Aim2 suppresses cigarette smoke-induced neutrophil recruitment, neutrophil caspase-1 activation and anti-Ly6G-mediated neutrophil depletion

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Abstract

Increased inflammasome responses are strongly implicated in inflammatory diseases; however, their specific roles are incompletely understood. Therefore, we sought to examine the roles of nucleotide-binding oligomerization domain–like receptor (NLR) family, pyrin domain–containing 3 (NLRP3) and absent in melanoma-2 (AIM2) inflammasomes in cigarette smoke–induced inflammation in a model of experimental chronic obstructive pulmonary disease (COPD). We targeted NLRP3 with the inhibitor MCC950 given prophylactically or therapeutically and examined $Aim2^{-/-}$ mice in cigarette smoke–induced experimental COPD. MCC950 treatment had minimal effects on disease development and/or progression. $Aim2^{-/-}$ mice had increased airway neutrophils with decreased caspase-1 levels, independent of changes in lung neutrophil chemokines. Suppressing neutrophils with anti-Ly6G in experimental COPD in wild-type mice reduced neutrophils in bone marrow, blood and lung. By contrast, anti-Ly6G treatment in $Aim2^{-/-}$ mice with experimental COPD had no effect on neutrophils in bone marrow, partially reduced neutrophils in the blood and had no effect on neutrophils or neutrophil caspase-1 levels in the lungs. These findings identify that following cigarette smoke exposure, Aim2 is important for anti-Ly6G–mediated depletion of neutrophils, suppression of neutrophil recruitment and mediates activation of caspase-1 in neutrophils.

INTRODUCTION

Inflammasomes are multiprotein signaling complexes that regulate homeostasis and are required for protection against foreign pathogens and tissue damage. Inflammasomes assemble in response to environmental stress, such as cigarette smoke exposure, cell damage or infections.[1] These stimuli can initiate oligomerization of proteins such as nucleotide-binding oligomerization domain–like receptor (NLR) family, pyrin domain–containing 3 (NLRP3) and

absent in melanoma-2 (Aim2), which are expressed in structural and immune cells.[2,3] Activation of these inflammasomes induces the recruitment of apoptosis-associated speck–like protein containing a pyrin domain containing a CARD (ASC) and pro-caspase-1, leading to caspase-1 activation, interleukin (IL)-1β and IL-18 production and secretion. IL-1β and IL-18 are potent proinflammatory cytokines that can recruit inflammatory cells, such as macrophages and neutrophils, and induce cell death, enlarge distal airspaces, increase mucus metaplasia and increase airway fibrosis, all of which are important cell types and features of respiratory diseases.[4,5] Strong clinical and experimental evidence has implicated excessive inflammasome responses in many respiratory diseases.[1]

Cigarette smoke has been widely used in respiratory models to mimic key features of chronic obstructive pulmonary disease (COPD) as cigarette smoke exposure is a major risk factor for the development and progression of COPD.[6,7] In addition to lung changes, smoke exposure can cause systemic inflammation and dysfunction at distal sites, such as the gastrointestinal and reproductive tracts.[8–10] Several studies have used short-term cigarette smoke exposure in mice to implicate the role of inflammasomes and their downstream effectors in pathogenic consequences.[11–13] Early evidence demonstrated roles for NLRP3 and ASC, but not NLRC4/IPAF and Aim2, in cigarette smoke-induced IL-1β/IL-18 release and partial decreases in lung tissue caspase-1 activity and bronchoalveolar lavage fluid neutrophilia in a 3-day whole-body cigarette smoke exposure model.[14] Furthermore, pulmonary inflammation is dependent on IL-1 and significantly attenuated by neutralizing IL-1α or IL-1β. Nlrp3- and caspase-1 (Casp1)-deficient (−/−) mice are not protected from cigarette smoke–induced airway inflammation following 4 weeks of whole-body smoke exposure.[15] Furthermore, Nlrp3−/− and Casp1−/− mice had reduced lung IL-1β levels,[15] suggesting a complex interplay between different cell types and inflammasome signaling following cigarette smoke exposure. In a well-established chronic 8-week cigarette smoke–induced model of experimental COPD that also has fibrotic and emphysematous changes in the lungs and impaired lung function,[8,13,16–27] we showed increased ASC specks.[17]

Targeting end effector molecules has proven ineffective in patients with COPD. Indeed, despite IL-1β being significantly increased in induced sputum of patients with COPD compared with never-smokers,[15] treatment with anti-IL-1β, anti-IL-1R1 and anti-IL-18 had no beneficial effects on lung function or disease progression in patients with COPD (clinical trial: NCT01322594).[28,29] These studies, however, do not demonstrate the potentially disease-causing role of aberrant inflammasome responses

in COPD, highlighting the need to better understand these processes in cigarette smoke–induced pathogenesis.

In this study, we used models of cigarette smoke–induced experimental COPD in mice and showed that targeting NLRP3 pharmacologically with MCC950 has minimal effects on disease when given prophylactically or therapeutically, despite cigarette smoke–induced inflammasome responses in airways. We then show that in the absence of active Aim2 using Aim2$^{Gt(CSG445)Byg}$ mice, which have truncated and inactive Aim2 (referred to as Aim2$^{−/−}$ mice throughout the manuscript), that inflammation is increased, particularly airway neutrophil numbers, and that these neutrophils have reduced caspase-1 activation. We show in Aim2$^{−/−}$ mice exposed to cigarette smoke that neutrophil production, recruitment and caspase-1 activation were not affected by anti-Ly6G treatment, demonstrating that Aim2 plays important roles in the recruitment of neutrophils to the lungs in experimental COPD and potentially mediates Ly6G antibody–induced neutrophil death.

RESULTS

MCC950 prophylactic and therapeutic treatment in experimental COPD has minimal effects on the development and progression of disease

Smoke exposure has been associated with increased NLRP3 inflammasome responses,[14,15] therefore we examined whether prophylactic or therapeutic targeting with the specific NLRP3 inhibitor MCC950[30] could inhibit the development and/or progression of disease. Prophylactic treatment with MCC950 for 1 week trended toward reducing smoke-induced total leukocytes but significantly reduced airway neutrophils (Figure 1a). Prophylactic treatment over 8 weeks had no effects on smoke-induced inflammation, emphysema or changes in lung function (Figure 1b–d). Therapeutic treatment of MCC950 (weeks 8–12) after disease was established at 8 weeks had no effect on airway inflammation (Figure 1e), emphysema (Figure 1f) or lung compliance (Figure 1g) but did reduce smoke-induced increases in central airways resistance (Figure 1g). MCC950 therapeutic treatment had no effect on smoke-induced increases in Nlrp3, Asc or Casp1 (Figure 1h). Thus, prophylactic treatment for 1 week, but not 8 weeks, or therapeutic treatment with MCC950 significantly reduced airway neutrophils in smoke-induced experimental COPD.

Aim2$^{−/−}$ mice with or without smoke exposure have increased airway inflammation

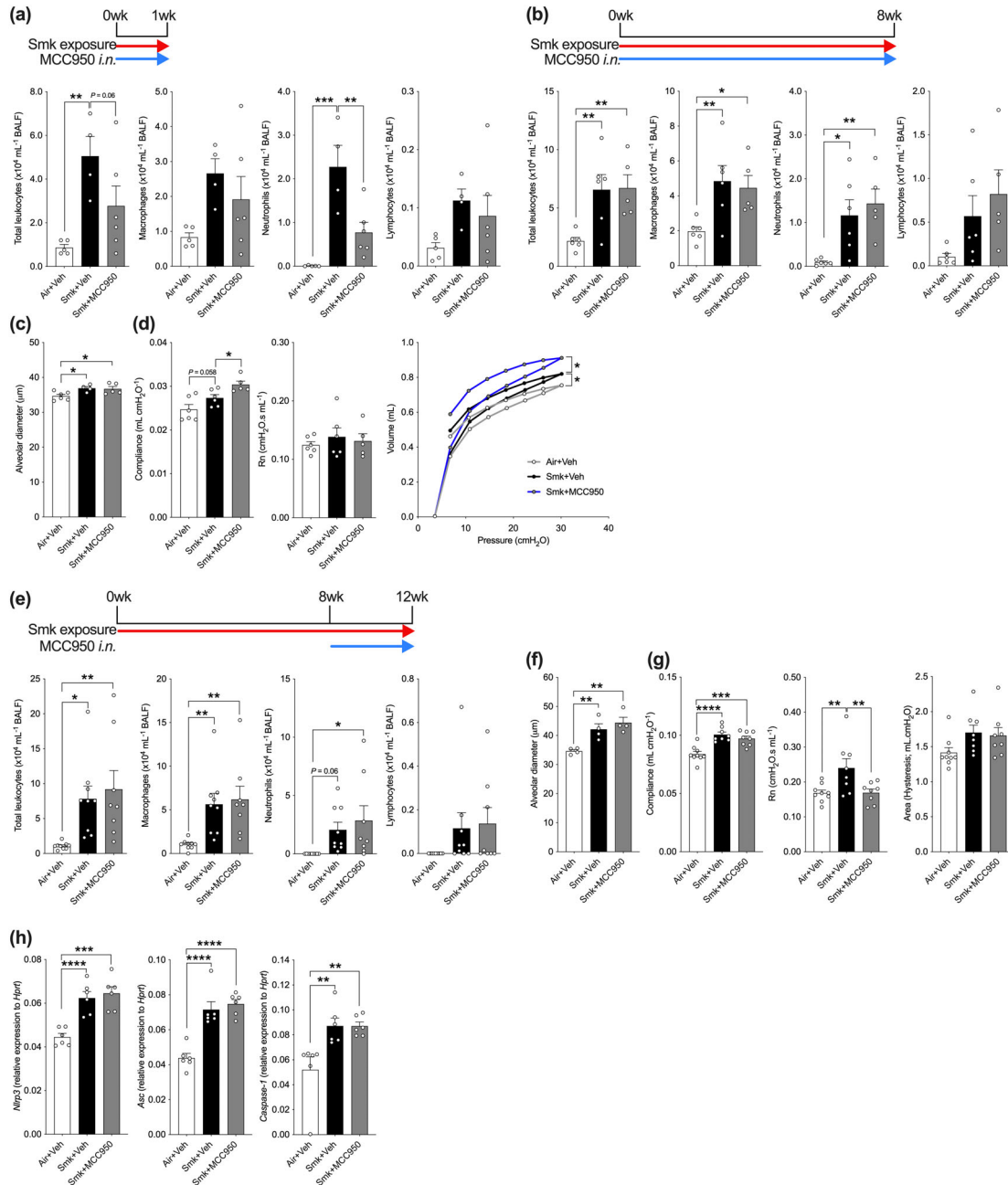We next examined the role of Aim2, as this inflammasome is activated independently of Nlrp3, but converges on ASC

