

Increased poly(ADP-ribose) polymerase (PARP)-1 expression and activity are associated with inflammation but not goblet cell metaplasia in murine models of allergen-induced airway inflammation

Thomas Havranek,¹ Pawandeep K. Aujla,² Tracey J. Nickola,² Mary C. Rose,³ and Louis M. Scavo⁴

¹*Division of Neonatology, Children's National Medical Center, Washington, DC, USA; and Department of Pediatrics, George Washington University, Washington, DC, USA*

²*Research Center for Genetic Medicine, Children's National Medical Center, Washington, DC, USA*

³*Research Center for Genetic Medicine, Children's National Medical Center, Washington, DC, USA; and Departments of Pediatrics and of Biochemistry and Molecular Biology, George Washington University, Washington, DC, USA*

⁴*Division of Neonatology, Children's National Medical Center; and Department of Pediatrics, George Washington University, Washington, DC, USA*

ABSTRACT

Inflammation plays a key role in lung injury and in the pathogenesis of asthma. Two murine models of allergic airway inflammation—sensitization and challenge to ovalbumin (OVA) and intratracheal exposure to interleukin-13 (IL13)—were used to evaluate the expression of poly(ADP-ribose) polymerase-1 (PARP-1) in allergic airway inflammation. Inflammation is prominent in OVA-induced allergic asthma, but this inflammation is greatly reduced by a PARP-1 inhibitor and almost eliminated when PARP-1 knockout mice are subjected to the OVA model. The present study temporally evaluated PARP-1 protein expression, localization, and activity, as well as inflammation and goblet cell metaplasia (GCM), in murine lungs following a single OVA challenge or IL13 exposure. Following OVA challenge PARP-1 protein expression and activity were greatly increased, being maximal at 3 to 5 days following OVA exposure and beginning to decrease by day 8. These changes correlated with the timing and degree of inflammation and GCM. In contrast, PARP-1 protein or activity did not change following single IL13 exposure, though GCM was manifested without inflammation. This study demonstrates that both PARP-1 protein and activity are increased by allergen-activated inflammatory mediators, excluding IL13, and that PARP-1 increase does not appear necessary for GCM, one of the characteristic markers of allergic airway inflammation in murine models.

KEYWORDS Asthma, goblet cell metaplasia, inflammation, interleukin-13, ovalbumin, poly(ADP-ribose) polymerase

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The present address of Thomas Havranek is Department of Pediatrics, Saint Louis University, Cardinal Glennon Children's Medical Center, St. Louis, Missouri, USA.

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Address correspondence to L. M. Scavo, MD, Department of Pediatrics, Division of Neonatology, Children's National Medical Center, 111 Michigan Avenue, NW, Washington, DC 20010, USA. E-mail: Lscavo@cnmc.org

Airway inflammation, hyperresponsiveness, and an increased number of goblet cells and increased airway mucus are typical features of human asthma [1–4] and these pathologies are also observed in murine models of allergen-induced airway inflammation developed to shed light on mechanisms of allergic asthma [5–8]. In response to allergens, inflammatory cells in the airways and alveolar spaces release a number of inflammatory mediators and reactive oxygen species (ROS) [9–11]. ROS are potent inducers of breaks in DNA [12–14], which in turn

activate poly(ADP-ribose) polymerase-1 (PARP-1) [15], which then ADP-ribosylates histones, transcription factors, and PARP-1 itself [16, 17], thus promoting an inflammatory response. A pathophysiological role for PARP-1 has been demonstrated in a number of pulmonary inflammatory disease models [18–21], as well as in other inflammatory models [18, 20, 22–24]. It is also involved in non-pathologic processes such as DNA repair [25] and replication [26, 27], cell cycle regulation and cell proliferation [28, 29], differentiation [30, 31], cell death [32, 33], and mammalian development [22, 34–36].

Several murine models of allergen-induced airway inflammation that manifest varying degrees of lung inflammation have been developed. In the ovalbumin (OVA) sensitization and challenge model, inflammation is prominent and eosinophils (60%), lymphocytes, neutrophils, and monocytes are seen in the bronchoalveolar lavage (BAL) fluid [6, 7]. Interleukin-13 (IL13) is a central mediator in the pathogenesis of allergic asthma [37–39] and is thought to initiate the pathophysiology of allergic asthma by its actions on epithelial and smooth muscle cells, rather than through traditional effector pathways involving eosinophils and immunoglobulin E (IgE)-mediated events [40]. In contrast to the OVA model, minimal inflammation is observed following exposure of murine lungs to IL13 [41, 42]. In the BAL fluid eosinophils greatly predominate for the first 24 hours following IL13 exposure but decrease to baseline levels after 72 hours [37].

