Interleukin-6 neutralization alleviates pulmonary inflammation in mice exposed to cigarette smoke and poly(I:C)

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Abstract

Increased systemic and pulmonary levels of IL-6 (interleukin-6) are associated with the severity of exacerbations and decline of lung function in patients with COPD (chronic obstructive pulmonary disease). Whether IL-6 is directly involved or plays a bystander role in the pathophysiology of COPD remains unclear. Here we hypothesized that neutralizing circulating levels of IL-6 would modulate episodes of acute pulmonary inflammation following CS (cigarette smoke) exposure and virus-like challenges. For this purpose, we used a model where C57BL/6 mice were exposed to CS twice daily via a nose-only system, and concomitant periodic intranasal challenge with poly(I:C), a synthetic ligand for TLR3 (Toll-like receptor 3) that mimics the encounter with double stranded RNA that is carried by influenza-like viruses. This protocol recapitulates several aspects of acute pulmonary inflammation associated with COPD, including prominent airway neutrophilia, insensitivity to steroid treatment and increased levels of several inflammatory cytokines in BAL (bronchoalveolar lavage) samples. Although IL-6-deficient mice exposed to CS/poly(I:C) developed pulmonary inflammation similar to WT (wild-type) controls, WT mice exposed to CS/poly(I:C) and treated intraperitoneally with IL-6-neutralizing antibodies showed significantly lower blood counts of lymphocytes and monocytes, lower BAL levels of IL-6 and CXCL1 (CXC chemokine ligand 1)/KC (keratinocyte chemoattractant), as well as reduced numbers of BAL neutrophils, lymphocytes and macrophages. Our results thus indicate that the systemic neutralization of IL-6 significantly reduces CS/poly(I:C)-induced pulmonary inflammation, which may be a relevant approach to the treatment of episodes of acute pulmonary inflammation associated with COPD.

Key words: chronic obstructive pulmonary disease, inflammation, interleukin-6, neutralizing antibody

INTRODUCTION

COPD (chronic obstructive pulmonary disease) affects approximately 10% of the world population, with an increased prevalence up to 50% among cigarette smokers. Importantly, because of sustained smoking habits in developing countries such as India and China, COPD is expected to move from the fourth to the third leading cause of global deaths by 2020 [1]. COPD is a complex disease that encompasses divergent pathologies of the lung including emphysema and chronic bronchitis. In distal lung tissues, the progressive destruction of alveolar walls leads to a reduction of gas exchange while in the small airways chronic inflammation, mucus metaplasia and fibrotic remodelling increasingly impair the respiratory function [2]. Most of the burden of COPD resulting in more rapid lung function decline is because of episodes of acute inflammation known as exacerbations. These can often be life-threatening, accelerate disease progression, and can represent a large proportion of the healthcare cost of COPD [1]. Exacerbations of COPD are associated with increased airway levels of several chemokines [e.g. IL (interleukin)-8/CXCL8 (CXC chemokine ligand 8)] and inflammatory cells (e.g. neutrophils), as well as higher systemic concentrations of certain inflammatory cytokines such as IL-6 and TNFα (tumour necrosis factor α), possibly as an overspill from the lungs [3]. Indeed, elevated systemic levels of IL-6 are a hallmark of COPD severity [4,5] and correlate with the degree of lower airway inflammation [6], the decline of lung function [7,8], as well as the development of COPD-associated pulmonary hypertension.

Abbreviations: AE, acute exacerbation; BAL, bronchoalveolar lavage; CCL, CC chemokine ligand; COPD, chronic obstructive pulmonary disease; CS, cigarette smoke; CXCL, CXC chemokine ligand; Dex, dexamethasone; GM-CSF, granulocyte/macrophage colony-stimulating factor; H&E, haematoxylin and eosin; IFNγ, interferon-γ; IL, interleukin; IR interferon-γ-inducible protein; KC, keratinocyte chemoattractant; MIP, macrophage inflammatory protein; RANTES, regulated upon activation, normal T-cell expressed and secreted; TLR3, Toll-like receptor-3; TNFα, tumour necrosis factor α; WT, wild-type.

