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Facilitation of IL-22 production from innate lymphoid cells by prostaglandin E\(_2\) prevents experimental lung neutrophilic inflammation

Jennifer M Felton, Rodger Duffin, Calum T Robb, Siobhan Crittenden, Stephen M Anderton, Sarah E M Howie, Moira K B Whyte, Adriano G Rossi, Chengcan Yao

ABSTRACT

Acute lung injury is a neutrophil-dominant, life-threatening disease without effective therapies and better understanding of the pathophysiological mechanisms involved is an urgent need. Here we show that interleukin (IL)-22 is produced from innate lymphoid cells (ILC) and is responsible for suppression of experimental lung neutrophilic inflammation. Blocking prostaglandin E\(_2\) (PGE\(_2\)) synthesis reduces lung ILCs and IL-22 production, resulting in exacerbation of lung neutrophilic inflammation. In contrast, activation of the PGE\(_2\) receptor EP4 prevents acute lung inflammation. We thus demonstrate a mechanism for production of innate IL-22 in the lung during acute injury, highlighting potential therapeutic strategies for control of lung neutrophilic inflammation by targeting the PGE\(_2\)/ILC/IL-22 axis.

INTRODUCTION

Acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) is a life-threatening neutrophil-dominant disease with a significant morbidity and mortality for which effective therapies are currently lacking. Excessive pulmonary neutrophil recruitment mediates lung tissue damage, despite the beneficial role of neutrophils in promoting tissue repair. Therefore, there is a need for more effective medicinal agents for use in these severe and often lethal lung injury syndromes.

Interleukin (IL)-22 is an IL-10 family member cytokine that is produced by both adaptive and innate immune cells. Recently, type 3 innate lymphoid cells (ILC3s) have been identified as the main source of innate IL-22. IL-22 is expressed in healthy human lung tissue, and patients with sarcoidosis and ARDS have decreased IL-22 levels. Innate IL-22 is protective against acute epithelial damage and inflammation in the lung as neutralisation of IL-22 exacerbates bacterial and viral infections and exogenous IL-22 attenuates bacterial pneumonias.

Non-steroidal anti-inflammatory drugs (NSAID) inhibit cyclooxygenase activity and subsequent production of prostaglandins and are generally used to manage many inflammatory conditions. Use of NSAIDs worsens lung bacterial infections and is a risk factor for severe sepsis. In contrast, prostaglandins including prostaglandin E\(_2\) (PGE\(_2\)) are used for treating critical lung diseases with improved oxygenation and decreased pulmonary artery pressures. PGE\(_2\) exerts its biological actions through engagement of its four receptors, namely EP1–4. We have recently found that PGE\(_2\) promotes IL-22 production from both T cells and ILC3s.

In this study, we have investigated the hypothesis that PGE\(_2\) inhibits acute lung neutrophilic inflammation through modulating lung ILC3 production of IL-22.

METHODS

Wild-type C57BL/6 mice were purchased from Harlan UK. Rag1\(^{-/-}\) mice and mice with selective EP4 deficiency in T cells (Lck\(^{Cre}\)EP4\(^{-/-}\) mice by crossing Lck\(^{Cre}\) mice to EP4-flox mice\(^{9}\)) were maintained under specific pathogen-free conditions in accredited animal facilities. Mice were aged >7 weeks old at the beginning of use. All experiments were conducted in accordance with the UK Scientific Procedures Act of 1986 and had local institutional ethical approval. The ALI mouse model was induced by intratracheal injection of 10\(\mu\)g of lipopolysaccharide (LPS) in combination with recombinant IL-22, EP2 and/or EP4 agonists, or indomethacin when indicated. After 24 hours, bronchoalveolar lavage (BAL) fluid and lung tissues were collected. Immune cells and cytokine production were measured by flow cytometry or ELISA. All data were expressed as mean±SD. The Student’s t-test or Mann-Whitney U test was used for statistical analyses by Prism V6 (GraphPad) and p<0.05 was considered statistically significant.

RESULTS

To study the cellular sources of innate IL-22 in the lung, we administered a low dose (10\(\mu\)g) of LPS to Rag1\(^{-/-}\) mice, which have no adaptive T and B cells, and analysed IL-22 production from various lung immune cells 24 hours later using flow cytometry. In naïve mice, very few (~0.1%) CD11b\(^{+}\)CD11c\(^{+}\)Ly-6G\(^{-}\)mononuclear phagocytes (MNP) or CD11b\(^{+}\)Ly-6G\(^{-}\) neutrophils produced IL-22, while ~0.4% CD11b\(^{+}\)CD11c\(^{+}\)Ly-6G\(^{-}\)CD90\(^{+}\) ILCs expressed IL-22. In naïve mice, very few (~0.1%) CD11b\(^{+}\)CD11c\(^{+}\)Ly-6G\(^{-}\)mononuclear phagocytes (MNP) or CD11b\(^{+}\)Ly-6G\(^{-}\) neutrophils produced IL-22, while ~0.4% CD11b\(^{+}\)CD11c\(^{+}\)Ly-6G\(^{-}\)CD90\(^{+}\) ILCs expressed IL-22 (figure 1A,B and online supplementary figure). Intratracheal administration of LPS to the lung strikingly increased IL-22 production from ILCs by 10-fold to ~4\(\%\) (n=3, p=0.004), but not from MNPs or neutrophils (figure 1A,B and online supplementary figure). The mean fluorescence intensity of IL-22...
was significantly enhanced by LPS from ILCs (p=0.00007), suggesting that IL-22 production was also increased at the single cell level (figure 1C). Consistently, in response to appropriate stimulus lung innate immune cells produced IL-22 (figure 1D). IL-22 has been demonstrated to protect against inflammation at mucosal sites including the lung. To test whether IL-22 protects against ALI, we administered recombinant IL-22 intratracheally into mice immediately before LPS challenge. Exogenous IL-22 significantly suppressed LPS-induced acute lung neutrophilic inflammation (p=0.006) but had little effect on BAL total protein levels (figure 1E, n=3–5).

