

Phosphatidylinositol 3-kinases pathway mediates lung caspase-1 activation and high mobility group box 1 production in a toluene-diisocyanate induced murine asthma model



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HIGHLIGHTS

- TDI-induced AHR and allergic airway inflammation are mediated by PI3K.
- TDI induces HMGB1 production and nucleocytoplasmic translocation through PI3K signaling.
- PI3K also mediates caspase-1 activation in TDI-induced asthma.

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ABSTRACT

We have previously demonstrated that downregulating HMGB1 decreases airway neutrophil inflammation in a toluene-diisocyanate (TDI)-induced murine asthma model, yet how HMGB1 is regulated in the lung remains uncertain.

In this study, we intended to explore whether PI3K signaling pathway mediates pulmonary HMGB1 production in TDI-induced asthma model and the possible roles of NLRP3 inflammasome and caspase-1 in this process.

BALB/c mice were sensitized and challenged with TDI to establish a TDI-induced asthma model. LY294002, a specific inhibitor of PI3K, was given intratracheally 1 h before each challenge.

Here we showed that airway hypersensitivity, airway infiltration of neutrophils and eosinophils, serum IgE and IL-4 in supernatant of cervical lymphocytes in TDI induced asthmatic mice were all markedly decreased by LY294002, accompanied by suppressed pulmonary expression of HMGB1. At the same time, we observed elevated protein levels of cleaved caspase-1 and IL-1 β after TDI challenge, as well as increased immunoreactivity in lung, all of which were significantly recovered by LY294002. While both the protein expression and immunodistribution of NLRP3 in the lung stayed unchanged.

These data suggest that PI3K mediates lung caspase-1 activation and HMGB1 production in TDI-induced murine asthma model.

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1. Background

Occupational asthma (OA) accounts for nearly 10% of all adult-onset asthma, with diisocyanates the most commonly reported

cause because isocyanates are widely used in industrial manufacturing (Johnson et al., 2005). We have established a toluene-diisocyanate (TDI)-induced asthma model characterized by Th2-dominated airway inflammation along with neutrophil and eosinophil infiltrating into the airways (Song et al., 2013).

High mobility group box 1 (HMGB1) is a DNA-binding protein that belongs to the alarmins family. It acts as a key molecule of innate immunity, and an important downstream effector of persistent tissue injury, which orchestrates inflammation, stem

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cell recruitment/activation, eventually leading to tissue remodeling. These processes can occur during cell activation as well as cell death. So it is regarded as a critical regulator of innate immunity (Castiglioni et al., 2011; Gauley and Pisetsky, 2009; Harris and Raucci, 2006). Recently, higher level of HMGB1 has also been reported to be associated with impaired lung function and more severe disease in subjects with asthma (Hou et al., 2011; Watanabe et al., 2011). In a previous study, we have demonstrated a relationship between increased pulmonary expression of HMGB1 and allergic airway inflammation in TDI-induced murine asthma model (Tang et al., 2014). Yet how HMGB1 is regulated in the lung remains largely uncertain.

Inflammasome is currently emerging as a key mediator in inflammation and innate immunity, and there has been a growing body of evidence that the NLRP3 inflammasome is involved in airway inflammation of asthma (Ather et al., 2011; Besnard et al., 2011; Tran et al., 2012). Inflammasome controls autoactivation of its effector caspase-1, which cleaves proIL-1 β and proIL-18 to mature forms and promotes release of IL-1 β , IL-18 (Keller et al., 2008; Lamkanfi et al., 2010) followed by initiation and amplification of various inflammatory responses. Recently, HMGB1 was identified as another important downstream target of inflammasome and caspase-1 (Denes et al., 2012; Keyel, 2014; Lamkanfi et al., 2010; Lu et al., 2012). Also several researches have implied a role for caspase-1 in allergic diseases (Bauer et al., 2012; Simpson et al., 2014). Currently an increasing number of studies (Martin et al., 2013; Simpson et al., 2014) have indicated that inflammasome, caspase-1 and HMGB1 are associated with neutrophil inflammation.

Phosphatidylinositol 3-kinases (PI3Ks) are a family of proteins that phosphorylate the 3-OH position of the inositol ring of phosphatidylinositols, which locate in the cellular membrane, resulting in the formation of phosphoinositide (3,4,5) triphosphate. PI3Ks play a prominent role in all eukaryotic cellular responses, including cell survival, proliferation, differentiation and cell migration as phosphoinositide (3,4,5) triphosphate activates various downstream signaling pathways (Toker and Cantley, 1997). PI3Ks are thought to be involved in a number of pathological states such as cancer, inflammation and allergy. Studies also showed that PI3K pathway plays a crucial role in the pathogenesis of asthma (Kampe et al., 2012; Ro et al., 2013; Takeda et al., 2010). In vitro, PI3K was required for activation of caspase-1, secretion of IL-1 β and IL-18, as well as nucleocytoplasmic translocation of HMGB1 (Oh et al., 2009; Tapia-Abellan et al., 2014). However, whether it is also involved in caspase-1 activation and downstream IL-1 β and HMGB1 production in asthma remains to be investigated.

Based on the above evidence, we hypothesized that PI3K signaling pathway mediates pulmonary HMGB1 production in TDI-induced asthma model and intended to explore the possible roles of NLRP3 inflammasome and caspase-1 in this process.

2. Materials and methods

2.1. Animals and reagents

Six-week old specific-pathogen-free male BALB/c mice (20–22 g) were purchased from Southern Medical University. The mice were housed in a SPF house (temperature $23 \pm 2^\circ\text{C}$, humidity range 40–70%, 12 h light/dark cycle [lighting: 7:00–19:00]). Food and UV-sterilized tap water were provided ad libitum. experimental animals used in this study were treated under a protocol approved by the committee of Southern Medical University on the use and care of animals. Standard guidelines for laboratory animal care followed the Guide for the Care and Use of Laboratory Animals. TDI, methacholine, acetone were obtained from Sigma–Aldrich (Shanghai, China). The vehicle (AOO) used to dissolve TDI consists of a mixture of 2 volumes of acetone and 3 volumes of olive oil

(Selection de Almazara, Carbonell, Madrid, Spain) for dermal sensitization, and 1 volume of acetone and 4 volumes of olive oil for challenge. Pentobarbital was obtained from Weijia Biotechnology Company (Guangzhou, China).

