel bivalent interaction with the coactivator CBP/p300. Mol. Cell. 1, 661–671 (1998).
16. Leff, J. A. et al. Post-insult treatment with interleukin-1 receptor antagonist decreases oxidative lung injury in rats given intratracheal interleukin-1. Am. J. Respir. Crit. Care Med. 150, 109–112 (1994).
17. Dinarrello, C. A., Simon, A. & van der Meer, J. W. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. Nat. Rev. Drug Discov. 11, 633–652 (2012).
18. Liu, Z. et al. NLRP3 inflammasome activation is essential for paraxanth-induced acute lung injury. Inflammation 38, 433–444 (2015).
19. Faccioli, L. H., Souza, G. E., Cunha, F. Q., Poole, S. & Ferreira, S. H. Recombinant interleukin-1 and tumor necrosis factor induce neutrophil migration ‘in vivo’ by indirect mechanisms. Agents Actions 30, 344–349 (1990).
20. Monteiro, A. P. et al. Pivotal role of the 5-lipoxygenase pathway in lung injury after experimental sepsis. Am. J. Respir. Cell Mol. Biol. 50, 87–95 (2014).
21. Eun, J. C. et al. The 5-lipoxygenase pathway is required for acute lung injury following hemorrhagic shock. Shock 37, 599–604 (2012).
22. Kalinski, P. Regulation of immune responses by prostaglandin E2. J. Immunol. 188, 21–28 (2012).
23. Birrell, M. A. et al. Anti-inflammatory effects of PGJ2 in the lung: role of the EP4 receptor subtype. Thorax 70, 740–747 (2015).
24. Gauvreau, G. M., Watson, R. M. & O’Byrne, P. M. Protective effects of inhaled PGE2 on allergen-induced airway responses and airway inflammation. Am. J. Respir. Crit. Care Med. 159, 31–36 (1999).
25. Menezes-de-Lima, Jr O., Kassuya, C. A., Nascimento, A. F., Henriques, M. & Calixto, J. B. Lipoxin A4 inhibits acute edema in mice: implications for the anti-edematogenic mechanism induced by aspirin. Prostaglandins Other Lipid Mediat. 80, 123–135 (2006).
26. Magnacca, S. & Faccioli, L. H. The anti-oedemagenic effect of SRS as an additional factor in the mode of action of non-steroidal anti-inflammatory drugs. Eur. J. Pharmacol. 112, 153–160 (1985).
27. Medeiros, A. I. et al. Blockade of endogenous leukotrienes exacerbates pulmonary histoplasmosis. Infect. Immun. 72, 1637–1644 (2004).
28. Byrum, R. S., Goulet, J. L., Griffiths, R. J. & Koller, B. H. Role of the 5-lipoxygenase-activating protein (FLAP) in murine acute inflammatory responses. J. Exp. Med. 185, 1065–1075 (1997).
29. Lee, G. S. et al. The calcium-sensing receptor regulates the NLRP3 inflammasome through Ca2+ and AMP. Nature 492, 123–127 (2012).
30. Sorgi, C. A. et al. Histoplasma capsulatum cell wall [beta]-glucan induces lipid body formation and role in HIV-1 infection. J. Immunol. 182, 4025–4035 (2009).
31. Coffey, M. J., Phare, S. M., Cinti, S., Peters-Golden, M. & Kazanjian, P. H. Granulocyte-macrophage colony-stimulating factor upregulates reduced 5-lipoxygenase metabolism in peripheral blood monocytes and neutrophils in acquired immunodeficiency syndrome. Blood 94, 3897–3905 (1999).
32. Sakhnovskaya, S. et al. Cryopyrin activates the inflammasome in response to toxins and ATP. Nature 440, 228–232 (2006).
33. Eltoum, S. et al. Role of the inflammasome-caspase-11/IL-1/18 axis in cigarette smoke driven airway inflammation: an insight into the pathogenesis of COPD. PloS ONE 9, e128294 (2014).
34. Mariathasan, S. et al. Differential activation of the inflammasome by caspase-1 and NLRP3. J. Biol. Chem. 280, 213–218 (2005).
35. Funk, C. D., Matsumoto, T., Hoshiko, S., Radmark, O. & Samuelsson, B. Characterization of the human 5-lipoxygenase gene. Adv. Prostaglandin Thromboxane Leukot. Res. 19, 470–473 (1989).
56. Marim, F. M., Silva, T. N., Lima, Jr D. S. & Zamboni, D. S. A method for generation of bone marrow-derived macrophages from cryopreserved mouse bone marrow cells. PloS ONE 5, e15263 (2010).

