Tissue-resident macrophages actively suppress IL-1beta release via a reactive prostanoid/IL-10 pathway

Natacha Ipseiz1,†, Robert J Pickering1,†, Marcela Rosas1, Victoria J Tyrrell1, Luke C Davies1, Selinda J Orr1,2, Magdalena A Czubala1, Dina Fathalla1,3, Avril AB Robertson4, Clare E Bryant5,6,*, Valerie O’Donnell1 & Philip R Taylor1,3,*

Abstract

The alarm cytokine interleukin-1β (IL-1β) is a potent activator of the inflammatory cascade following pathogen recognition. IL-1β production typically requires two signals: first, priming by recognition of pathogen-associated molecular patterns leads to the production of immature pro-IL-1β; subsequently, inflammasome activation by a secondary signal allows cleavage and maturation of IL-1β from its pro-form. However, despite the important role of IL-1β in controlling local and systemic inflammation, its overall regulation is still not fully understood. Here we demonstrate that peritoneal tissue-resident macrophages use an active inhibitory pathway, to suppress IL-1β processing, which can otherwise occur in the absence of a second signal. Programming by the transcription factor Gata6 controls the expression of prostacyclin synthase, which is required for prostacyclin production after lipopolysaccharide stimulation and optimal induction of IL-10. In the absence of secondary signal, IL-10 potently inhibits IL-1β processing, providing a previously unrecognized control of IL-1β in tissue-resident macrophages.

Keywords IL-10; IL-1beta; macrophages; prostacyclin

Introduction

Interleukin-1β (IL-1β) is a pro-inflammatory cytokine, an alarmin which, once released into the extracellular milieu, triggers the inflammatory response. It is commonly accepted that a two-step mechanism is required for IL-1β production in mouse macrophages (MΦ). First, pathogen-associated molecular pattern (PAMP) recognition induces transcription and translation of the inactive pro-form of IL-1β (pro-IL-1β). A secondary signal, such as reactive oxygen species (ROS) (Nakahira et al, 2011; Zhou et al, 2011), crystals (Hornung et al, 2008) or potassium efflux (Petrilli et al, 2007), is then needed to induce the classical inflammasome assembly, composed of NOD-like receptor family, pyrin domain containing 3 (Nlrp3) and apoptosis-associated speck-like protein containing a CARD (ASC), also called PYCARD. Once assembled, the NLRP3 inflammasome activates caspase1 which in turn cleaves pro-IL-1β into its mature IL-1β form (Bryant & Fitzgerald, 2009; Dowling & O’Neill, 2012; Latz et al, 2013; Lamkanfi & Dixit, 2014). Despite intensive research, the mechanisms regulating IL-1β maturation and release (Lopez-Castejon & Brough, 2011; Martin-Sanchez et al, 2016) are still under discussion (Cullen et al, 2015; Evavold et al, 2018; Monteleone et al, 2018). Dysregulated IL-1β production has been associated with the development of many inflammatory and autoimmune inflammatory diseases (Lamkanfi & Dixit, 2012, 2014; Yao et al, 2016; Mayer-Barber & Yan, 2017) such as cryopyrin-associated periodic syndromes (CAPS), type 2 diabetes (Jourdan et al, 2013), increased susceptibility to Crohn’s disease (Villani et al, 2009) and intestinal inflammation (Shouval et al, 2016), gout (Joosten et al, 2010) and rheumatoid arthritis (Pascual et al, 2005).

Macrophages are part of the immune system’s first line of defence. Initially simply categorized as phagocytes, evidence of their complexity has accumulated over the years (Ley et al, 2016). Resident peritoneal macrophages (pMΦ), a well-studied tissue macrophage population, have essential functions, including modulation of the inflammatory response after pathogen recognition (Dioszeghy et al, 2008; Spight et al, 2008; Leendertse et al, 2009) or injury
(Uderhardt et al., 2019), phagocytosis of pathogens (Ghosn et al., 2010) and dying cells (Fond & Ravichandran, 2016), liver repair (Wang & Kubes, 2016; Reherrmann, 2017) and maintenance of self-tolerance (Russell & Steinberg, 1983; Mukundan et al., 2009; Munoz et al., 2010; Uderhardt et al., 2012; Ipseiz et al., 2014; Majai et al., 2014; Carlucci et al., 2016). pMΦs are part of the first wave of response during peritonitis (Khameneh et al., 2017) and help ensure the survival of the host and the optimal clearance of the infection. Their efficiency is coupled to their optimal cytokine and chemokine secretion which have to be finely tuned, including IL-1β (Topley et al., 1996; Hautem et al., 2017). After the first inflammatory burst following PAMP recognition, macrophages dampen their inflammatory processes by producing anti-inflammatory molecules, such as IL-10 (Bogdan et al., 1991; Berlato et al., 2002; Saraiva & O’Garra, 2010). IL-10 protects against acute inflammation (Howard et al., 1993), and its loss has dramatic effects as observed in IL-10 deficient mice, which develop chronic enterocolitis (Kuhn et al., 1993; Krause et al., 2015). However, the regulatory control of IL-10 production by pMΦ (Liao et al., 2016) as well as its mode of action remain unclear. Additionally, after microbial stimulation, peritoneal macrophages rapidly release prostanooids, such as prostaglandin I2 (PGI2), also called prostacyclin (Brock et al., 1999). PGI2 is known to be generated in peritoneal macrophages following inflammatory stimulation, although it is poorly studied and neglected in this context (Yokode et al., 1988; Stewart et al., 1990; Wightman & Dallob, 1990). Despite the paradoxical role of PGI2 in inflammatory diseases (Stitham et al., 2011), synthetic analogues can decrease tumour necrosis factor (TNF) and induce IL-10 in human peripheral mononuclear cells in vitro (Eisenhut et al., 1993; Luttmann et al., 1999) and inhibit function of murine dendritic cells (Zhou et al., 2007), suggesting an active control of inflammation.

Here we have studied the inflammatory response of resident pMΦ that lack their specialized tissue-programming as a consequence of deletion of the tissue-specific transcription factor Gata6 (Rosas et al., 2014). While wild-type (WT) pMΦ need a secondary signal after lipopolysaccharide (LPS) stimulation to produce mature IL-1β, we show that the Gata6-deficient pMΦ do not, and they exhibit aberrant production of IL-1β after LPS stimulation. Using Gata6-KO pMΦ, we identified a Gata6-PGI2-IL-10 axis as a major regulator of IL-1β processing in resident pMΦ. This axis actively inhibits IL-1β processing during response to a microbial stimulus in the absence of a second signal and thus ensures proportionate and finely regulated production of IL-1β in response to LPS.