2.2. TDI-induced asthma and PI3K inhibitor administration

The TDI-induced asthma model was prepared by a modification of Hoet and Lee's method (Hirota et al., 2012; Lee et al., 2001). All mice were randomized to the following 4 groups: (1) AOO-sensitized, AOO-challenged, and PBS-treated mice (AOO group); (2) TDI-sensitized, TDI-challenged and PBS treated mice (TDI group); (3) TDI-sensitized, TDI-challenged and LY294002-treated mice (TDI+LY294002 group) and (4) TDI-sensitized, TDI-challenged and DMSO-treated mice (TDI+DMSO group). Briefly, on days 1 and 8, mice were dermally treated with 0.3% TDI dropped on the dorsum of both ears (20 μL /ear). On days 15, 18, and 21 the mice were challenged for 3 h each time via the airways with 3% TDI by means of compressed air nebulization. Challenging was performed by placing mice in a plexiglas box, and an nebulizer (NE-C28; Omron, Tokyo, Japan) driven by compressed air was used to aerosolize TDI, with operating air flow rate of 3.5 L/min and mass median aerodynamic diameter (MMAD) of 3 μm . As a control, mice was sensitized and challenged by the same procedures with the same amount of acetone and olive oil. LY294002 (1.5 mg/kg per mouse per time; Enzo life sciences, Farmingdale, NY, USA) was dissolved in DMSO and diluted with 0.9% NaCl. LY294002 in a volume of 50 μL was given intratracheally 1 h before each challenge. By comparison, control mice received the same volume of vehicle (0.9% NaCl containing DMSO).

2.3. Airway hyperresponsiveness (AHR)

AHR to methacholine was assessed 24 h after the third challenge. Briefly, mice were placed in a barometric plethysmographic chamber (Buxco Electronics, Troy, NY) and challenged with vehicle (sterile saline) followed by increasing concentrations of methacholine (1.25, 2.5, 5, and 10 mg/mL) by means of ultrasonic nebulization. Aerosols were generated with an matching ultrasonic nebulizer (Aeroneb Laboratory Nebulizer; Buxco Electronics, Inc., Troy, NY), and then nebulized into the main chamber through its inlet for 3 min. Pressure fluctuations caused by breathing of the mice were continuously monitored for 3 min after each nebulization, and subsequently these pressure fluctuations were quantified using the algorithm for enhanced pause (Penh), which represented an accurate index of airway resistance (Lundblad et al., 2002).

2.4. Animal euthanasia and necropsy

The mice were sacrificed with an overdose of sodium pentobarbital (pentobarbital sodium, 100 mg/kg body weight, administered intraperitoneally). Blood samples were taken from the retro-orbital plexus/sinus. Cervical lymph nodes were dissected and processed for each mouse separately (as described in Section 2.5). The chest cavity was exposed, then the trachea was carefully intubated and the catheter secured with ligatures. Lungs were lavaged in situ, three times with 0.8 mL prewarmed sterile saline (0.9% NaCl, 37°C), which was slowly infused into the lungs and withdrawn. The left lungs were infused with 4% neutral buffered formalin (0.2 mL) then removed and immersed in 4% neutral buffered formalin to fixed for pathological section, while the right lungs were removed and stored at -80°C for western blot.

2.5. Preparing lymph node cells

Dissected cervical lymph nodes were kept on ice in RPMI-1640 medium and cell suspensions were obtained by pressing the

lymph nodes through a cell strainer (40 μ m) (BD Bioscience, Erembodegem, Belgium) and rinsed with 10 mL tissue culture medium (RPMI-1640). Cells were counted using a Bürker hemocytometer. Lymphocytes were then washed three times and suspended (10^7 cells/mL) in complete tissue culture medium (RPMI-1640 supplemented with 10% heat-inactivated FBS). Cells were seeded into 48-well culture plates at a density of 10^6 cells/mL and incubated in complete RPMI-1640 medium for 43 h with 5 mg/mL of concanavaline A (Sigma–Aldrich, Shanghai, China), then centrifuged ($1000 \times g$, 4 °C, 10 min) and the supernatants were stored at -80°C for further measurement of IL-4 by ELISA (BoShiDe Biological Engineering Company, Wuhan, China).

2.6. Total serum IgE

Blood samples collected were rest for 1 h at room temperature, then centrifuged ($3000 \times g$, 20 min) and supernatants were harvested and stored at -80°C . Total serum IgE was measured by ELISA (BD Bioscience) according to the manufacturer's instructions.

2.7. Bronchoalveolar lavage (BAL)

The recovered BAL fluid was pooled. Total cells in the BAL fluid were counted, and the BAL fluid was centrifuged ($1000 \times g$, 10 min). The supernatant was frozen (-80°C) for further analysis. For differential cell counts, a cytospin sample (Shandon Scientific, Runcorn, UK) was prepared and stained using hematoxylin and

eosin (H&E) (Nanjing Jiancheng, China). For each sample, a total of 200 cells were counted for the numbers of macrophages, eosinophils, neutrophils and lymphocytes.

2.8. Lung histopathology

The left lungs were embedded in paraffin. Lung sections (4 μ m) were cut with a Leica microtome 2030 (Leica Microsystems Nussloch GmbH, Nussloch, Germany). Lung tissues were stained with hematoxylin and eosin to assess pathological changes. Lung inflammation was semiquantified as described in our previous study (Tang et al., 2014).

2.9. Immunohistochemistry and western blot

Immunohistochemistry and western blot for HMGB1, caspase-1, IL-1 β and NLRP3 were performed as previously described (Tang et al., 2014). Primary antibodies for HMGB1, caspase-1 and NLRP3 were obtained from Abcam (Hong Kong, China), and IL-1 β from Santa Cruz (CA, USA).