In contrast to the different inflammatory cell profiles seen in these 2 models of allergic airway inflammation, they both show similar levels of goblet cell metaplasia (GCM), which is seen as an increased number of goblet cells in the conducting airway epithelium, [6, 37, 38, 41, 42].

It had been reported that PARP-1 knockout mice are resistant to OVA-induced airway inflammation and that 3-aminobenzamide, an inhibitor of PARP-1, greatly reduces airway inflammation elicited by OVA [43]. Recently, it was also shown that PARP-1 inhibition using the phenanthridinone-based inhibitor PJ-34 suppressed inflammatory cell migration in the murine OVA model, with down-regulation of selective chemokines and cytokines but no effect on IL13 or IL5 levels [44]. Because these reports indicate that PARP-1 is involved in an inflammatory response in the OVA model, we investigated the temporal expression of PARP-1 protein and PARP-1 activity in both the OVA and IL13 murine models of allergic asthma to determine whether PARP-1 effects contributed to GCM as well as inflammation.

MATERIALS AND METHODS

Mice

Male Balb/C mice, 4 to 6 weeks old, were obtained from Harlan Sprague-Dawley (Indianapolis, IN) and housed in the Children's National Medical Center (CNMC) environmentally controlled pathogen-free animal facility accredited by the American Association of Laboratory Animal Care. The handling of mice was carried out in accordance with institutional protocols, based on National Institutes of Health (NIH) guidelines for laboratory animal research. To facilitate pulmonary aspiration with intranasal (IN) as well as intratracheal (IT) instillation, mice were anesthetized using isoflurane (Baxter Healthcare, Deerfield, IL).

Models of allergen-induced airway inflammation

Intranasal OVA challenge in sensitized mice

Forty-eight male Balb/C mice were challenged and sensitized as previously reported [41] and divided into 4 groups. Half of each group was sensitized with OVA in phosphate-buffered saline (PBS); the remaining half (control mice) received PBS only. Mice were sensitized by intraperitoneal injection (days 0, 4, 7, 11, 14) and IN instillation (day 14). Experimental animals were challenged IN with OVA (100 μ g/0.50 mL of PBS), whereas control animals were challenged with 0.5 mL of PBS on day 21. Mice were sacrificed by carbon dioxide asphyxiation on days 1, 3, 5 and 8 following challenge. Each of the 4 groups had 6 experimental and 6 control animals corresponding to the day of sacrifice after challenge. Lungs were dissected and frozen immediately in liquid nitrogen and stored at -80°C prior to protein analyses or fixed in 10% formalin for histological or immunohistochemical analyses.

Intratracheal IL13 exposure

Forty-eight male Balb/C mice were divided into 4 groups, each having 6 experimental and 6 control animals. For intratracheal (IT) instillation, mice were placed on a 45° inclined board and the trachea was transilluminated indirectly to allow its visualization through the oral cavity. A canula attached to a Hamilton syringe containing either PBS (50 μ L) or recombinant murine IL13 (R&D Systems, Minneapolis, MN) (5 μ g/50 μ L of PBS) was inserted into the trachea for delivery of sample. Mice were sacrificed by carbon dioxide asphyxiation and lungs were harvested

for histological and immunohistochemical analyses at 12, 48, 72, and 96 hours after a single IT instillation of IL13 or PBS.