Results

Gata6-deficient peritoneal macrophages exhibit dysregulated IL-1β release

We and others previously identified the transcription factor Gata6 as a major key regulator of tissue-resident peritoneal macrophage (pMΦ) specialization (Gautier et al., 2014; Okabe & Medzhitov, 2014; Rosas et al., 2014). To determine its role in the inflammatory function of pMΦ, we analysed the response of Gata6-WT and Gata6-KO pMΦ after toll-like receptor (TLR) ligand stimulation. Surprisingly, we observed that ultra-pure LPS, a specific TLR4 agonist, induced the production of IL-1β by Gata6-KO pMΦ in the absence of an exogenous secondary signal (Fig 1A). LPS also induced significantly higher production of TNF by Gata6-KO pMΦ as observed by ELISA (Fig 1B) and flow cytometry (Figs EV1 and 2A). Additionally, the effect of LPS on IL-1β and TNF production was found to be concentration (Fig 1C and D) and time-dependent (Fig 1E and F). Interestingly, inhibiting TNF with etanercept (a fusion protein composed of TNFR2 connected to a human IgG1 Fc tail) slightly reduced IL-1β production from Gata6-KO pMΦ and blocking the IL-1 receptor antagonist (IL1-ra) when stimulating the cells with purified LPS did not dramatically change IL-1β secretion by Gata6-WT or Gata6-KO pMΦ (Fig 1G). Stimulating the cells with recombinant TNF (recTNF) did not induce IL-1β production from either Gata6-WT or Gata6-KO pMΦ (Fig 1H). These data suggest that neither IL-1 receptor nor TNF signalling greatly affected IL-1β production.

Activation of the NLRP3 inflammasome by classical stimuli is independent of Gata6 programming

To investigate the mechanism behind the aberrant release of IL-1β by Gata6-KO pMΦ following LPS stimulation, we analysed the components of the classical NLRP3 inflammasome pathway. Gata6-WT and KO pMΦ showed similar levels of toll-like receptors (TLR) expression, with an exception for Tlr13 which appeared to be reduced in Gata6-KO cells. Tlr4 and Cdl4, the receptor and co-receptor, respectively, for LPS were similarly expressed by Gata6-WT and KO cells (Fig 2A). Cell surface expression analysis by flow cytometry however showed an increase in TLR2 and TLR4 in KO cells (Figs 2B and EV2B). Gata6-WT and KO pMΦ exhibited similar Il1b mRNA expression (Fig 2C) and comparable production of pro-IL-1β (Figs 2D and EV3A), 3 and 6 h after LPS stimulation, respectively. Interestingly, Gata6-KO cells showed a significantly upregulated Tnf expression (Fig 2C), indicating a direct regulation of TNF production on a mRNA level rather than on a protein level. The pro-IL-1β expression was confirmed by flow cytometry analysis (Fig 2E). These results suggest that, despite an increased TLR4 expression in Gata6-KO pMΦ, both Gata6-WT and KO pMΦ have a similar response capacity to the primary signal LPS regarding the initiation of IL-1β production and that the aberrant release observed in the Gata6-KO cells was likely due to a downstream dysregulation in pro-IL-1β processing. Further investigation revealed that Gata6-KO pMΦ did not have increased Nlrp3 mRNA expression compared to WT pMΦ (Fig 2F), as well as similar protein levels (Figs 2G and EV3B). To determine whether the classical Nlrp3 inflammasome was responsible for the IL-1β release in Gata6-KO pMΦ, we stimulated Gata6-WT and KO pMΦ with LPS in the presence of the specific Nlrp3 inhibitor MCC950 (Coll et al., 2015, 2019). We observed an abrogation of IL-1β secretion from Gata6-KO cells (Fig 2H and I), confirming the essential role of Nlrp3 in the production of IL-1β by Gata6-KO pMΦ. In addition, Gata6-KO cells showed upregulated mRNA expression of caspase1 (Casp1) (Fig 2I) but protein expression showed no significant difference (Figs 2K and EV3C). The selective caspase1 inhibitor Ac-YVAD-cmk blocked IL-1β secretion by LPS-stimulated Gata6-KO pMΦ (Fig 2L). Interestingly, when first primed with LPS for 3 h and then stimulated with a secondary signal (ATP or nigericin) for 30 min, both Gata6-WT and KO pMΦ
pMΦ released comparable levels of IL-1β (Fig 2M). Confocal immunofluorescence analysis showed that both cell types were able to form ASC specks, a hallmark of the classical NLRP3 inflammasome assembly, when stimulated with LPS and ATP (Fig 2N). Overall, these data indicate a similar NLRP3 inflammasome capacity of both Gata6-WT and KO<sup>mye</sup> pMΦ, suggesting that the aberrant IL-1β release observed in Gata6-KO<sup>mye</sup> cells after LPS stimulation might be due to alteration of the regulatory mechanisms and not to the inflammasome machinery itself.

