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Perillyl alcohol suppresses antigen-induced immune responses in the lung

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ABSTRACT

Perillyl alcohol (POH) is an isoprenoid which inhibits farnesyl transferase and geranylgeranyl transferase, key enzymes that induce conformational and functional changes in small G proteins to conduct signal production for cell proliferation. Thus, it has been tried for the treatment of cancers. However, although it affects the proliferation of immunocytes, its influence on immune responses has been examined in only a few studies. Notably, its effect on antigen-induced immune responses has not been studied. In this study, we examined whether POH suppresses Ag-induced immune responses with a mouse model of allergic airway inflammation. POH treatment of sensitized mice suppressed proliferation and cytokine production in Ag-stimulated spleen cells or $CD4^+$ T cells. Further, sensitized mice received aerosolized OVA to induce allergic airway inflammation, and some mice received POH treatment. POH significantly suppressed indicators of allergic airway inflammation such as airway eosinophilia. Cytokine production in thoracic lymph nodes was also significantly suppressed. These results demonstrate that POH suppresses antigen-induced immune responses in the lung. Considering that it exists naturally, POH could be a novel preventive or therapeutic option for immunologic lung disorders such as asthma with minimal side effects.

1. Introduction

The mevalonate pathway is a metabolic cascade which produces cholesterol. Cholesterol is intensively involved in cardiovascular diseases. Although cholesterol synthesis is thought to be conducted mainly in the liver, the mevalonate pathway is ubiquitous in living cells, and nonsterol products of this pathway also play an essential role in cell-signaling. Farnesyl diphosphate and geranylgeranyl diphosphate are intermediate metabolites of the mevalonate pathway, and are transferred to the C-terminal cysteines of target proteins by farnesyl transferase or by geranylgeranyl transferase, through farnesylation and geranylgeranylation, respectively [1]. Farnesylation and geranylgeranylation modulate various functions of proteins including the small GTPases Ras, Rac, and Ras homologue (Rho), by promoting the attachment of these proteins to cell membranes. For example, farnesylated Ras moves from the cytoplasm to cell membrane, then the activation of signal transduction pathways necessary for cell proliferation begins.

In normal sterologenic cells, there is a post-transcriptional feedback mechanism by which these nonsterol products down-regulate the overgrowth of cells through suppression of HMG-CoA reductase activity [2]. In contrast, tumor cells become resistant to this feedback mechanism, so up-regulation of HMG-CoA reductase results in continuous activation of the mevalonate pathway, which eventually leads to overgrowth of the cells [2]. Therefore, normalization of this pathway could become a novel strategy for cancer therapy. So far, several candidates including HMG-CoA reductase inhibitors (statins), inhibitors of mevalonic acid pyrophosphate decarboxylase, and inhibitors of farnesyl transferase, have been tried for treatment of cancers [2].

Among them, perillyl alcohol (POH) has attracted great attention. POH is a naturally occurring monoterpene, which exists in plants in a pure or mixed form. The mechanism by which POH suppresses cell growth has been studied intensively. POH acts as an inhibitor of farnesyl transferase [3,4] and geranylgeranyl transferase [5,6], which leads to suppress activities of various small GTPases [7,8]. Further, as these studies have confirmed that POH is relatively safe in terms of toxicity, POH has been considered a strong candidate for cancer treatment. In fact, phase I studies for

Abbreviation: POH, perillyl alcohol.

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refractory solid tumors have been reported [9,10]. Further, phase II trials have been conducted in prostate cancer [11], ovarian cancer [12], and metastatic colon cancer [13]. A recent study also showed that intranasal administration of POH (440 mg daily) increased the overall survival of patients with recurrent glioblastoma [14].

As POH suppresses the proliferation of cancer cells, it might affect the proliferation of immunocompetent cells as well. In addition, it might affect other functions of immnocompetent cells such as cytokine production and antibody production, thus modulating immune responses. So far, several in vitro studies have been conducted to examine the effect on T cells [15,16]. On the other hand, the effect of POH on immune cells in vivo has been examined only in a few studies with animal models of lupus [17], transplant coronary artery disease [17,18], and liver injury [19]. However, the effect on other types of immune responses, especially on allergic immune responses, has not been studied.

The purpose of the present study is to examine whether POH suppresses antigen-induced immune responses. We prepared a mouse model with OVA-induced allergic airway inflammation, and then examined the effect of POH.

2. Materials and methods

2.1. Mice

Male BALB/c mice 7 weeks of age were obtained from Charles River Japan (Kanagawa Japan). They were maintained under conventional animal housing conditions in a specific pathogen-free setting. All animal experiments were approved by the Animal Research Ethics Board of the Department of Allergy and Rheumatology, University of Tokyo (Tokyo, Japan).