Western analysis

For protein extraction, frozen lung tissue was transferred to 3× volumes of homogenization buffer (0.5% NP-40/0.3 NaCl/0.1 mM EDTA/20 mM HEPES/10% glycerol, 1 mM dithiothreitol [DTT], and 2% protease inhibitor cocktail [Sigma]) and homogenized on ice using a Dounce homogenizer. Samples were sonicated for 20 seconds, then incubated at 4°C for 60 minutes. Samples were then centrifuged for 5 minutes at 14,000 rpm, and an aliquot of the supernatant was used to determine protein concentration using Bio-Rad DC assay (Bio-Rad, Richmond, CA) and the remainder was stored at -80°C. For electrophoresis, equal amounts of protein extract (10 µg) were mixed with 2× sample loading buffer (6 M urea/0.35 M Tris HCl/4% sodium dodecyl sulfate [SDS]/20% glycerol/2.0% β-mercaptoethanol, pH 6.8) (Invitrogen, Carlsbad, CA) and incubated for 5 minutes at 90°C. Electrophoresis was carried out on precast 4–12% Tris-Glycine gel (Invitrogen) in Novex apparatus system. PARP-1 enzyme (Biomol, Plymouth Meeting, PA) was used as a positive control. Proteins were transferred electrophoretically onto 0.45-micron nitrocellulose (MSI, Westboro, MA) using a Novex apparatus. After blocking in freshly prepared 5% milk-buffer solution (PBS, pH 7.4), the blots were incubated with C 2–10 anti-PARP monoclonal antibody (Pharmingen, San Diego, CA) (dilution 1:250 in 2.5% milk-PBS) overnight at 4°C on a rotator. The blots were washed extensively with PBS-Tween D20 (0.1%) and incubated with anti-mouse immunoglobulin G (IgG), horseradish peroxidase-conjugated secondary antibody (Jackson ImmunoResearch, West Grove, PA), diluted 1:40,000 in 2.5% milk-PBS, for 1 hour at room temperature. Membranes were washed with PBS-Tween D20 (0.1%), then visualized using Chemiluminescence Reagent Plus (NEN Life Science Products, Boston, MA). Images were developed on Biomax MS film (Eastman Kodak, Rochester, NY). Electrophoresis of samples was repeated multiple times using different samples. Protein loading, electrophoresis run veracity, and transfer efficiency verified by Ponceau staining. Some membranes were stripped and reprobed with monoclonal anti-β-actin primary antibody (clone AC-15; Sigma-Aldrich, St. Louis, MO).

Histology

Formalin-fixed tissues were dehydrated through graded ethanol solutions, embedded in paraffin, and

cut into 5-µm sections (American Histoserv, Germantown, MD). Slides were stained with hematoxylin and eosin (H&E) to evaluate general morphology and with Alcian blue/periodic acid-Schiff (AB/PAS) to identify the presence of mucin glycoconjugates and goblet cells (stained as purple magenta). Briefly, slides were incubated with AB (pH 2.5) for 30 minutes, rinsed with PBS, incubated with 0.5% periodic acid for 10 minutes, rinsed with PBS, incubated in Schiff reagent (Sigma) for 10 minutes, washed in tap water, and counterstained with Harris hematoxylin and eosin (Sigma).

Immunohistochemical analysis of PARP-1 protein and activity

Paraffin-embedded tissues were cut into 5-µm sections and mounted onto poly-L-lysine slides (American HistoServ). All procedures were carried out at room temperature unless otherwise stated. Sections were deparaffinized in 100% xylene, rehydrated through graded ethanol series, and rinsed for 5 minutes in tap water. Antigen recovery was performed using Dako target retrieval solution for 10 minutes at 95°C, followed by cooling for 20 minutes in PBS. Endogenous peroxidase activity was quenched by incubation with 0.3% hydrogen peroxide for 5 minutes followed by a PBS wash. PARP-1 was detected in tissue sections using C 2–10 anti-PARP monoclonal antibody (dilution 1:250 in 2.5% milk-PBS). Amplification of the primary antibody is performed using a mouse Vectastain ABC kit (Vector Laboratories, Burlingame, CA) and visualization is performed with 3',3'-diaminobenzidine (Sigma) as the substrate for the peroxidase-linked secondary antibody. The nuclei were counterstained with Gill's hematoxylin (Sigma), air dried, and mounted with Aquamount (Lerner Laboratories, Pittsburgh, PA). Immunohistochemical detection was repeated at least 3 times using sections from different mice.