Peritoneal-resident macrophages actively suppress IL-1β production

Based on our findings above, we next hypothesized that the Gata6-KO<sup>mye</sup> pMΦ had a defect in an inhibitory pathway, present in the Gata6-WT cells, that basally constrains further processing of pro-IL-1β after LPS stimulation. To investigate this hypothesis, we co-cultured Gata6-WT and KO<sup>mye</sup> cells and observed a nearly complete inhibition of IL-1β secretion by the Gata6-KO<sup>mye</sup> pMΦ (Fig 3A). This
observation suggested that the Gata6-WT cells actively inhibited IL-1β processing by the Gata6-KO<sup>myo</sup> pMΦ. To determine whether this inhibition was due to direct contact between the cells or to a soluble factor secreted by the Gata6-WT pMΦ, we performed a Transwell experiment (Fig 3B). In this setting, the Gata6-WT pMΦ significantly inhibited IL-1β production from the Gata6-KO<sup>myo</sup> pMΦ, however to a lesser extent than in the direct co-culture experiments. These data may suggest that the Gata6-WT pMΦ might be secreting a soluble molecule, albeit with a short half-life based on the reduced effect observed in the Transwell setting, inhibiting the pro-IL-1β processing pathway. Transcriptomic analysis of Gata6-WT and Gata6-KO<sup>myo</sup> pMΦ (Rosas et al, 2014) showed alterations in many such potential candidate molecules (GEO: GSE47049); however, one of the greatest differentially expressed genes was prostaglandin I<sub>2</sub> synthase (Ptgs) which converts prostaglandin H<sub>2</sub> (PGH<sub>2</sub>) into prostacyclin (PGI<sub>2</sub>). Naïve Gata6-KO<sup>myo</sup> pMΦ expressed significantly reduced amount of Ptgs mRNA (Fig 3C) and protein (Fig 3D) and significantly upregulated thromboxane A<sub>2</sub> synthase 1 (Txbas1) (Fig 3C), a direct competitor to Ptgs for the conversion of PGH<sub>2</sub> into thromboxane A<sub>2</sub> (TXA<sub>2</sub>). The expression of the two other enzymes implicated in the processing pathway of arachidonic acid (AA), cyclooxygenase 1 (Ptgs1) converting AA into prostaglandin G<sub>2</sub> (PGG<sub>2</sub>) followed by PGH<sub>2</sub> and prostaglandin E synthase 2 (Ptges2) processing PGH<sub>2</sub> into prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) was less dramatically changed between Gata6-WT and Gata6-KO<sup>myo</sup> pMΦ (Fig 3C). PGI<sub>2</sub> has a very short half-life (< 2 min in vivo) and is rapidly hydrolysed to form 6-keto-prostaglandin F<sub>1α</sub> (6-keto-PGF<sub>1α</sub>), a metabolite that is readily detectable by mass spectrometry (Kunze & Vogt, 1971; Hamberg & Samuelsson, 1973; Jogee et al, 1983; Lewis & Dollery, 1983; Sítham et al, 2011). Therefore, to assess the impact of the Ptgs deficiency in Gata6-KO<sup>myo</sup> cells on prostaglandin production, we performed mass spectrometric analysis of the oxylipins in supernatants from Gata6-WT and KOG<sup>myo</sup> pMΦ cultured with or without 100 ng/ml LPS for 3 h. As expected, a significant reduction of 6-keto-PGF<sub>1α</sub> coupled with increased thromboxane B<sub>2</sub> (TXB<sub>2</sub>) was observed from Gata6-KO<sup>myo</sup> pMΦ after LPS stimulation (Fig 3E). Notably, prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) was also significantly decreased in Gata6-KO<sup>myo</sup> pMΦ after LPS stimulation although the expression of the enzyme regulating its production (Ptges2) was unchanged (Fig 3C). These results confirmed an imbalanced prostaglandin response in Gata6-KO<sup>myo</sup> cells upon LPS stimulation (Fig 3F). It is important to note that both TXB<sub>2</sub> and PGE<sub>2</sub> were produced at much lower levels when compared to 6-keto-PGF<sub>1α</sub>, in WT LPS-stimulated cells (approximately 10% of the levels). This suggests that PGI<sub>2</sub> might normally be the dominant effector on downstream signalling in pMΦ (Norris et al, 2011). Altogether, these data suggest that Gata6-WT pMΦ are actively inhibiting the processing of IL-1β upon LPS stimulation and that a candidate for this effect may be PGI<sub>2</sub> produced by the Gata6-dependent Ptgs enzyme.

**Prostacyclin inhibits IL-1β production via IL-10 induction in a Gata6-dependant pathway**

PGI<sub>2</sub> and LPS have both been previously shown to induce IL-10 production, including in MΦ (Fiorentino et al, 1991; Luttmann et al, 1999; Zhou et al, 2007). It is also known that pMΦ are predisposed to the production of IL-10 after stimulation with microbial products (Liao et al, 2016). Here, we observed that Gata6-KO<sup>myo</sup> pMΦ produced significantly less IL-10, compared to Gata6-WT cells, after LPS stimulation (Fig 4A). To determine if this could be a consequence of reduced PGI<sub>2</sub> levels, the impact of beraprost, cicaprost and iloprost (PGI<sub>2</sub> analogues with various IP receptor affinities) was assessed (Fig 4B). When combined with LPS, all three analogues significantly increased IL-10 production in Gata6-KO<sup>myo</sup> pMΦ (Fig 4B) compared to LPS and vehicle control (DMSO). In addition, all three analogues inhibited IL-1β production from Gata6-KO<sup>myo</sup> pMΦ (Fig 4C). These data indicate that LPS-mediated induction of IL-10 in pMΦ is driven via a Gata6-PGI<sub>2</sub>-dependent pathway that, in turn, controls IL-1β production. As beraprost has been previously shown to have the most specific binding to IP receptor (Clapp & Gurung, 2015), we chose to conduct further experiments with this agonist only. Given the general imbalance in prostaglandin content in Gata6-KO<sup>myo</sup> pMΦ, we wondered if other prostanooids could also play a similar role in the control of IL-10 and IL-1β in pMΦ. To test this hypothesis, we stimulated the cells with beraprost, PGE2, U46619 (TXA2 receptor agonist) and picotamide (TXA2 receptor and Txbas1 inhibitor) and analysed their IL-10 and IL-1β production (Fig 4D and E). PGE2 was able to induce IL-10 at a similar level to beraprost in Gata6-KO<sup>myo</sup> pMΦ (Fig 4D) and also block IL-1β production (Fig 4E). However, neither U46619 nor picotamide affected IL-10 or IL-1β production in LPS-stimulated cells. These results indicate that prostaglandin balance, especially the prevalence of PGE2 and PGI<sub>2</sub> in pMΦ, controls the activation status of the cells and their response to inflammatory stimuli such as LPS. To investigate if the reduced amount of PGI2-derived IL-10 in Gata6-KO<sup>myo</sup>
Figure 2.
The EMBO Journal
Natacha Ipseiz et al