Figure 1. Prophylactic and therapeutic treatment with MCC950 has minimal effects on cigarette smoke (Smk)-induced disease features. MCC950 (10 mg per kg intranasal 5 days/week) administered to C57BL/6 prior to daily cigarette Smk exposure. **(a)** Total and differential leukocyte enumeration in bronchoalveolar lavage fluid (BALF) following 1 week of Air/Smk exposure. **(b)** Total and differential leukocyte enumeration in BALF following 8 weeks of air/Smk exposure. **(c)** Alveolar diameter measured in hematoxylin and eosin sections following 8 weeks of air/Smk exposure. **(d)** Lung function parameters measured following 8 weeks of air/Smk exposure using the forced oscillations technique. **(e)** Total and differential leukocyte enumeration in BALF following 12 weeks of air/Smk exposure. **(f)** Alveolar diameter measured in hematoxylin and eosin sections following 12 weeks of air/Smk exposure. **(g)** Lung function perturbations (including airway resistance [Rn]) measured following 12 weeks of air/Smk exposure using the forced oscillations technique. **(h)** Messenger RNA (mRNA) expression measured in whole lungs following 12 weeks of air/Smk exposure. Data are expressed as mean ± s.e. of the mean and each symbol represents one mouse. $n = 4$ to 9 independent mice per experimental group analyzed across one experiment. **(a–h)** Data were analyzed by one-way ANOVA and Fisher's least significant difference *post hoc* test. **(d)** Pressure–volume curves analyzed by two-way ANOVA with Tukey's *post hoc* test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ comparing groups as indicated in the figure. NS, not significant; Veh, vehicle.

and caspase-1. Smoke exposure for 10 weeks increased total leukocytes composed of macrophages, neutrophils and lymphocytes in the bronchoalveolar lavage fluid of wild-type (WT) mice (Figure 2a). Smoke-exposed *Aim2*$^{-/-}$ mice had further increased total leukocytes, macrophages, neutrophils and lymphocytes compared with smoke-exposed WT and normal air-exposed *Aim2*$^{-/-}$ mice (Figure 2a). Smoke exposure increased lung macrophage chemokine *Ccl3* messenger RNA responses that were comparable between WT and *Aim2*$^{-/-}$ mice (Figure 2b). *Tnfa* messenger RNA responses, as additional lung macrophage cytokine, were increased in smoke-exposed WT mice, and were markedly increased in air- and smoke-exposed *Aim2*$^{-/-}$ mice compared with their WT counterparts (Figure 2b). The assessment of neutrophil chemokine responses showed that smoke exposure increased *Cxcl1* and *Cxcl2* messenger RNA responses in WT mice that was comparable in smoke-exposed *Aim2*$^{-/-}$ mice, suggesting that Aim2 is not critical for these responses. Smoke exposure resulted in emphysema-like increased alveolar diameter and tissue destructive index in WT and *Aim2*$^{-/-}$ mice (~20% increase; Figure 2d). Furthermore, smoke exposure of both WT and *Aim2*$^{-/-}$ mice decreased lung function measured by increases in compliance, total lung capacity and hysteresis (Figure 2e). Together, these data suggest that while Aim2 plays a key role in suppressing smoke-induced inflammation, this does not exacerbate smoke-induced structural or function changes.

Aim2$^{-/-}$ smoke-exposed mice have decreased caspase-1 activation

We next examined the effect of Aim2 deficiency on caspase-1 responses following 10 weeks of smoke exposure (Figure 3a). Smoke exposure for 10 weeks increased parenchymal, but not airway, caspase-1 responses in WT mice (Figure 3b). Smoke-exposed *Aim2*$^{-/-}$ mice had reduced parenchymal caspase-1 activity. To decipher which cell types were contributing to the reduced caspase-1 activity, FAM-FLICA staining (to label active caspase-1) of lung cells was measured (Figure 3c). Smoke induced similar increases in caspase-1 activity in monocytes in WT and *Aim2*$^{-/-}$ mice. Active caspase-1^{+} macrophages were increased in *Aim2*$^{-/-}$ mice irrespective of air or smoke exposure compared with WT mice. Interestingly, active caspase-1^{+} neutrophils were increased with smoke exposure in WT mice but were significantly reduced in smoke-exposed *Aim2*$^{-/-}$ compared with WT mice. Lung IL-1β protein levels were increased with smoke exposure and there were no differences between WT and *Aim2*$^{-/-}$ mice (Figure 3d). These data suggest that reduced parenchymal caspase-1 in *Aim2*$^{-/-}$ smoke-exposed mice is in part because of reduced caspase-1^{+} neutrophils.

Anti-Ly6G treatment during smoke exposure reduces hematopoietic progenitor cells in bone marrow but not blood

Given that *Aim2*$^{-/-}$ smoke-exposed mice had increased airway neutrophils but reduced caspase-1^{+} neutrophils, we next investigated the role that neutrophils were playing in driving disease features in *Aim2*$^{-/-}$ mice. To do this, WT and *Aim2*$^{-/-}$ mice were exposed to smoke in the absence or presence of anti-Ly6G to deplete mice of neutrophils (Figure 4a). We assessed whether anti-Ly6G treatment affected the production of hematopoietic progenitor cells in bone marrow as this may impact the replenishment of tissue-resident myeloid cells that are recruited to the lungs (Figure 4b). Smoke exposure in WT mice increased Lin^{-}CD117^{+} hematopoietic progenitors, which were reduced with anti-Ly6G treatment. By contrast, *Aim2*$^{-/-}$ smoke-exposed mice did not have increased Lin^{-}CD117^{+} cells; however, anti-Ly6G treatment also significantly reduced their levels to similar numbers in WT anti-Ly6G–treated smoke-exposed mice. Smoke exposure and/or anti-Ly6G treatment had no effect on blood hemopoietic progenitors in WT mice (Figure 4c). However, there were increased hemopoietic progenitors in *Aim2*$^{-/-}$ air-exposed mice compared with both WT air-exposed mice and *Aim2*$^{-/-}$ smoke-exposed mice. These data suggest, under normal conditions, that Aim2 might be an important regulator of the release of hematopoietic progenitor cells from the bone marrow to the blood.

Anti-Ly6G treatment has minimal effects on smoke-induced monocytes and macrophages in WT and *Aim2*$^{-/-}$ mice

