European Journal of Pharmacology 775 (2016) 78–85

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Immunopharmacology and inflammation

IκB kinase β inhibitor, IMD-0354, prevents allergic asthma in a mouse model through inhibition of CD4$^+$ effector T cell responses in the lung-draining mediastinal lymph nodes

Tomasz Maślanka [a,*], Iwona Otrocka-Domagała [b], Monika Zuśka-Prot [a], Mateusz Mikiewicz [b], Jagoda Przybysz [a], Agnieszka Jasiecka [a], Jerzy J. Jaroszewski [a]

[a] Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Oczapowskiego Street 13, 10-719 Olsztyn, Poland

[b] Department of Pathological Anatomy, Faculty of Veterinary Medicine, University of Warmia and Mazury, Oczapowskiego Street 13, 10-719 Olsztyn, Poland

ARTICLE INFO

Article history:
Received 2 October 2015
Received in revised form
28 January 2016
Accepted 8 February 2016
Available online 9 February 2016

Keywords:
Asthma
NF-κB
CD4$^+$ T cells
Treg cells
MLNs
Lungs

ABSTRACT

IκB kinase (IKK) is important for nuclear factor (NF)-κB activation under inflammatory conditions. It has been demonstrated that IMD-0354, i.e. a selective inhibitor of IKKβ, inhibited allergic inflammation in a mouse model of ovalbumin (OVA)-induced asthma. The present study attempts to shed light on the involvement of CD4$^+$ effector (Teff) and regulatory (Treg) T cells in the anti-asthmatic action of IMD-0354. The animals were divided into three groups: vehicle treated, PBS-sensitized/challenged mice (PBS group); vehicle treated, OVA-sensitized/challenged mice (OVA group); and IMD-0354-treated, OVA-sensitized/challenged mice. The analyzed parameters included the absolute counts of Treg cells (Foxp3$^+$CD25$^+$CD4$^+$), activated Teff cells (Foxp3$^-$CD25$^+$CD4$^+$) and resting T cells (CD25$^-$CD4$^+$) in the mediastinal lymph nodes (MLNs), lungs and peripheral blood. Moreover, lung histopathology was performed to evaluate lung inflammation. It was found that the absolute number of cells in all studied subsets was considerably increased in the MLNs and lungs of mice from OVA group as compared to PBS group. All of these effects were fully prevented by treatment with IMD-0354. Histopathological examination showed that treatment with IMD-0354 protected the lungs from OVA-induced allergic airway inflammation. Our results indicate that IMD-0354 exerts anti-asthmatic action, at least partially, by blocking the activation and clonal expansion of CD4$^+$ Teff cells in the MLNs, which, consequently, prevents infiltration of the lungs with activated CD4$^+$ Teff cells. The beneficial effects of IMD-0354 in a mouse model of asthma are not mediated through increased recruitment of Treg cells into the MLNs and lungs and/or local generation of inducible Treg cells.

1. Introduction

Asthma is a heterogeneous disorder defined as a variable and largely reversible airway obstruction usually accompanied by airway hyperreactivity. In general, inhaled glucocorticoids remain the first line treatment for most patients, however, these therapies are not always sufficiently effective and can be associated with undesired side effects and steroid resistance (Edwards et al., 2009). Currently, nuclear factor (NF)-κB is regarded as a promising target for the development of a novel therapeutic strategy in asthma treatment because asthmatic airway inflammation is mediated, at least in part, by NF-κB signaling pathways (Edwards et al., 2009). NF-κB is a transcription factor expressed in numerous cell types and plays a key role in the expression of many pro-inflammatory genes (Hoffmann and Baltimore, 2006). In unstimulated cells, NF-κB is bound to IκB and retained in the cytosol in its inactive form. Phosphorylation of IκB by the IκB kinase (IKK) complex leads to the degradation of this protein, thereby releasing the active form of NF-κB which translocates to the nucleus and up-regulates gene expression (Edwards et al., 2009). Studies by Sugita et al. (2009) showed that IMD-0354, i.e. a selective inhibitor of IKKβ, inhibited allergic inflammation in an acute mouse model of ovalbumin (OVA)-induced asthma. In subsequent studies (Ogawa et al., 2011), these investigators demonstrated, using a mouse model of chronic asthma, that IMD-0354 inhibited the pathological features of airway remodeling.

