International Immunopharmacology 60 (2018) 121–131

Contents lists available at ScienceDirect

International Immunopharmacology

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

Progressive increase in allergen concentration abrogates immune tolerance in ovalbumin-induced murine model of chronic asthma

Gurupreet S. Sethi, Amarjit S. Naura*

Department of Biochemistry, Panjab University, Chandigarh 160014, India

ARTICLE INFO

Keywords:
Airway inflammation
Airway remodeling
Chronic asthma
Dexamethasone
Immune tolerance
OVA

ABSTRACT

Persistent inflammation and remodeling of airways are the major hallmarks of asthma. Though airway inflammation diminishes in ovalbumin (OVA)-based mouse model of chronic asthma owing to immune-tolerance linked with repeated allergen exposure, which limits the application of the disease model. Accordingly, the present study was designed to develop a murine model of chronic asthma which presents persistent airway inflammation coupled with remodeling traits. Herein, OVA-sensitized BALB/c mice were challenged with increasing (modified protocol) or constant concentration (conventional protocol) of the allergen for 6 weeks; 3 times/week. The results, indeed, revealed that mice subjected to modified protocol demonstrate an improved response to the allergen as reflected by the significant increase in inflammatory cells particularly, eosinophils in bronchoalveolar lavage fluid compared to conventional protocol. Moreover, the expression of Th2 cytokines and their responsible transcription factors (GATA-3 and STAT-6) was markedly enhanced in lungs. The increase in inflammation was further accompanied by a marked increase in mucus production, collagen deposition, and the expression of allied factors (Muc5ac, Col1α1, and α-SMA). Interestingly, pre-treatment of dexamethasone, a corticosteroid (0.5 mg/kg b.wt., i.p.), suppressed the allergen-induced airway inflammation and mucus production without altering collagen deposition. Failure of dexamethasone seems to be related to their ineffectiveness to modulate the expression of TGF-β, MMP-9, COL1α1, and α-SMA. Overall, our results strongly suggest that mice underwent modified chronic protocol bears more resemblance with asthmatics as it imitates persistent airway inflammation allied with steroid-refractory remodeling traits; hence, may be useful for the evaluation of new/alternative drugs in steroid-refractory asthmatic conditions.

1. Introduction

Asthma is a chronic disorder of lung airways characterized by persistent airway inflammation and remodeling that leads to the obstruction of airways [1,2]. It is a major health problem as around 300 million people are affected by it, globally [3]. The annual death rate due to asthma is around 2,50,000 and the majority of data come from low and middle-income countries (GINA, [57]). Currently, corticosteroids are the most effective way to control the asthmatic airway inflammation [4]. However, the efficacy of steroids is limited to airway remodeling traits such as basement membrane thickness, type I and III collagen deposition, and TGF-β expression [5–7]. Statistically, 5–10% of patients do not respond well to steroid therapy and tagged as steroid-resistant [8,9]. Besides the vast efforts to cure or control the disease, this data indicating that still there is an unmet clinical need.

An animal model of the disease is an excellent tool to obtain insights into the underlying disease pathways and to test the new therapies before their clinical trials [10]. Ovalbumin (OVA), a chicken egg protein, is widely used to elicit asthma-like symptoms in animal models. OVA-induced murine model of acute asthma is the classical model of the disease which reflects only the initial events of the disease mainly airway inflammation [11]. Conversely, human asthma is a chronic condition which is manifested by both inflammation and remodeling of airways. Thus, there is a need to develop an improved animal model that closely mimics the chronic settings of asthma which are resistant to steroid therapy. In comparison to acute model (generally requires the allergen exposure of few days), the chronic models incorporate several weeks to months of allergen challenges in sensitized animals [12,13].

Abbreviations: ANOVA, Analysis of variance; ASM, Airway smooth muscle; BALF, Bronchoalveolar lavage fluid; cDNA, Complementary DNA; COL1α1, Collagen, type I, alpha 1; CON, Conventional chronic model/protocol; H&E, Hematoxylin and eosin; IL, Interleukin; MMP-9, Matrix metalloproteinase-9; MOD, Modified chronic model/protocol; MT, Masson's trichrome; Muc5ac, Mucin 5AC; OVA, Ovalbumin; PAS, Periodic acid-Schiff; PBS, Phosphate-buffered saline; PCR, Polymerase chain reaction; RNA, Ribonucleic acid; SEM, Standard error of mean; STAT-6, Signal transducer and activator of transcription protein; TGF-β, Transforming growth factor beta; Th2, Type 2 helper T cells; α-SMA, α-Smooth muscle actin

* Corresponding author.
E-mail address: anaura@pu.ac.in (A.S. Naura).

https://doi.org/10.1016/j.intimp.2018.04.047
Received 9 March 2018; Received in revised form 27 April 2018; Accepted 28 April 2018
1567-5769/ © 2018 Elsevier B.V. All rights reserved.

Previously, animal models of chronic asthma have been developed by increasing the number of OVA challenges over many weeks [14–16]. It has been evident that prolonged OVA exposure replicates some of the features of human asthma but the reduction in airway inflammation and airway hyper-responsiveness (AHR) indicates the development of immune tolerance to allergen [13,17–19]. Indeed, no animal model is available that mimics all the symptoms of chronic asthma except the murine model developed by Kumar and co-workers which imitates most of the asthmatic symptoms. However, the technique used by Kumar et al. was very expensive [15,20].