We also assessed the effects of anti-Ly6G treatment on circulating and lung-resident monocytes and macrophages. In the bone marrow, total myeloid-derived suppressor cells numbers were increased in anti-Ly6G-treated smoke-exposed WT mice compared with smoke-exposed isotype-treated WT controls (Supplementary figure 1a) and it was notable that anti-Ly6G treatment in WT mice reduced monocytes. Anti-Ly6G treatment in smoke-exposed *Aim2*$^{-/-}$ mice had no effect on total myeloid-derived suppressor cells or monocytes compared with isotype-treated *Aim2*$^{-/-}$ smoke- or air-exposed controls. There was no effect of smoke and/or anti-Ly6G treatment in the blood (Supplementary figure 1b). In the lung, there was no effect of smoke or anti-Ly6G treatment on proinflammatory, Ly6C intermediate or patrolling monocytes in WT or *Aim2*$^{-/-}$ mice (Supplementary figure 1c). However, there was a significant increase in patrolling monocytes in air-
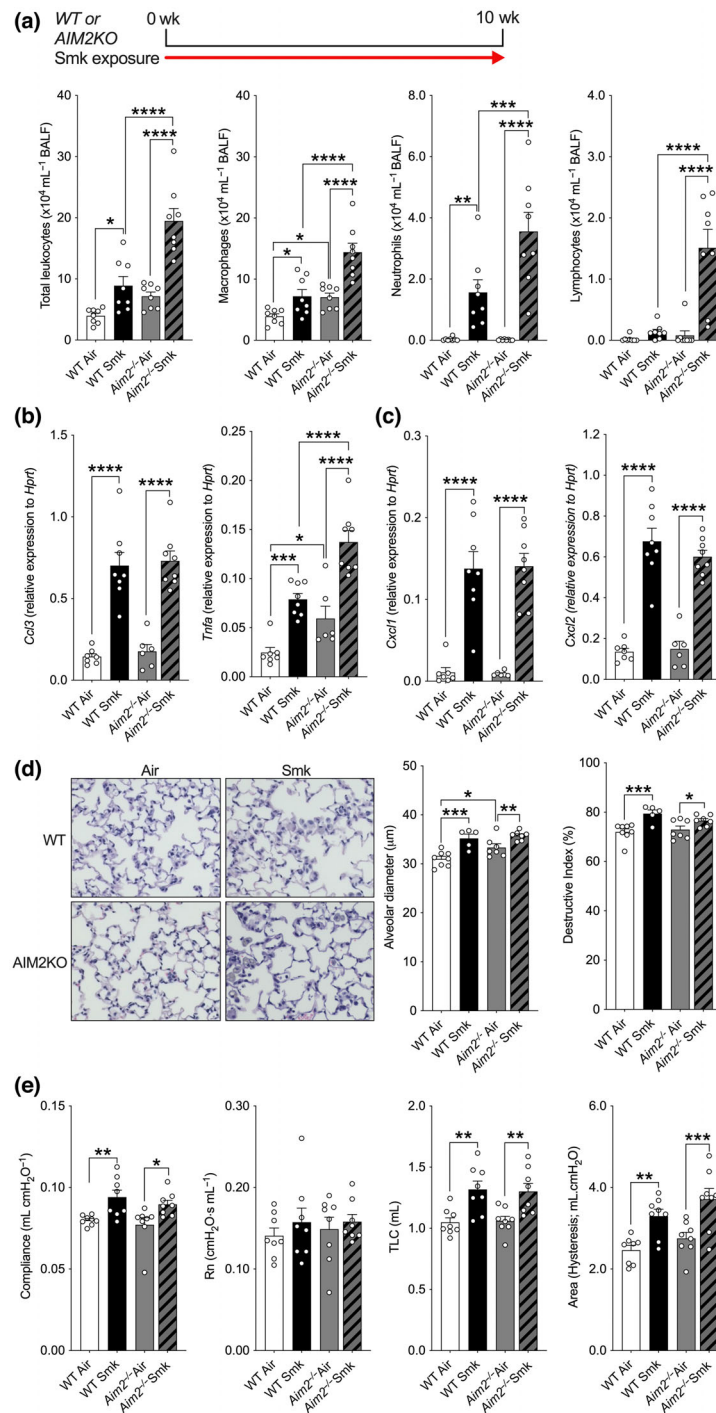
Figure 2. $Aim2^{-/-}$ mice with or without smoke (Smk) exposure have increased airway inflammation. Wild-type C57BL/6 and $Aim2^{-/-}$ mice were exposed to cigarette Smk for 10 weeks. **(a)** Total and differential leukocyte enumeration in bronchoalveolar lavage fluid (BALF) following 10 weeks of air/Smk exposure. **(b)** Macrophage chemokine messenger RNA (mRNA) expression in whole lungs following 10 weeks of air/Smk exposure. **(c)** Neutrophil chemokine mRNA expression measured in whole lungs following 10 weeks of air/Smk exposure. **(d)** Alveolar diameter and destructive index measured in hematoxylin and eosin sections following 10 weeks of air/Smk exposure. **(e)** Lung function parameters (including airway resistance, [Rn] and total lung capacity [TLC]), measured following 8 weeks of air/Smk exposure using the forced oscillations technique. Data are expressed as mean ± s.e. of the mean and each symbol represents one mouse, $n = 5$ to 8 independent mice per experimental group analyzed across one experiment. Data were analyzed by one-way ANOVA and Fisher's least significant difference *post hoc* test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ comparing groups as indicated in the figure.
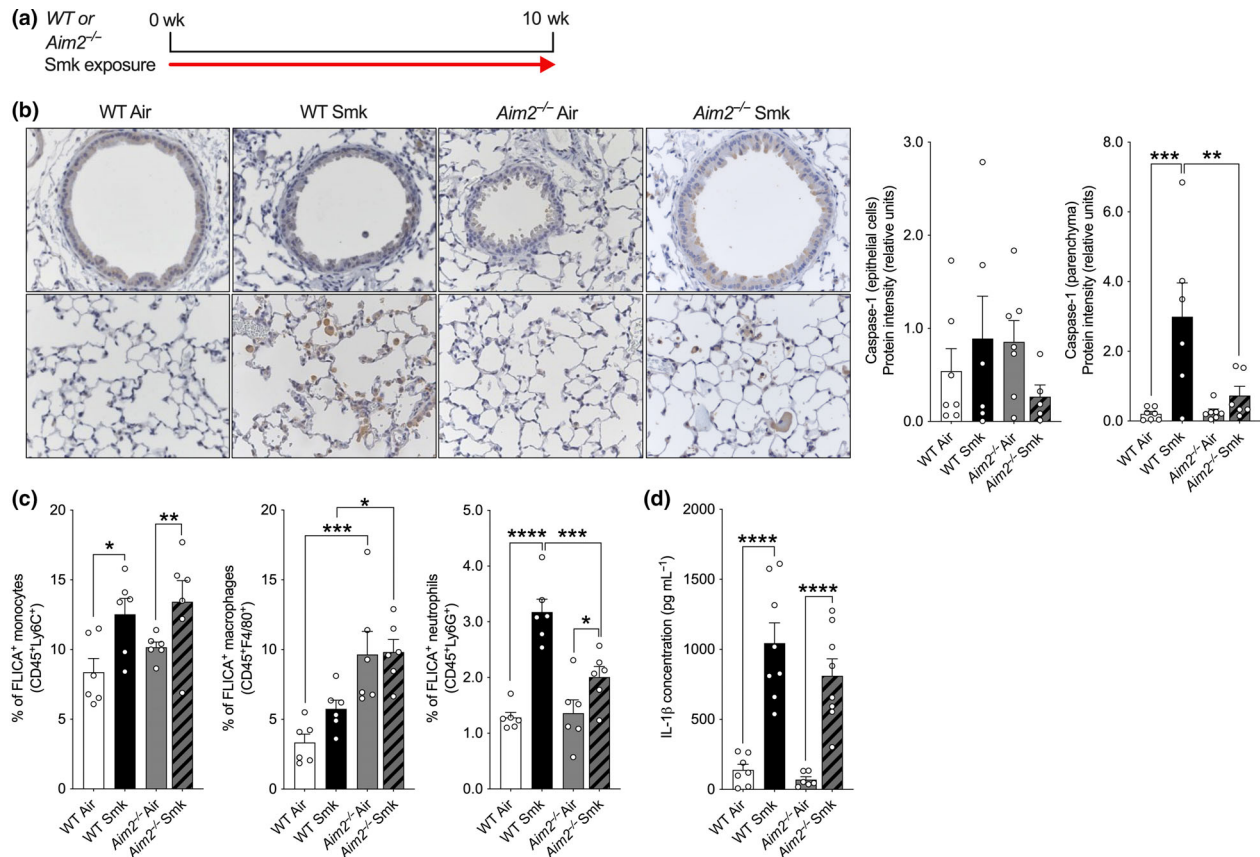
Figure 3. $Aim2^{-/-}$ mice exposed to smoke (Smk) have reduced caspase-1 activation in lung parenchyma and neutrophils. **(a)** Wild-type (WT) C57BL/6 and $Aim2^{-/-}$ mice were exposed to cigarette Smk for 10 weeks. **(b)** Caspase-1 staining (brown) and DAB counterstain (blue) measured in lung sections analyzed with ImageJ. **(c)** Percentage of fluorochrome-labeled inhibitor of caspases (FLICA)+ monocytes, macrophages and neutrophils in the lung measured by flow cytometry. **(d)** IL-1β protein measured in homogenized whole lung samples by ELISA. Data are expressed as mean ± s.e. of the mean and each symbol represents one mouse, n = 5 or 8 independent mice per group analyzed cumulatively across two independent experiments. Data were analyzed by one-way ANOVA and Fisher's least significant difference *post hoc* test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ comparing groups as indicated in the figure. DAB, 3,3′-Diaminobenzidine.

exposed $Aim2^{-/-}$ mice compared with air-exposed WT controls. Smoke exposure increased interstitial macrophages in WT mice compared with air-exposed controls; however, there was no effect of anti-Ly6G treatment (Supplementary figure 1d). Air-exposed $Aim2^{-/-}$ mice had increased interstitial macrophages compared with air-exposed WT controls, and there was no effect of smoke and/or anti-Ly6G treatment. There was also no effect of smoke or anti-Ly6G treatment on alveolar macrophages in WT mice; however, in smoke-exposed $Aim2^{-/-}$ mice there was a significant reduction in alveolar macrophages that was restored and increased with anti-Ly6G treatment compared with isotype-treated smoke-exposed $Aim2^{-/-}$ and anti-Ly6G smoke-exposed WT mice. Collectively, these data show that anti-Ly6G treatment and $Aim2^{-/-}$ have minimal effects on smoke-induced changes in monocytes and interstitial

macrophages, but that alveolar macrophages are altered by anti-Ly6G treatment in $Aim2^{-/-}$ mice.