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Materials and Methods

Animals and nose-only CS exposure combined with poly(I:C) challenge
All animal studies were conducted under Institutional Animal Care and Use Committee (IACUC)-approved protocols, in accordance with the Animal Welfare Act. Female C57BL/6 WT mice 8–12-week-old, were obtained from Taconic Farms. Age-matched IL-6-deficient female C57BL/6 mice bearing a targeted mutation for the IL-6 gene (B6:129S2-I6m1Kopf/J) were obtained from the Jackson Laboratory. Figure 1(A) summarizes the protocol of CS exposure, poly(I:C) challenge and anti-inflammatory treatment. Reference cigarettes 3R4F were obtained from the University of Kentucky, College of Agriculture, Lexington, KY, U.S.A. On days 0–4 and 7–10, the mice were exposed to either air or CS for 42 min twice daily (mornings and afternoons) using a Baumgartner-Jaeger CSM 2080 cigarette smoking machine from CH Technologies connected to a 64 port nose-only system. On days 0, 3 and 7, these mice were also challenged intranasally with either PBS or 50 μg of poly(I:C) in PBS (Invivogen) prepared daily using a BioRad thermocycler to induce annealing. On day 11 of the protocol all the mice were killed by CO2 asphyxiation and tissue samples were collected to evaluate modulation of the disease phenotype.

Steroid and antibody treatment
On days 2, 4, 7 and 9 of the protocol (Figure 1), the mice were injected intraperitoneally with saline alone (vehicle controls), Dex (dexamethasone) in PBS (3 mg/kg of body weight; Sigma–Aldrich), anti-mouse IL-6 in saline (25 mg/kg of body weight, rat IgG1, clone MP520F3; Bio X Cell) or matching isotype control in saline (25 mg/kg of body weight, rat IgG1; Bio X Cell). These antibodies were shown previously to modulate inflammation in vivo in a CIA (collagen-induced arthritis) model [16]. Note that in preliminary experiments that evaluated a range of antibody concentrations from 5 to 25 mg/kg of body weight, we observed no evidence of a dose–response effect. However, we found the dose at 25 mg/kg of body weight to provide more consistent results, which is the reason why we focused on this dose to generate the data shown in the present study.

Evaluation of systemic and airway markers of inflammation
BAL (bronchoalveolar lavage) samples were collected for the assessment of airway cellularity and cytokine levels. Blood samples were collected for the assessment of whole blood cellularity and serum levels of cytokines. Total BAL cellularity, total leucocyte numbers and specific blood counts of lymphocytes, neutrophils, monocytes, basophils and eosinophils were measured using a Cell-Dyn 3700 haematology analyser (Abbott Diagnostics). Cytokines were measured using ELISA kits for IFNα (interferon α; Life Technologies) according to the manufacturer’s instruction, and multiplex protein kits from Millipore that were analysed with a Luminex 100.

Histopathology
Lung tissues collected from at least five animals per group per study were formalin-fixed and paraffin embedded. Tissue sections (5-μm-thick) were stained with H&E (haematoxylin and eosin), and were evaluated by a board-certified pathologist. Single sections from each left caudal lung lobe were scored according to the system described in Table 1. Histopathology was evaluated using an Olympus BX51 microscope containing Olympus UPlanAPO objectives in ×4, ×10, ×20 and ×40 magnification and representative images were captured with a SPOT Insight 2.0 mp colour camera and associated software. All images were identically post-processed to increase brightness, contrast and sharpening using Adobe Photoshop CS 8.0.

Statistical analysis
Differences between treatments were analysed with Prism 5 (GraphPad) using a non-parametric Mann–Whitney U test for the comparison of unpaired groups. A P < 0.05 was considered significant. Results are means ± S.E.M., except for histopathological scoring shown as individual values with means.

