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Interleukin-33 (IL-33) Increases Hyperoxia-Induced Bronchopulmonary Dysplasia in Newborn Mice by Regulation of Inflammatory Mediators

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Background: Interleukin-33 (IL-33) has been reported to affect chronic inflammation of the lungs, but its impact on hyperoxia-injured lungs in newborns remains obscure. This study aimed to investigate the role of IL-33 in the lungs of neonatal mice with hyperoxia-induced bronchopulmonary dysplasia (BPD).

Material/Methods: Twenty-four C57BL/6 baby mice were randomly separated into three groups: the on-air group (N=16); the O₂ group (N=8); and the O₂ + anti-IL-33 group (N=8). Forced mechanical ventilation with oxygen-rich air (MV-O₂) was used in 16 mouse pups. The mouse pups were incubated in containers with either air or 85% O₂ for 1, 3, 7, 14, 21, and 28 days after birth. At the end of the treatment period, the mouse lungs were studied by histology, Western blot, and quantitative real-time polymerase chain reaction (qRT-PCR) to examine the expression of the pro-inflammatory mediators, including interleukin (IL)-1 β , chemokine (CC motif) ligand 1 (CXCL-1), and monocyte chemoattractant protein-1 (MCP-1).

Results: Following forced MV-O₂, increased levels of IL-33 in whole mouse lungs were associated with impaired alveolar growth and with changes consistent with BPD, including reduced numbers of enlarged alveoli, increased apoptosis, and increased expression of IL-1 β , CXCL-1, and MCP-1. IL-33 inhibition improved alveolar development in hyperoxia-impaired lungs and suppressed IL-1 β and MCP-1 expression and was associated with increased transforming growth factor- β (TGF- β) signaling, reduced pulmonary NF- κ B activity and decreased expression of the TGF- β inhibitor SMAD-7 in forced MV-O₂ exposed mouse pups.

Conclusions: IL-33 increased hyperoxia-induced BPD in newborn mice by regulation of the expression of inflammatory mediators.

MeSH Keywords: **Bronchopulmonary Dysplasia • Hyperoxia • Inflammation**

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Background

Bronchopulmonary dysplasia (BPD) is a common chronic pulmonary disease in preterm infants [1]. The progression of BPD is associated with respiratory tract infections, lung damage, and pulmonary dysfunction [2]. Although a variety of factors are associated with BPD, the major causes are a combination of lung immaturity and the effects of ventilation-mediated pulmonary injury [3], long-term oxygen exposure [4], and abnormal epithelial repair following lung injury. Pulmonary injury triggered by mechanical ventilation (MV) with oxygen (O₂) is characterized by inflammation driven by pro-inflammatory cytokines, as well as an increase in levels of alveolar macrophages and neutrophils [5–7].

Previous studies have shown that an increase in pro-inflammatory cytokines, including interleukin (IL)-1 β and transforming growth factor- β (TGF- β), are important for the progression of BPD [8]. There are several cytokines essential for alveolar development that have been shown to be down-regulated in patients with BPD [9]. Multiple stimuli, including high oxygen pressure, have been reported to be associated with lung inflammation and with edema, neutrophil infiltration, and protein effusion into the lung alveoli in mouse models of BPD [4,10]. In a previously published study using a mouse model, forced mechanical ventilation with oxygen (MV-O₂) was shown to trigger BPD and to cause both the progression of the development of alveolar damage and inflammation [11].

Interleukin-33 (IL-33) is a recently described cytokine that is a member of the IL-1 family, which participates in Th2-associated immune reactions [12–14], and has been shown to be associated with a variety of inflammatory diseases, including atopic asthma and rhinitis, and ulcerative colitis [15]. ST2 is a receptor for the IL-1 family, including for IL-33, with a transmembrane isoform (ST2L) and a soluble isoform (sST2) [14]. IL-1R auxiliary protein facilitates the interaction between IL-33 and its receptor ST2, contributing to the production of IL-4, IL-5, IL-10 and IL-13 by eosinophils, mast cells, and Th2 lymphocytes [14]. IL-33 coordinates with IL-4 *in vitro* to promote macrophage monokine production [16], including the secretion of TGF- β and IL-10.