2.10. Statistical analysis

Statistical analysis was performed using SPSS version 19.0. Data were expressed as mean $\pm$ standard error (SE) and comparisons among groups were analyzed by one-way analysis of variance (ANOVA) accompanied by Bonferonni post hoc test for multiple comparisons. $p < 0.05$ was considered statistically significant.

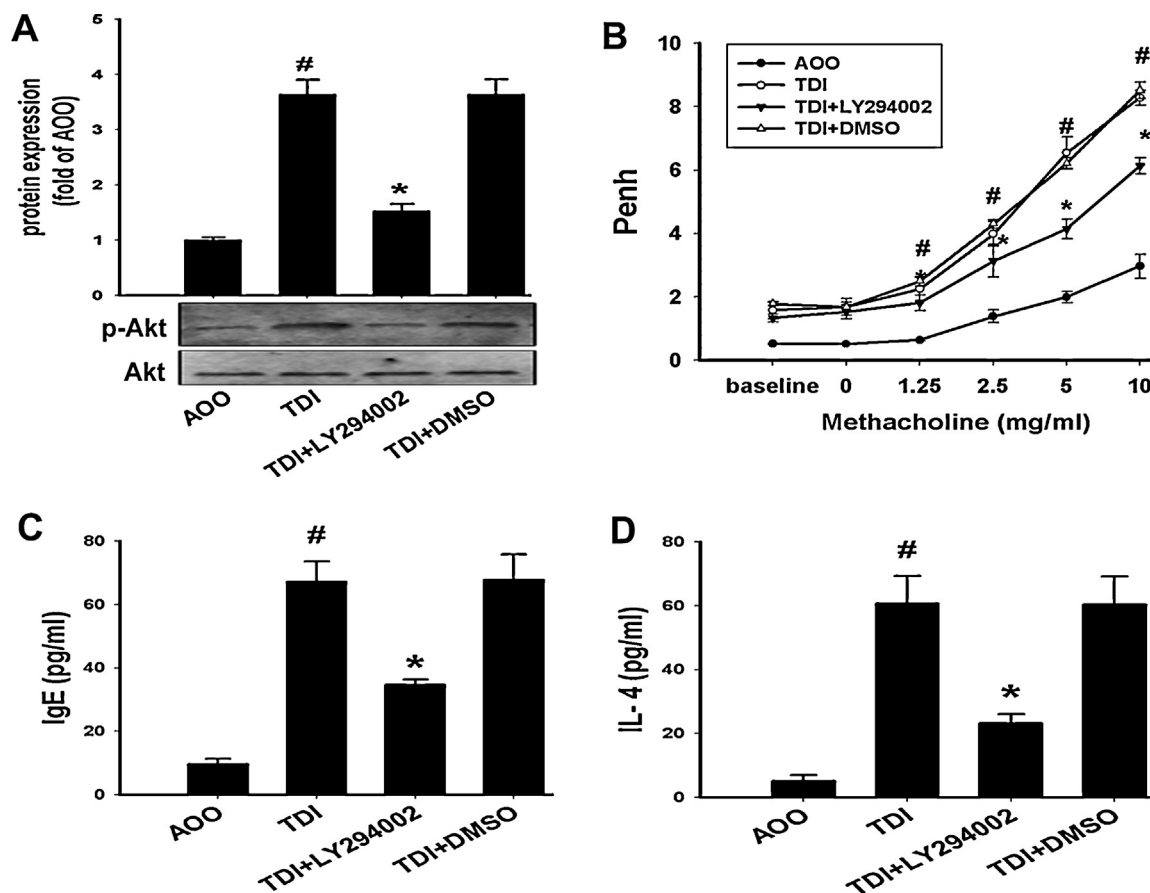


Fig. 1. Effects of LY294002 on TDI-induced AHR and airway inflammation. (A) Western blot analysis of p-Akt in the lung. Histogram represents results of densitometric analysis of p-Akt normalized to Akt. (B) AHR to methacholine 24 h after the last challenge. (C) Serum total IgE was measured using ELISA. (D) IL-4 in supernatant of cultured lymphocytes was measured using ELISA. Data are presented as mean $\pm$ SE ($n = 6-8$ per group). # $p < 0.05$ compared with AOO group; * $p < 0.05$ compared with TDI group.

3. Results

3.1. Effect of LY294002 on PI3K signaling in TDI-induced asthmatic mice

To confirm the effect of LY294002 on PI3K signal pathway, we detected p-Akt, the most important downstream of PI3K signal pathway. As shown in Fig. 1A, TDI sensitization and challenge upregulated the expression of p-Akt compared with AOO group ($p < 0.05$); while administration of LY294002 abolished this TDI induced elevated p-Akt expression ($p < 0.05$). The vehicle DMSO alone did not affect p-Akt expression compared with TDI group ($p > 0.05$).

3.2. TDI-induced AHR and allergic airway inflammation are mediated by PI3K

Effects of PI3K on TDI induced AHR are shown in Fig. 1B. Compared with AOO group, Penh values in TDI group were significantly increased when stimulated by methacholine (1.25, 2.5, 5, and 10 mg/mL) ($p < 0.05$), all of which were partly recovered after LY294002 treatment ($p < 0.05$). The release of total serum IgE was significantly increased in TDI group compared with AOO group ($p < 0.05$), as well as IL-4 ($p < 0.05$) in supernatant of cultured lymphocytes, and both were markedly decreased by LY294002 ($p < 0.05$) (Fig. 1C and D).