Results
Combined exposure to CS and poly(I:C) synergistically induces airway neutrophilia and inflammatory cytokines
In contrast with protocols evaluating the effects of long-term CS exposure, which stimulate background levels of pulmonary
Figure 1  Characterization of the 2-week CS and poly(I:C) model
(A) CS/poly(I:C) exposure and treatment regimen. (B) Modulation of BAL cellularity in response to CS, poly(I:C) or CS/poly(I:C) exposure. (C) Comparison of BAL levels of GM-CSF, IFNα, IL-1β, IL-6, CXCL10/IP-10, CXCL1/KC, CCL3/MIP-1α,
inflammation that progressively lead to COPD-like emphysema in mice [17], in the present study we used an abbreviated version of a 4-week model originally developed by Kang et al. [15]. This protocol recapitulates aspects of acute airway inflammation as seen during exacerbations of COPD. WT C57BL/6 female mice submitted to the 2-week protocol presented in Figure 1(A) developed robust airway inflammation with a significant increase in total BAL cellularity compared with mice exposed to CS alone (Figure 1B, \( P = 0.0046 \)). This increase in BAL cellularity was mainly due to a preponderance of airway neutrophilia in mice exposed to CS/poly(I:C) compared with mice exposed to CS alone (\( P = 0.0012 \)), or mice challenged with poly(I:C) only (\( P = 0.0001 \)). Although the increase in BAL macrophage numbers did not reach statistical significance, BAL lymphocyte numbers were also found to be significantly increased in mice exposed to CS/poly(I:C) compared with mice exposed to CS alone (\( P = 0.0001 \)). Figure 1(C) shows that several cytokines commonly associated with exacerbations of COPD were increased in the BAL of mice exposed to CS/poly(I:C), including IFN\( \alpha \) (\( P = 0.0079 \)), IL-1\( \beta \) (\( P = 0.0005 \)), IL-6 (\( P = 0.0303 \)), CXCL10/IP-10 (interferon-\( \gamma \)-inducible protein)-10 (\( P = 0.0001 \)), KC (keratinocyte chemoattractant) (\( P < 0.0001 \)), CCL3/MIP-1\( \alpha \) (CC chemokine ligand/macrophage inflammatory protein-1\( \alpha \)) (\( P = 0.0002 \), CCL5/RANTES (regulated upon activation, normal T-cell expressed and secreted) (\( P = 0.0012 \)) and TNF\( \alpha \) (\( P = 0.0004 \)). Consistent with the concept that cytokine overproduction in the lung may overspill into the systemic compartment [3], only the chemokine found at the highest level in BAL samples, CXCL10/IP-10, was also found significantly elevated in serum samples (Figure 1D, \( P = 0.0079 \)). Other BAL cytokines seemingly remained at baseline levels or were undetectable in serum samples (results not shown).

### IL-6-deficient mice are susceptible to the combined exposure to CS and poly(I:C) similarly to WT mice

Pauwels et al. [14] have reported that IL-6-deficient mice exposed to CS alone for 4–24 weeks develop pulmonary inflammation independently of increased levels of IL-6 protein and RNA in the lungs. These results suggested that IL-6 plays a limited role in chronic aspects of COPD but did not rule out the contribution of IL-6 to episodes of acute pulmonary inflammation associated with exacerbations. Thus we sought to evaluate whether IL-6 deficiency would protect from, or modulate, the acute pulmonary inflammation triggered by exposure to CS and poly(I:C). Figure 2(A) shows that WT mice exposed to CS/poly(I:C) experienced a significant reduction in total blood leucocytes (\( P = 0.0079 \)), mainly driven by decreased lymphocyte counts (\( P = 0.0079 \)). IL-6-deficient mice exposed to CS/poly(I:C) did not show such a substantial reduction in total circulating leucocytes or lymphocytes, perhaps due to seemingly lower lymphocyte counts at baseline. Conversely, IL-6-deficient mice exposed to CS/poly(I:C) showed significantly decreased blood neutrophil counts (\( P = 0.0317 \)), which was not observed in WT animals. On the other hand, WT mice exposed to CS/poly(I:C) demonstrated significantly lower blood basophil counts (\( P = 0.0079 \)), whereas IL-6-deficient mice did not, probably because these mutant mice showed intrinsically lower blood basophil counts at baseline (\( P = 0.0317 \)). Variations of blood monocytes and eosinophils were observed, but remained below significance in all groups. Histologically, the lungs of IL-6-deficient mice exposed to CS/poly(I:C) were comparable with those of WT animals despite a trend towards decreased inflammatory infiltrates around the airways and blood vessels, albeit with a slightly increased thickening of the alveolar walls (Figure 2B). These subtle variations did not translate into significant differences in terms of pathological