ST2 secreted by fibroblasts has been shown to act as a decoy receptor by binding with IL-33 [17]. Upregulation of ST2, which can be measured in the serum, has been shown to be upregulated in diseases including sepsis and acute myocardial infarction [18].

Given the findings of these previous studies, and the possibility that IL-33 expression may control the upregulation of pro-inflammatory cytokines in the lungs, the aim of this study was to investigate the role of IL-33 in the lungs of neonatal mice with hyperoxia-induced BPD.

Material and Methods

Mice groups studied and inhibition of interleukin-33 (IL-33)

At 48 h post-acclimatization, 24 C57BL/6 baby mice were randomly separated into three groups: the on-air group (N=16); the O₂-treated group (N=8); and the O₂ + anti-IL-33 group (N=8). Forced mechanical ventilation with oxygen-rich air (MV-O₂) was used in 16 mouse pups. The mouse pups were incubated in containers with either air or 85% O₂ for 1, 3, 7, 14, 21, and 28 days after birth. The mice were moved daily between air-filled and O₂-filled containers to prevent oxygen intoxication. For the O₂ + anti-IL-33 group, to study the effects of IL-33 blocking, eight mice exposed to O₂ were injected subcutaneously with an antibody against IL-33 (10 mg/kg/day) along with isotype controls, every three days from day 14. The mice were euthanized after O₂ exposure for 28 days. The study was approved by the Ethics Committee of Animal Experiments of the First Affiliated Hospital of Chongqing Medical University, China.

Mouse lung histology

The mouse lungs were fixed by intratracheal infusion with 4% paraformaldehyde overnight. The size of the processed lungs was determined using the fluid displacement method. Following embedding the lungs in paraffin wax and tissue sectioning at 4 μ m, the tissue sections were routinely stained with hematoxylin and eosin (H&E). Using light microscopy, quantitative evaluation of the alveoli, size of the alveolar spaces, and the alveolar walls were evaluated using three tissue samples for each mouse. The number of alveoli in a radial pattern was counted in 30 randomly selected histological fields selected from three random lung tissue samples per mouse.

Western blot

Tissues were lysed in a buffer containing a protease inhibitor. Protein concentrations were determined using a bicinchoninic acid (BCA) protein quantitation kit. Protein separation was performed using a 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel, and the resolved proteins were transferred to polyvinylidene difluoride (PVDF) (0.45 μ m), which were blocked with 5% bovine serum albumin (BSA) in phosphate buffered saline containing Tween 20 (PBST) for 1 hour at room temperature. Immunoblotting was performed overnight at 4°C with primary antibodies to IL-33, Bcl-2, Bax, cleaved caspase-3, IL-1 β , CXCL-1, and MCP-1 antibodies. Membranes were treated for one hour at room temperature with either goat anti-mouse or goat anti-rabbit peroxidase-labeled secondary antibodies. Western blot immunoreactivity was imaged using a C-DiGit Western Blot Scanner.

Figure 1. Expression of interleukin-33 (IL-33) during lung development and hyperoxic exposure in mouse lungs. **(A)** Comparison of mRNA expression of IL-33 in mouse pup lungs with 85% O₂ exposure during the indicated time course. Gene expression was calculated relative to the housekeeping gene 18S rRNA and further normalized to the P1 control group. **(B)** Western blot analysis showed that the protein levels of IL-33 in the mouse lungs with on-air or O₂ exposure for 28 days. Lower panel: quantitative data for IL-33 were calculated relative to β -actin. ** Represents $p < 0.01$, compared with the on-air group.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from cells of the lungs from the three mouse groups using Trizol reagent. The levels of IL-33, BCL-2, BAX, IL-1 β , CXCL-1, MCP-1, TGF- β , and SMAD-7 mRNA were determined using SYBR Green and a LightCycler 480 quantitative real-time polymerase chain reaction (qRT-PCR) system (Roche). GAPDH was used as a control. The qRT-PCR was conducted in a volume of 20 μ L with SYBR Green PCR Master Mix at a high temperature of 95°C for ten minutes and 40 cycles at 95°C for 15 s, 60°C, and 72°C each for 30 s. The amount of target was calculated ($2^{-\Delta\Delta CT}$) by standardizing to the internal reference and comparison with calibration using the control.