pMΦ was responsible for their aberrant IL-1β production, we stimulated the cells with either beraprost or IL-10 together with LPS. Both beraprost and IL-10 significantly reduced the IL-1β production from Gata6-KO^mye cells after LPS stimulation, to levels that were comparable to those of the WT cells (Fig 4F). Lastly, we incubated the cells with an anti-IL-10 receptor (αIL-10R) neutralizing antibody, or an isotype-matched control, together with LPS and beraprost. As expected, the blockade of the IL-10 receptor, and thereby its signalling pathway, completely abrogated the effect of beraprost on IL-1β release (Fig 4G). Strikingly, IL-10R-inhibition, in the absence of any exogenous second signal, resulted in IL-1β production from Gata6-WT cells that was comparable to aberrant behaviour of Gata6-deficient counterparts. Together, these data demonstrate that WT cells actively block IL-1β production via a Gata6-PCG12-IL-10-dependent pathway, that is induced by LPS stimulation. To further understand the mechanism regulating this pathway, we performed a similar experiment, including the MCC950 inhibitor. When combined with αIL-10R treatment, MCC950 still inhibited the production of IL-1β after LPS stimulation, but only in Gata6-KO^mye pMΦ (Fig 4H), which coincided with a decrease in IL-10 production from these cells (Fig 4I). Notably, MCC950 had no effect on IL-1β and IL-10 production from Gata6-WT cells incubated with αIL-10R and LPS compared to αIL-10R and LPS-stimulated cells. These data suggest that the blockade of the IL-10 pathway in Gata6-WT pMΦ activates alternative pathways leading to increased stress and production of IL-1β in a non-canonical Nlrp3-independent pathway, in the absence of a secondary signal. As IL-10 had such a potent effect on IL-1β production in pMΦ after primary stimulation, we wondered if it could also affect IL-1β production in the presence of a secondary signal. We incubated Gata6-WT and Gata6-KO^mye pMΦ with LPS and IL-10 for 16 h, followed by a 30 min pulse with ATP. IL-10 was sufficient to significantly inhibit IL-1β production from both Gata6-WT and KO^mye cells (Fig 4J), consistent with its major role in the control of inflammation.

Deficiency in Gata6 does not lead to dysfunctional mitochondria

Previous work has shown that IL-10 is essential to maintain mitochondrial integrity in bone-marrow-derived macrophages (BMDM) (Ip et al, 2017). IL-10-deficient BMDM accumulate ROS which is known to activate NLRP3 inflammasome assembly leading to IL-1β production (Zhou et al, 2011). To investigate whether the aberrant IL-1β production we observed was mediated by mitochondrial dysfunction, we first analysed the mitochondrial membrane potential (MMP) and mitochondrial mass from Gata6-WT and KO^mye pMΦ in vitro by intraperitoneal injection of MitoTracker Green (MT green, total mitochondrial mass, independent of MMP) and MitoTracker Red (MT red, live mitochondria, dependent on MMP) to naïve mice before harvesting the peritoneal cells (Fig 5A). Both Gata6-WT and Gata6-KO^mye mitochondria showed similar mitochondrial mass characterized by a similar MT green staining but the Gata6-KO^mye pMΦ had significantly more MMP characterized by an increased MT red staining. We then investigated the effect of LPS, IL-10 and αIL-10R antibody on the mitochondria by performing in vitro experiments (Fig 5B). Unstimulated Gata6-KO^mye pMΦ had a significantly higher amount of mitochondrial mass and respiring mitochondria; however, addition of LPS reduced this phenotype to that of Gata6-WT pMΦ level. Inhibition of IL-10 signalling using αIL-10R resulted in lower mitochondrial mass of Gata6-WT pMΦ stimulated with LPS compared to Gata6-KO^mye cells. No significant difference in MMP could be observed between Gata6-WT and Gata6-KO^mye pMΦ (Fig 5B, right). Altogether, these data confirmed that deletion of Gata6 was not detrimental to mitochondria health, in naïve or LPS-stimulated pMΦ. In addition, staining with MitoSOX, a mitochondria-specific ROS indicator, showed that Gata6-WT and KO^mye pMΦ produced similar levels of superoxide (Fig 5C), regardless of the stimulation. To confirm that mitochondrial ROS were not implicated in the aberrant IL-1β production observed in the Gata6-KO^mye pMΦ, we treated the cells with the...
**Figure 3.**

**A** Co-culture

**B** Co-culture Transwell

**C** Normalised expression

**D** Gata6-WT

**E** LPS

**F**

- Down in Gata6-KO
- Up in Gata6-WT
- Not measured
- Lipid
- Enzyme

![Diagram](image-url)
antioxidants MitoTEMPO, N-acetyl-L-cysteine (NAC) and mito-quinone (MitoQ) together with LPS and analysed their IL-1β production. All three antioxidants were unable to inhibit IL-1β production from Gata6-KO<sup>mye</sup> cells (Fig 5D), confirming that mitochondrial ROS were not responsible for IL-1β production by Gata6-KO<sup>mye</sup> pMΦ.