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**Table 1 Histopathological scoring system**

Scores were determined by examining H&E-stained 5-μm-thick tissue sections from each left caudal lung lobe of lung tissues collected from at least five animals per group per study.

<table>
<thead>
<tr>
<th>Lung region</th>
<th>Grade of tissue inflammation</th>
<th>Pathological score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchovascular</td>
<td>Scattered inflammatory infiltrates around large airways</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Thicker inflammatory infiltrates around large airways, some mid-sized airways and around rare large vessels</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Thicker inflammatory infiltrates around large airways, mid-sized airways, most large vessels and some mid-sized vessels, occupy &lt;30% of parenchyma</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Similar to 3 but affects large/mid/some small airways, large/mid/some small vessels, and extend into alveoli, occupy 30–50% of parenchyma</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Similar to 4 but most airways and vessels are surrounded and infiltrates occupy &gt;50% of parenchyma</td>
<td>5</td>
</tr>
<tr>
<td>Alveolar</td>
<td>Scattered cells in alveoli lumen/walls, with rare focal aggregates</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Many alveoli have small numbers of cells in lumen/walls, with few focal aggregates</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Most alveoli have small numbers of cells in lumen/walls and aggregates are frequent</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Most alveoli have moderate numbers of cells in lumen/walls, foci are frequent and coalesce, small foci of consolidation may be present</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Severe consolidation</td>
<td>5</td>
</tr>
</tbody>
</table>

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CCL5/RANTES and TNF\( \alpha \) between mice exposed to air and mice exposed to CS/poly(I:C). (D) Comparison of serum levels of CXCL10/IP-10 between mice exposed to air and mice exposed to CS/poly(I:C). Results in (B–D) are means ± S.E.M. of \( n = 5–10 \) mice per group. For statistical analysis (Mann–Whitney), \( *P < 0.05, **P < 0.01 \) or ***\( P < 0.001 \).
Figure 2  IL-6 deficiency does not protect from acute pulmonary inflammation triggered by the combined exposure to CS and poly(I:C)
scores (Figure 2C). Although IL-6-deficient mice exposed to CS/poly(I:C) did not release detectable levels of BAL IL-6 (Figure 2D), these animals developed airway inflammation that remained quite similar to that found in WT mice (Figure 2E), despite a trend towards decreased airway neutrophilia (non-significant). Thus, consistent with Pauwels et al. [14] and despite a few intrinsic differences in blood leucocyte counts, IL-6-deficient mice exposed to CS/poly(I:C) developed acute pulmonary inflammation similarly to WT animals.