We have previously shown that PGE₂ promotes intestinal ILC3 activation and IL-22 production. To examine whether PGE₂ similarly regulates lung ILC3s and whether this leads to suppression of acute lung inflammation, we treated naïve mice with indomethacin, a cyclooxygenase inhibitor that inhibits endogenous PGE₂ synthesis. Administration of indomethacin significantly reduced IL-22-producing ILC3s in the lung (figure 2A, n=4, p=0.048). In agreement with an increase in IL-22-producing ILC3s (figure 1A–C), LPS also induced accumulation of lung RAR-related orphan receptor gamma T (RORγT)⁺ ILC3s (p=0.027) and elevated BAL IL-22 levels (p=0.015, figure 2B,C). Coadministration of indomethacin decreased LPS-induced accumulation of RORγT⁺ ILC3s (p=0.019, n=4–8) and IL-22 production (p=0.023, n=3–9) in the lung (figure 2B,C). This reduction of ILC3s and IL-22 was associated with augmented LPS-induced acute lung neutrophilic inflammation (figure 2D, p=0.035, n=3–4). Inhibition of endogenous PGE₂, downregulated genes responsible for IL-22 signalling (eg, IL-22, IL-22Rα) and lung epithelial barrier function (eg, ZO-1, Occludin) but upregulated S100A8/S100A9, which mediate neutrophilic lung inflammation (figure 2E).

then examined whether exogenous activation of PGE2 signalling prevented ALI using selective EP2 or EP4 agonists. The EP4, but not EP2, agonist markedly suppressed LPS-induced neutrophil accumulation (figure 2F, n=5–6, p=0.005), and coactivation of EP2 and EP4 receptors reduced total protein levels in the BAL (figure 2E, p=0.007), suggesting that PGE2-EP4 signalling limits neutrophilic lung inflammation. Because PGE2, also promotes adaptive IL-22 production from T cells, we examined whether T cells are involved in PGE2-modulated innate IL-22 production in mice administered with vehicle or indomethacin for 5 days (n=4 for each group). Lung cells were restimulated with IL-23 ex vivo for 4 hours before staining. (B-E) Mice were administered with indomethacin (Indo) or vehicle control (Veh) via drinking water for 4 days and then challenged intratracheally with PBS or LPS. Lung tissue and BAL fluid were harvested at 24 hours after LPS or PBS challenge. Lung RORγt+ ILC3s (B, n=8, 8, 3), BAL IL-22 levels (C, n=8, 9, 3) and neutrophils (D, n=3, 4, 3) in the BAL were measured by flow cytometry. (E) Summarised gene expression in lungs determined by quantitative real-time PCR. Expression level for each gene in the vehicle group was set as 1 (n=4 for each group). (F) BAL neutrophil numbers and total protein levels in mice administered intratracheally with PBS or LPS plus an EP2 agonist (Butaprost), an EP4 agonist (L-902,688) or both (n=5–6 mice per group). (G) BAL neutrophil numbers and total protein levels in EP4fl/fl Lck Cre (n=7) or control EP4fl/fl (n=5) mice administered intratracheally with LPS. P values are calculated by Student’s t-tests or Mann-Whitney U tests. (H) Proposed mechanistic schematic diagram for PGE2 functions on restriction of acute lung neutrophilic inflammation through amplifying the ILC3/IL-22 pathway. BAL, bronchoalveolar lavage; IL, interleukin; ILC, innate lymphoid cell; PBS, phosphate buffered saline; PGE2, prostaglandin E2.

**Figure 2** PGE2 promotes innate IL-22 production from lung ILC3s and inhibits acute lung injury. (A) IL-22-producing ILC3s in the lung from mice administered with vehicle or indomethacin for 5 days (n=4 for each group). Lung cells were restimulated with IL-23 ex vivo for 4 hours before staining.
Despite these important novel findings, we acknowledge this report has several limitations. First, the numbers of animals used in experiments were limited, so further validation of the findings may be needed by increasing mice numbers. Second, therapeutic effects of PGE₂ analogues and rIL-22 on established neutrophilic lung inflammation induced by LPS or by other stimuli were not examined. Third, we have shown that exogenous IL-22 prevented ALI and that blocking PGE₂ signalling reduced numbers of IL-22⁺ ILC3s in the lung. However, given the multiple roles of IL-22 in lung homeostasis as well as injury/inflammation, further studies to determine direct roles of endogenous IL-22 in ALI are warranted. Fourth, the protective actions of the PGE₂/ILC3/IL-22 axis have not been examined in human patients with ALI/ARDS, although findings from our mouse studies could have important translational implications.

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Contributors  CY and AGR conceived the project and designed the experiments. JMF, SC, RD and CTR performed the experiments. JMF, AGR and CY wrote the manuscript. SMA, SEMH and MKBW provided guidance and edited the manuscript.

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REFERENCES
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