Total cell counts, as well as numbers of macrophages, neutrophils, eosinophils and lymphocytes were assessed in BAL fluid (Fig. 2A and B). In agreement with total cell counts, higher

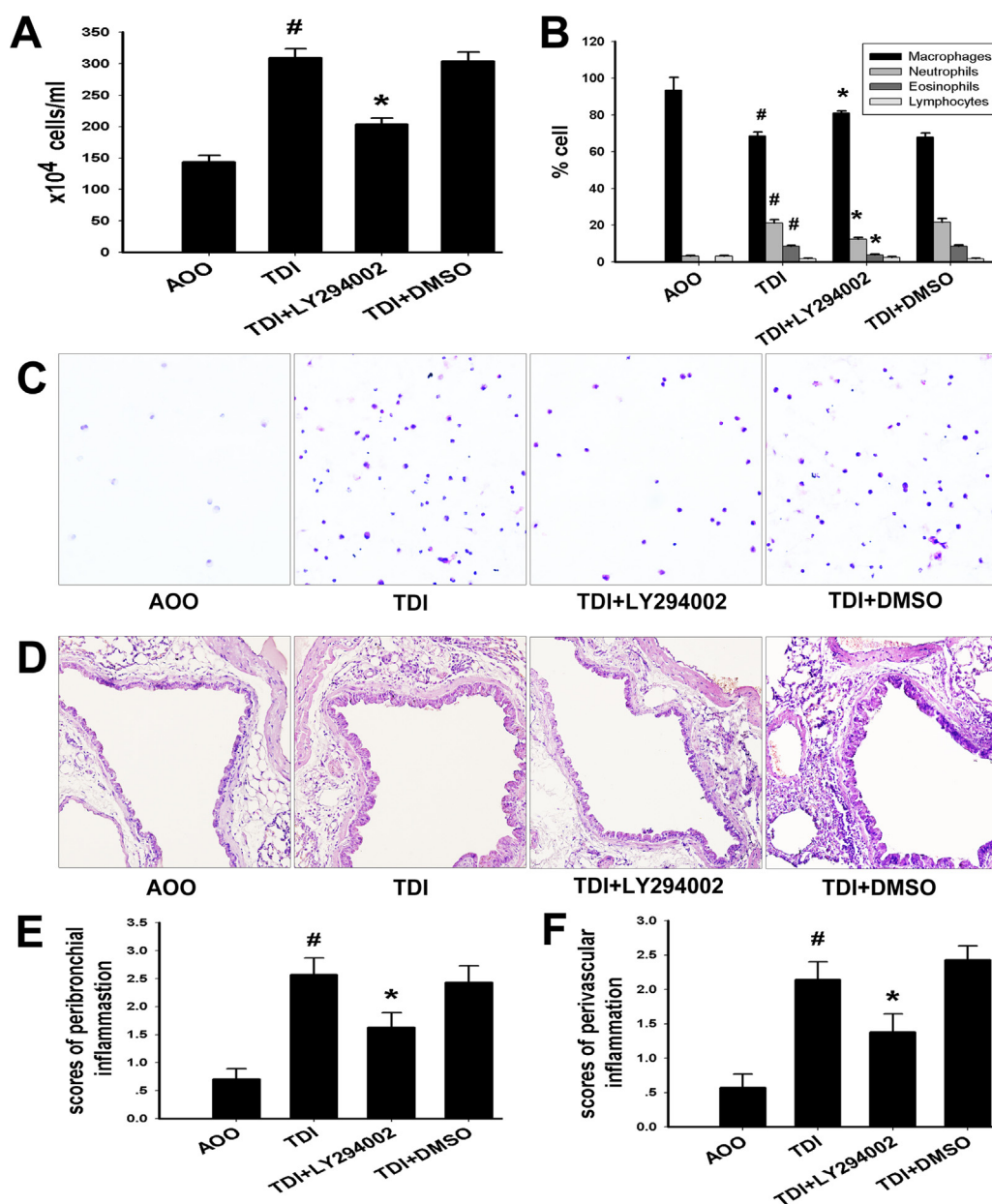


Fig. 2. Effects of LY294002 on TDI-induced inflammatory infiltration in BALF and pulmonary histological changes. (A) Total cell counts and (B) differential cell counts of macrophages, neutrophils, eosinophils and lymphocytes in BALF. Data are expressed as mean $\pm$ SEMs ($n = 6-8$). [#] $p < 0.05$ compared with AOO group; ^{*} $p < 0.05$ compared with TDI group. (C) Hematoxylin and eosin staining of cell smears of BALF at $\times 200$ magnification. (D) Hematoxylin and eosin staining of lung tissue at $\times 200$ magnification. (E) Results of histological scoring for peribronchial inflammatory changes and (F) perivascular inflammatory changes of lung sections from different groups. Representative images from 5 experiments.

amounts of neutrophils and eosinophils were found after TDI stimulation ($p < 0.05$). Pulmonary histological examination showed similar results. The TDI-exposed mice had heavier infiltration of inflammatory cells and more notable epithelial proliferation than the AOO-exposed (Fig. 2C–F). Changes in both BALF and lung tissue were partly reversed after LY294002 treatment ($p < 0.05$).

3.3. PI3K is required for HMGB1 production and nucleocytoplasmic translocation

In accordance with AHR and allergic airway inflammation described above, the protein level of HMGB1 in TDI group was significantly increased compared with that in AOO group (Fig. 3A). Similar results were obtained by immunohistochemistry. In AOO group, HMGB1 was detected almost only in the nuclei, however, after TDI challenge, we found that HMGB1 was translocated from the nuclei to the cytoplasm and scattered in bronchial epithelium, alveolar epithelium, airway fibroblasts and

infiltrating inflammatory cells (Fig. 3B–D). Notably, both the elevated protein expression and nucleocytoplasmic translocation of HMGB1 were largely alleviated after LY294002 administration ($p < 0.05$) (Fig. 3A–D).