**IL-6 neutralization alleviates acute pulmonary inflammation in mice exposed to CS and poly(I:C)**

The lack of differences between IL-6-deficient mice and WT animals exposed to CS/poly(I:C) was consistent with previous results obtained with mice chronically exposed to CS alone [14]. However, with deficient mice there exists a concern about developmental compensation mechanisms, particularly with a protein/cytokine as pleitropic as IL-6. Hence we investigated the modulation of IL-6 in normal animals by administering monoclonal rat anti-mouse IL-6 antibodies intraperitoneally (clone MP520F3) to WT mice exposed to CS/poly(I:C) and administered intraperitoneally with saline (vehicle). Control treatment groups included steroids (Dex) and control IgG1 antibodies. Figure 3(A) shows that a significant reduction in total circulating leucocytes was observed in mice exposed to CS/poly(I:C) (P = 0.0011), similarly because of decreased lymphocyte counts (P = 0.0013). Besides confirming the results obtained with IL-6-deficient mice, these studies also showed that intraperitoneal administration of Dex induced an even more substantial reduction of blood leucocytes (P = 0.0080), driven by a greater decrease in blood lymphocyte counts (P = 0.0027) compared with CS/poly(I:C) controls. Notably, Dex treatment also led to increased blood monocyte and basophil counts compared with CS/poly(I:C) controls (P = 0.0028 and 0.0080 respectively). In contrast, IL-6 neutralization stimulated the reduction of blood leucocytes compared with CS/poly(I:C) (P = 0.0070), due to a significant decrease in both lymphocytes (P = 0.0070) and monocytes counts (P = 0.0091). Changes in blood neutrophil and eosinophil counts remained non-significant in these studies.

This model of acute pulmonary inflammation triggered by the combined exposure to CS and poly(I:C) led to marked tissue inflammation around the blood vessels, airways and alveoli, albeit with limited evidence of alveolar enlargement (Figure 3B). On the basis of morphology and immunohistochemistry (results not shown), bronchovascular inflammation was determined to be predominantly lymphocytic, whereas interstitial and alveolar cellular infiltrates comprised a mix of lymphocytes, fewer granulocytes (e.g. neutrophils) and infrequent macrophages. When tissue sections were scored blindly, no significant difference was found in terms of bronchovascular inflammation between control mice exposed to CS/poly(I:C) and any of the treatment groups. No significant effect was detected in the alveoli, despite a trend towards reduced inflammation in all treatment groups, including IgG1-treated controls (Figure 3C).

Conversely, IL-6 neutralization by intraperitoneal injection of monoclonal antibodies resulted in the robust modulation of airway/BAL inflammation in this model (Figure 4A). Indeed, total BAL cellularity was reduced by 59% in mice treated with IL-6 neutralizing antibodies compared with CS/poly(I:C)-challenged, vehicle-treated controls (P < 0.0001). Consistent with this finding, mice treated with IL-6 neutralizing antibodies showed reduced BAL numbers of neutrophils (−49%, P = 0.0031), lymphocytes (−56%, P < 0.0001) and macrophages (−78%, P < 0.0001). The effects of neutralizing IL-6 on BAL cellularity were specific since IgG1-treated animals did not show any statistically significant reduction of any cell type. Moreover, anti-IL-6-treated mice showed consistently reduced numbers of inflammatory cells compared with IgG1-treated mice in terms of total BAL cellularity (P = 0.0004), BAL neutrophils (P = 0.0004), BAL lymphocytes (P = 0.0004) or BAL macrophages (P = 0.0006) respectively. It is important to note here that mice exposed to CS/poly(I:C) were largely insensitive to steroid treatment, thus systemic administration of Dex did not significantly modulate total BAL cellularity or BAL neutrophilia, whereas Dex treatment did reduce the number of BAL lymphocytes (P = 0.0473) and macrophages (P = 0.0090). The modulation of BAL cellularity was associated with limited yet significant differences in several cytokines and chemokines in BAL samples (Figure 4B). Although the levels of GM-CSF (granulocyte/macrophage colony-stimulating factor), CCL3/MIP-1α, CCL5/RANTES and TNFα remained comparable with those found in CS/poly(I:C) animals (vehicle controls), IL-6 neutralization depleted BAL levels of IL-6 and was associated with significantly decreased levels of BAL KC (−45%, P = 0.0220). It is worth noting that the depletion of IL-6 in BAL samples seemed largely specific (compared with IgG1-treated animals, P = 0.0007), although there was no statistically significant difference between IgG1- and anti-IL-6-treated animals in terms of BAL levels of KC. Furthermore, Dex treatment did not significantly reduce BAL levels of any cytokines/chemokines but in fact it was associated with increased levels of GM-CSF (P = 0.0021). Our results thus show that in WT animals producing normal cytokine levels in response to CS/poly(I:C), the systemic neutralization of IL-6 was sufficient to significantly alleviate acute airway inflammation, in particular BAL cellularity.