Anti-Ly6G treatment reduces neutrophils in bone marrow, blood and lungs in smoke-exposed WT, but not $Aim2^{-/-}$ mice

We next assessed neutrophil responses in bone marrow, blood and lungs. In bone marrow, there was no effect of smoke exposure on neutrophil numbers in WT or $Aim2^{-/-}$ mice compared with their air-exposed controls (Figure 5a). Anti-Ly6G treatment resulted in a reduction in neutrophil numbers in WT mice ($P = 0.056$); however, treatment in $Aim2^{-/-}$ smoke-exposed mice had no effect on neutrophil numbers. In the blood, there was an increase in neutrophils in smoke-exposed WT mice compared with air-exposed controls ($P = 0.055$,
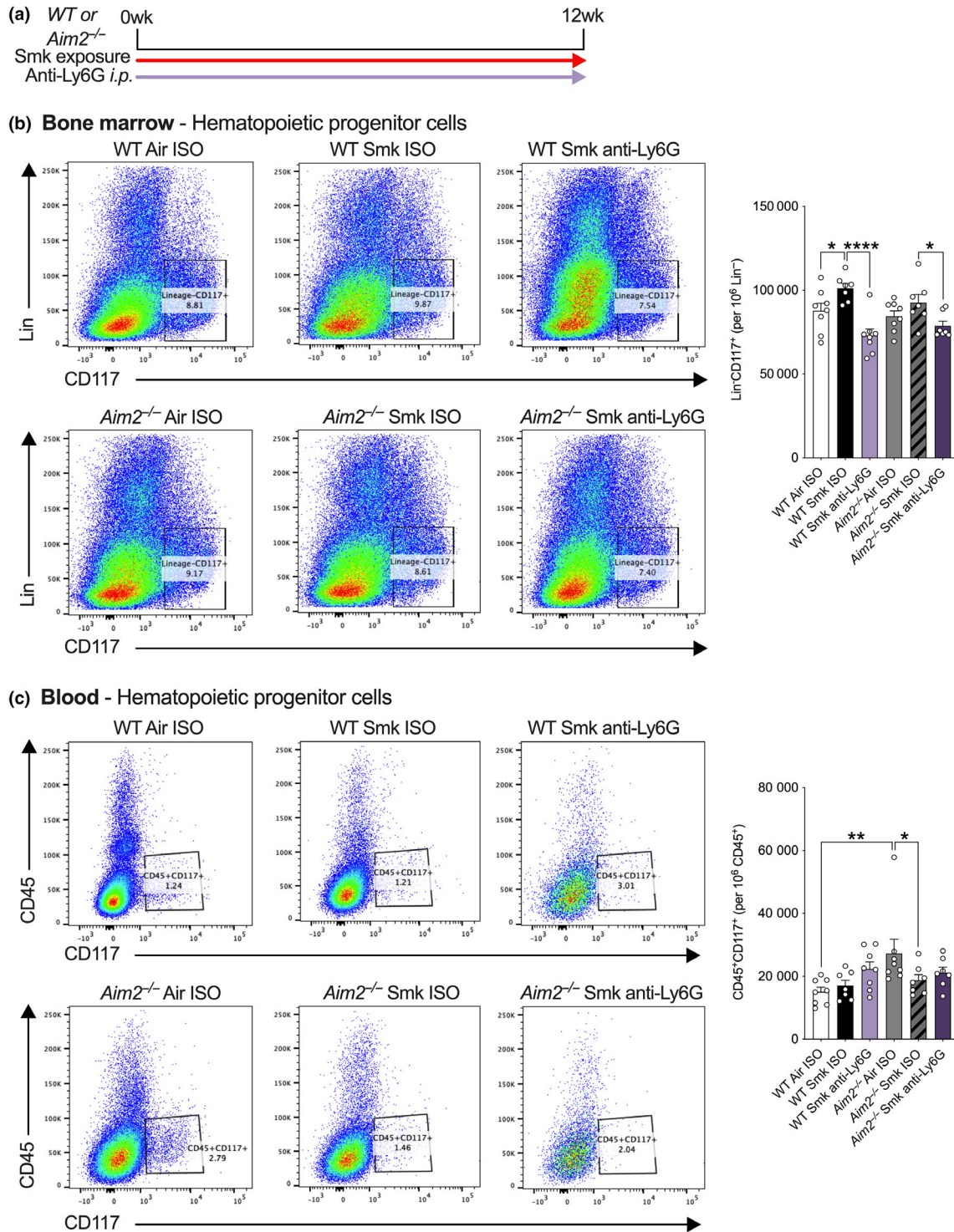
Figure 4. Anti-Ly6G treatment reduces hematopoietic progenitor cells in bone marrow but not blood during cigarette smoke (Smk) exposure. **(a)** Wild-type (WT) C57BL/6 and *Aim2*^{−/−} mice were exposed to cigarette Smk for 12 weeks and treated with isotype (ISO) or anti-Ly6G antibody up to 3 days/week for 12 weeks. **(b)** Lin^{−}CD117^{+} hematopoietic progenitor cells in bone marrow measured using flow cytometry. **(c)** CD45^{+}CD117^{+} hematopoietic progenitor cells in blood measured using flow cytometry. Data are expressed as mean ± s.e. of the mean and each symbol represents one mouse, $n = 7$ or 8 independent mice per experimental group analyzed across one experiment. Data were analyzed by one-way ANOVA and Fisher's least significant difference *post hoc* test. *$P < 0.05$, **$P < 0.01$, ****$P < 0.0001$ comparing groups as indicated in the figure.

(a) Bone marrow - Neutrophils
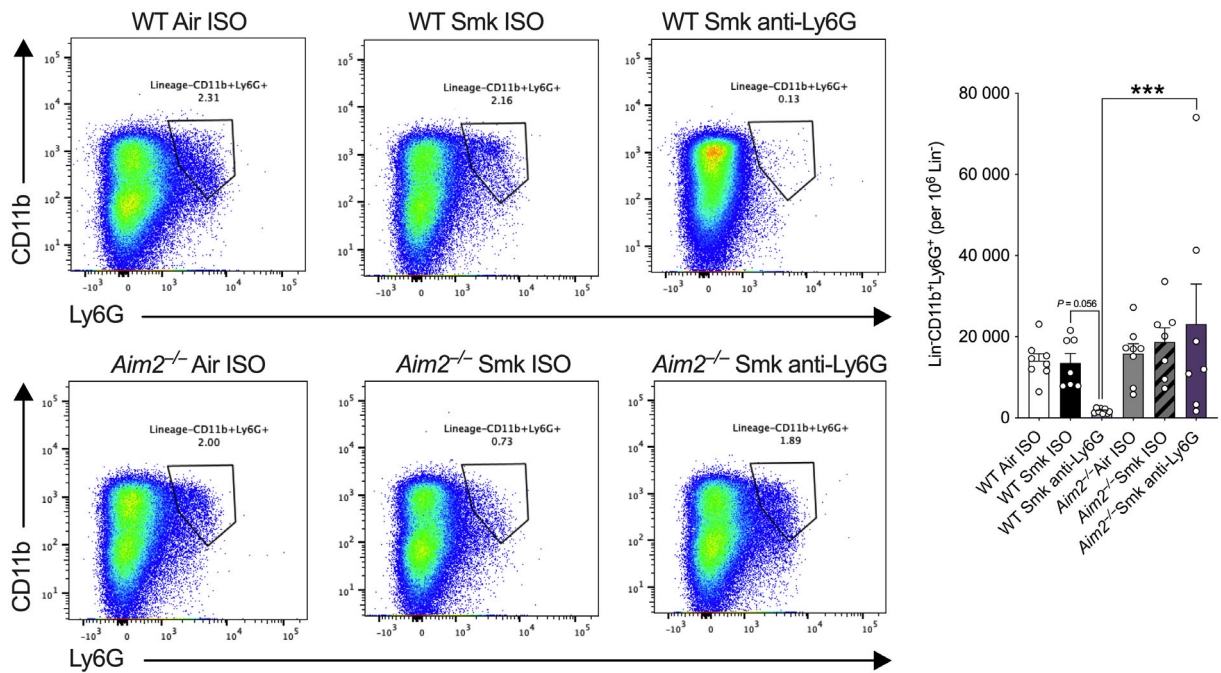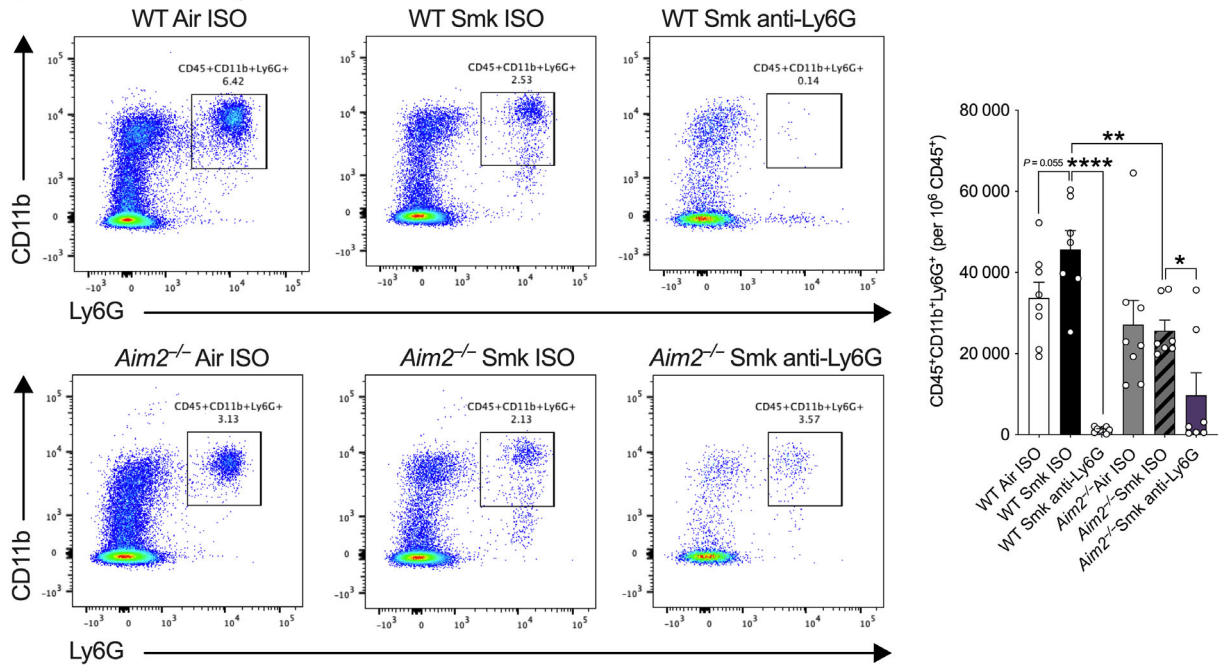