For immunohistochemical detection of PARP-1 activity, paraffin sections (3 µm) were deparaffinized in xylene and rehydrated in decreasing concentrations of ethanol, followed by rinsing with PBS (pH 7.4). Sections were treated with 0.3% hydrogen peroxide for 5 minutes to block endogenous peroxidase activity and then rinsed briefly in PBS. Nonspecific binding was blocked by incubating the slides for 1 hour in PBS containing 2% horse serum. A monoclonal anti-poly(ADP-ribose) (anti-PAR) antibody (Alexis, San Diego, CA) at a 1:600 dilution was applied for 1 hour at room temperature. Immunoreactivity was detected with a biotinylated anti-mouse secondary antibody and the avidin-biotin-peroxidase complex, both supplied in the Vector Elite Kit (Vector Laboratories).

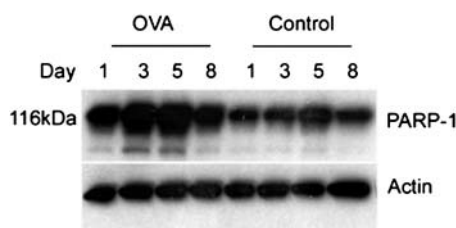


FIGURE 1 Effects of OVA sensitization and challenge on PARP-1 protein expression in mouse lung. A representative Western blot from 3 experiments shows PARP-1 and actin levels in OVA-challenged mice as compared to control mice on days 1, 3, 5, and 8 following intranasal OVA or PBS challenge of OVA-sensitized or PBS-exposed mice, as described in the Materials and Methods. Ten micrograms of protein was loaded in each lane.

Color was developed using the peroxidase substrate SG kit (Vector Laboratories) for 10 minutes. Sections were counterstained with Meyer's hematoxylin (Sigma).

RESULTS

PARP-1 protein expression and localization in an OVA sensitization and challenge model of allergen-induced airway inflammation

Western analysis

Expression of PARP-1 protein in lung tissues obtained on days 1, 3, 5, and 8 following OVA or PBS challenge was examined by Western blot analysis. PARP-1 protein levels increased at day 1, peaked at day 3 and day 5, and decreased by day 8 post OVA challenge (Figure 1). Minimal changes in PARP-1 baseline levels were observed in control groups, which were administered only PBS. Actin levels were unchanged in the lungs of control or OVA-challenged mice at the time points examined.

Histological analysis

In order to correlate PARP-1 protein expression changes with changes in both inflammation and GCM at the aforementioned time points, 3 types of histological analyses were performed on tissue sections of control (PBS-challenged) and experimental (OVA-sensitized and -challenged) lungs. Immunohistochemistry for PARP protein was used to profile both expression levels and location of that protein; H&E staining was used to identify lung structural and inflammatory changes, and AB/PAS staining was used to identify GCM (representative examples in Figure 2). Minimal PARP-1 expression was observed in the lungs of PBS-exposed mice at all time points;

a representative field from day 5 post challenge is shown in 2 magnifications in Figure 2A and C. It is most clear in Figure 2C that all nuclei are either very deep or pale blue, negative for PARP-1 protein. In contrast, abundant PARP-1 protein expression was detected in the alveoli and in the conducting airways (bronchi and bronchioles) of OVA-sensitized mice, which peaked on days 3 and 5 post OVA challenge, as observed by Western analysis. A representative field from day 5 post OVA challenge is shown in 2 magnifications in Figure 2B and D. It is most clear in Figure 2D that many nuclei are either very deep or pale brown (PARP-1 positive), whereas others are blue (PARP-1 negative). The PARP-1 staining showed predominantly nuclear localization in epithelial and endothelial cells as well as in the alveolar macrophages, eosinophils and neutrophils.

Neither inflammatory changes nor GCM were observed in the lungs of PBS-treated mice on any of the days studied. Representative examples for day 5 post PBS exposure are shown in Figure 2E and G, respectively. In contrast, in OVA-sensitized and -challenged mice, inflammatory changes, characterized by thickening of the alveolar septa, inflammatory cell infiltrate, and airway smooth muscle hypertrophy, were observed at days 1, 3, 5, and 8 and were most prominent on days 3 to 5. GCM was observed at days 1, 3, 5, and 8 and was most prominent on days 3 to 5. Representative examples from day 5 post OVA challenge are shown for inflammatory changes (Figure 2F) and for GCM staining (deep blue epithelial staining; Figure 2H).