**DISCUSSION**

Episodes of acute pulmonary inflammation in COPD patients are often triggered by respiratory infections, or other environmental challenges, and may require hospitalization time. Exacerbation
Figure 3  Effects of IL-6 neutralization in the systemic and lung tissue compartment
(A) Modulation of blood cellularity in response to CS/poly(I:C) exposure in controls (vehicle) and mice administered intraperitoneally with either Dex (3 mg/kg of body weight), rat IgG1 antibodies (25 mg/kg of body weight) or rat anti-IL-6.
of COPD represent the most dramatic – and costly – aspect of the disease [1]. Specifically, bacterial and viral infections account for up to 80% of all COPD exacerbations [3], with sputum IL-1β and serum CXCL10/IP-10 being the most accurate biomarkers identifying each clinical phenotype, respectively. Although there exists a clear relationship between levels of IL-6 and exacerbation of COPD, the direct contribution of this cytokine to CS-induced pulmonary inflammation has remained elusive [13]. IL-6 is a pleiotropic cytokine that is rapidly released during the acute phase of various inflammatory responses. As a key orchestrator of the immune response and depending on the nature of the initial stimulus, IL-6 is capable of promoting either the resolution or maintenance of inflammation [18]. In BAL and serum samples from COPD patients, the presence of significantly higher levels of IL-6 compared with smokers with normal lung function and healthy controls seems difficult to interpret [4,5,7] whether IL-6 directly contributes to the pathophysiology of COPD or merely stands as an ubiquitous biomarker remains elusive [13,19].

Despite interspecies biological variations, animal models of COPD such as rodent CS exposure protocols provide invaluable flexibility to examine a variety of pathological pathways and evaluate novel therapeutic approaches. This applies particularly to treatments aiming to address the early stage of the disease, and/or episodes of acute pulmonary inflammation [17,20]. Indeed, because most of the burden of COPD ensues from exacerbations triggered by bacterial, viral or toxic agents [3], we focused on evaluating an anti-IL-6 therapeuti protocol in an AE (acute exacerbation) model in which C57BL/6 female mice are challenged intranasally with the synthetic TLR3 agonist poly(I:C), which mimics the encounter with influenza-like viruses while being daily exposed to CS. This 2-week protocol was derived from a 4-week model originally published by Kang et al. [15], and stimulates the development of pulmonary inflammation that recapitulates several key features of COPD, including the recruitment of neutrophils and lymphocytes to the airways as well high levels of IL-6, CCL3/MIP-1α, CCL5/RANTES, CXCL1/KC and CXCL10/IP-10 locally (BAL) and/or systemically (serum).

Pauwels et al. [14] have reported that IL-6-deficient mice develop pulmonary and systemic inflammation comparably with WT mice exposed to CS for 4–24 weeks. One key concern is that mutant mice unable to produce a cytokine as critical for the immune system as IL-6 must develop compensatory mechanisms in response to pro-inflammatory stimuli. Indeed, consistent with the findings by Pauwels et al. [14] when we submitted IL-6-deficient mice to our protocol we noticed that, despite minor differences in blood leucocyte counts, mutant animals developed a pattern of pulmonary inflammation similar to that found in WT controls. Several differences exist, however, between our model and the one used by Pauwels et al. [14], such as whole-body against nose-only CS exposure and the critical addition of an exacerbating agent [poly(I:C)] in our model, which dramatically enhances the release of IL-6. This is a major difference since in the studies conducted by Pauwels et al. levels of BAL IL-6 remained strikingly low (<15 pg/ml), even in WT control mice exposed to CS for up to 24 weeks, suggesting that IL-6 can play only a limited role in this model.