(b) Blood - Neutrophils

Figure 5. Anti-Ly6G treatment during cigarette smoke (Smk) exposure reduces neutrophils in the bone marrow and blood in wild-type (WT) mice, but not in the bone marrow of $Aim2^{-/-}$ mice and only partially reduces neutrophils in the blood. WT C57BL/6 and $Aim2^{-/-}$ mice were exposed to cigarette Smk for 12 weeks and treated with isotype (ISO) or anti-Ly6G antibody up to 3 days/week for 12 weeks. **(a)** Lin$^-$CD11b$^+$Ly6G$^+$ neutrophils in bone marrow measured using flow cytometry. **(b)** CD45$^+$CD11b$^+$Ly6G$^+$ neutrophils in blood measured using flow cytometry. Data are expressed as mean ± s.e. of the mean and each symbol represents one mouse, $n = 7$ or 8 independent mice per experimental group analyzed across one experiment. Data were analyzed by one-way ANOVA and Fisher's least significant difference *post hoc* test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ comparing groups indicated.

Figure 5b), which were almost completely abolished with anti-Ly6G treatment. By contrast, smoke exposure in $Aim2^{-/-}$ mice had no effect on neutrophils compared with air-exposed $Aim2^{-/-}$ mice; however, there was a 50% reduction with anti-Ly6G treatment. In the lungs, smoke exposure increased neutrophils in both WT and $Aim2^{-/-}$ mice (Figure 6a). Anti-Ly6G treatment reduced neutrophil numbers back to air-exposed controls in WT mice. Surprisingly, treatment had no effect on neutrophil numbers in smoke-exposed $Aim2^{-/-}$ mice. Interestingly, similar trends were observed with caspase-1 activation (Figure 6b), suggesting that in the absence of active Aim2, neutrophils can still have activated caspase-1 even following anti-Ly6G treatment and this caspase-1 activation may be a result of Nlrp3 activation. There was no effect of smoke and/or anti-Ly6G treatment in WT or $Aim2^{-/-}$ mice on eosinophils, T cells, endothelial or epithelial cells in the lungs (Supplementary figure 2a–c). Collectively, these data demonstrate that anti-Ly6G treatment reduces neutrophils in smoke-exposed WT mice and that $Aim2^{-/-}$ neutrophils are not amenable to anti-Ly6G depletion, suggesting that Aim2 plays an important role in antibody-mediated depletion of neutrophils.

DISCUSSION

In this study, we describe a role for Aim2 in neutrophil recruitment and caspase-1 activation and anti-Ly6G–mediated neutrophil depletion following cigarette smoke exposure. We show that cigarette smoke increases hematopoietic progenitor responses in bone marrow, but not blood, and increases caspase-1 activation and neutrophil numbers in the lung in WT mice. Importantly, $Aim2$ deletion in mice results in increased numbers of airway neutrophils, but decreased caspase-1 activation in the parenchyma and lung neutrophils. Treatment with anti-Ly6G depletes neutrophils in bone marrow, blood and lungs in WT mice and we show that anti-Ly6G treatment has no effect on neutrophil responses in $Aim2^{-/-}$ mice, suggesting that Aim2 plays a critical role in neutrophil recruitment and activation and anti-Ly6G–mediated neutrophil death (Figure 7). These data add to the increasing body of evidence that strongly implicates increased inflammasome responses and the cell types involved in pathogenic inflammatory processes including in COPD. The identification of the specific inflammasome pathways involved, and the development of novel therapeutic strategies for targeting the important aspects of these pathways, will advance this field because inhibition of the major downstream effector molecules (i.e. IL-1β and IL-18) has not proven to be beneficial in COPD (clinical trial: NCT01322594).[28,29] Furthermore, there is an identified risk that this approach may result in severe infections.[31] Importantly, the data from these clinical trials indicated that targeting end effectors of inflammasome pathways after disease is established has limited efficacy; however, this does not discount the disease-causing contribution that inflammasomes responses, including their effects on pyroptosis,[21] may play during disease development. Therefore, there is a need to examine the inflammasome/s responses that are activated during pathogenesis and assess their specific contributions. Given that inflammasomes also play important roles in controlling infections, increasing knowledge of inflammasome-mediated disease-causing processes in COPD will refine any inflammasome-targeting therapies for this disease.

We show that prophylactic and therapeutic inhibition of NLRP3 with MCC950 has limited effects on pathophysiological features of cigarette smoke–induced experimental COPD. Our findings with 1 week of smoke exposure with MCC950 treatment are similar to those from an acute (3 day) cigarette smoke model, which demonstrated decreased neutrophils in $Nlrp3^{-/-}$ mice compared with controls.[14] Our 8-week prophylactic MCC950 treatment data are similar to a different 4-week whole-body smoke exposure model that used $Nlrp3^{-/-}$ mice that were not protected against cigarette smoke–induced airway inflammation.[15] Interestingly, and similar to our findings, there were also limited effects of $Nlrp3$ deletion on caspase-1 responses, whereby 3-day smoke exposure increased caspase 1 activity and Nlrp3 deletion had no effects on these responses.[14] Together, these data demonstrate that NLRP3 is not critical in driving the development or progression of COPD; however, because the responses of this inflammasome are important in protecting against infection, it is feasible that it may play a role in exacerbations of COPD with viral and/or bacterial agents. Nevertheless, these data implicate the involvement of an NLRP3-independent, upstream driver of caspase-1 activation in response to cigarette smoke exposure. Thus, because NLRP3 and Aim2 pathways converge at caspase-1 activation, we examined the role of the Aim2 inflammasome.

Surprisingly, smoke-exposed $Aim2^{-/-}$ mice had increased airway macrophage responses. Aim2 is required for detection of double-stranded DNA.[32] In the absence of active Aim2, macrophages may not be able to detect extracellular double-stranded DNA derived from cell death that occurs through cigarette smoke–induced necroptosis or apoptosis.[21] This could lead to defective macrophage activation/efferocytosis responses and subsequent clearance of dying cells, which may lead to the accumulation of airway inflammatory cells in these mice. Another possibility is that Aim2 is exerting inflammasome-independent effects in this context and may alter macrophage responses as observed in other

(a) Lungs - Neutrophils
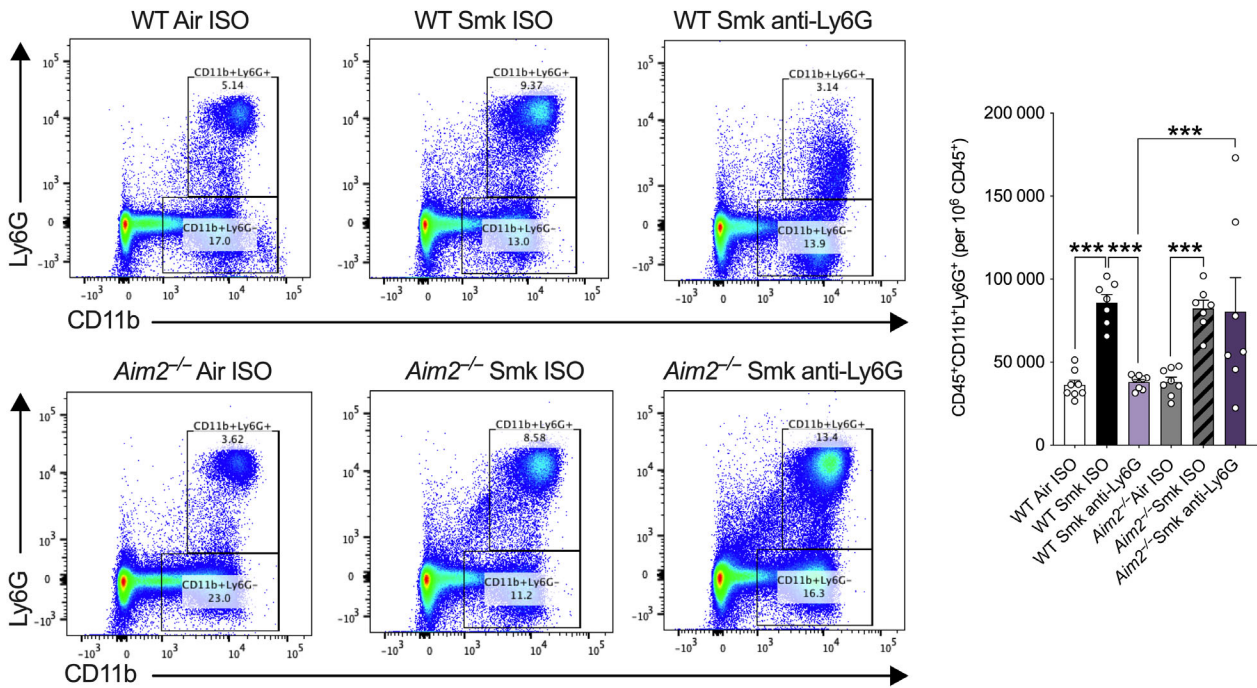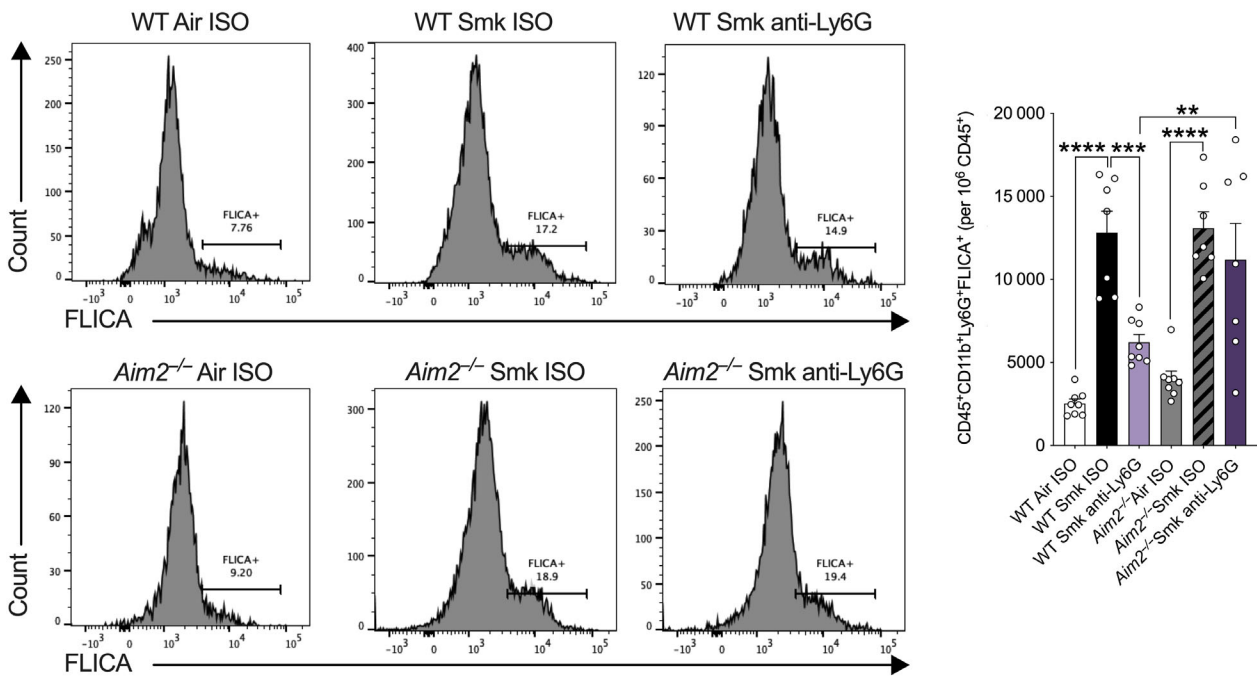