Immunofluorescence (IF)

Tissue sections were treated with 4% paraformaldehyde for 1 hour at room temperature and permeabilized in 0.5% Triton X-100 in PBST at room temperature for 10 minutes. Tissue sections were then blocked with 0.4% BSA in PBST at 37°C for 1 hour, and incubated with polyclonal anti-Bcl-2 and anti-cleaved-caspase-3 antibodies diluted in PBST containing 0.2% BSA at 37°C for 1 hour. After washing in PBST (1 hour), cells were incubated with tetramethylrhodamine (TRITC) bright orange fluorescent dye-tagged goat anti-rabbit antibody in PBST (0.2% BSA) at 37°C (1 hour), and then washed in PBST (1 hour).

Cells were stained with 4',6-diamidino-2-phenylindole (DAPI) for 5 min. Colocalization of Bcl-2 and cleaved-caspase-3 was observed using an Olympus LSCMFV500 confocal fluorescence scanning microscope.

Statistical analysis

Numerical data were recorded as the mean $\pm$ standard deviation (SD). Group comparisons were made using one-way analysis of variance (ANOVA) or two-tailed t-tests. Statistically significant differences were considered to be $p < 0.05$.

Results

Interleukin-33 (IL-33) expression in mice with forced mechanical ventilation with oxygen (MV-O₂)

To investigate the production of IL-33 in pulmonary tissues in a mouse model of bronchopulmonary dysplasia (BPD), the expression of IL-33 was evaluated in triplicate (P1, P14, and P21). As the baby mice matured, IL-33 mRNA expression increased progressively, while maintaining a relatively low overall level. Mice treated with 85% O₂ showed upregulation in IL-33 mRNA expression during the period from between 1–21 days. With longer exposure to MV-O₂, IL-33 mRNA expression continued to rise progressively up to time point P21 (Figure 1A). Consistent

Figure 2. Mechanical ventilation with oxygen (MV-O₂) shows an impaired alveolar structure in mouse lungs and MV-O₂ for 8 hours increased airspace size and decreased the number of alveoli. **(A)** Photomicrographs of representative lung tissue sections from 6-day-old to 7-day-old mice after treatment with mechanical ventilation with oxygen-rich air (MV-O₂) for 8 h, showing increased airspace size when compared with the on-air group. Magnification ×200. **(B)** Quantitative image analysis shows that the alveolar area of lung tissue sections increased after MV-O₂ for 8 h compared with on-air group. **(C)** Quantitative image analysis for radial alveolar counts. * Represents p<0.05, compared with the on-air group.

with the mRNA expression results for IL-33, the protein levels of IL-33 were also increased in mice treated with MV-O₂ when compared with the control group (Figure 1B).

MV-O₂ impaired alveolar development in mouse lungs

Quantitative lung tissue morphometry showed that mice treated with O₂ for 8 hours had damaged the pulmonary structure, including an increase in the size of the lung alveolar terminal spaces and a decrease in the number of radiating alveoli in the O₂-treated group when compared with the on-air group (Figure 2A–2C).

MV-O₂ increased apoptosis in mouse lungs

To investigate the impact of MV-O₂ on apoptotic cell levels in lung tissue, Bcl-2 and Bax expression and cleaved caspase-3 was evaluated by Western blot and quantitative real-time polymerase chain reaction (qRT-PCR). MV-O₂ treatment reduced Bcl-2 levels while promoting Bax levels when compared with the

on-air group (Figure 3A, 3B). These data were confirmed by the mRNA expression levels of the BCL-2 and BAX genes, as shown in Figure 3D and 3E. These findings were also confirmed by the 5-fold to 6-fold increase of caspase-3 in the lungs of MV-O₂ mice compared with the on-air treated mice (Figure 3C, 3F). Dual immunofluorescence (IF) assay for Bcl-2 and caspase-3 showed increased expression in mouse primary lung myofibroblasts in the lungs of the MV-O₂ group (Figure 3G).