In WT animals we found that the combined challenge with CS and poly(I:C) led to lower numbers of blood lymphocytes. This apparent cell depletion in the periphery may be related to the local recruitment of leucocytes into the lung compartment exposed to a potent inflammatory stimulus. A similar pattern was observed in blood samples from IL-6-deficient mice, suggesting that the absence of IL-6 does not directly disrupt the trafficking of peripheral leucocytes in response to the exposure to CS and poly(I:C). Conversely, if WT animals exposed to CS/poly(I:C) and treated with steroids or anti-IL-6 antibodies were expected to show decreased pulmonary inflammation, the impact of these treatments was less clear at the systemic level. Although Dex-treated animals displayed decreased numbers of blood lymphocytes, Dex administration also led to increased counts of circulating monocytes and basophils, which was an expected profile post-steroid injection. This mixed effect of steroid treatment was associated with the finding that Dex failed to significantly diminish total BAL cellularity post-CS/poly(I:C) challenge, which was largely due to a lack of effect on airway neutrophilia. However, Dex treatment did significantly reduce BAL lymphocyte and macrophage numbers. Thus the limited effect of Dex treatment on CS/poly(I:C)-induced pulmonary inflammation is consistent with the underlying insensitivity of COPD patients to even high doses of inhaled or oral steroids [21]. Moreover, Dex treatment did not lead to the reduction of any of the cytokines analysed, but rather enhanced their release to significantly higher levels, for example, GM-CSF. This is quite an undesirable effect in the case of a growth factor considered to be an important mediator of smoke-induced inflammation [22,23]. The overall insensitivity to steroid treatment in mice exposed to CS plus poly(I:C) supports the validity of our model since it is consistent with the limited benefits from such intervention in COPD patients, in particular the modulation of cytokines released by alveolar macrophages [24,25].

It is worth emphasizing that our model focuses on acute, exacerbation-like airway inflammation in the context of daily CS exposure, which leads to the release of high levels of IL-6, in parallel with robust airway inflammation dominated by steroid-resistant neutrophilia. When mice received anti-IL-6 antibodies, lower counts of blood leucocytes were associated with the substantial reduction in BAL cellularity, i.e. decreased numbers of neutrophils, lymphocytes and macrophages in the airways. In contrast to steroid treatment, the modulation of cellular inflammation by IL-6 neutralization was also associated with an apparent decrease in BAL CCL3/MIP-1α and TNFα, albeit non-significant, as well as the significant reduction in CXCL1/KC levels, which partially drives airway neutrophilia. Therefore the main outcome of neutralizing IL-6 levels in this model seems to be the reduction of the immune response and depending on the nature of the initial stimulus, IL-6 is capable of promoting either the resolution or maintenance of inflammation [18]. In BAL and serum samples from COPD patients, the presence of significantly higher levels of IL-6 compared with smokers with normal lung function and healthy controls seems difficult to interpret [4,5,7] whether IL-6 directly contributes to the pathophysiology of COPD or merely stands as an ubiquitous biomarker remains elusive [13,19].

Despite interspecies biological variations, animal models of COPD such as rodent CS exposure protocols provide invaluable flexibility to examine a variety of pathological pathways and evaluate novel therapeutic approaches. This applies particularly to treatments aiming to address the early stage of the disease, and/or episodes of acute pulmonary inflammation [17,20]. Indeed, because most of the burden of COPD ensues from exacerbations triggered by bacterial, viral or toxic agents [3], we focused on evaluating an anti-IL-6 therapeutic approach in an AE (acute exacerbation) model in which C57BL/6 female mice are challenged intranasally with the synthetic TLR3 agonist poly(I:C), which mimics the encounter with influenza-like viruses while being daily exposed to CS. This 2-week protocol was derived from a 4-week model originally published by Kang et al. [15], and stimulates the development of pulmonary inflammation that recapitulates several key features of COPD, including the recruitment of neutrophils and lymphocytes to the airways as well high levels of IL-6, CCL3/MIP-1α, CCL5/RANTES, CXCL1/KC and CXCL10/IP-10 locally (BAL) and/or systemically (serum).

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Figure 4  Effects of IL-6 neutralization in the airways

(A) Modulation of BAL cellularity in response to CS/poly(I:C) exposure in controls (vehicle) and mice administered intraperitoneally with either Dex (3 mg/kg of body weight), rat IgG1 antibodies (25 mg/kg of body weight) or rat anti-IL-6 antibodies (25 mg/kg of body weight).  