(b) Lungs - Neutrophils (FLICA⁺)

Figure 6. Anti-Ly6G treatment during cigarette smoke (Smk) exposure reduces neutrophils and their caspase-1 activation in the lungs in wild-type (WT) but not $Aim2^{-/-}$ mice. WT C57BL/6 and $Aim2^{-/-}$ mice were exposed to cigarette Smk for 12 weeks and treated with isotype (ISO) or anti-Ly6G antibody up to 3 days/week for 12 weeks. **(a)** $CD45^+CD11b^+Ly6G^+$ neutrophils in lungs measured using flow cytometry. **(b)** Caspase-1^+ fluorochrome-labeled inhibitor of caspases (FLICA) neutrophils measured in lungs measured using flow cytometry. Data are expressed as mean ± s.e. of the mean and each symbol represents one mouse, $n = 7$ or 8 independent mice per experimental group analyzed across one experiment. Data were analyzed by one-way ANOVA and Fisher's least significant difference *post hoc* test. **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ comparing groups indicated.
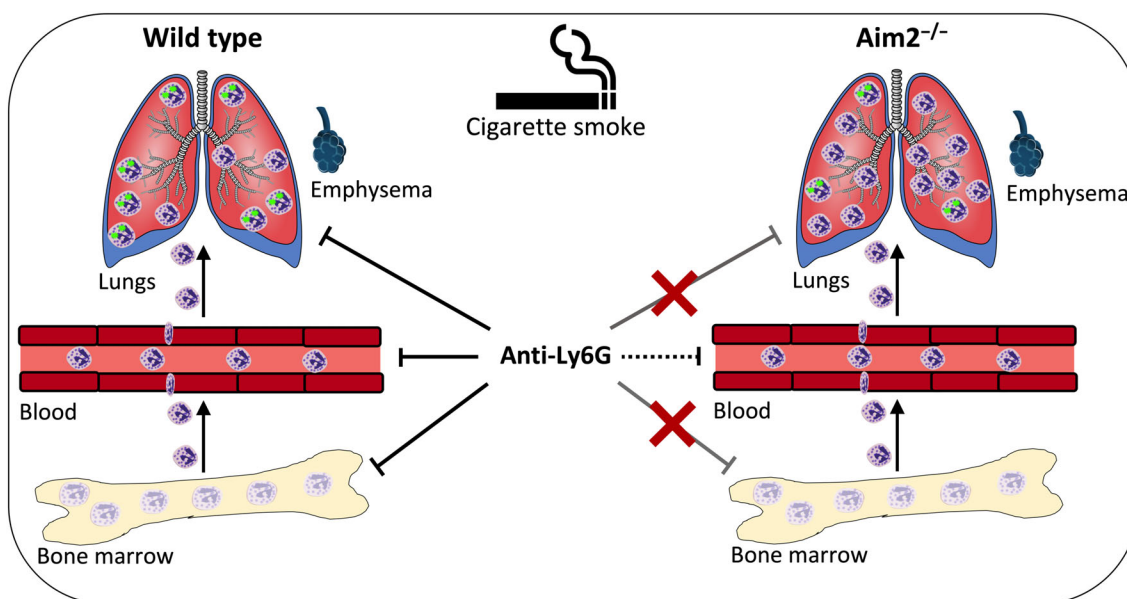
Figure 7. Aim2 plays important roles in lung neutrophil recruitment and caspase-1 activation following cigarette smoke exposure. Cigarette smoke increases hematopoietic progenitor responses in bone marrow, but not blood, and increases caspase-1 activation and neutrophil numbers in the lung in wild-type mice. Treatment with anti-Ly6G–depleted neutrophils in bone marrow, blood and lungs in wild-type mice did not reduce neutrophils in the bone marrow or lungs, and only partially depleted neutrophils in the blood of smoke-exposed $Aim2^{-/-}$ mice. Thus, Aim2 mediates lung neutrophil recruitment and caspase-1 activation following smoke exposure.

mucosal sites.[33] These potential relationships in macrophages warrant further investigation.

Notably, lung expression of macrophage inflammatory protein-1α/$Ccl3$ was similar in WT and $Aim2^{-/-}$ mice and circulating monocyte numbers were unaltered, suggesting that the recruitment of monocytes to the lung in response to smoke exposure is not dependent on Aim2. However, Aim2 may play a role in permitting the movement of recruited monocytes/macrophages from the lung tissue into the airway lumen during smoke exposure and this warrants further investigation.

Smoke-exposed $Aim2^{-/-}$ mice also had increased airway neutrophil responses despite having similar lung neutrophil chemoattractant ($Cxcl1$ and $Cxcl2$) responses to WT mice. We also show that lung neutrophilic inflammation is similar in WT and $Aim2^{-/-}$ mice; however, $Aim2^{-/-}$ neutrophils have reduced caspase-1 activity. This suggests that Aim2, unlike NLRP3, plays an important role in regulating lung neutrophilic inflammation and caspase-1 activation in these cells following recruitment in smoke-induced experimental COPD. Our findings agree with previous human studies that identified an association between Aim2 and neutrophilic inflammation[34]; however, no studies have demonstrated the direct involvement of Aim2 in the recruitment of neutrophils and their activation of caspase-1.

It is important to examine the role of Aim2 in neutrophil responses in the hematopoietic and circulatory compartments following smoke exposure to obtain a holistic understanding of the systemic effects of Aim2 depletion on lung recruitment and subsequent caspase activation of these cells. Thus, we also assessed the potentially disease-causing role of neutrophil responses in the absence of active Aim2 by administering anti-Ly6G neutrophil-depleting antibody *in vivo* and examined the effects on the lung and major extrapulmonary compartments. Anti-Ly6G robustly reduced smoke-induced hematopoietic progenitor responses in the bone marrow in WT and $Aim2^{-/-}$ mice but had no effect on these cells in the blood, showing that long-term treatment with anti-Ly6G may suppresses neutrophil responses during the early stages of production and/or that systemic neutrophil depletion results in negative feedback signals that suppress *de novo* production in the bone marrow. Interestingly, this effect occurred in conjunction with a reduction in monocytes in the bone marrow in WT but not $Aim2^{-/-}$ mice, suggesting that smoke-induced systemic neutrophil responses may coordinate with monocyte production and that Aim2 is a key regulator of this process. Interestingly, proinflammatory, Ly6C$^+$ intermediate and patrolling monocytes were not affected by smoke or anti-Ly6G treatment in the lung in WT or $Aim2^{-/-}$ mice. However, we did observe an increase in smoke-induced interstitial macrophages in WT mice compared with air-exposed controls. It is important to note that $Aim2^{-/-}$ mice had elevated lung interstitial macrophages irrespective of smoke

exposure or anti-Ly6G treatment, suggesting that Aim2 may exert effects as an endogenous suppressor of interstitial macrophages in the lung. Thus, it is important to consider the differences in local lung *versus* systemic effects of Aim2 responses following cigarette smoke exposure.