Considering this, the present study was designed to develop an OVA-induced murine model of chronic asthma that closely reflect the persistent airway inflammation and steroid refractory remodeling features in a cost-effective manner. For this, mice were challenged with increasing concentration of aerosolized OVA for six-weeks (termed as modified chronic model; MOD) to overcome the associated immune tolerance. Thereupon, the modified chronic model was compared to conventional chronic as well as the acute model to analyze persistent airway inflammation and remodeling traits such as mucus production and collagen deposition. Further, the efficacy of dexamethasone, an immunosuppressive drug; was examined to validate the effectiveness of steroid class of drug on airway inflammation and remodeling in the present model.

2. Materials and methods

2.1. Animals

Male BALB/c mice weighing 15–18 g (4–5 weeks) were procured from the central animal house of Panjab University, Chandigarh. All the animals were housed in polypropylene cages and were allowed unlimited access to sterilized chow and water. The animals were housed, cared, and used for experiments in accordance with the “Guidelines for the Care and Use of Experimental Animals” approved by Institutional Animal Ethics Committee (PU/IAEC/S/14/36). Animals were acclimatized to the laboratory conditions for one or two weeks prior to start experiments.

2.2. Drugs and chemicals

All the chemicals used in the study were of analytical grade. OVA, aluminium hydroxide, and dexamethasone were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals needed to be were purchased either from Hi-media Laboratories, Mumbai, India or Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Antibodies used for western blot were purchased from Santa Cruz Biotechnology (Finnell St, Dallas, Texas) or Sigma (St. Louis, MO, USA).

2.3. Experimental design

The mice were randomly segregated into the following five groups (each having 5–6 animals):

2.3.1. Control group

No allergen and drug were given to them.

2.3.2. Acute group

Mice were first sensitized and then challenged with 3% concentration of aerosolized OVA for 3 consecutive days *i.e.* on 28th, 29th and 30th day (see the detail of sensitization and challenge protocol; given below) (Fig. 1a).

2.3.3. Conventional chronic group (CON)

Sensitized mice were challenged with a constant concentration of aerosolized OVA (*i.e.* 3% OVA) for 6 weeks; 3 consecutive days/week (see the detail of sensitization and challenge protocol; given below) (Fig. 1b).

2.3.4. Modified chronic group (MOD)

Sensitized mice were challenged with the gradually increasing concentration of aerosolized OVA (*i.e.* 1% OVA for first two weeks, 2% OVA for 3rd and 4th week and 3% OVA for 5th and 6th week) for 6 weeks; 3 consecutive days/week (see detail of sensitization and challenge protocol; given below) (Fig. 1c).

2.3.5. Dexa group

Dexamethasone at a dose of 0.5 mg/kg (*i.p.*) was given to OVA-sensitized/challenged mice 24 h prior to first OVA challenge and 1 h prior to every OVA challenge (Fig. 1c).

2.4. Sensitization and challenge

Six to seven-week-old mice (except control group) were sensitized intraperitoneally by injecting a mixture containing 10 μg OVA and 2 mg aluminium hydroxide in saline on 0th and 14th day. From 28th day to onwards, mice were challenged with aerosolized OVA (diluted in saline solution) for six-weeks (three consecutive days/week). For this, mice were placed in plexiglass chamber for 30 min which is further connected to the nebulizer that generates aerosolized OVA. The concentration of aerosolized OVA was constant (for the conventional chronic model) or increased after every couple of weeks (for the modified chronic model) to avoid the immune tolerance. The animals were euthanized after 48 h from last challenge by cervical dislocation and bronchoalveolar lavage fluids (BALF), as well as lung tissues, were processed for subsequent analysis. A pictorial diagramme showing the treatment details is shown in Fig. 1.

2.5. BALF procurement and quantification of total and differential inflammatory cells

For BALF procurement, the thoracic cavity of mice was opened and upper part of trachea was cannulated, carefully. The lungs were lavaged twice with 750 μL ice-cold phosphate-buffered saline (PBS, pH 7.4). The pooled BALF was centrifuged at 1700 rpm for 8 min at 4 °C. The cell pellet was re-suspended in fresh PBS which further subjected to hemocytometer for the assessment of total count of inflammatory cells. A smear of BAL cells was prepared using cytospin, to quantify differential cells (eosinophils, neutrophils, and lymphocytes) smear were stained with hematoxylin and eosin (H&E) [21].

2.6. Histopathology

Lung tissues were fixed in 10% buffered formalin and slides of 5 μm thick sections were prepared [22]. The sections (n = 5–6 per group) were then subjected to H&E, periodic acid-Schiff (PAS), and Masson's trichrome (MT) stain to examine inflammatory cell infiltration, mucus production, and collagen deposition, respectively. The sections were examined in a blinded manner and score was assigned. The images of the stained sections were evaluated at the magnification of 200 × (H&E and MT stained slides) and 400 × (PAS stained slides) magnification. At least 6 bronchioles were randomly scanned in each slide, and their average scores were calculated. Briefly, goblet cells were counted in PAS-stained lung sections using an arbitrary scoring system (0: < 5% goblet cells; 1: 5–25%; 2: 25–50%; 3: 50–75%; 4: > 75%), as described by Grunig et al. [23]. Collagen deposition in peribronchial region was detected using 0–3 scoring system (0, no collagen deposition; 1, a thin layer of collagen; 2, a cluster of collagen; and 3, a thick layer of collagen) as described by Valentin et al. [24].

2.7. RNA isolation and conventional reverse transcriptase-PCR

Total RNA was isolated from lung tissue of different groups using

Download English Version:

https://daneshyari.com/en/article/8531129

Download Persian Version:

https://daneshyari.com/article/8531129

Daneshyari.com