PARP-1 protein expression and localization in the IL13 model of allergen-induced airway inflammation

Western analysis

Expression of PARP-1 protein was examined by Western blot analysis. In lung tissues obtained on days 0.5, 2, 3, and 4 following intratracheal (IT) instillation of PBS (control) or IL13 (experimental). A representative immunoblot of 3 exposure experiments is shown in Figure 3. PARP-1 protein was expressed at low and comparable levels at all time points in both IL13-exposed and PBS control groups. Actin expression was minimally altered by IL13 exposure. These data demonstrated that IL13 exposure to murine lungs did not increase PARP-1 protein expression.

Histological analysis

Using the same methods as for the OVA model of murine asthma, lung tissue of the IL13-treated control lungs were examined for PARP-1 protein levels and location, lung structural and inflammatory

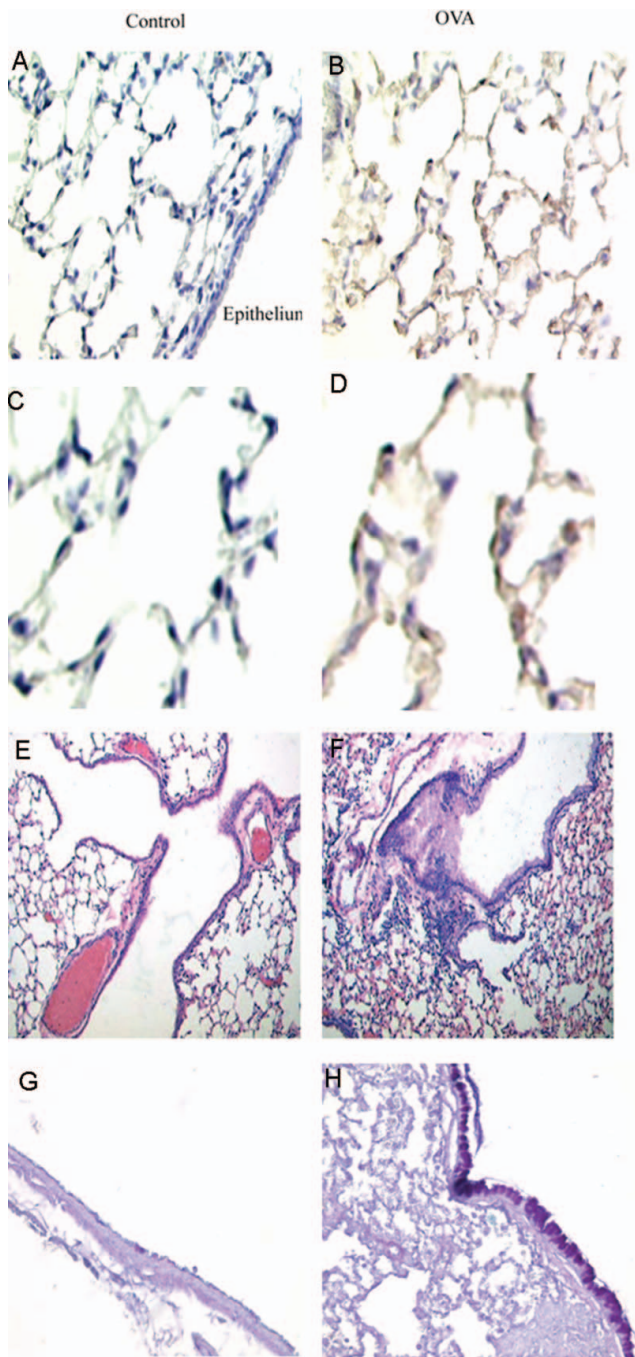


FIGURE 2 Immunohistochemistry and histopathology of lungs of control and OVA-sensitized/challenged Balb/C mice. *Top two rows:* Immunohistochemical analysis of PARP-1 protein. Lung sections from day 5 following PBS exposure (**A**) or OVA challenge (**B**). (**C**) and (**D**) are from the same slides as **A** and **B**, respectively, but at higher magnification. Marked PARP-1 protein expression (*brown*) was observed in the alveolar epithelium of the OVA-sensitized and -challenged mice (**B** and **D**), which was minimal in controls (**A** and **C**). Magnification 200 $\times$ for first row and 450 $\times$ for second row. *Third row:* Lung sections stained with hematoxylin and eosin for general morphology showed thickening of the alveolar septa, inflammatory cell infiltrate, and airway smooth muscle