The present study attempts to shed light on the involvement of CD4$^+$ effector (Teff) and Foxp3$^+$CD25$^+$CD4$^+$ regulatory (Treg) T cells in the anti-asthmatic action of IMD-0354. CD4$^+$ Teff cells have a central role in the pathogenesis of asthma. Following

* Corresponding author.
E-mail address: tomasz.maslanka@uwm.edu.pl (T. Maślanka).

http://dx.doi.org/10.1016/j.ejphar.2016.02.023
0014-2999/

antigen presentation by dendritic cells to recirculating naïve T cells (Tn) in the mediastinal lymph nodes (MLNs), specific $CD4^+$ T cells are activated and differentiated into Th2 effector type cells, which migrate to the lungs and orchestrate pulmonary immune responses (Lambrecht and Hammad, 2003). Moreover, Foxp3-expressing Treg cells have been discovered as another pivotal subset of $CD4^+$ T cells involved in the pathogenesis of asthma (Provoost et al., 2009; Barczyk et al., 2014). There are numerous indications suggesting that the anti-allergic and anti-inflammatory effects of IKK inhibition in mouse models of asthma may be mediated, in a large part, through controlling the levels of $CD4^+$ Teff and Treg cells in the MLNs and lungs. For example, it has been demonstrated that NF-κB plays a critical role in Th2 differentiation in allergic airway inflammation (Das et al., 2001). A beneficial effect of NF-κB inhibition in a rat model of severe pulmonary arterial hypertension was accompanied by a markedly increased abundance of Treg cells in the lungs (Farkas et al., 2014a, 2014b). Therefore, we hypothesized that one of the mechanisms underlying the anti-asthmatic action of IKK inhibitors can be the following effects: (a) preventing an allergen-induced increase in the number of $CD4^+$ Teff cells in the MLNs, (b) inhibition of the recruitment of $CD4^+$ Teff cells into the lungs, and (c) an increase in the number of Treg cells in the lungs and/or MLNs.

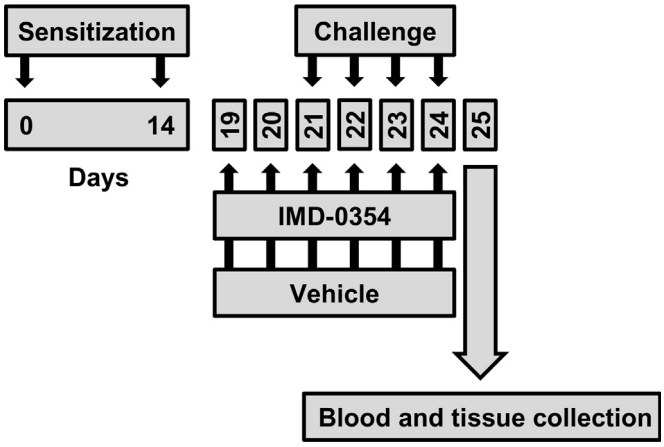

Fig. 1. Experimental study design scheme. Mice were sensitized to ovalbumin (OVA) by two intraperitoneal (i.p.) injections on days 0 and 14 with OVA absorbed on aluminum hydroxide. Subsequently, mice were challenged intranasally with OVA (OVA and OVA+IMD-0354 groups) on days 21, 22, 23, and 24. Mice in the negative control group (PBS group) received only aluminum hydroxide in PBS (sensitization) or PBS alone (challenge). Different groups of mice were treated i.p. with vehicle (PBS and OVA groups) or IMD-0354 (OVA+IMD-0354 group).