(B) Comparison of BAL levels of GM-CSF, IL-6, CXCL1/KC, CCL3/MIP-1α, CCL5/RANTES and TNFα between controls (vehicle) and treatment groups. Results are means ± S.E.M. of n = 10–15 mice per group. For statistical analysis (Mann–Whitney), * P < 0.05, ** P < 0.01 or *** P < 0.001.
in airway neutrophilia and related mediators, which represents a primary objective in the context of COPD.

Similarly to TNFα, IL-6-driven pulmonary inflammation is thought to contribute to emphysema [26,27] and other aspects of airway remodelling in COPD [28,29]. Our 2-week model of acute, exacerbation-like pulmonary inflammation with daily CS exposure probably provides a more limited insight into COPD-like tissue remodelling compared with long-term CS exposure protocols. In our studies, lung sections obtained from mice exposed to CS and poly(I:C) showed a comparable grade of mild to moderate bronchovascular inflammation in vehicle-treated animals as well as treatment groups. In addition, only a subtle trend towards decreased alveolar inflammation was found across all treatment groups including mice treated with isotype control antibodies. These results suggest a limited, non-specific effect of IL-6 neutralization on inflammatory infiltrates. It is not clear, however, whether these data contradict the robust modulation of BAL inflammation described above since the absence of correlation between cell profiles in sputum, BAL, and biopsy samples is well-documented in published articles comparing COPD patients with healthy subjects [30], or smokers with non-smokers [31]. In fact it is likely that, in addition to the robust release of IL-6 in the airways, presumably by the respiratory epithelium and alveolar macrophages [25,32], other mediators also account for the accumulation of inflammatory cells into the airway wall. Costly, long-term studies aimed to evaluate IL-6 neutralization in WT mice exposed to CS for 3–6 months, with or without poly(I:C), would thus be more suitable to determine the therapeutic effect of antibody treatment on tissue inflammation and remodelling.

In summary, the studies presented here demonstrate that a two week protocol, which exposes mice to CS with periodic poly(I:C) challenges, can recapitulate several key features of COPD-like pulmonary exacerbations, including steroid-insensitive airway neutrophilia and high BAL levels of IL-6 and CXCL10/IP-10 that can also be detected in serum samples. Importantly, we found that the systemic administration of IL-6 neutralizing antibodies can alleviate overt lung inflammation, particularly airway neutrophilia. Although it is likely that both TNFα and IL-6 play a role in CS-induced pulmonary inflammation and thus COPD, TNFα blockade is thought to increase the risk of infections, CVD (cardiovascular disease) and certain types of cancers [33]. On the other hand, IL-6 is known to promote lung carcinogenesis and tumour growth [34,35]; therefore we propose that anti-IL-6 treatment could not only alleviate acute pulmonary inflammation in COPD patients, but could also mitigate the risk of lung cancer in a highly susceptible patients’ population.

CLINICAL PERSPECTIVES

- Episodes of AE are largely responsible for the morbidity and mortality associated with COPD. Although IL-6 is a pro-inflammatory cytokine consistently found at high levels in patients experiencing AE, its role remains unclear in this particular context.
- In the present study we describe a pre-clinical model of CS/poly(I:C) exposure that recapitulates several features of AE-like pulmonary inflammation, including increased BAL levels of pro-inflammatory cytokines as well as steroid-insensitive airway neutrophilia. WT mice exposed to this protocol and treated with IL-6 neutralizing antibodies showed significantly reduced numbers of BAL neutrophils, lymphocytes and macrophages, along with decreased BAL levels of CXCL1/KC.
- This murine model of CS/poly(I:C)-induced pulmonary inflammation suggests that IL-6 indeed contributes to pulmonary inflammation during AE. Thus IL-6 neutralization may be a relevant approach to the treatment of AE in COPD.

REFERENCES

IL-6 and smoke-induced inflammation


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