**PGI2-derived IL-10 controls IL-1β processing**

IL-10 has been reported to control the production of pro-inflammatory cytokines, such as TNF, by inhibiting their transcription (Murray, 2005). Here, to investigate how the PGI2-derived IL-10 regulates IL-1β in pMΦ in the absence of a secondary signal, we stimulated Gata6-WT and KO<sup>mye</sup> pMΦ with beraprost, IL-10, zIL-10R or isotype control together with LPS for 3 h and analysed IL1b mRNA expression. IL-10 only slightly downregulated IL1b expression (Fig 6A) and zIL-10R increased it, most notably in Gata6-WT cells. In the presence of LPS, beraprost and IL-10 did not significantly affect Nlrp3 or Casp1 expression. However, when combined with LPS, zIL-10R significantly increased Nlrp3 mRNA expression in both Gata6-WT and KO<sup>mye</sup> pMΦ, compared to LPS and isotype-matched control. The treatments had no significant effect on Casp1 expression, except for a small but significant downregulation induced by beraprost alone. Downstream analysis revealed that the protein level of the inflammasome components also remained quite stable following the various treatments (Fig 6B). LPS stimulation of Gata6-WT and KO<sup>mye</sup> pMΦ for 6 h strongly induced pro-IL-1β and Nlrp3 protein level, and co-stimulation with IL-10 repeatedly decreased them. The various treatments did not dramatically affect caspase1 or ASC protein level, although it is interesting to note that ASC protein level showed strong variation between experiments. Caspase1 activity was evaluated using the FAM FLICA reagent on cells stimulated for 16 h. Gata6-KO<sup>mye</sup> pMΦ showed a significant increase in caspase1 activity compared to Gata6-WT cells, independently of the stimulation (Fig 6C). LPS further increased caspase1 activity in Gata6-KO<sup>mye</sup> pMΦ, and this could not be rescued by any other additional stimulation. Thorough analysis of pro-IL-1β by flow cytometry confirmed that LPS induced pro-IL-1β production in both Gata6-WT and KO<sup>mye</sup> pMΦ and that co-stimulation with IL-10 decreased it in WT cells (Figs 6D and 6E), in accordance with the mRNA and Western blot data. Blockade of the IL-10 pathway with zIL-10R increased pro-IL-1β, as observed on mRNA. Overall, these data indicate a direct regulation of pro-IL-1β and Nlrp3 mRNA and protein synthesis via an IL-10-dependent pathway in pMΦ. It is interesting to note that a short time in vitro culture of pMΦ (16 h) was sufficient to strongly affect the baseline production of pro-IL-1β. Indeed, we could not detect any pro-IL-1β in freshly isolated and stained cells, compared to cells cultured unstimulated for 16 h (Figs 6D and EV4A and 5B). Despite all precautions taken to ensure optimal cell culture and minimizing activation of the cells, both Gata6-WT and Gata6-KO<sup>mye</sup> pMΦ cultured for 16 h showed elevated pro-IL-1β content, compared to freshly isolated cells. This could, in part, explain the variation observed between samples and experiments. It is interesting to note that when challenged with LPS, Gata6-WT and KO<sup>mye</sup> mice showed similar neutrophil recruitment to their peritoneal cavity (Fig EV5A) and increased pro-IL-1β content in pMΦ (Fig EV5B) as well as an increased IL-1β content in the peritoneal fluid, the latter of which was higher in the Gata6-KO<sup>mye</sup> mice than the Gata6-WT mice (Fig EV5C). Although previous work have shown that IL-1β can directly modulate neutrophil recruitment (Martinson et al, 2006), it does not seem to be the case in our model. This discrepancy could possibly be explained by the fact that the defect we observe is localized to a single-cell population, which is reduced in number. Additionally, preliminary attempts to regulate neutrophil recruitment with anakinra had no effect (Fig EV5D). Further in vitro analyses showed that LPS upregulated Il10 expression in Gata6-WT pMΦ but to a much lower extent in Gata6-KO<sup>mye</sup> cells (Fig 6E). Interestingly, the addition of beraprost to LPS stimulation rescued Il10 expression in Gata6-KO<sup>mye</sup> cells, confirming the essential role of PGI2 in the induction of Il10 in pMΦ after LPS stimulation. Direct stimulation with IL-10 was not able to rescue the Il10 expression in Gata6-KO<sup>mye</sup> pMΦ, suggesting that, in this setting, IL-10 does not control its own transcription. However, blocking IL-10 signalling using zIL-10R boosted Il10 expression in Gata6-WT pMΦ stimulated with LPS, underlying the fact that IL-10 is an essential response to LPS stimulation and is at least partially dependent on PGI2. In contrast, Tnf expression in Gata6-KO<sup>mye</sup> pMΦ after LPS stimulation was significantly downregulated by co-treatment with beraprost or IL-10 and zIL-10R antibody strongly induced Tnf expression in both Gata6-WT and Gata6-KO<sup>mye</sup> cells (Fig 6E), indicating a direct transcriptional regulation of Tnf expression. Overall, these results show a clear differential regulation of classical pro-inflammatory cytokines production, such as TNF, and IL-1β in pMΦ. PGI2-derived IL-10 at least partially

---

**Figure 3. Prostanoid production is imbalanced in Gata6-KO<sup>mye</sup> pMΦ.**

- **A** IL-1β ELISA analysis of supernatants of Gata6-WT and Gata6-KO<sup>mye</sup> pMΦ in monoculture or co-cultured (ratio 1:1) and stimulated for 24 h with 100 ng/ml LPS. Data shown are pooled from three independent experiments. One-way ANOVA statistical analysis with Tukey’s multiple comparison test was performed.
- **B** IL-1β ELISA analysis of supernatants of Gata6-WT and Gata6-KO<sup>mye</sup> pMΦ co-cultured in the same well (co-culture) or using Transwell system (ratio 1:1) and stimulated for 24 h with 100 ng/ml LPS. Data shown are pooled from three independent experiments. One-way ANOVA statistical analysis with Tukey’s multiple comparison test was performed.
- **C** Microarray analysis of Ptgis, Tbxas1, Pgss2 and Ptgs2 expression from Gata6-WT and Gata6-KO<sup>mye</sup> pMΦ isolated from naive mice. Data are shown as mean ± SEM from three biological replicates. Statistical significance was determined using empirical Bayesian statistic corrected for false discovery rate by the Benjamini–Hochberg procedure.
- **D** Western blot analysis of Ptgis protein level of unstimulated pMΦ from Gata6-WT and Gata6-KO<sup>mye</sup> mice.
- **E** Mass spectrometry analysis of 6-keto-PGFα, TXB2 and PGE2 content of Gata6-WT and Gata6-KO<sup>mye</sup> pMΦ unstimulated (−) or stimulated for 3 h with 100 ng/ml LPS. n = 6. Two-way ANOVA statistical analysis with Tukey’s multiple comparison post-test was performed.
- **F** Representation of the variation of the prostanoid synthesis pathway in Gata6-KO<sup>mye</sup> pMΦ created using Cytoscape software. Circle shape represent lipids, diamond shape enzymes, yellow downregulation and purple upregulation of the expression/production in Gata6-KO<sup>mye</sup> cells. The size of the circles represents relative levels observed in Gata6-KO<sup>mye</sup> cells.

Data information: Data is shown as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001.
impaired the production of pro-IL-1β and Nlrp3 but not caspase1 nor ASC. Our data suggest that pMΦ possess an active inhibitory mechanism able to tightly control IL-1β processing in the absence of secondary signal. This inhibitory pathway not only regulates pro-IL-1β production but also maturation and possibly its subsequent processing (Fig 6F).
Discussion

Our study here shows that, in the absence of secondary signal, WT tissue-resident pMΦ actively block IL-1β processing via a Gata6-PGI2-IL-10-dependent pathway. The absence of this inhibitory pathway leads to a significant production of IL-1β, as observed in the Gata6-KO

\textsuperscript{mice} tissue-resident pMΦ or when using xyl-10R antibody in Gata6-WT pMΦ. IL-1β production and secretion is a complex mechanism, still partially uncharacterized. Upon LPS stimulation, the immature pro-IL-1β form accumulates and its processing is restricted, unless permitted by a secondary signal. The role of IL-1β as an alarmin makes it an extremely potent cytokine, whose production must be tightly controlled to avoid unnecessary inflammatory damage to the surrounding area. The concept of specific mechanisms restraining activation of the inflammasome in the absence of secondary signal therefore seems essential. Recent work showed that tissue MΦ actively attempt to suppress inflammation and that pMΦ promote local injury repair (Wang & Kubes, 2016; Uderhardt et al., 2019). Our work provides a mechanism for constraint of pro-inflammatory responses at the cellular level, both autocrine and paracrine, supporting the idea that upon pro-inflammatory signal recognition, tissue-resident pMΦ are programmed to first control and dampen inflammation. In the presence of a secondary insult, such as in our case inflammasome-activating molecules, this first controlling response may be over-ruled and pMΦ would then actively promote inflammation by the release of alarmins such as IL-1β and recruitment of neutrophils (Martinon et al., 2006).