3.4. Effect of LY294002 on caspase-1 activation

To further explore how PI3K regulates HMGB1 production and release in TDI-induced airway response, we measured its well-established upstream caspase-1. Firstly, using immunohistochemistry, we found that after TDI challenge, the production of caspase-1 was remarkably increased with dense staining throughout the cytoplasm of bronchial and alveolar epithelium as well as infiltrating inflammatory cell, while inhibition of PI3K by LY294002 largely attenuated this TDI-induced caspase-1 upregulation (Fig. 4A–C). Secondly, we measured the protein levels of procaspase-1 (45 kD) and cleaved caspase-1 (20 kD) in lung tissue by western blot. The protein level of cleaved caspase-1 rather than procaspase-1 was significantly elevated in TDI group compared

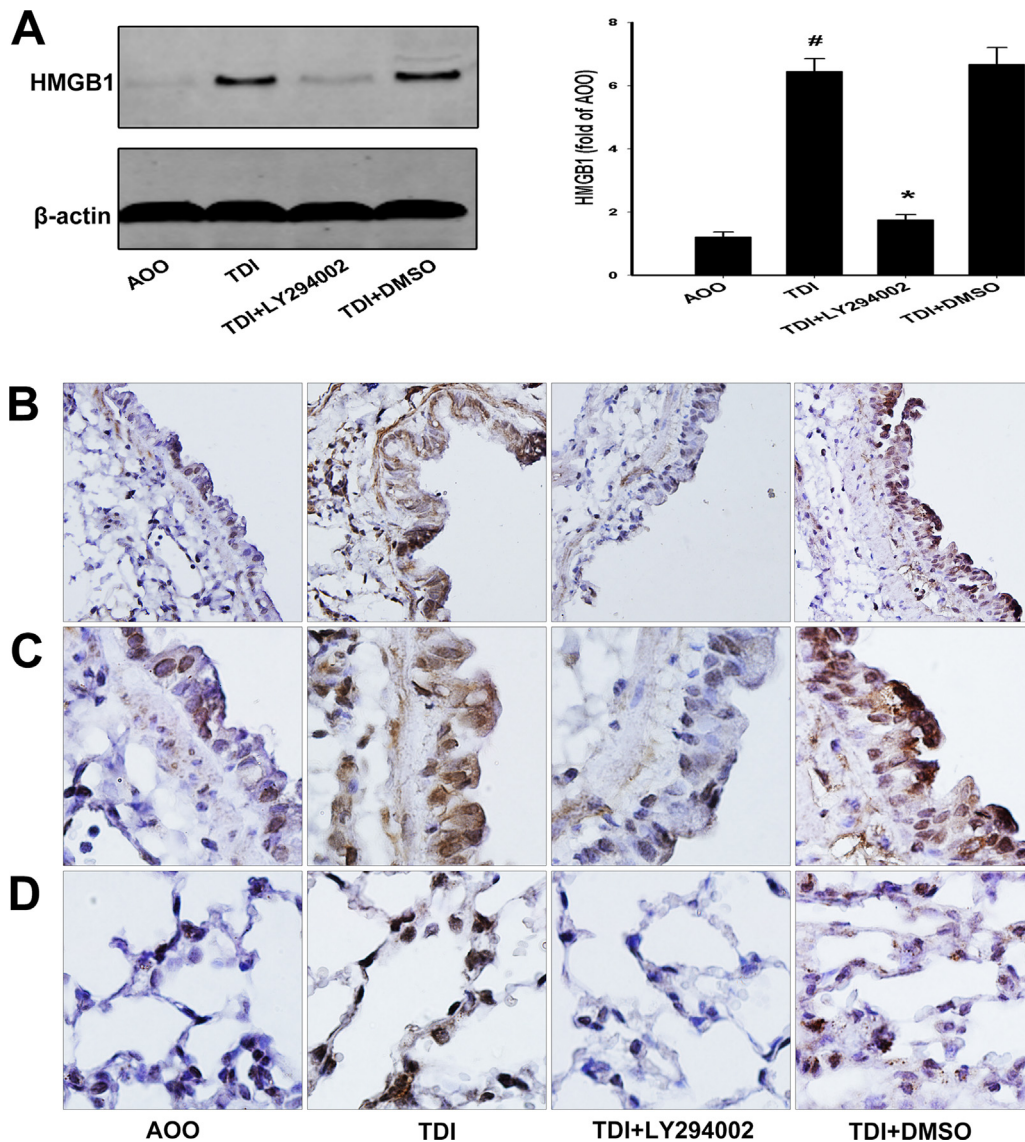


Fig. 3. Effects of LY294002 on HMGB1 production in the lung. (A) Western blot analysis of HMGB1. Histogram represents results of densitometric analysis of HMGB1 normalized to β -actin. Data are expressed as mean $\pm$ SEMs ($n=5$). [#] $p < 0.05$ compared with AOO group; ^{*} $p < 0.05$ compared with TDI group. (B) HMGB1 immunohistochemistry (brown) with hematoxylin counterstaining (blue) in airway epithelium at $\times 400$ magnification. (C) HMGB1 immunohistochemistry with hematoxylin counterstaining in airway epithelium and (D) in alveolar cells at $\times 1000$ magnification. Representative images from 5 experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

