RG-II from *Panax ginseng* C.A. Meyer suppresses asthmatic reaction

In Duk Jung1,2, Hye Young Kim3, Jin Wook Park1, Chang-Min Lee1, Kyung Tae Noh1, Hyun Kyu Kang1, Deok Rim Heo1, Su Jung Lee1, Kwang Hee Son1, Hee-ju Park3, Sung Jae Shin4, Jong-Hwan Park5, Seung-Wook Ryu6 & Yeong-Min Park1,2,*

1Department of Microbiology and Immunology, School of Medicine, Pusan National University, 2Research Institute of Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Yangsan 626-770, 3Department of Pediatrics, Pusan National University Hospital, Pusan 602-739, 4Department of Microbiology, College of Medicine, Chungnam National University, Daejeon 301-747, 5Department of Biochemistry, College of Medicine, Konyang University, Daejeon 302-731, 6Cell Signaling and Bioimaging Laboratory, Department of Bio and Brain Engineering, KAIST, Daejeon 305-701, Korea

In asthma, T helper 2 (Th2)-type cytokines such as interleukin (IL)-4, IL-5, and IL-13 are produced by activated CD4+ T cells. Dendritic cells played an important role in determining the fate of naive T cells into either Th1 or Th2 cells. We determined whether RG-II regulates the Th1/Th2 immune response by using an ovalbumin-induced murine model of asthma. RG-II reduced IL-4 production but increased interferon-gamma production, and inhibited GATA-3 gene expression. RG-II also inhibited asthmatic reactions including an increase in the number of eosinophils in bronchoalveolar lavage fluid, an increase in inflammatory cell infiltration in lung tissues, airway luminal narrowing, and airway hyperresponsiveness. This study provides evidence that RG-II plays a critical role in ameliorating the pathogenic process of asthmatic inflammation in mice. These findings provide new insights into the immunotherapeutic role of RG-II in terms of its effects in a murine model of asthma. [BMB reports 2012; 45(2): 79-84]

INTRODUCTION

Asthma is a chronic inflammatory disease affecting the airways that is characterized by recurring symptoms, including reversible airflow obstruction and bronchospasm (1). Asthma episodes are thought to be caused by a combination of genetic and environmental factors such as allergens, tobacco smoke, and emotional stress (2, 3). As a model, ovalbumin (OVA)-induced asthma is characterized by airway hyperresponsiveness (AHR) and airway inflammation (4), and is closely associated with the accumulation of eosinophils, neutrophils, and lymphocytes in the bronchial lumen and lung tissues (4). These cellular infiltrates release various chemical mediators capable of inducing AHR (5, 6). Additionally, recruitment of these inflammatory cells from the blood to sites of inflammation is regarded as a central event in the development and prolongation of airway inflammation (7).

The roots of *P. ginseng* are precious since the plant requires 4-6 years to harvest, whereas the leaves can be harvested every year. If the leaves of *P. ginseng* had similar pharmacological activity as the roots, much more of the therapeutic compounds could be available for clinical use. Previous studies have reported that polysaccharides from the leaves of *P. ginseng* possess potent anti-complementary (8) and anti-ulcer activities (9), indicating the potential clinical value of the leaves.

Antigen-activated CD4+ T cells are able to differentiate into different types of effector cells, each with distinct functional properties conferred by cytokines (10, 11). The Th2 helper 2 (Th2)-type cytokines interleukin-4 (IL-4), IL-5, and IL-13, all of which are expressed by activated CD4+ T cells, have critical roles in the pathogenesis of asthma by controlling immunoglobulin E (IgE) production, mast cell growth, as well as differentiation and activation of mast cells and eosinophils (12, 13). In contrast, Th1 cytokines such as interferon-gamma (IFN-γ) and IL-12, which downregulate the Th2 response, inhibit the development of allergic lung inflammation (14, 15). Therefore, therapeutic interventions that simultaneously inhibit Th2 cytokine production while enhancing Th1 cytokine production may be useful in treating allergic asthma (16).