The fact that the active inhibitory mechanism we describe here has not been previously reported is likely explained by the lack of tools to study IL-1β regulation. Although genetic defects leading to spontaneous IL-1β secretion by MΦ have been reported (Saitoh et al., 2008; Vince et al., 2012; Duong et al., 2015), its maturation and release are usually investigated in the presence of highly potent secondary signals such as ATP and nigericin.

Previous work (Ip et al., 2017) has shown that deficiency in IL-10 in BMDM leads to dysfunctional mitochondria releasing mitochondrial ROS which act as a secondary signal, activating the inflammasome and leading to the aberrant release of IL-1β. Our results clearly show that although Gata6-KO

\textsuperscript{mice} pMΦ produce a much lower amount of PGI2-derived IL-10 compared to Gata6-WT pMΦ, their mitochondria remain healthy and functional upon LPS stimulation. This discrepancy can first be explained by the fact that different types of MΦ have been used (BMDM versus tissue-resident pMΦ). Previous work (Norris et al., 2011) showed that BMDM and pMΦ differently utilize the enzymes implicated in the AA pathway and thereby produce differential amount of prostanooids. Upon TLR4 activation, pMΦ synthesize PGI2, among others, while BMDM do not, due to the absence, or really low expression of Ptgis. Our results clearly show a strong preference for PGI2 production in pMΦ, approximately 10-fold higher levels of PGI2 are produced than PGF2. When comparing tissue-resident pMΦ, BMDM, thioglycolate-induced inflammatory peritoneal MΦ and RAW 264.7 MΦ cell line, the authors showed that tissue-resident pMΦ had the highest expression of Ptgis. Altogether, these data suggest that depending on their origin, MΦ differentially respond to TLR4 stimulation by favouring the production of specific prostanooid species. Their response to TLR4 ligand should therefore not be directly compared, at least from a prostanooid synthesis point of view. Next, it is important to note that the Gata6-KO

\textsuperscript{mice} pMΦ are still able to produce some IL-10 (about 3–4 times less than Gata6-WT pMΦ) after LPS stimulation, whereas Ip and colleagues (Ip et al., 2017) used IL-10-deficient MΦ. IL-10 is known to play an important role in homeostasis (Shouval et al., 2014; Zigmond et al., 2014; Girard-Madoux et al., 2016), inflammation regulation (Mossner & Zhang, 2008) and tissue regeneration (Siqueira Mietto et al., 2015) and a complete loss of IL-10 could explain cell instability and mitochondria dysfunction. The amount of IL-10 produced must be finely tuned, and although a small amount seems to be sufficient for tissue-resident pMΦ mitochondria health, it is less effective for IL-1β regulation.

It is interesting to note that blockade of the IL-10 signalling pathway using xyl-10R antibody induced both mRNA and protein expression of IL-1β and Nlrp3, suggesting that a complete blockade of the signalling pathway directly affects IL-1β and inflammasome components production and that the small amount of IL-10 still produced in Gata6-KO

\textsuperscript{mice} pMΦ is sufficient to avoid this direct impact. We also show that PGI2-derived IL-10 inhibits the production of both classically produced cytokines such as TNF and IL-1β but via two completely different mechanisms, TNF being inhibited directly at the mRNA level and IL-1β during a later stage of processing. These data point out that the anti-inflammatory effect of IL-10 is dependent on its target and emphasizes the fact that careful...
consideration should be taken when modulating the response to inflammation.

Marketed therapeutics targeting IL-1β are mostly focused on blocking its receptor (Dinarello et al., 2012) and thereby its signaling. Although proven efficient in reducing symptoms in some contexts (Cunnane et al., 2001; Bresnihan et al., 2004; Goldbach-Mansky et al., 2006; Ridker et al., 2017), these drugs do not treat the cause of aberrant IL-1β production and need to be constantly taken to prevent overwhelming inflammation. Our work provides insight into IL-1β regulation, which will be necessary to provide new therapeutic options directed to the source of the dysfunction and improve IL-1β control to ameliorate diseases.

**Figure 5.** The aberrant IL-1β production by Gata6-KOmye pMΦ is not led by mitochondria dysfunction.

A Flow cytometry analysis of Mitotracker (MT) Green and Red integration in Gata6-WT and Gata6-KOmye naïve pMΦ (gated on F4/80hi Tim-1hi) in vivo, 30 min after intraperitoneal injection (i.p.) of 1 μM of Mitotracker Green and Red. Data shown are representative of 3-5 independent mice of each genotype and are expressed as mean ± SEM. Student’s t-test analysis was performed.

B Flow cytometry analysis of Mitotracker Green and Red staining in Gata6-WT and Gata6-KOmye pMΦ (gated on F4/80hi Tim-1hi) unstimulated (-) or after LPS (100 ng/ml), IL-10 (10 ng/ml), αIL-10R (5 μg/ml) or isotype (5 μg/ml) stimulation for 16 h in vitro. Data shown are pooled from three independent experiments and normalized to WT unstimulated.

C Flow cytometry analysis of MitoSox staining in Gata6-WT and Gata6-KOmye pMΦ (gated on F4/80hi Tim-1hi) unstimulated (-) or after LPS (100 ng/ml), IL-10 (10 ng/ml), αIL-10R (5 μg/ml) or isotype (5 μg/ml) stimulation for 16 h in vitro. Data shown are pooled from three independent experiments and normalized to WT unstimulated.

D IL-1β ELISA of supernatants of Gata6-WT and Gata6-KOmye pMΦ unstimulated (-) or stimulated with 100 ng/ml LPS, 500 μM MitoTEMPO, 10 mM NAC, 0.5 μM MitoQ for 16 h. Data are representative of at least 3 experiments.

Data information: Data are expressed as mean ± SEM, and two-way ANOVA statistical analysis with Tukey’s multiple comparison post-test was performed unless otherwise stated. *P < 0.05, **P < 0.001.
Figure 6.
Materials and Methods

**Mice**

Lysozyme M Cre-recombinase “knock-in” congenic mice on the C57BL/6 background (‘Lyz2Cre’, B6.129P2-Lyz2tm1(cre)Ivf/J) and conditional “floxed” Gata6-deficient mice (‘Gata6F1, Gata6fltn1(cre)Ifo/J’) were obtained from the Jackson Labs and bred in our animal facilities as previously described (Rosas et al., 2014). All mice were sex-matched and between 6–12 weeks of age at the time of use, unless otherwise stated. All animal work was conducted in accordance with Institutional and UK Home Office guidelines.