MV-O₂ increased pro-inflammatory cytokines in the mouse lung

Pulmonary inflammatory cytokines were evaluated using Western blot and qRT-PCR, including interleukin (IL)-1β, chemokine (CC motif) ligand 1 (CXCL-1), and monocyte chemoattractant protein-1 (MCP-1). Protein expression levels of these three cytokines increased significantly after MV-O₂ treatment when compared with the on-air group (Figure 4A). These data were further confirmed with qRT-PCR data (Figure 4B–4D).

Figure 3. Mechanical ventilation with oxygen (MV-O₂) increased apoptosis in mouse lungs. **(A–C)** Western blot was performed to assess Bcl-2 and Bax expression and cleaved caspase-3 protein expression in newborn mice compared with the on-air group after 8 h of mechanical ventilation with oxygen-rich air (MV-O₂). **(D, E)** Quantitative real-time polymerase chain reaction (qRT-PCR) data shows that Bcl-2 and Bax mRNA expression was altered by MV-O₂. **(F)** Quantitative analysis of the cleaved caspase-3 band intensity in each group. **(G)** Immunofluorescence (IF) analysis of lung tissue shows increased dual staining for cleaved caspase-3 (red) and Bcl-2 (green) in the lungs of 6-day-old to 7-day-old mice after 8 h MV-O₂ compared with the on-air group. Magnification ×400. ** Represents $p < 0.01$. *** Represents $p < 0.001$, compared with the on-air group.

Figure 4. Mechanical ventilation with oxygen (MV-O₂) increased cytokine expression in mouse lungs. **(A)** Western blot results to assess interleukin (IL)-1β, chemokine (CC motif) ligand 1 (CXCL-1), and monocyte chemoattractant protein-1 (MCP-1) expression in newborn mice treated with MV-O₂, compared with the on-air group. **(B–D)** Pulmonary mRNA expression of IL-1β, CXCL-1, and MCP-1 is increased in newborn mice after day 1, 14, and 28 of MV-O₂, compared with the on-air group. ** Represents p<0.01. *** Represents p<0.001, compared with the on-air group.

Effect of IL-33 inhibition on alveolar development, apoptosis, and inflammation in mouse pups following O₂ exposure

The effect of IL-33 inhibition on the development of alveolar damage resulting from O₂ exposure was studied. Without inhibition of IL-33, following O₂ exposure for 21 days, mouse pup lungs showed abnormal alveolar development. IL-33 inhibition resulted in a histological appearance that was almost indistinguishable from that of lung tissue from the on-air group (Figure 5A). Quantification of the distal airspace size and in radial alveolar counts showed that IL-33 inhibition restored the O₂-induced alveolar damage (Figure 5B, 5C).

The level of lung apoptosis was measured following IL-33 inhibition in the O₂-treated group. Protein and mRNA expression patterns of Bcl-2, Bax, and cleaved caspase-3 were examined using Western blot and qRT-PCR. The data showed that IL-33 inhibition caused upregulation of Bcl-2 and down-regulation for Bax at both the protein and mRNA level (Figure 6A–6C). There was also a decrease in the amount of cleaved caspase-3.

These findings indicated that IL-33 inhibition could, to some extent, recover hyperoxia-induced apoptosis in mouse lung tissue. To examine whether hyperoxia-associated expression of pro-inflammatory cytokines could also be regulated by IL-33, three cytokines, IL-1β, CXCL-1, and MCP-1, were evaluated by Western blot and qRT-PCR. The results showed that the expression of these three cytokines was significantly decreased following IL-33 inhibition, at both the protein and mRNA levels, indicating that the decrease in levels of pulmonary inflammatory cytokines could be attributed to IL-33 inhibition.