GATA-3, a member of the GATA family of transcription factors, is a transcription factor that binds to the T cell receptor-alpha (TCR-α) gene enhancer (17). Specifically, GATA-3 is induced through the action of the STAT6 protein upon binding of IL-4 to its receptor and plays a critical role in regulating Th1 and Th2 cell differentiation. GATA-3 specifically regulates Th2 cytokine expression at the transcriptional level.

*Corresponding author. Tel: +82-51-510-8097; Fax: +82-55-382-8090; E-mail: immunpym@pusan.ac.kr
http://dx.doi.org/10.5483/BMBRep.2012.45.2.79

Received 16 January 2012, Revised 16 January 2012, Accepted 17 January 2012

**Keyword:** Asthma, GATA-3, Rhamnogalacturonan II (RG-II), T helper 2 (Th2), T-bet

http://bmbreports.org
by binding directly to the promoters of IL-5 and IL-13, as well as by affecting chromatin remodeling, resulting in opening of the IL-4 locus (18). The T-box transcription factor T-bet (Tbx21) has emerged as a key regulator of dendritic cells as well as the type 1 immune response, playing an essential role in establishing effector cell fate in T and B lymphocytes (19). T-bet expression is induced in Th1 cells but not Th2 cells, upon signal transduction, and acts as a potent transactivator of the IFN-γ gene in Th1, NK, and B cells (20). Therefore, we investigated the effects of RG-II on T-bet and GATA-3 expression in a murine model of asthma.

In this study, administration of RG-II before the final airway OVA challenge resulted in significant inhibition of asthmatic reactions, suggesting that RG-II could play a critical role in the improvement of the pathogenic processes of asthma in mice.

RESULTS

RG-II inhibits AHR, lung inflammation, and inflammatory cell infiltration

Airway responsiveness was measured as a Penh value in response to increasing doses of methacholine. In OVA-sensitized and -challenged mice, the dose-response curve of the Penh value was shifted to the left compared to that of control mice (Fig. 1A). In addition, the Penh value produced by methacholine administration (at doses ranging from 2.5-50 mg/ml) was significantly higher in the OVA-sensitized and -challenged mice compared to controls. In OVA-sensitized and -challenged mice treated with RG-II, the dose-response curve of the Penh value was shifted to the right compared to that of untreated OVA-sensitized and -challenged mice. Moreover, the shift was dose-dependent.

Histological analyses revealed the typical pathological features of asthma in OVA-exposed mice compared to control mice, with the OVA-exposed mice displaying numerous inflammatory cells, including infiltrated eosinophils around the bronchioles (Fig. 1B). Mice treated with RG-II showed a marked decrease in inflammatory cell infiltration in the peribronchial and perivascular regions (Fig. 1B). Therefore, the increases in total lung inflammation and cell infiltration were significantly inhibited by administration of RG-II. These results suggest that RG-II inhibits OVA-induced airway hyperresponsiveness and antigen-induced inflammation in the lungs, including the influx of eosinophils.

RG-II reduces Th2 cytokine levels in lung tissues of OVA-sensitized and -challenged mice

BAL fluids were obtained 24 hours after the final airway challenge with OVA, the levels of IL-4, IL-5, and IL-13 were significantly higher than those in control mice. Administration of RG-II reduced the secretion of IL-4 (Fig. 2A), IL-5 (Fig. 2B), and IL-13 (Fig. 2C). However, the levels of the Th2 cytokines IL-4, IL-5, and IL-13, as well as those of the Th1 cytokines IFN-γ (Fig. 2D) and IL-12 (Fig. 2E) were higher in OVA-sensitized and OVA-challenged mice compared to cytokine levels in saline-sensitized and -challenged control mice. These results indicate that RG-II treatment reduces Th2 cytokine levels, such as IL-4, IL-5, and IL-13, in BAL fluids.

RG-II decreases IgE levels in the serum and the number of inflammatory cells in BAL fluid

Since Th2 cytokines promote airway inflammation in asthma...