The International Journal of Biochemistry & Cell Biology 73 (2016) 53–62

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

TLR-7 agonist attenuates airway reactivity and inflammation through Nrf2-mediated antioxidant protection in a murine model of allergic asthma

Ahmed Nadeem[a,*], Nahid Siddiqui[b], Naif O. Al-Harbi[a], Mohammed M. Al-Harbi[a], Sheikh F. Ahmad[a]

[a] Department of Pharmacology & Toxicology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia
[b] Amity Institute of Biotechnology, Amity University, Noida, India

ARTICLE INFO

Article history:
Received 2 February 2016
Accepted 3 February 2016
Available online 3 February 2016

Keywords:
Airway inflammation
TLR-7 agonist
NADPH oxidase
Nrf2
Cu/Zn SOD

ABSTRACT

Toll-like receptors (TLRs) through innate immune system recognize pathogen associated molecular patterns and play an important role in host defense against bacteria, fungi and viruses. TLR-7 is responsible for sensing single stranded nucleic acids of viruses but its activation has been shown to be protective in mouse models of asthma. The NADPH oxidase (NOX) enzymes family mainly produces reactive oxygen species (ROS) in the lung and is involved in regulation of airway inflammation in response to TLRs activation. However, NOX-4 mediated signaling in response to TLR-7 activation in a mouse model of allergic asthma has not been explored previously. Therefore, this study investigated the role TLR-7 activation and downstream oxidant–antioxidant signaling in a murine model of asthma. Mice were sensitized with ovalbumin (OVA) intraperitoneally and treated with TLR-7 agonist, resiquimod (RSQ) intranasally before each OVA challenge from days 14 to 16. Mice were then assessed for airway reactivity, inflammation, and NOX-4 and nuclear factor E2-related factor 2 (Nrf2) related signaling [inducible nitric oxide synthase (iNOS), nitrotyrosine, lipid peroxides and copper/zinc superoxide dismutase (Cu/Zn SOD)]. Treatment with RSQ reduced allergen induced airway reactivity and inflammation. This was paralleled by a decrease in ROS which was due to induction of Nrf2 and Cu/Zn SOD in RSQ treated group. Inhibition of MyD88 reversed RSQ-mediated protective effects on airway reactivity/inflammation due to reduction in Nrf2 signaling. SOD inhibition produced effects similar to MyD88 inhibition. The current study suggests that TLR-7 agonist is beneficial and may be developed into a therapeutic option in allergic asthma.

1. Introduction

Asthma is one of the most common airway diseases characterized by eosinophilic/neutrophilic inflammation and airway hyperreactivity. ROS signaling plays an important role in the initiation and perpetuation of asthma pathogenesis. In response to allergens, there is increased production of ROS from inflammatory and structural cells in the airways of asthmatics that is associated with airway inflammation/remodeling and mucus hypersecretion (Lee and Yang, 2012; Nadeem et al., 2003, 2014a; Zuo et al., 2013). Excess ROS production overpowers the normal antioxidative capacity of the lung thus leading to impairment in the function of different lung components such as epithelium, endothelium and airway smooth muscle. Upregulation of antioxidants may be required to counteract increased ROS production in asthma (Boueiz and Hassoun, 2009; Nadeem et al., 2008, 2014a).

The main source of cellular ROS production is the family of NADPH oxidases (NOXs), which consists of seven different isoforms (Bedard and Krause, 2007; Katsuyama et al., 2012; Nadeem et al., 2014a). Different NOXs play important roles in the lung under both physiological and pathological conditions. For example, phagocytic NOX-2 is the main isoform responsible for the ROS generation under inflammatory conditions in the lung. Overproduction of ROS by NOX-2 been shown to be associated with allergen and virus-induced airway inflammation (Fink et al., 2008; Nadeem et al., 2014b; Vlahos et al., 2011). Another important isoform of NOX in the lung is NOX-4. NOX-4 has been shown to be upregulated in

Abbreviations: BAL, bronchoalveolar lavage; DETC, diethylthiocarbamate; iNOS, inducible nitric oxide synthase; i.n., intranasal; MyD88-IP, MyD88 inhibitory peptide; Nrf2, nuclear factor E2-related factor 2; NOX, NADPH oxidase; ROS, reactive oxygen species; RSQ, resiquimod; SOD, superoxide dismutase; TLR, Toll-like receptor.

* Corresponding author. Tel.: +966 553013401; fax: +966 4677200.
E-mail address: anadeem@ksu.edu.sa (A. Nadeem).

http://dx.doi.org/10.1016/j.biocel.2016.02.004
1357-2725/

asthmatics as compared to control subjects (Sutcliffe et al., 2012). NOX-4 is also involved in pulmonary fibrosis as well as virus-/bacteria-mediated lung injury (Amatore et al., 2015; Fu et al., 2013; Jarman et al., 2014). Together, these findings suggest that these two NOX isoforms play an important role in the regulation of airway inflammation.

TLRs present on innate immune cells recognize pathogen associated molecular patterns and lead to a variety of responses which serve to protect the host from invading pathogens. Emerging evidence suggests that NOX-derived ROS contribute to diverse signaling processes, including TLR-induced inflammation (Amatore et al., 2015; Nadeem et al., 2015; Yang et al., 2013). For example, it has been demonstrated that NOX-2 plays an important role in TLR-2 as well as TLR-3 dependent inflammatory responses and antimicrobial activity against pathogens. Other TLRs involved in sensing the presence of pathogens in the airways also play an important role in airway inflammation through different isoforms of NOXs (Lee et al., 2008; Ryu et al., 2013; Yang et al., 2013). However, role of NOX-2/NOX-4 in response to TLR-7 activation has not been explored in a mouse model of allergic asthma previously.