2.2. Medium for cell culture

Complete DMEM was used as the medium for cell incubation. Complete DMEM consisted of DMEM with glucose (4.5 g/L), pyridoxine HCl (4.0 mg/l), and sodium pyruvate (110 mg/l), which contained FBS (10%, w/v), HEPES buffer sodium (0.01 M), MEM nonessential amino acid solution (0.1 mM), 2-ME (50 μM: Sigma–Aldrich), penicillin (100 U/ml), and streptomycin (100 μg/ml).

2.3. Quantification of cytokines and cell proliferation

Cytokine concentrations in BALF, serum, and cell culture supernatants were determined by Enzyme-linked immunosorbent assay (ELISA). Concentrations of mouse IL-4, IL-5, IL-10, IFN-γ, IgE (BD pharmingen), and IL-13 (R&D Systems) were measured using commercial ELISA kits. We measured concentrations of mouse IL-17 by ELISA as previously reported [20]. Cell proliferation was measured by BrdU incorporation using a BrdU cell proliferation ELISA kit (Roche, Mannheim, Germany). The data were analyzed with Microplate Manager III, version1.45 (Bio-Rad).

2.4. Effects of POH on spleen cells in vitro

BALB/c mice were immunized with 2 μg of OVA (Sigma–Aldrich)/2 mg of alum i.p. on days 0. On day 10, spleen cells were obtained and cultured (2.5×10^6 cells/ml) with OVA (100 μg/ml) in the absence or presence of different concentrations of POH (0.1 or 1 μM) (Wako, Osaka, Japan). Then, cell proliferation and cytokine production were measured.

2.5. Preparation of single cell suspensions of spleen and lymph node cells

Spleens were collected and incubated at 37 °C for 15 min after treatment with 0.1% (w/v) collagenase (Sigma–Aldrich)/complete DMEM solution, then minced. Lymph node cells were collected and minced, then incubated at 37 °C for 30 min with 0.033% collagenase/complete DMEM solution. Single-cell suspensions were prepared by passing through a cell strainer. RBCs were removed by hypotonic lysis using RBC lysing buffer (Sigma–Aldrich). After two washes with HBSS, spleen or lymph node cells were used for experiments.

2.6. Spleen and lymph node cell responses to OVA

Spleen or lymph node cells (2.5×10^6/ml) were cultured in a 96-well, flat-bottomed microtiter assay plate with OVA (100 μg/ml) in an incubator (37 °C, 5% CO_2, 90% humidity). Cell Proliferation was measured on day 3. On day 4, cytokine production was measured by ELISA.

2.7. Animal preparation for ex vivo analyses

BALB/c mice were immunized with 2 μg of OVA/2 mg of alum i.p. on days 0 and 11. On days 11–17, mice were treated with POH (75 mg/kg body weight) dissolved in 0.5 ml of vehicle or the vehicle alone by i.p. injection. The vehicle for POH was Tricaprylin [17] (Sigma–Aldrich). The control mice received only saline injections on days 0 and 11. On day 18, spleens were collected and cell proliferation and cytokine production were measured as described above.

2.8. Effects of POH on $CD4^+$ T cells ex vivo

BALB/c mice were immunized with OVA/alum i.p. on days 0 and 11. On days 11–17, mice were treated with POH (75 mg/kg body weight) dissolved in 0.5 ml of vehicle or vehicle alone by i.p. injection. $CD4^+$ T cells were selected from spleen cells of the OVA-sensitized mice on day 18. To obtain $CD4^+$ T cells, we used monoclonal anti-mouse CD4 magnetic particles (BD Biosciences) following the manufacturer's protocol. The purity of $CD4^+$ cells, confirmed by flow cytometry, was >95%. Then $CD4^+$ cells (2.5×10^5/ml) were cocultured with freshly isolated mitomycin-C (Sigma–Aldrich)-treated splenocytes (2.5×10^5 cells/ml) under OVA stimulation (30 μg/ml). After 48 h, proliferation was assessed with a cell proliferation ELISA BrdU kit. After 72 h, the cytokine concentration in the supernatant was measured by ELISA.

2.9. Induction of allergic airway inflammation and POH treatment

BALB/c mice were immunized with OVA/alum i.p. on days 0 and 11. Control mice received i.p. injections of saline instead of the OVA/alum solution. Mice were challenged with an aerosolized solution of 3% w/v OVA in PBS for 10 min from day 18 to day 20. POH (75 mg/kg) dissolved in 0.5 ml of vehicle or the vehicle alone was given by i.p. injection from days 0 to 17. The control mice received PBS by inhalation on days 18–20. On day 21, samples of serum and bronchoalveolar lavage fluid (BALF) were obtained. The lungs were cut out and fixed with 10% neutralized buffered formalin. Thoracic lymph nodes were also obtained and used for analyses as described above. Cell counts and cell differentials in BALF were determined as previously reported [21]. Concentrations of IL-13 in BALF and IgE in sera were measured by ELISA.