Significantly, in smoke-exposed WT mice, anti-Ly6G treatment robustly depleted neutrophils in the lung and the blood, and strongly trended toward neutrophil depletion in the bone marrow. However, these effects of treatment were not present in $Aim2^{-/-}$ mice with the exception of a partial reduction of blood neutrophils. This suggests that $Aim2^{-/-}$ neutrophils exhibit deficits in death and/or clearance compared with WT neutrophils and/or may be replenished at an increased rate by systemic progenitors that overcomes the depletive effects of anti-Ly6G treatment rather than by depletion escapes. Our observations align with previous studies that demonstrate the critical role of Aim2 in promoting cell death decisions[35,36] that together suggest that $Aim2^{-/-}$ neutrophils are not dying appropriately, leading to their accumulation in the lung. The examination of the contribution of caspase-8 activation and other cell death pathways[21] in this context would be of value. Furthermore, these data support our proposal that depleting mature neutrophils may have a suppressive effect on progenitor responses in the bone marrow. Importantly, $Aim2^{-/-}$ neutrophils that remain in the lung following anti-Ly6G–mediated depletion have sustained increases in caspase-1 activation that are similar to levels observed in smoke exposure alone, which further supports the concept that Aim2 is an endogenous suppressor of inflammasome-mediated, caspase-1 responses in smoke exposure–recruited lung neutrophils.

Furthermore, increased tumor necrosis factor alpha (TNFα) can recruit neutrophils into sites of injury. However, our data show that increased TNFα in $Aim2^{-/-}$ air-exposed mice is not associated with increases in airway neutrophilic inflammation. Moreover, we show that $Aim2^{-/-}$ smoke-exposed mice have further increases in TNFα and airway neutrophils compared with WT smoke-exposed controls. This suggests that smoke-induced TNFα may recruit additional neutrophils into the lungs in the absence of Aim2 or it is feasible that there are more TNFα-expressing neutrophils present in the lungs. While we did not assess the levels of TNFα following anti-Ly6G treatment in $Aim2^{-/-}$ smoke-exposed mice, it would be informative to assess the contribution of TNFα in anti-Ly6G–mediated neutrophil depletion.

In our study, Aim2 expression or protein levels were not assessed in experimental or human COPD. A recent study, however, showed that Aim2 levels are not altered in whole-lung samples from experimental COPD and patients with COPD. However, levels were increased in lung dendritic cells and macrophages following 4 and 16 weeks of experimental smoke

exposure, respectively,[37] and in alveolar macrophages after 24 weeks of exposure.[38] Furthermore, increased AIM2 protein levels in alveolar macrophages and bronchiolar epithelium positively correlated with GOLD (Global Initiative for Obstructive Lung Disease) stage in patients.[38] Thus, it will be important to measure Aim2 in specific cell types in future studies. Moreover, gasdermin D, which is also downstream of NLRP3 and Aim2, may be playing a role in smoke-induced experimental COPD but this has not been examined and warrants further exploration. It is also important to consider the potential contribution that passenger mutations can have on the phenotypes of mice; however, we do not consider that such mutations would have had any significant impact on the outcomes we have reported because the experiments were performed over 5 years and in two different sites and yielded the same outcomes each time. Furthermore, some experiments were performed once with the relevant controls and the outcomes observed could be further validated in future studies.

Our study is the first to show that Aim2 plays a critical role in the recruitment and activation of neutrophils following cigarette smoke exposure and contributes to caspase-1 activation. Our data also suggest the potential for crosstalk between monocytes and neutrophils in extrapulmonary compartments that may play an important role in cigarette smoke–induced disease and future studies that examine the potential mechanistic interplay are warranted.

METHODS

Mice

C57BL/6 mice were purchased from Australian Bioresources (ABR; Moss Vale, NSW, Australia). $Aim2^{-/-}$ mice [$Aim2^{Gt(CSG445)Byg}$][39] on a C57BL/6 background were purchased from The Jackson Laboratory (USA) and bred at ABR or Centenary Institute (Camperdown, Sydney, NSW, Australia). The WT C57BL/6 controls used in this study were not littermate controls as our colony is homozygous. All experiments were appropriately controlled for age, sex, breeding and experimental conditions throughout, as outlined below. All studies were performed under protocols approved by the Animal Care and Ethics Committee at the University of Newcastle and Sydney Local Health District.

Induction of cigarette smoke–induced inflammation and experimental COPD

Mice were exposed to cigarette smoke *via* a purpose-designed custom-built nose-only exposure system (CH Technologies, Westwood, NJ, USA) up to 12 weeks as previously described.[8,13,16,18–22] In some experiments, mice were treated with MCC950 (10 mg/kg, intranasal, 5 days per week) for up to 8 weeks as previously described.[40] In some other experiments, mice were treated with anti-mouse Ly6G (Clone

1A8; InVivoMAb; catalog number BE0075-1; BioXcell, Lebanon, NH, USA) or isotype control (catalog number BP0089; BioXcell, Lebanon, NH, USA)[40] intraperitoneally up to three times per week for 12 weeks.

Bronchoalveolar lavage

Bronchoalveolar lavage fluid was collected as previously described.[8,13,16,18–22] In brief, mice were cannulated via the trachea and left lobe of the lung flushed with $2\times$ 0.5 mL of Hanks buffered salt solution (Life Technologies, Australia). Red blood cells were lysed, and remaining cells counted by trypan blue exclusion and cells were spun onto slides stained with May–Grunwald–Giemsa and differentially enumerated. A minimum of 200 cells were counted per sample.

Measurement of emphysematous changes

Formalin-fixed paraffin-embedded lungs were sectioned (4 μm) and stained with hemoxylin and eosin. A minimum of 10 random parenchymal images (no airways or blood vessels present) were overlayed with 11 lines and mean linear intercept counts were conducted as previously described.[13,16,18–20]

Lung function

Lung function was assessed using the forced oscillation technique as previously described.[13,16,18–20] Data presented in Figure 1 were analyzed on a SCIREQ flexiVent FX1 machine (Montreal, QC, Canada). Data presented in Figure 2 onward were analyzed on a SCIREQ flexiVent FX2 machine with FEV extension.

Quantification of messenger RNA expression by real-time quantitative PCR

Total RNA was isolated from homogenized lung tissue with TRIzol Reagent (Invitrogen/Thermo Fisher Scientific, Adelaide, SA, Australia). Random-primed reverse transcriptions were performed followed by real-time quantitative PCRs. Gene expression was normalized to the expression of the transcript of the housekeeping gene hypoxanthine guanine phosphoribosyltransferase (Hprt). Primer sequences are provided in Supplementary table 1. All reactions were performed using BioScript reverse transcriptase in $1\times$ first-strand buffer according to the manufacturer's instructions (Bioline, Eveleigh, NSW, Australia). Real-time quantitative PCR assays were performed with SYBR Green Supermix (KAPA Biosystems, Merck, Sydney, NSW, Australia) and a Mastercycler ep RealPlex2 system (Eppendorf South Pacific, Macquarie Park, NSW, Australia).

ELISA

Mouse lung IL-1β protein levels were measured in lung tissue homogenates using anti-mouse ELISA kits (R&D Systems, Minneapolis, Minnesota, USA) according to the manufacturer's instructions.

Caspase-1 immunohistochemistry

Formalin-fixed paraffin-embedded lungs were sectioned (4 μm) and stained with a caspase-1 p10 subunit antibody (1:500 dilution, GTX123675; Sapphire Biosciences, Redfern, NSW, Australia) as per the manufacturer's instructions.

Single-cell suspension and flow cytometric analysis

Lung single-cell suspensions were prepared as previously described[13,18] and resuspended in 250 μL of 2% fetal bovine serum/phosphate-buffered saline. For blood single-cell suspensions, 500 μL of blood was collected by cardiac puncture per mouse, red cell lysis buffer added (20 mL, 1:40 dilution, 10 min), centrifuged ($300g$, 10 min) and resuspended in 200 μL of 2% fetal bovine serum/phosphate-buffered saline. For bone marrow, one tibia and one femur were flushed, and cell pellets treated with red cell lysis buffer (200 μL, 5 min), centrifuged ($300g$, 10 min) and resuspended in 500 μL of 2% fetal bovine serum/phosphate-buffered saline. All cells were then stained with antibodies (Supplementary table 2) and a FAM-FLICA caspase-1 assay kit (Sapphire Biosciences, Redfern, NSW, Australia) as per the manufacturer's instructions. Cells were then fixed in 4% paraformaldehyde (PFA) and analyzed the following day using a BD Fortessa. Full gating strategies are presented in Supplementary figure 3.

Statistical analysis

Comparisons between multiple groups were made using a one-way ANOVA and Fisher's least significant difference post hoc test where appropriate. Comparisons between pressure–volume curves were made using two-way ANOVA and Tukey's post hoc test where appropriate. Analyses were performed using GraphPad Prism software (version 9.1.2). All data shown are representative of individual mice. No data have been pooled.

CONFLICT OF INTEREST

KS is a co-inventor on patent applications for NLRP3 inhibitors that have been licensed to Inflazome Ltd, a company

headquartered in Dublin, Ireland. Inflazome is developing drugs that target the NLRP3 inflammasome to address unmet clinical needs in inflammatory disease. KS also served on the Scientific Advisory Board of Inflazome in 2016–2017, and serves as a consultant to Quench Bio, USA and Novartis, Switzerland. All other authors declare no competing interests.