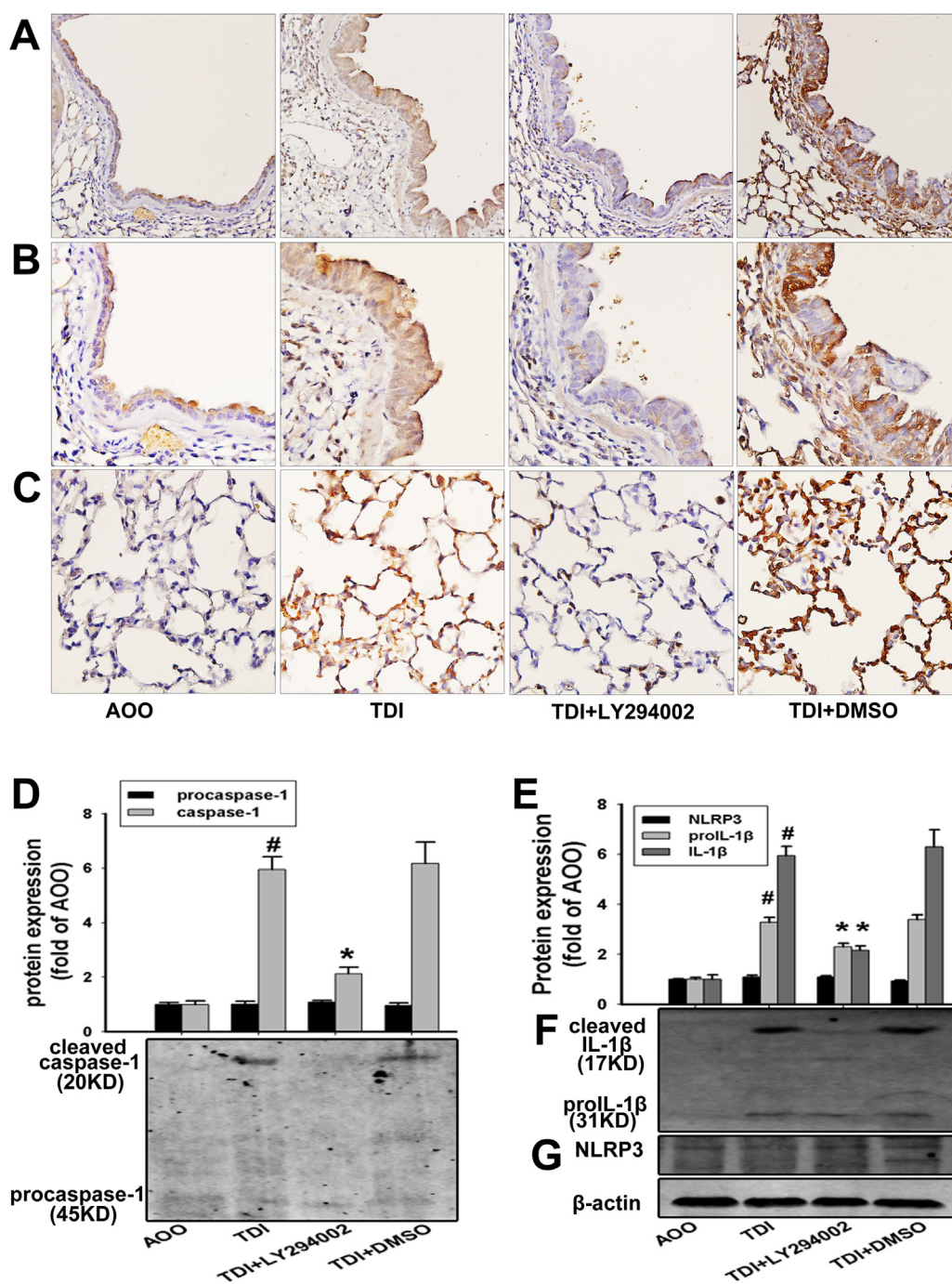


Fig. 4. Effects of LY294002 on caspase-1 activation in the lung. (A) Caspase-1 immunohistochemistry (brown) with hematoxylin counterstaining (blue) in airway epithelium at $\times 200$ magnification. (B) Caspase-1 immunohistochemistry with hematoxylin counterstaining in airway epithelium and (C) in alveolar cells at $\times 400$ magnification. Representative images from 5 experiments. (D) Western blot analysis of cleaved caspase-1 and procaspase-1. Histogram represents results of densitometric analysis of cleaved caspase-1 and procaspase-1 normalized to β -actin. (E) Results of densitometric analysis of cleaved IL-1 β , proIL-1 β and NLRP3 normalized to β -actin. (F) Western blot analysis of cleaved IL-1 β and proIL-1 β . (G) Western blot analysis of NLRP3. (D–G) share the same internal control β -actin. Data are expressed as mean $\pm$ SEMs ($n = 5$). [#] $p < 0.05$ compared with AOO group; ^{*} $p < 0.05$ compared with TDI group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with that in AOO group ($p < 0.05$) (Fig. 4D), indicating that the TDI-induced caspase-1 production observed in immunohistochemistry above mainly resulted from caspase-1 cleaving; we further found that this increased cleaved caspase-1 production was mostly reversed when administrating LY294002 before challenge, while the expression of procaspase-1 stayed nearly unchanged in all groups ($p > 0.05$) (Fig. 4D). These data demonstrated that the lung caspase-1 was activated in TDI induced asthma and PI3K activity was crucial for its maturation.

3.5. Effect of LY294002 on IL-1 β production

To reconfirm the role of PI3K in caspase-1 activation and explore the downstream mechanism involved in TDI-induced asthma. We detected IL-1 β , a proinflammatory factor well known as the downstream target of caspase-1. Immunohistochemistry (Fig. 5A and B) showed faint expression of IL-1 β in the lung in AOO group, while TDI treatment made more IL-1 β thinly scattered in the airway infiltrating inflammatory cells, alveolar epithelium,

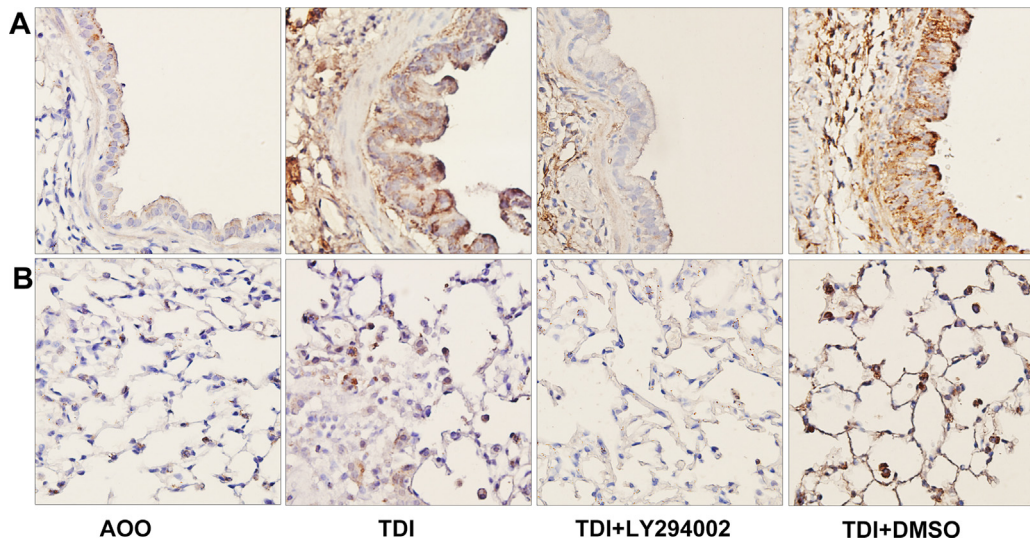


Fig. 5. Effects of LY294002 on pulmonary IL-1 β production in immunohistochemistry. (A) IL-1 β immunohistochemistry (brown) with hematoxylin counterstaining (blue) in airway epithelium and (B) in alveolar cells at $\times 400$ magnification. Representative images from 5 experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