TLR-7 signaling has been shown to be protective in murine models of allergic asthma. Several mechanisms have been postulated to contribute to TLR-7 mediated anti-inflammatory effect. For example, one study has shown suppression of Th2 cytokines whereas others have shown anti-inflammatory effect through suppression of both Th1 and Th2 cytokines in response to TLR-7 activation (Grela et al., 2011; Camateros et al., 2007; Moisan et al., 2006). However, no study so far has investigated the effect of TLR-7 activation on nuclear factor E2-related factor 2 (Nrf2) signaling in murine model of asthma. This pathway may be one of the mechanisms by which TLR-7 activation contributes to anti-inflammatory effects in the lung.

Nrf2 is a transcription factor that is activated by ROS and responsible for detoxification through induction of various antioxidant genes (Lee and Yang, 2012; Liu et al., 2015; Spiess et al., 2013; Yao et al., 2007). One of the antioxidants that may be induced through Nrf2 is superoxide dismutase (SOD). SOD may provide protection against excess ROS generated from inflammatory cells in asthmatic lung (Eggler et al., 2008; Nadeem et al., 2014a; Zhu et al., 2005). These observations are also supported by studies that show exacerbation of pulmonary inflammation in Nrf2 or SOD knockout mice (Cho et al., 2013; Kwon et al., 2012; Rangasamy et al., 2005).

In this study, we tested the hypothesis that TLR-7 agonist triggers activation of anti-oxidant pathways that prevent excessive airway inflammation in a murine model of allergic asthma. Our study shows that treatment of allergen sensitized and challenged mice with TLR-7 agonist, resiquimod leads to Nrf2-mediated antioxidant protection against excessive ROS production.

2. Materials and methods

2.1. Animals

Male Balb/c mice, 8 to 10 weeks of age (20–25 g), free of specific pathogens, were used in the experiments. The animals were obtained from Experimental Animal Care Center, College of Pharmacy, King Saud University. The animals were kept under standard laboratory conditions of 12-h light–dark cycle and 24–26 °C ambient temperature. All experimental animals used in this study were under a protocol approved by Animal Care and Research Committee of College of Pharmacy, King Saud University.

2.2. Mice sensitization and challenge

Sensitization was performed according to the protocol described earlier (Nadeem et al., 2014b; Rievaj et al., 2012). Mice were sensitized on days 1 and 6 with intraperitoneal injections of 10 μg ovalbumin (grade V), adsorbed to 4 mg alum. Non-sensitized control animals received only alum with the same volumes. Two weeks after 1st sensitization, the mice were challenged intransally (i.n.) under light anesthesia with 100 μg ovalbumin once only on days 14, 15, and 16. To assess the role of TLR-7 during allergic responses, a synthetic TLR-7 ligand, resiquimod (RSQ; Tocris, UK) was administered i.n. before each allergen challenge at a concentration of 20 μg/mouse. MyD88 inhibitory peptide (MyD88-IP; InvivoGen, USA) or diethylthiocarbamate (DETC) was administered i.n. at a concentration of 100 μg/mouse and 5 mg/mouse before RSQ to block MyD88 and SOD mediated effects, respectively, in allergic/control groups.

Mice were divided into following groups: Control group (CON): mice received only vehicles for sensitization and challenge; Control group administered RSQ or MyD88-IP (CON + RSQ or CON + MyD88-IP), respectively: mice received only vehicles for sensitization and challenge, and RSQ or MyD88-IP was administered i.n. on days 14, 15 and 16; Sensitized and challenged group (SEN^{CHAL}): mice were sensitized and challenged with ovalbumin using the same protocol described above; Sensitized and challenged group administered RSQ (SEN^{CHAL} + RSQ): mice were sensitized and challenged with ovalbumin using the same protocol described above and RSQ was administered before each allergen challenge; Sensitized and challenged group administered MyD88-IP before RSQ (SEN^{CHAL} + MyD88-IP + RSQ): mice were sensitized and challenged with ovalbumin using the same protocol described above and MyD88-IP was administered before every RSQ treatment; Sensitized and challenged group administered DETC before RSQ (SEN^{CHAL} + DETC + RSQ): mice were sensitized and challenged with ovalbumin using the same protocol described above and DETC was administered every RSQ treatment.

2.3. Measurement of airway reactivity in vivo

Six hours after final allergen challenge, airway reactivity to methacholine in conscious, unrestrained mice were assessed by a whole-body noninvasive plethysmograph (Buxco Electronics Inc.) as described earlier (Nadeem et al., 2014b, 2015). Baseline Penh was determined by exposing mice to nebulized saline. The mice were then exposed to increasing concentrations of aerosolized methacholine dissolved in saline (0–32 mg/ml) to obtain a dose response and Penh values were recorded at each dose.

2.4. Bronchoalveolar lavage (BAL)

The mice were sacrificed by isoflurane anesthesia and the trachea was cannulated to perform BAL one day after final allergen challenge; phosphate-buffered saline was introduced into the lungs via the tracheal cannula and the total cells were counted manually in a hemocytometer chamber followed by spinning of cells onto glass slides for differential count. A differential count of at least 300 cells was made according to standard morphologic criteria on cytocentrifuged Diff-Quik stained slides. The number of cells recovered per mouse was calculated and expressed as mean ± SE per ml for each group. The level of total protein concentration as a measure of lung permeability/injury in BAL fluid (BALF) was determined using a commercial kit (Bio-Rad, Hercules, CA) using the Bradford method.

2.5. Real-time PCR

Total RNA was isolated by TRIzol reagent (Invitrogen, USA) from the tracheas/lungs of different groups as described previously (Nadeem et al., 2014b) and checked for purity by Nanodrop 1000 (Thermo Scientific, USA). This was followed by conversion of