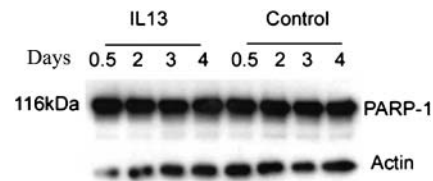


FIGURE 3 The effects of IL13 on PARP-1 protein expression in Balb/C murine lung. A representative Western blot from 3 experiments comparing the effects of PARP-1 and actin expression in murine lungs at 12, 48, 72, and 96 hours following intratracheal instillation of IL13 or PBS to murine airways.

changes, and GCM at each of the posttreatment time points. Representative examples from day 2 of treatment are shown in Figure 4. This time point was chosen because maximal airway GCM had previously been observed at 48 hours following a single IT exposure of IL13 [42].

Minimal positive staining for PARP-1 was observed in both the experimental and control groups at all of the time periods evaluated. Representative sections from the PBS controls (Figure 4A) and IL13-treated lungs (Figure 4B) are shown. Results from both the PBS- and IL13-treated lungs were similar to those observed with the PBS controls from the OVA experiments (Figure 2A). Thus, direct exposure of murine lungs to IL13 did not increase PARP-1 protein expression or localization. This stands in contrast to the OVA mouse model of allergic asthma wherein PARP-1 protein levels were increased strongly following OVA challenge.

By H&E staining, no appreciable inflammatory changes were observed at day 2 following PBS or IL13 exposure (Figure 4C, D, respectively). However, on AB/PAS staining, robust GCM (deep blue epithelial staining) was observed at day 2 following IL13 exposure (Figure 4F).

PARP-1 activity in murine models of allergen-induced airway inflammation

In order to determine whether the increased PARP-1 protein levels observed following OVA challenge

hypertrophy in OVA-exposed mice (**F**) when compared to controls (**E**) on day 5 following PBS exposure or OVA challenge. Magnification 100 $\times$. *Fourth row:* AB/PAS staining showed marked GCM in murine airways on day 5 following OVA challenge of OVA-sensitized mice (**H**) when compared to control mice (**G**). Magnification 100 $\times$.