bronchial epithelium and airway fibroblasts. Likewise, LY294002 administration remarkably reduced this TDI induced IL-1 β expression in the cytoplasm. Similar result was obtained by western blot (Fig. 4E and F). The TDI-induced elevated cleaved IL-1 β (17 kD) production ($p < 0.05$) was nearly abolished when treated with LY294002 ($p < 0.05$). We also observed increased expression of proIL-1 β (31 kD) after TDI stimulation ($p < 0.05$) which was decreased after LY294002 treatment ($p < 0.05$). But we failed to detect IL-1 β in BALF by ELISA (data not shown) in all enrolled groups. In addition, we measure NLRP3, the sensor of NLRP3 inflammasome and the most well-established upstream of caspase-1. We found that although NLRP3 was fully expressed in the bronchial epithelium, alveolar epithelium as well as infiltrating inflammatory cell (Fig. 6A and B), the distribution (Fig. 6A and B) and protein level (Fig. 4E and G) of NLRP3 remained unchanged either after TDI challenge or LY294002 treatment ($p > 0.05$).

4. Discussion

To our knowledge, this is the first report demonstrating that PI3K pathway mediates caspase-1 activation and HMGB1 production in TDI-induced murine asthma model.

Evidence suggests that HMGB1 acts as a key molecule of innate immunity that is passively released upon cell necrosis/pyroptosis and is actively produced by immune cells (Castiglioni et al., 2011) and other non-myeloid cells such as neurons (Rauvala and Rouhiainen, 2010) as well as airway epithelium (Ullah et al., 2014). To exert alarmin activities, HMGB1 must transit from the nucleus, through the cytoplasm, to the outside of the cell. This nucleocytoplasmic translocation is an important process for HMGB1 secretion (Bonaldi et al., 2003; Gauley and Pisetsky, 2009). In our study, after TDI challenge, pulmonary HMGB1 expression was apparently elevated and became

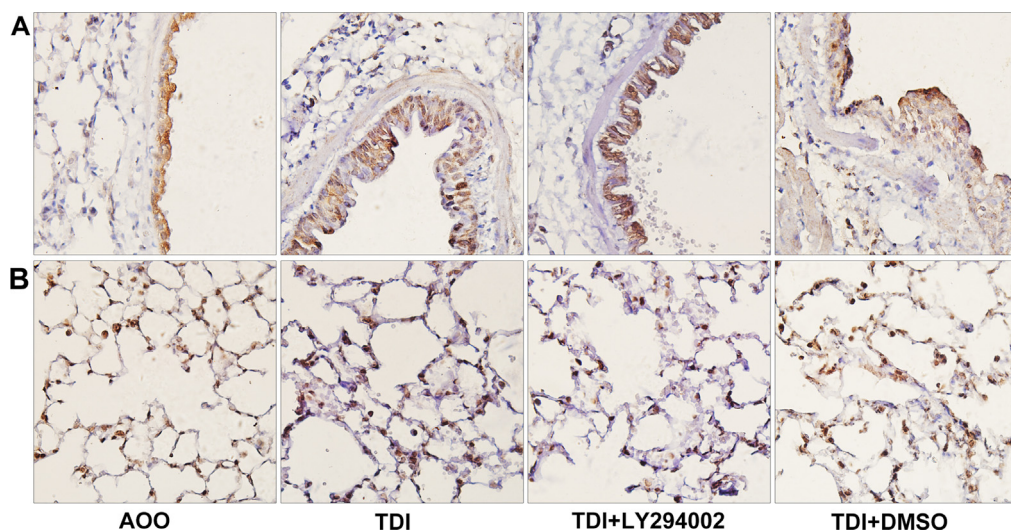


Fig. 6. Effects of LY294002 on pulmonary NLRP3 expression in immunohistochemistry. (A) NLRP3 immunohistochemistry (brown) with hematoxylin counterstaining (blue) in airway epithelium and (B) in alveolar cells at $\times 400$ magnification. Representative images from 5 experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

predominantly cytoplasmic, which provided increased evidence for active HMGB1 nucleocytoplasmic translocation especially in airway and alveolar epithelium (Ullah et al., 2014). This diffused release of HMGB1 in the lung indicated an active release from activated lung epithelium.

However, the molecular mechanisms of HMGB1 translocation from nuclear to cytoplasm in response to various infectious agents or endogenous danger signals remain largely unknown (Kang et al., 2009; Lu et al., 2013). Our current data showed that PI3K pathway mediated HMGB1 translocation to the cytoplasm as well as its expression in the whole lung along with AHR and allergic airway inflammation in TDI induced asthmatic mice. This was in consistent with the finding in macrophages that after LPS exposure, cytoplasmic cPKC (classical protein kinase C), a downstream target of PI3K, translocates to the nucleus, where it binds to and directly phosphorylates HMGB1 for secretion (Oh et al., 2009). Other in vitro studies (Kang et al., 2009; Oh et al., 2011; Zhou et al., 2013) also supported that PI3K/PKC pathway mediated HMGB1 release.