The effects of IL-33 inhibition on the transforming growth factor-beta (TGF-β) pathway and NFκB activation

To further investigate crosstalk between IL-33 and two inflammation-associating factors, TGF-β and NFκB, Western blot and qRT-PCR were performed. The activity of the TGF-β pathway was supported by the finding of reduced pSMAD2 protein expression in the lungs of mice following IL-33 inhibition (Figure 7A, 7B), and an increase in TGF-β mRNA expression (Figure 7C). These findings also indicated a decrease in

Figure 5. Interleukin-33 (IL-33) inhibition restored alveolar structure in mouse lungs. Treatment with IL-33 neutralizing antibody for 8 h recovered airspace size and the number of lung alveoli. **(A)** Photomicrographs of representative sections of lung tissue sections (200×) from 6-day-old to 7-day-old mice in the O₂-treated group after treatment of IL-33 antibody, showing decreased airspace size when compared with the O₂-treated group. Magnification ×200. **(B)** Quantitative image analysis of the alveolar area in lung tissue sections shows a decrease of alveolar area after IL-33 inhibition. **(C)** Quantitative image analysis of radial alveolar counts, an index of alveolar number, in lung tissue sections from O₂-treated and on-air treated mice after IL-33 inhibition. * Represents $p < 0.05$, compared with the on-air group. # Represents $p > 0.05$, compared with the O₂-treated group.

IκB phosphorylation (Figure 7D, 7E), indicating a reduction in NFκB activation in the lungs of O₂-ventilated pups following treatment with the IL-33 inhibitor. The evaluation of the TGF-β inhibitor SMAD-7 also showed a significant decrease in protein and mRNA expression in the lungs of O₂-ventilated pups treated with IL-33 inhibitor compared with non-treated O₂-ventilated pups (Figure 7F, 7G).

Discussion

In the present study, a mouse model of bronchopulmonary dysplasia (BPD) was studied, which showed increased expression of interleukin-33 (IL-33) in neonatal mice treated with forced mechanical ventilation with oxygen-rich air (MV-O₂). In this model, changes in alveolar development were similar to those described in hyperoxia-induced BPD. Interleukin-33 (IL-33) has previously been shown to be expressed in chronic inflammatory diseases, and the mouse model of BPD used in this study was highly sensitive to modification in the levels of IL-33. The use of an anti-IL-33 neutralizing antibody to

induce IL-33 blockade in the MV-O₂-injured newborn lung reduced the progression of BPD through inhibition of lung tissue apoptosis and the expression of pro-inflammatory cytokines. Blocking of IL-33 reduced the size of the alveolar airspaces and alveolar count of O₂-treated mouse pups, reduced cell apoptosis and reduced the expression of pro-inflammatory cytokines. Also, blockade of IL-33 inhibited the TGF-β signaling pathway and the activation of NFκB. These results, in a mouse model of BPD, support that IL-33 might have a role in the pathogenesis of BPD pathogenesis by its effects on alveolar cell apoptosis and inflammation.

Previous studies in animal models have shown that exposure to above normal physiological levels of oxygen alone can induce a lung phenotype that is similar to BPD, including impaired alveolar development [19]. Also, while endotracheal administration of recombinant superoxide dismutase to premature human infants has not been shown to reduce the rates of BPD, long-term pulmonary outcomes were improved, which supports the contribution of oxidative injury in BPD [20]. Increased levels of oxygen that are above normal physiological

Figure 6. Effect of interleukin-33 (IL-33) inhibition of apoptosis and pulmonary inflammation. **(A)** Western blot analysis shows protein expression patterns of Bcl-2, Bax, and cleaved caspase-3 in the lungs of 12-day-old mouse pups in each group. **(B, C)** Quantitative real-time polymerase chain reaction (qRT-PCR) data shows that Bcl-2 and Bax mRNA expression was altered after treatment with mechanical ventilation with oxygen-rich air MV-O₂ and IL-33 inhibition. **(D)** Quantitative analysis of cleaved caspase-3 band intensity in each group. **(E)** Western blot analysis comparing the concentrations of three cytokines (IL-1β, CXCL-1, and MCP-1) in the lungs of pups treated as indicated. **(F–H)** Comparison of interleukin (IL)-1β, chemokine (CC motif) ligand 1 (CXCL-1), and monocyte chemoattractant protein-1 (MCP-1) mRNA expression, expressed relative to β-actin mRNA, in 14-day-old pups treated as indicated. * Represents $p < 0.05$. ** Represents $p < 0.01$, compared with the on-air group. # Represents $p < 0.05$. ## Represents $p < 0.01$, compared with the O₂ group.