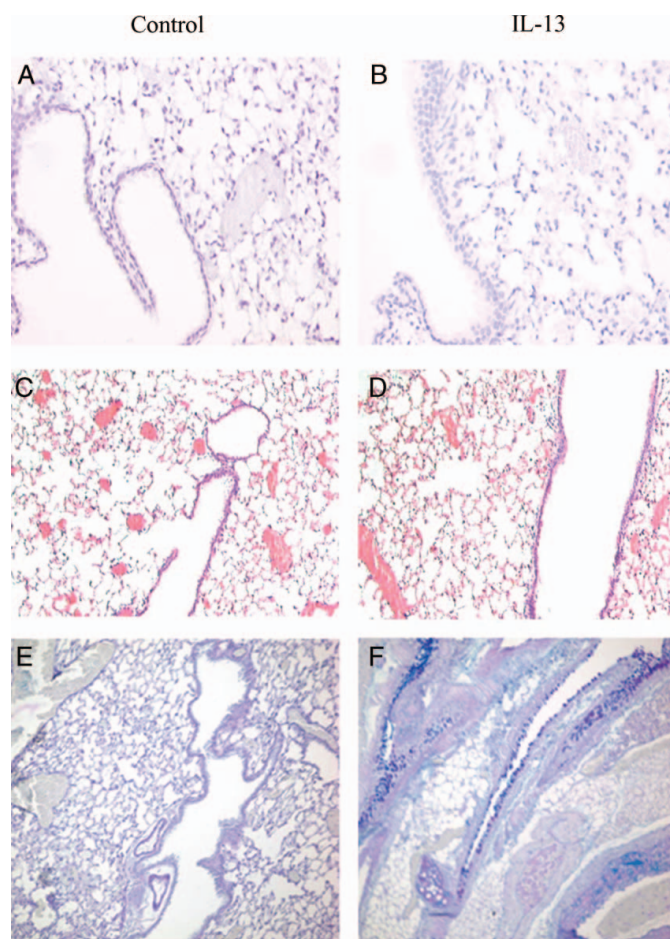


FIGURE 4 Lung immunohistochemistry and histopathology of control and IL13-exposed Balb/C mice. *First row:* Immunohistochemical analysis of PARP-1 protein in an IL13 model of allergic asthma. Lung sections from control (**A**) and IL13-exposed (**B**) mice show minimal PARP-1 expression at 48 hours following IL13 or PBS exposure. Magnification 200 $\times$. *Second row:* Histopathology of murine lungs 48 hours after exposure to PBS or IL13. Lung sections were stained with H&E for general morphology. Minimal inflammatory changes are seen in control (**C**) or experimental (**D**) mice. Magnification 100 $\times$. *Third row:* Histochemical analysis of murine lungs following exposure to PBS or IL13. Lung tissues were stained with AB/PAS. Lung sections from IL13-exposed mice (**F**) showed marked goblet cell metaplasia at 48 hours when compared to control mice (**E**) following intratracheal instillation of IL13 or PBS to murine airways. Magnification 100 $\times$.

to OVA-sensitized mice were also associated with an increase in PARP-1 activity, immunohistochemical detection of poly(ADP-ribose) (PAR) moieties as a marker of PARP-1 enzyme activity was employed. Lung tissues were evaluated following exposure to OVA for OVA-sensitized mice, or to IL13 or PBS in naïve mice. For the OVA-challenged mice, the temporal pattern of PARP-1 activity was similar to that observed for PARP-1 protein expression, as shown

above by Western blot (Figure 1) and immunohistochemical (Figure 2B) analyses. PAR moieties were observed in abundance in the nuclei of cells in the small conducting airways and alveolar walls on days 3 to 5 following OVA exposure. An example from day 5 post challenge is shown in Figure 5A. In contrast, PAR was minimally detected in IL13-exposed (Figure 5B) or PBS control (Figure 5C) murine lungs.

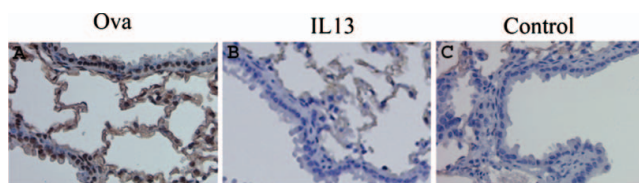


FIGURE 5 Immunohistochemical detection of PARP-1 activity following OVA, IL13 and PBS exposure. Lung tissues show marked PARP-1 activity after OVA exposure on day 3 (A), when compared to murine lungs 2 days following IL13 (B) or PBS (C) exposure. Magnification 200 $\times$.

DISCUSSION

Increased PARP-1 enzymatic activity has been shown to play a role in a growing number diseases with an inflammatory component, including lipopolysaccharide (LPS)-induced septic shock [22], colitis [23], streptozocin-induced pancreatic inflammation [24], and multiple organ system failure [45]. Increased PARP-1 activity has also been shown to occur with lung inflammation triggered by acute respiratory distress syndrome [20], hyperoxic lung injury [21], distant (i.e., limb) tissue injury [18, 19], as well as the allergic reaction seen in the murine OVA sensitization and challenge model of asthma studied here [43]. The responses of cells and tissues in such *in vivo* models are many and diverse, including inflammatory cell infiltration, the secretion of a multitude of cytokines, changes in redox state, and cellular responses ranging from cell death to cell replication and differentiation. Though global inflammatory changes are mitigated by PARP-1 inhibition in the above-mentioned models, the specific cellular responses regulated by this enzyme have not been elucidated. Moreover, PARP-1 has been implicated in the regulation of cell growth and differentiation during development in the complete absence of inflammation [30, 34]. With regard to lung changes seen in OVA allergic challenge models of airway inflammation, one question that arose, then, was whether PARP-1 activity was increased in and important to the inflammatory process, to the epithelial cell differentiation changes, or to both aspects of the allergic response seen in this model.

The specific tissue changes seen in the lungs of mice sensitized to and then challenged with OVA include a complex inflammatory cell infiltrate including eosinophils, monocytes, and neutrophils; the generation of ROS; the release of a host of cytokines including IL4, IL5, IL10, and IL13; and epithelial differentiation that results in abundant GCM [6]. IL13, which is increased in BAL fluid following OVA challenge [40], is a central mediator of a number of the lung responses to the allergic challenge, including GCM/mucus hypersecretion [39], airway hyper re-

sponsiveness, eosinophilic infiltration, and subepithelial fibrosis [6, 40, 46]. However, the degree of generalized inflammation involving neutrophils and monocytes observed following exposure to IL13 is minimal [41, 42]. Herein, we have shown that although OVA challenge elicits a marked increase in PARP-1 activity and protein levels in murine lungs, direct exposure of lungs of naïve mice to IL13 did not induce changes in either PARP-1 enzymatic activity or protein levels, which were both low and similar to those of sham-treated mice. One can conclude that PARP-1 is not involved in the subset of changes induced by exposure to IL13, including in the abundant GCM seen in the IL13-treated mice.