Notably, recent studies (Keller et al., 2008; Lamkanfi et al., 2010; Lamkanfi and Dixit, 2010) in vitro demonstrated that not only the actively noncanonical secretion of HMGB1 through proteolytic activation of an unknown secretion apparatus, but also its passive release through cell lysis during pyroptosis may be mediated by caspase-1, the effector of inflammasome. Thus we further hypothesized that caspase-1 activation was involved in PI3K-dependent HMGB1 release during TDI-induced airway inflammation. As previously described, a broad spectrum caspase inhibitor attenuated allergic airway inflammation in a murine asthma model (Iwata et al., 2003). Elevated expression of the NLRP3 inflammasome and caspase-1 were detected in neutrophilic asthma (Simpson et al., 2014) and caspase-1 activation led to neutrophilic inflammation (Satoh et al., 2013). So caspase-1 activation may be associated with the prominent neutrophil infiltration in TDI induced asthma. In our current study, pulmonary expression of caspase-1 was tested and results showed that caspase-1 was activated after TDI exposure, while PI3K inhibitor LY294002 could almost recover the TDI-induced expression and abnormal distribution of cleaved caspase-1 (20 kD) but not procaspase-1 (45 kD), implying that PI3K was mainly required in the cleavage process of caspase-1 rather than increasing its protein expression. As far as we know, plenty of evidence suggested that PI3K signaling plays a pivotal role downstream of oxidative stress in airway inflammation (Marwick et al., 2009) and in pulmonary epithelial cells (Papaiahgari et al., 2006). Oxidative stress was an vitally important mechanism involved in TDI induced asthma (Shin et al., 2013) as well as the most well-established upstream of caspase-1 (Kim et al., 2014). Therefore, it is possible that ROS/PI3K signal pathway mediates TDI induced caspase-1 activation, which deserves our future investigation.

Intriguingly, immunohistochemistry showed this PI3K-mediated increased caspase-1 was detected in the cytoplasm of bronchial and alveolar epithelium coinciding with PI3K-dependent nucleocytoplasmic translocation of HMGB1. Peeters PM et al. also showed that crystalline silica led to maturation of proIL-1 β to secreted IL-1 β , and significantly increased the release of HMGB1 through activation of caspase-1 and NLRP3 inflammasome in lung epithelial cells (Peeters et al., 2013). Taken together, these findings suggested that PI3K/caspase-1 pathway may be responsible for TDI induced HMGB1 release in the lung, which needs to be further clarified (Fig. 7).

Meanwhile, we measured IL-1 β in the lung and BALF because in general, proIL-1 β is cleaved to mature IL-1 β in a caspase-1 dependent manner (Andrei et al., 2004). As expected, we found that protein level of cleaved IL-1 β in the lung was upregulated after TDI challenge. Interestingly, in accordance with caspase-1,

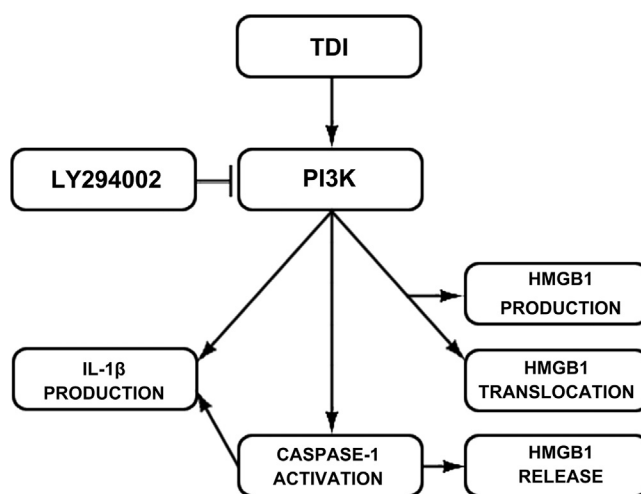


Fig. 7. The possible PI3K/caspase-1/HMGB1 pathway involved in TDI-induced asthma.

IL-1 β was mainly located in infiltrating inflammatory cell, bronchial and alveolar epithelium, while a remarkably decreased level of IL-1 β was detected after LY294002 treatment, indicating that proIL-1 β might be cleaved in a PI3K/caspase-1 dependent manner (Andrei et al., 2004). However, unlike procaspase-1, the expression of proIL-1 β was remarkably reduced after LY-294002 administration, implying that PI3K was not only involved in the caspase-1 dependent IL-1 β cleavage, but also in the expression of this cytokine, which could be attributed to the pluripotent functions of PI3K in the initiation of inflammatory response including asthma (Lee et al., 2006; Ro et al., 2013; Zhang et al., 2013). In addition we failed to detect IL-1 β in BALF using ELISA. These data supported the findings in vitro that caspase-1 may play alternative roles in nonmyeloid cells, including airway epithelial cells, which secrete relatively lower levels of IL-1 β (Bauer et al., 2012). In fact, caspase-1 itself represents a platform of enormous potential in innate immunity. Besides processing of IL-1 β , it exerts important and a conserved roles in activation of NF- κ B signaling, cell death, cellular metabolism, and cell repair (Lamkanfi, 2011), which may involve other non-inflammasome related functions that contribute to innate immunity. And IL-1 β is just the tip of the ICEberg of caspase-1 (Denes et al., 2012).

Unexpectedly, both the protein level and distribution of NLRP3, the sensor of NLRP3 inflammasome and the most well-established upstream of caspase-1, stayed unchanged, which supported the possibility of inflammasome independent activation of caspase-1 in this model as well. At the same time, it is worth noting that NLRP3 represents a bioactive receptor of various ligands and maybe its gene or protein expression need not altered in a disease state (Milutinovic et al., 2012). However, in Peeters' study (Peeters et al., 2013), cristobalite silica induced increased NLRP3 mRNA levels (2.5 fold change) in lung epithelial cells, and small interfering RNA experiments knocking down NLRP3 indeed revealed the pivotal role of inflammasome in the release of IL-1 β and HMGB1.

5. Conclusion

We primarily found that caspase-1 was activated accompanied by increased HMGB1 production and translocation through PI3K signal pathway in the TDI-induced murine asthma model. The results suggest PI3K/caspase-1 as a potential therapeutic target for TDI induced airway inflammation and caspase-1 mediated inflammatory response.

Conflict of interest statement

There are no conflicts of interest to report.

Acknowledgments

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