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Dossier : Present status of drug from marine origin

Effects of *Ecklonia cava* ethanolic extracts on airway hyperresponsiveness and inflammation in a murine asthma model: Role of suppressor of cytokine signaling

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Abstract

Ecklonia cava (EC) is a brown alga that evidences radical scavenging activity, bactericidal activity, tyrosinase inhibitory activity, and protease inhibitory activity. However, its anti-allergic effects remain poorly understood. In the current study, we attempted to determine whether pretreatment with EC induces a significant inhibition of asthmatic reactions in a mouse asthma model. Mice sensitized and challenged with ovalbumin (OVA) evidenced typical asthmatic reactions, as follows: an increase in the number of eosinophils in bronchoalveolar lavage fluid; a marked influx of inflammatory cells into the lung around blood vessels and airways, and airway luminal narrowing; the development of airway hyperresponsiveness; the detection of tumor necrosis factor-alpha (TNF-α) and Th2 cytokines, including IL-4 and IL-5 in the bronchoalveolar lavage (BAL) fluid; and the detection of allergen-specific immunoglobulin E (IgE) in the serum. However, the administration of EC extract prior to the final airway OVA challenge resulted in a significant inhibition of all asthmatic reactions. We also demonstrated that EC extracts treatment resulted in significant reductions on matrix metalloproteinase-9 (MMP-9) and Suppressor of cytokine signaling-3 (SOCS-3) expression and a reduction in the increased eosinophil peroxidase (EPO) activity. The treatment of animals with EC extracts resulted in a significant reduction in the concentrations of the Th2 cytokine (IL-4 and IL-5) in the airways, without any concomitant increase in the concentration of Th1 cytokines. These findings indicate that EC extracts may prove useful as an adjuvant therapy for allergic airway reactions via the inhibition of the Th2 response. Accordingly, this study may provide evidence that EC extract performs a critical function in the amelioration of the pathogenetic process of asthma in mice.

Keywords: Asthma; Suppressor of cytokine signaling; *Ecklonia cava*; Eosinophilia; Airway hyperresponsiveness

1. Introduction

Asthma is an inflammatory disease characterized by bronchial hyperresponsiveness, which can proceed to life-threatening airway obstructions. The T helper 2 (Th2)-type cytokine interleukins (IL)-4, IL-5, and IL-13, which are generated by activated

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$CD4^+$ T cells, play a central role in asthma pathogenesis [1,2], by controlling the key process of immunoglobulin E (IgE) production, the growth of mast cells, and the differentiation and activation of mast cells and eosinophils [3–5]. Genetic and environmental factors influence the development of Th1 or Th2 cells. The direction of Th cell differentiation is determined by the cytokine environment at the site of initial antigenic activation. It has been well documented that the presence of IL-4 during the induction phase accounts for a predominance of Th2 cells [6,7], which subsequently determine the allergic inflammatory responses. Th2 cells are the predominant lymphocyte population that infiltrates the airways of asthmatics, and the cytokine products of Th2 cells perform essential roles in airway eosinophilia, AHR, and serum IgE in animal models [2]. Eosinophils are generated in the bone marrow, and recent observations in both mice and humans indicate that pulmonary allergen exposure results in both an increased output of eosinophils from hemopoietic tissues and an increased migration of these cells to the lung [8,9]. It is the accumulation of activated eosinophils during the late-phase response to allergen exposure that ultimately induces progressive inflammatory tissue damage. Thus, interventions that inhibit Th2 cytokines via the augmentation of Th1 cytokine production may prove useful in the treatment of allergic asthma.