2. Materials and methods

2.1. Animals

All of the procedures were approved by the Local Ethics Commission (Ethic permission No. 10/2015). The experiments were carried out on 6-week-old Balb/c mice. Mice were bred and maintained under standard lab conditions [12/12 h light/dark cycle, controlled temperature (21 ± 2 °C) and humidity (55 ± 5%), and ad libitum access to food and water] in the Animal Facility of the Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn.

2.2. IMD-0354

The specificity of IMD-0354 (N-[3,5-bis-trifluoromethyl-phenyl]-5-chloro-2-hydroxy-benzamide) and its inhibitory action against IKKβ have been demonstrated previously (Onai et al., 2004; Tanaka et al., 2007). IMD-0354 powder was dissolved in 0.5% carboxymethylcellulose (both from Sigma-Aldrich, Munich, Germany) and administered intraperitoneally (i.p.) at a dose of 20 mg/kg/day for 6 days. This dose was chosen according to our preliminary studies and published reports (Ogawa et al., 2011; Sugita et al., 2009) indicating the anti-asthmatic effect of IMD-0354 at this dosage.

2.3. Sensitization, challenge, and treatment protocol

Mice were divided into three groups, namely PBS group (PBS-sensitized and -challenged mice treated with vehicle, i.e. negative control group), OVA group (OVA-sensitized and -challenged mice treated with vehicle, i.e. mice with OVA-induced model of allergic asthma), and OVA+IMD-0354 group (OVA-sensitized and -challenged mice treated with IMD-0354). The mice were sensitized on days 0 and 14 *via* i.p. injection of 20 μg OVA (Grade V) emulsified in 2 mg aluminum hydroxide (both from Sigma-Aldrich) in total volume of 200 μl PBS. On days 21, 22, 23 and 24, mice were challenged intranasally (i.n.) with 100 μg of OVA in 50 μl of PBS. Mice in PBS group received only aluminum hydroxide in PBS (sensitization) or PBS alone (challenge).

IMD-0354 or vehicle (0.5% carboxymethylcellulose) administration was started 48 h prior to i.n. challenge (i.e. on day 19 after the initial sensitization) and continued for 5 consecutive days; the test substance or placebo were given 3 h before challenge. Mice were euthanized (by asphyxiation with CO_2) 24 h after the last administration. The experimental study design scheme is shown in Fig. 1.

2.4. Lung histology

Lungs taken during necropsy were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Lung histological Sections (3–4 μm) were stained with haematoxylin and eosin (H&E) (Merck, Germany) for detection of inflammatory infiltrates and with periodic acid-Schiff (PAS) (Sigma-Aldrich) for goblet cells and mucus production visualization. Sections were analyzed using a light microscope (Olympus BX51, Japan) with digital camera (ColorView, Olympus, Japan) and CellB software (Olympus, Japan). Airway inflammation was quantified in the peribronchial region of 5 different medium-sized bronchi per slide on the basis of a scoring system (Table 1) previously described and used in similar studies (Wittke et al., 2004). The results were averaged and totaled for each mouse, and thereafter, the mean [± standard deviation (S.D.)] score per group was calculated. Epithelial thickness (as the area between the luminal cell membrane and the basement membrane) was measured at 4 sites for 5 different medium-sized bronchi per slide. All measurements were averaged, giving the mean (± S.D.) epithelial thickness per group.

2.5. Cell recovery

2.5.1. MLNs

MLNs were removed and subjected to dounce homogenization. The resulting cell suspensions were filtered through nitex fabric (Fairview Fabrics, Hercules, USA), washed with Facs (fluorescence-activated cell-sorting) buffer [FB; Dulbecco's PBS devoid of Ca^{2+} and Mg^{2+} with 2% (v/v) heat-inactivated FBS (both from Sigma-Aldrich)], and centrifuged (300g for 5 min at 5 °C; the same parameters were used for all cell-washing procedures). Cells were re-suspended in FB, counted and stained for flow cytometric analysis.