**Reagents**

Ultra-pure LPS-EB from E. coli O111:B4, Pam3CSK4, Poly(I:C) (HMW), Flagellin, R848, and CpG ODN 1826 were purchased from InvivoGen. MitoTEMPO, N-acetyl-L-cysteine, Ac-YVAD-cmk, nigericin, ATP and picotamide were purchased from Sigma. Beraprost, cicaprost, iloprost, U46619 and mitoquine were purchased from Cambridge Bioscience. PGE2 was purchased from R&D. MCC950 sodium salt was a kind gift from Prof. Avril Robertson (University of Queensland). Recombinant IL-1ra was purchased from R&D System. Recombinant IL-10 and TNF were purchased from PeproTech and the anti-IL-10R Antibody (clone 1B1.3A, Bio X Cell). Etanercept was purchased from Sigma-Aldrich. GolgiBlock was purchased from BD Biosciences.

**Resident peritoneal macrophages (pMφs) isolation and culture**

Resident peritoneal macrophages (pMφs) were obtained via peritoneal lavage with 5 ml lavage solution (PBS (Invitrogen) supplemented with 5 mM EDTA and 4% foetal calf serum (FCS)). Lavages of the same genotype were pooled and resuspended in complete medium (RPMI 1640 supplemented with 10% FCS, 100 U/ml penicillin, 10 μg/ml streptomycin and 400 μM L-glutamine (Invitrogen)). Typically, the cells were plated and left to adhere for 3 h at 37°C, 5% CO₂ before being washed two times with warm complete medium and further stimulated as indicated. When needed, cells were plated on Transwell permeable supports (Corning).

**Flow cytometry**

The following antibodies were used for flow cytometry analysis: CD11b (clone M1/70) was purchased from BD Biosciences; MHCII (clone M5/114.15.2), Tim4 (clone RMT4-54), F4/80 (clone BM8), Ly6G (clone IA8) from BioLegend; pro-IL-1β (clone NJTEN3) and matching isotype control antibody were purchased from eBioScience. For extracellular staining, cells were collected and resuspended in flow cytometry buffer (4% FCS in PBS) containing 4 μg/ml of Fc receptor blocking antibody 2.4G2 (homemade) for 15 min on ice. Cells were then stained for 30 min with the indicated antibodies. For intracellular staining, cells were first fixed for 15 min with 2% paraformaldehyde (PFA) and then permeabilized and blocked at 4°C for 30 min in permeabilization buffer (0.5% bovine serum albumin (BSA), 5 mM EDTA, 2 mM NaN₃ and 0.5% saponin) containing 4 μg/ml 2.4G2 blocking antibody (homemade). Cells were then stained with the indicated antibodies for 1 h at 4°C in permeabilization buffer. Flow cytometry was performed on Cyan (Beckman Coulter) or Attune (Thermo Fisher) flow cytometer and analysed with FlowJo software.

**Cytokine measurement**

Supernatants were collected at the indicated time points, transferred to V-bottom 96-well plate and centrifuged at 500 × g for 5 min. The supernatant was carefully removed and placed into a fresh 96-well V-bottom plate (cell-free supernatant). If the supernatants were not assessed for cytokine production immediately, samples were stored at −80°C until use. Supernatants were assayed for IL-1β (R&D Systems), TNF and IL-10 (BD OptEIA™, BD Biosciences) following the manufacturer’s instructions.

**Caspase1 activity measurement**

Caspase1 activity was evaluated using the FAM FLICA™ Caspase-1 Kit (Bio-Rad), following the manufacturer’s instructions.

**Mitochondrial content measurement**

MitoTracker Green FM, MitoTracker DeepRed and MitoSOX Red mitochondrial superoxide indicator were purchased from Thermo Fisher. In vitro staining was performed following the manufacturer’s instructions. For in vivo staining, naïve mice were intraperitoneally (i.p.) injected with 1 μM MitoTracker Green FM or MitoTracker DeepRed diluted in PBS. Mice were sacrificed 30 min after injection, and peritoneal cells were collected as described above. The cells were then stained for cell surface antigens and subsequently analysed by flow cytometry.
Immunoblotting

Proteins were isolated using RIPA buffer (Santa Cruz), following manufacturer’s instructions. Cell-free culture supernatants were concentrated using methanol/chloroform precipitation. Briefly, one volume of methanol and one-quarter volume of chloroform were added to the supernatant, vortexed vigorously for 20 s and then centrifuged at 20,000 × g for 10 min. After centrifugation, the upper phase was aspirated and one volume of methanol was added, vortexed for 20 s and centrifuged at 20,000 × g for 5 min. The supernatant was removed, and the pellets briefly dried in a heat block set at 55°C for 10 min. After drying, the pellet was resuspended in 1 × laemml buffer (Bio-Rad), vortexed and heated to 95°C for 5 min. Proteins were run on SDS–PAGE gels and transferred to PVDF membranes (Bio-Rad). The primary antibodies used were goat anti-mouse IL-1β (catalogue number AF-401-NA; R&D Systems), rat anti-human/mouse Nlrp3 (clone 768319; R&D Systems), mouse anti-mouse caspase1 p20 (clone Casper-1; Adipogen), rabbit anti-mouse ASC (clone AL177, Adipogen), rabbit anti-mouse Ptgis (catalogue number ab23668, Abcam) and mouse anti-mouse β-actin (clone AC-74, Sigma). The secondary antibodies used were rabbit anti-goat (Dako), goat anti-rabbit (Dako), sheep anti-mouse (GE healthcare life sciences) and rabbit anti-rat (GE healthcare life sciences). Western blots were quantified using ImageJ.

Quantitative real-time PCR

Total RNA was isolated from cells using RNeasy Mini or Micro Kit (Qagen) following the manufacturer’s instructions, and at least 350 ng were reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). mRNA levels were quantified using a ViiA™ 7 Real-Time PCR System (Applied Biosystem) and Power SYBR Green PCR Master Mix (Thermo Fisher). The gene expression values were normalized to Ywhaz expression and normalized to WT control. The primers used were as follows: Il1b 5'-ATGAAGGGCTCTTCCAAAAC-3' and 5'-ATGTCGTGTCGCGAGAA TTGTG-3'; Nlrp3, 5'-TGGGCCAAATAAATGATCGGC-3' and 5'-TTTC ACCAAACGTAGGGCTC-3'; Tnf, 5'-TACGCCAAGGGCTAGAAGAAA C-3' and 5'-ACAAGGTACACACCATCAGG-3'; Casp1, 5'-CACCGCTC TGGAGATGGTA-3' and 5'-CTTCCAAGCTTGGGCACCT-3'; Il10, 5'-GGTTGCCAAGGCTTATCGGA-3' and 5'-GAGAAATCGGTAGACAG CGCC-3'; Ywhaz 5'-TTGACAGCAAGACGAGGT-3' and 5'-GAA GCATTGGGGATCAAGAA-3'.