AUTHOR CONTRIBUTIONS

Chantal Donovan, Richard Y Kim, Philip M Hansbro and Jay C Horvat wrote the original draft, reviewed and edited the revised versions of the manuscript, and prepared the figures (visualization). Chantal Donovan, Richard Y Kim and Philip M Hansbro conceptualized the project, methodology, supervision, software and project administration. Chantal Donovan and Philip M Hansbro acquired funding for this project. Chantal Donovan, Richard Y Kim, Izabela Galvao, Andrew G Jarnicki, Alexandra C Brown, Bernadette Jones-Freeman, Henry M Gomez, Ridhima Wadhwa, Elinor Hortle, Ranjith Jayaraman, Haroon Khan, Sophie Pickles, Priyanka Sahu, Vrushali Chimankar, Xiaofan Tu, Md Khadem Ali, Jemma R Mayall, Duc H Nguyen, Kurtis F Budden and Vinod Kumar performed investigation, data curation, validation and formal analysis of the *in vivo* experimental studies. Kate Schroder, Peter AB Wark and Brian G Oliver provided access to resources, reagents and intellectual input on the role of inflammasome-associated inflammatory responses. Avril AB Robertson and Matthew A Cooper provided resources, synthesized the NLRP3 inhibitor for *in vivo* experimental studies and provided intellectual input on the role of NLRP3-associated inflammatory responses. All authors contributed to writing - review & editing, and approved the final manuscript.

REFERENCES

1. Kim RY, Pinkerton JW, Gibson PG, Cooper MA, Horvat JC, Hansbro PM. Inflammasomes in COPD and neutrophilic asthma. *Thorax* 2015; **70**: 1199–1201.
2. Donovan C, Liu G, Shen S, *et al.* The role of the microbiome and the NLRP3 inflammasome in the gut and lung. *J Leukoc Biol* 2020; **108**: 925–935.
3. Schroder K, Tschopp J. The inflammasomes. *Cell* 2010; **140**: 821–832.
4. Lappalainen U, Whitsett JA, Wert SE, Tichelaar JW, Bry K. Interleukin-1beta causes pulmonary inflammation, emphysema, and airway remodeling in the adult murine lung. *Am J Respir Cell Mol Biol* 2005; **32**: 311–318.
5. Imaoka H, Gauvreau GM, Watson RM, *et al.* Interleukin-18 and interleukin-18 receptor-α expression in allergic asthma. *Eur Respir J* 2011; **38**: 981–983.
6. Fricker M, Deane A, Hansbro PM. Animal models of chronic obstructive pulmonary disease. *Expert Opin Drug Discov* 2014; **9**: 629–645.
7. Jones B, Donovan C, Liu G, *et al.* Animal models of COPD: What do they tell us? *Respirology* 2017; **22**: 21–32.
8. Fricker M, Goggins BJ, Mateer S, *et al.* Chronic cigarette smoke exposure induces systemic hypoxia that drives intestinal dysfunction. *JCI Insight* 2018; **3**: e94040.
9. Yanbaeva DG, Dentener MA, Creutzberg EC, Wesseling G, Wouters EF. Systemic effects of smoking. *Chest* 2007; **131**: 1557–1566.
10. Sobinoff AP, Beckett EL, Jarnicki AG, *et al.* Scrambled and fried: cigarette smoke exposure causes antral follicle destruction and oocyte dysfunction through oxidative stress. *Toxicol Appl Pharmacol* 2013; **271**: 156–167.
11. Kang MJ, Homer RJ, Gallo A, *et al.* IL-18 is induced and IL-18 receptor alpha plays a critical role in the pathogenesis of cigarette smoke-induced pulmonary emphysema and inflammation. *J Immunol* 2007; **178**: 1948–1959.
12. Botelho FM, Nikota JK, Bauer CM, *et al.* Cigarette smoke-induced accumulation of lung dendritic cells is interleukin-1α-dependent in mice. *Respir Res* 2012; **13**: 81.
13. Donovan C, Starkey MR, Kim RY, *et al.* Roles for T/B lymphocytes and ILC2s in experimental chronic obstructive pulmonary disease. *J Leukoc Biol* 2019; **105**: 143–150.
14. Eltom S, Belvisi MG, Stevenson CS, *et al.* Role of the inflammasome-caspase1/11-IL-1/18 axis in cigarette smoke driven airway inflammation: an insight into the pathogenesis of COPD. *PLoS One* 2014; **9**: e112829.
15. Pauwels NS, Bracke KR, Dupont LL, *et al.* Role of IL-1α and the Nlrp3/caspase-1/IL-1β axis in cigarette smoke-induced pulmonary inflammation and COPD. *Eur Respir J* 2011; **38**: 1019–1028.
16. Beckett EL, Stevens RL, Jarnicki AG, *et al.* A new short-term mouse model of chronic obstructive pulmonary disease identifies a role for mast cell tryptase in pathogenesis. *J Allergy Clin Immunol* 2013; **131**: 752–762.
17. Franklin BS, Bossaller L, De Nardo D, *et al.* The adaptor ASC has extracellular and 'prionoid' activities that propagate inflammation. *Nat Immunol* 2014; **15**: 727–737.
18. Haw TJ, Starkey MR, Nair PM, *et al.* A pathogenic role for tumor necrosis factor-related apoptosis-inducing ligand in chronic obstructive pulmonary disease. *Mucosal Immunol* 2016; **9**: 859–872.
19. Haw TJ, Starkey MR, Pavlidis S, *et al.* Toll-like receptor 2 and 4 have opposing roles in the pathogenesis of cigarette smoke-induced chronic obstructive pulmonary disease. *Am J Physiol Lung Cell Mol Physiol* 2018; **314**: L298–L317.
20. Liu G, Cooley MA, Jarnicki AG, *et al.* Fibulin-1 regulates the pathogenesis of tissue remodeling in respiratory diseases. *JCI Insight* 2016; **1**: e86380.
21. Lu Z, Van Eeckhoutte HP, Liu G, *et al.* Necroptosis signalling promotes inflammation, airway remodelling and emphysema in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2021; **204**: 667–681.
22. Starkey MR, Plank MW, Casolari P, *et al.* IL-22 and its receptors are increased in human and experimental COPD and contribute to pathogenesis. *Eur Respir J* 2019; **54**: 1800174.
23. Hansbro PM, Hamilton MJ, Fricker M, *et al.* Importance of mast cell Prss31/transmembrane tryptase/tryptase-γ in lung function and experimental chronic obstructive pulmonary disease and colitis. *J Biol Chem* 2014; **289**: 18214–18227.

24. Hsu AC, Dua K, Starkey MR, et al. MicroRNA-125a and -b inhibit A20 and MAVS to promote inflammation and impair antiviral response in COPD. JCI Insight 2017; 2: e90443.

25. Hsu AC, Starkey MR, Hanish I, et al. Targeting PI3K-p110α suppresses influenza virus infection in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2015; 191: 1012–1023.

26. Schanin J, Gebremeskel S, Korver W, et al. A monoclonal antibody to Siglec-8 suppresses non-allergic airway inflammation and inhibits IgE-independent mast cell activation. Mucosal Immunol 2021; 14: 366–376.

27. Skerrett-Byrne DA, Bromfield EG, Murray HC, et al. Time-resolved proteomic profiling of cigarette smoke-induced experimental chronic obstructive pulmonary disease. Respirology 2021; 26: 960–973.

28. Rogliani P, Calzetta L, Ora J, Matera MG. Canakinumab for the treatment of chronic obstructive pulmonary disease. Pulm Pharmacol Ther 2015; 31: 15–27.

29. Calverley PMA, Sethi S, Dawson M, et al. A randomised, placebo-controlled trial of anti-interleukin-1 receptor 1 monoclonal antibody MEDI8968 in chronic obstructive pulmonary disease. Respir Res 2017; 18: 153.

30. Coll RC, Hill JR, Day CJ, et al. MCC950 directly targets the NLRP3 ATP-hydrolysis motif for inflammasome inhibition. Nat Chem Biol 2019; 15: 556–559.

31. Cabral VP, Andrade CA, Passos SR, Martins MF, Hokerberg YH. Severe infection in patients with rheumatoid arthritis taking anakinra, rituximab, or abatacept: a systematic review of observational studies. Rev Bras Reumatol Engl Ed 2016; 56: 543–550.

32. Hornung V, Ablasser A, Charrel-Dennis M, et al. AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. Nature 2009; 458: 514–518.

33. Wilson JE, Petrucelli AS, Chen L, et al. Inflammasome-independent role of AIM2 in suppressing colon tumorigenesis via DNA-PK and Akt. Nat Med 2015; 21: 906–913.

34. Bakele M, Joos M, Burdi S, et al. Localization and functionality of the inflammasome in neutrophils. J Biol Chem 2014; 289: 5320–5329.

35. Fernandes-Alnemri T, Yu JW, Datta P, Wu J, Alnemri ES. AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. Nature 2009; 458: 509–513.

36. Sagulenko V, Thygesen SJ, Sester DP, et al. AIM2 and NLRP3 inflammasomes activate both apoptotic and pyroptotic death pathways via ASC. Cell Death Differ 2013; 20: 1149–1160.

37. Colarusso C, Terlizzi M, Lamort AS, et al. Caspase-11 and AIM2 inflammasome are involved in smoking-induced COPD and lung adenocarcinoma. Oncotarget 2021; 12: 1057–1071.

38. Tran HB, Hamon R, Jersmann H, et al. AIM2 nuclear exit and inflammasome activation in chronic obstructive pulmonary disease and response to cigarette smoke. J Inflamm 2021; 18: 19.

39. Rathinam VA, Jiang Z, Waggoner SN, et al. The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. Nat Immunol 2010; 11: 395–402.

40. Kim RY, Pinkerton JW, Essilfie AT, et al. Role for NLRP3 inflammasome-mediated, IL-1beta-dependent responses in severe, steroid-resistant asthma. Am J Respir Crit Care Med 2017; 196: 283–297.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.