It is worth noting that the large increases in PARP protein levels and activity in the lungs of OVA-challenged mice are clearly seen prominently in the lung parenchyma and epithelial cells, even in areas where there is not an abundance of inflammatory cells (i.e., Figure 2B, OVA). Therefore, these increases did not derive solely from infiltrating neutrophils and monocytes. This suggests the possibility that increased PARP-1 protein and activity levels are secondary consequences of allergen-induced inflammation, which results in the release of a myriad of inflammatory and ROS mediators. Interestingly, it has recently been shown that lung injury occurs in response to the experimental induction of lower limb reperfusion injury and that this lung injury can be reduced by pretreatment with PARP-1 inhibitor [19]. This further supports the view that PARP activity in the lung can be mediated by factors produced at a distance that contribute to the consequent lung injury. The data presented in this study rule out IL13 as a modulator of PARP induction or activity, but other TH2 factors are candidates to be explored [47]. In a subsequent study, the investigators used the same PARP inhibitor TIQ-A 1 hour and 6 hours after the allergen challenge in their model of allergic inflammation, underlying the importance of temporal changes and interventions after the disease process is already developed [49].

Cells typically respond to signals (i.e., cytokines) or injury at several levels. PARP-1 is present in a quiescent state in most cells, albeit in widely varying and developmentally specific quantities [34]. Yet this enzyme can be activated very quickly, primarily but not exclusively by interaction with nicked DNA [48], which in inflammatory states is often due to increased ROS; this has been well demonstrated in the OVA sensitization and challenge model of asthma [43]. However, PARP protein levels are also highly regulated in many situations where differentiation is taking place, such as in developing chick limb buds [30] and developing lung [34]. In addition to confirming

the increase in PARP-1 enzymatic activity previously noted to occur in the OVA challenge model of asthma, the present investigation demonstrated that PARP-1 protein levels are greatly increased in that model and that the increase was maximal 3 to 5 days post challenge, correlating with the degree of inflammation observed in the airways and alveoli at days 3 to 5. The mechanisms by which PARP-1 protein levels are regulated are not well known, but in the OVA model system it could be a downstream consequence of the increased enzyme activity (positive feedback) or an independent cellular response to extraneous signals produced by inflammatory process. It is important to know that antigen challenge increases PARP-1 protein levels in concert with the activation of existing PARP-1 enzyme activity because this may affect the capacity of the lung to respond to the next antigenic challenge: increased PARP-1 protein could result in an exaggerated increase in enzymatic activity on subsequent challenges, thereby increasing the response to the challenge. Working out the kinetics of the protein response and its profile on repeated challenges at varying times could be very instructive and may point the way to finer tuned therapeutic interventions.

Despite recent advances in medicine, asthma remains a serious medical condition characterized by significant morbidity and mortality. The pathophysiology of most real world cases of asthma resembles that of the complex OVA challenge model rather than that of the selective IL13 exposure model. PARP-1 activity has been shown to play an important role in the pathophysiology of the former model and inhibiting PARP-1 activity shows promise as a possible mode of therapeutic intervention. To further the understanding of this component of the lungs complex response to immunologic challenge, we showed that IL13, a major mediator of several specific aspects of the overall allergic response, does not work through a PARP-1 pathway. We further demonstrated a temporal fluctuation in PARP-1 protein expression in response to allergic challenge, which correlates with increased PARP-1 enzymatic activity. We knew from prior studies that blocking PARP-1 activity reduced the pathologic response to allergic challenge [43]. New therapeutic interventions could result from gaining a more complete understanding of the signals in allergic asthma that regulate PARP-1 activity and protein levels. Pharmacologic modulation of these regulatory mechanisms alone or in combination with PARP-1 inhibitors might greatly enhance our ability to control this widespread and costly disease.

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