Lipid analysis

Peritoneal lavages of Gata6-WT and Gata6-KO mice were collected. Cells were washed with complete medium and plated for 3 h with or without 100 ng/ml LPS. Supernatants were collected, snap frozen and stored at −80°C prior to lipid extraction and analysis. Lipids were extracted by adding a 1.25 ml solvent mixture (1 M acetic acid/isopropanol/hexane; 2:20:30, v/v/v) to 0.5 ml supernatants in a glass extraction vial and vortexed for 30 s. 1.25 ml hexane was added to samples and after vortexing for 30 sec., tubes were centrifuged (500 g for 5 min at 4°C) to recover lipids in the upper hexane layer (aqueous phase), which was transferred to a clean tube. Aqueous samples were re-extracted as above by addition of 1.25 ml hexane. The combined hexane layers were dried in a RapidVap (Labconco) at 30°C, resuspended in 100 μl methanol and stored at −80°C. Lipids were then separated on a C18 Spherisorb ODS2, 5 μm, 150 × 4.6-mm column (Waters) using a gradient of 50–90% B over 20 min (A, water:acetonitrile:acetic acid, 75:25:0.1; B, methanol:acetonitrile:acetic acid, 60:40:0.1) with a flow rate of 1 ml/min. Products were quantitated by LC/MS/MS electrospray ionization on an Applied Biosystems 4000 Q-Trap using parent-to-daughter transitions of m/z 351.2–m/z 271.1 (PGE2), m/z 355.2–m/z 275.1 (PGE2-d4), m/z 369.2–m/z 169.1 (TXB2), m/z 369.2–m/z 245.1 (6-keto-PGF1α), all [M-H], with collision energies of −20 to −36 V. Source parameters: TEM 650, IS -5000, CUR -35, GS1 60, GS2 30, EP -10. Products were identified and quantified using PGE2, 6-Keto-PGF1α, TXB2 and PGE2-d4 (10 ng each was added to samples prior to extraction) standards run in parallel under the same conditions. Chromatographic peaks were integrated using Analyst software (Sciex). The criteria for assigning a peak were signal:noise of at least 3:1 and with at least 7 points across a peak. The ratio of analyte peak areas to internal standard was taken and lipids quantified using a standard curve made up and run at the same time as the samples and the amount of lipids were normalized to the number of pMΦ.

Immunofluorescence

Cells were plated in complete medium on cover glass (VWR, thickness no. 1) and left to adhere for 3 h before washing 3 times with complete medium. Following indicated treatment, cells were fixed for 15 min with 4% PFA and permeabilized and blocked for 30 min with PBS containing 0.1% Triton X-100 (Sigma), 10% FCS and 10% rabbit serum. The cells were then stained overnight with the following antibody: rabbit anti-mouse ASC (catalogue number AL177, Adipogen) and F4/80-AF488 (clone BM8, BioLegend). Following washes, secondary antibody (Goat anti-rabbit-AF594, Thermo Fisher) was applied for 1 h. DAPI (Thermo Fisher) was applied as counterstain, and cells were mounted on microscope slides (Thermo Scientific) using fluorescent mounting medium (Dako). Pictures were taken using LSM800 confocal laser scanning microscope (Zeiss).

Statistical analysis

Results are expressed as the mean ± SEM. Data were analysed with two-way ANOVA followed by post-tests, unless otherwise stated. A P < 0.05 was considered statistically significant (*P < 0.05, **P < 0.01, ***P < 0.001). All statistics were performed using GraphPad Prism 6 software.

Data availability

Microarray expression data from wild-type and Gata6-deficient tissue-resident peritoneal macrophages data are available via GEO (https://www.ncbi.nlm.nih.gov/geo/) (GSE47049).

Expanded View for this article is available online.

Acknowledgements

We thank the staff of our animal facilities for the care of the animals. P.R.T is funded by the Wellcome Trust Investigator Award (2107964/2/152/2) and the UK
Dementia Research Institute and L.C.D was funded by the Wellcome Trust (103973/2/14/2). C.E.B is funded by the Wellcome Trust Investigator Award (108045/2/15/2). S.J.O was funded by a Sir Henry Dale Fellowship jointly funded by the Wellcome Trust and the Royal Society (Grant Number 099953/2/12/2). We would like to thank Drs. Mathew Clement and Gareth Jones for assistance with reagents.

Author contributions
Conceptualization: NI, RJP, MR, LCD, CEB, PRT; Methodology: NI, RJP, MR, SJO; Experimentation: NI, RJP, MR, MAC, DF, VT; Resources: SJO, AABR, VO'D, PRT; Data analysis: NI, RJP, MR, CEB, VO'D, PRT; Writing & Editing: NI, PRT; Supervision: PRT All authors read and commented on the manuscript.

Conflict of interest
AABR is a co-inventor on granted patents (US 10,538,487, EP 3259253) and patent applications (WO2018215818, WO2017140778, WO2016131098) for NLRP3 inhibitors, which are licensed to Infazome Ltd, a company headquartered in Dublin, Ireland. Infazome is developing drugs that target the NLRP3 inflammasome to address unmet clinical needs in inflammmatory disease.

References


Khameneh HJ, Ho AW, Laudisi F, Derks H, Kandasamy M, Sivasankar B, Teng GG, Mortellaro A (2017) CsA regulates IL-1beta production and leukocyte recruitment in a murine model of monosodium urate crystal-induced peritonitis. Front Pharmacol 8: 10


Murray PJ (2005) The primary mechanism of the IL-10 regulated antiinflammatory response is to selectively inhibit transcription. Proc Natl Acad Sci USA 102: 8686 – 8691


Stewart AG, Dubbin PN, Harris T, Dusting GJ (1990) Platelet-activating factor as a second messenger in the release of lysosomal enzymes and superoxide anions from leukocytes and endothelial cells. Proc Natl Acad Sci USA 87: 3215 – 3219


License: This is an open access article under the terms of the Creative Commons Attribution 4.0 License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.