57. Canetti, C. L., Hoffer, M. H., Curtis, J. L. & Peters-Golden, M. Syk activation is a leukotriene B4-regulated event involved in macrophage phagocytosis of IgG-coated targets but not apoptotic cells. Blood 102, 1877–1883 (2003).

58. Serezani, C. H. et al. Macrophage dectin-1 expression is controlled by leukotriene B4 via a GM-CSF/PU.1 axis. J. Immunol. 189, 906–915 (2012).

59. Kim, S. H. et al. Distinct protein kinase A anchoring proteins direct prostaglandin E2 modulation of Toll-like receptor signaling in alveolar macrophages. J. Biol. Chem. 286, 8873–8883 (2011).

60. Fujihara, M., Muroi, M., Muroi, Y., Ito, N. & Suzuki, T. Mechanism of lipopolysaccharide-triggered junB activation in a mouse macrophage-like cell line (J774). J. Biol. Chem. 268, 14898–14905 (1993).

61. Case, C. L. et al. Caspase-11 stimulates rapid flagellin-independent pyroptosis in response to Legionella pneumophila. Proc. Natl Acad. Sci. USA 110, 1851–1856 (2013).

62. Horii, J. I., Pereira, M. S., Roy, C. R., Nagai, H. & Zamboni, D. S. Identification and functional characterization of K(+) transporters encoded by Legionella pneumophila kup genes. Cell. Microbiol. 15, 2006–2019 (2013).

63. Calil, I. L. et al. Lipopolysaccharide induces inflammatory hyperalgesia triggering a TRLA/MyD88-dependent cytokine cascade in the mice paw. PloS ONE 9, e90013 (2014).

64. Zoccal, K. F. et al. Ts6 and Ts2 from Tityus serrulatus venom induce inflammation by mechanisms dependent on lipid mediators and cytokine production. Toxicon 61, 1–10 (2013).

65. De Souza, G. E. et al. A comparative study of the antipyretic effects of indomethacin and dipyrone in rats. Inflammam. Res. 51, 24–32 (2002).

66. Li, W., Wan, L., Zhai, L. Y. & Wang, J. Effects of SC-560 in combination with cisplatin or taxol on angiogenesis in human ovarian cancer xenografts. Toxicon 51, 1851–1856 (2008).

67. Jiang, J. et al. Inhibition of the prostaglandin receptor EP2 following status epilepticus reduces delayed mortality and brain inflammation. Proc. Natl Acad. Sci. USA 110, 3591–3596 (2013).

68. Mancuso, P., Lewis, C., Serezani, C. H., Goel, D. & Peters-Golden, M. Intrapulmonary administration of leukotriene B4 enhances pulmonary host defense against pneumococcal pneumonia. Infect. Immun. 78, 2264–2271 (2010).

69. Cardoso, C. R. et al. IL-4 regulates susceptibility to intestinal inflammation in murine food allergy. Am. J. Physiol. Gastrointest. Liver Physiol. 296, G593–G600 (2009).

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Author contributions
K.F.Z. designed and performed the experiments, analysed the data, prepared the figures and wrote the manuscript. C.A.S. helped to design the study, performed the experiments, analysed the data, prepared the figures and revised the manuscript. J.I.H. helped to design the study and performed the experiments. C.H.S. and D.S.Z. discussed the hypotheses, helped with data interpretation and revised the manuscript. E.C.A. provided the scorpion venom and discussed the hypotheses. F.W.G.P. designed and performed the experiments. L.H.F. conceived and supervised the project, designed the experiments, helped with the data interpretation, participated in the data analysis and wrote the manuscript.

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