Figure 7. Interleukin-33 (IL-33) inhibition increased the activation of transforming growth factor-beta (TGF-β) signaling and decreased NFκB activation in lungs of pups treated with IL-33 antibody. **(A)** Western blot showing a significant increase of pSMAD2 and SMAD2 protein expression in the lungs of pups undergoing mechanical ventilation with oxygen-rich air MV-O₂ and/or IL-33 inhibition. **(B)** Quantitative image analysis of pSMAD2 staining per total tissue indicating significantly increased pSMAD2 expression in the lung of mouse pups treated with IL-33 inhibitor compared with the O₂-treated group. **(C)** Transforming growth factor-β(TGF-β) mRNA expression in indicated groups was examined by quantitative real-time polymerase chain reaction (qRT-PCR). **(D)** IL-33 inhibition reduced the expression of phosphorylated IκB, indicating a reduced activation of NFκB in the lungs of newborn mice compared with pups from the MV-O₂ group. **(E)** Quantitative analysis of phosphorylated IκB band intensity in the indicated groups. **(F, G)** Western blot and quantitative real-time polymerase chain reaction (qRT-PCR) analysis of the TGF-β inhibitor SMAD-7 expression at both the protein and mRNA level in the lungs of newborn mice in the IL-33 inhibition group compared with the O₂-treated group. * Represents p<0.05, compared with the O₂-treated group.

levels contribute to the increased generation of mitochondrial reactive oxygen species (ROS) resulting in increased susceptibility to oxidative stress and alveolar cell injury in the developing lung, which might also be increased by a deficiency in antioxidants and immature defenses of the newborn lung [21]. Animal models suggest that even brief exposure to high concentrations of oxygen can result in long-term morphologic and functional changes in the lung [22]. Also, a critical window of susceptibility to oxidative lung injury may exist in the immature lung [23]. Supporting these concerns are clinical data suggesting that even brief exposure to high oxygen levels during resuscitation increases the risk of BPD [24], and evidence of oxidative stress can be identified in exhaled breath condensate of infants born preterm [25].

Under normal conditions, IL-33 mRNA and protein are expressed constitutively and abundantly in many tissues in both mice and humans [26]. In the human airway, bronchial epithelial cells and endothelial cells of high endothelial venules are the primary sites for IL-33 production [27,28]. In contrast, mouse endothelial cells are not the major source for constitutive expression of IL-33, although its expression can be induced in mouse endothelial cells under chronic inflammatory stimuli [29,30]. Also, previous studies have shown that mouse IL-33 is predominantly expressed in lung alveolar type II pneumocytes, but human IL-33 is expressed by bronchial epithelial cells [31,32]. IL-33 plays a significant role in immunity, inflammation, and homeostasis of mucosal tissues and in severe human airway diseases such as asthma [16], allergic rhinitis [33], and chronic rhinosinusitis [34]. In the present study, in a mouse model, upregulation of IL-33 expression

was found during hyperoxia-induced BPD development, which is also considered a chronic lung disease. The data from this study support that IL-33 might be involved in pulmonary inflammation, caused by MV-O₂, in newborns.