Suppressor of cytokine signaling (SOCS) is a molecule that functions as a negative regulator of cytokine signaling, and is known to be involved in the pathogenesis of a host of inflammatory diseases [10–12]. The discovery of SOCS proteins has provided novel insights into the cytokine regulation of Th1 and Th2 immune responses. Eight members of the SOCS protein family have been identified thus far: cytokine-inducible SH2 domain-containing protein and SOCS-1 to -7. Among the SOCS proteins, SOCS-3 is expressed preferentially in Th2 cells and plays a crucial role in the regulation of the onset and maintenance of Th2-mediated allergic immune disease [13]. Serum IgE concentrations are also increased in patients evidencing high SOCS-3 expression. On the other hand, SOCS-5 is expressed preferentially in Th1 cells, and its expression can result in a reduction of Th2 differentiation as a consequence of the inhibition of IL-4 signaling [14]. It has been suggested that the inhibition of SOCS-3 expression may be a useful therapeutic approach to the treatment of Th2-dominant diseases, including allergic asthma.

Ecklonia cava (EC) is a brown alga (Laminariaceae) that is found abundantly in the subtidal regions of Jeju Island, Korea. Recently, an increasing amount of evidence has demonstrated that EC exhibits radical scavenging activity [15], matrix metalloproteinase inhibitory activity [16], bactericidal activity, protease inhibitory activity [17], antioxidative activity, and anti-inflammatory activity [18]. It has been demonstrated in many studies that antioxidants can reduce airway inflammation and hyperreactivity in an animal asthma model. This suggests that EC extracts may be associated with a reduced incidence of allergic asthma. However, no experimental evidence currently exists to support this notion.

In conclusion, it has been shown using animal models that allergic airway inflammation can be impaired by Th2 cytokine production and reduced Th1 cytokine production. In many allergic inflammatory diseases, elevations of the plasma IgE levels and attraction of eosinophils have been reported to correlate with the appearance of Th2 cells that generate IL-4 and IL-5 [19,20]. In this regard, we have attempted to investigate the role of the SOCS proteins as negative cytokine regulators in a murine asthma model. SOCS-3 and SOCS-5 are expressed predominantly in Th2 and Th1 cells, respectively. SOCS-3 and SOCS-5 are expected to be the principal modulators of allergic immune diseases, and they have been shown to reciprocally inhibit the Th1 and Th2 differentiation processes. It was recently shown that SOCS-3 expression is correlated with the pathology of Th2-mediated allergic immune diseases, including asthma. Thus, we utilized a murine asthma model to evaluate the effects of EC extracts on hyperresponsiveness and airway inflammation. In this study, we attempted to determine whether EC extracts evidences an inhibitory effect against OVA-induced asthma via the inhibition of suppressor of cytokine signaling-3 in mice.

2. Materials and methods

2.1. Preparation of EC extracts

The brown seaweed, EC extracts was collected along Jeju island coast of Korea during the period from October 2004 to March 2005. Fresh EC extract was washed 3 times with tap water to remove salt, epiphytes and sand attached to the surface of the samples and stored at −20 °C. The frozen samples were lyophilized and homogenized using a grinder before extraction. The dried *E. cava* powder (1 kg) was extracted with 95% EtOH (1:10 w/v) and evaporated in vacuo.

2.2. Animals

Female BALB/c mice were obtained from the Charles River Laboratories (Yokohama, Japan), and were kept in our animal facility for at least 1 week before use. All experimental animals used in this study were maintained under a protocol approved by the Institutional Animal Care and Use Committee of the Inje University Medical School. All mice were at 6–8 weeks of age.

2.3. Immunization and challenge

Mice were immunized intraperitoneally (i.p.) with 20 μg of OVA plus 1.0 mg aluminium hydroxide adjuvant on days 1 and 15. Mice were challenged via the airway with OVA (50 mg/ml of saline) each day from days 22 to 24 on consecutive days. Control mice were exposed to aerosolized saline. Aerosolization was performed for 20 min by placing the mice in a chamber (15 × 25 × 15 cm) connected to the ultrasonic nebulizer (NE-U12, Omron, Tokyo, Japan). Mice were injected i.p. with 20 mg/kg/day in 200 μl of EC each day from days 16 to 20 on consecutive days.