Activation of the TGF- β pathway has been shown to be the major cause of BPD through its modulating effect on elastic fiber deposition [35,36]. TGF- β signaling is triggered by the interaction of TGF- β and type II TGF- β receptor (T β RII), with subsequently binding with the type I receptor. The type I receptor transmits cellular signals through second-messenger Smad 1 to Smad 4 proteins [37]. The TGF- β signal pathway can negatively modulate the alveolar and airway branching [38] and septation [39] phases during lung development. For septation, it has been reported that adenoviral-mediated transfer of TGF- β 1 to the neonatal rat lung [39] or overexpression of TGF- β 1 between postnatal days 7 and 14 in the mouse [40] both induced histological changes analogous to BPD. However, SMAD3 knockout mice have been shown to exhibit delayed development of lung alveoli between days 7 and 28 [41], suggesting that TGF- β may also function as a positive regulator of septation, but also may have a bidirectional role in the development of lung alveoli. Smad3 deficiency in adult mice results in airspace enlargement and centrilobular emphysema in later life [42], also indicating an essential role for TGF- β signaling in both the formation of the alveoli and the maintenance of the alveolar structure. In the present study, TGF- β expression was increased and downstream phosphorylation of SMAD-2 was increased, while the expression of SMAD-7, the inhibitor of TGF- β , was reduced following IL-33 inhibition. Although the data from the present study did not demonstrate the precise mechanism of TGF- β activation by IL-33, there are two possible explanations for the study findings. Firstly, it is possible that IL-33 can directly upregulate TGF- β expression, but there is no direct evidence to support this viewpoint at this point. Secondly, it is possible that IL-33 could enhance TGF- β activity through IL-1 β activation resulting in neutrophil recruitment in pulmonary tissue.

There is known to be crosstalk between NF κ B and the TGF- β pathway [43]. NF κ B inhibition contributes to increased activation of TGF- β , facilitating the recruitment of inflammatory cells and promoting the production of pro-inflammatory cytokines [44,45]. NF κ B is a transcription factor that regulates the transcription of several genes related to the immune response to external stimuli and exerts a pivotal role in inducing the expression of type I interferons (IFNs), including IFN- α and IFN- β , and pro-inflammatory cytokines [46–48]. Recent studies have shown that NF κ B plays an important role in inflammation, cell survival, and developmental functions in the

newborn lung [49,50]. The deactivation of NF κ B observed in the present study might support that further reduction of the inflammation caused by MV-O₂ also enhanced the pro-apoptotic effects of TGF- β . Also, NF κ B deactivation in the mouse model used in the present study was associated with a reduction of SMAD-7 protein and mRNA levels. Therefore, it is possible to hypothesize that augmented NF κ B phosphorylation inhibited the development of the newborn lung and promoted excessive activation of the TGF- β pathway, enhancing the recruitment of inflammatory cells to the lung tissue in BPD [51].

The findings of this study, to investigate the role of IL-33 in the lungs of neonatal mice with hyperoxia-induced BPD, indicated that IL-33 expression was elevated by MV-O₂ exposure, and treatment with an IL-33 inhibitor restored MV-O₂-impaired alveolar development in the newborn mouse lung. The findings of the study also showed that aberrant expression of IL-33 resulted in dysplasia in the newborn mouse lung, and enhanced apoptosis and inflammation in newborn lung, via modulation of the TGF- β pathway and NF κ B phosphorylation. Further studies are required to support these findings and should focus on understanding the mechanism by which IL-33 activates the TGF- β pathway and results in NF κ B subunit phosphorylation during the development of BPD.

Conclusions

The findings of this study showed that increased expression of interleukin-33 (IL-33) in a mouse model of bronchopulmonary dysplasia (BPD) using forced mechanical ventilation with oxygen-rich air (MV-O₂) to create lung hyperoxia was associated with the expression of inflammatory mediators and lung changes consistent with alveolar damage. Treatment with an IL-33 inhibitor restored MV-O₂-impaired alveolar development in the newborn mouse lung. Also, increased expression of IL-33 was associated with apoptosis and inflammation in the newborn mouse lung, and with modulation of the TGF- β pathway and NF κ B phosphorylation. Further studies are required to investigate the mechanisms of the interactions between IL-33 activation, the TGF- β pathway, and NF κ B subunit phosphorylation during the development of BPD and to determine whether there might be clinical potential in the use of treatment approaches to block IL-33 to protect the newborn lung against hyperoxia-induced BPD.

Conflict of interests

None.

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