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Abrogation of the oral tolerance to ovalbumin in mice by citrus pectin

Daria S. Khramova, M.Sc., Sergey V. Popov, Ph.D.*, Viktoria V. Golovchenko, Ph.D., Feodor V. Vityazev, M.Sc., Nikita M. Paderin, M.Sc., and Yury S. Ovodov, Prof. Dr.

Institute of Physiology, Komi Science Centre, The Urals Branch of the Russian Academy of Sciences, Syktyvkar, Republic of Komi, Russia

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Abstract

Objective: We studied the effects of dietary pectins (citrus pectin [CP] and apple pectin) on oral tolerance in mice.

Methods: Pectins (1 mg/d) were administered orally for 2 wk. Tolerance was induced with 20 mg of ovalbumin (OVA). Levels of serum antibodies (immunoglobulin [Ig] G, IgG1, IgG2a, IgE) and delayed type hypersensitivity response determined in footpad tests were measured after subcutaneous injection of OVA with complete Freund's adjuvant. Concentrations of immunoreactive OVA in blood were measured by enzyme-linked immunosorbent assay after feeding the animals 20 mg of OVA. Adhesion and cytokine production (tumor necrosis factor-α, interferon-γ) were measured in peritoneal macrophages.

Results: Oral administration of CP was found to prevent the induction of immune hyporesponsiveness induced by OVA feeding. Animals fed OVA and CP were found to produce similar titers of antigen-specific serum IgG and levels of delayed type hypersensitivity response as those animals not fed OVA. CP increased levels of serum IgG1 and IgE. CP was found to enhance the penetration of immunogenic OVA into the serum. CP (1 mg/d) administered orally for 1 wk was also observed to enhance the adhesion and production of cytokines (tumor necrosis factor-α, interferon-γ) in peritoneal macrophages.

Conclusion: CP administered orally was shown to inhibit oral tolerance. Enhancement of protein antigen penetration to the blood and activation of macrophages were found to precede the inhibitory effect and appeared to mediate it.

Keywords: Pectins; Citrus pectin; Oral tolerance; Ovalbumin; Anti-ovalbumin antibodies; Peritoneal macrophages; Inflammatory cytokines; Adhesion

Introduction

Oral tolerance (OT) has long been recognized as a physiologic mechanism of immune unresponsiveness to dietary and bacterial antigens that maintain tissue integrity. Breakdown of OT may be the underlying cause of different food-sensitive enteropathies and inflammatory bowel disease [1]. Therefore, the identification of factors that are able to regulate OT is of great interest. It has been hypothesized that food components may affect the immunogenic potential of ingested proteins [2].

Pectic polysaccharides, which are major constituents in the plant cell wall, are present in the diet and are widely used in the food industry as gelling agents. Moreover, pectins have been shown to possess some immunomodulatory activity [3] and an ability to influence nutrient absorption [4]. Oral administration of the polysaccharide fraction of a dried extract (TJ-48) of the Japanese herb *kampo* to mice resulted in changes in the intestinal immune system [5]. Furthermore, an acidic polysaccharide fraction prepared from a constituent herb of *juzen-taiho-to* has been shown to have protective activity against *Candida* infection in mice [6]. Continuous feeding of the pectin bupleuran, which was prepared from the roots of *Bupleurum falcatum* L., led to enhancement of proliferation of spleen cells [7].

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* Corresponding author: Tel./fax: +7-8212-241001.

E-mail address: popov@physiol.komisc.ru (S. V. Popov).

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Citrus pectin (CP), one of the most widely consumed dietary pectins, is known for its ability to decrease plasma cholesterol concentrations [8] and for its influence on metastasis growth [9]. However, the effect of CP on OT has not been studied.

The aim of the present investigation was to study the effect of CP and apple pectin (AP) on OT in mice.

Materials and methods

Materials

Ovalbumin from egg whites (OVA; grade V), AP, thioglycolate, lipopolysaccharide (*Escherichia coli* 0111:B4), *ortho*-phenylene-diamine, phorbol-12-myristate-13-acetate (PMA), and RMPI-1640 medium were purchased from Sigma, St. Louis, MO, USA. CP, complete Freund's adjuvant, bovine serum albumin (BSA), phosphate buffered saline (PBS), and horseradish peroxidase (HRP)-labeled goat anti-mouse immunoglobulin (Ig) G antibodies (Abs) were purchased from MP Biomedicals, Solon, OH, USA.

The galacturonic acid contents of CP and AP were 87% and 75%, respectively, accordingly to certificates of analysis. The degrees of esterification were 56% and 73%, respectively.

Induction of OT and pectin administration

The structure of this study and animal experimental procedures were approved by the ethical committee of the Komi Science Center of the Russian Academy of Sciences on animal care and use.

The mice were fed the cereal-based diet which consisted of 12.7% protein, 5.6% fat and 54.1% carbohydrate, with a total fiber content of 3.7%. The diet was supplemented with a vitamin–mineral premix according to the recommendation of the American Institute of Nutrition (AIN-93M diet) [10].

In experiment 1, female A/HeJ mice (20–25 g) were immunized with 100 μg of OVA in 50 μL of complete Freund's adjuvant subcutaneously at the base of the tail. To induce OT, 7 d before subcutaneous immunization, mice were intragastrically given 20 mg of OVA dissolved in 0.2 mL of PBS (tolerant group, $n = 21$) or PBS only (control group, $n = 7$) [11]. Pectins were dissolved in PBS (pH 7.2) up to a concentration of 5 mg/mL. CP and AP solutions obtained were pH 6.7 and 6.6, respectively, and were clear with no visible particulates. Aliquots of the pectic solutions (200 μL) were administered once daily in the morning to tolerant mice intragastrically through a polyethylene tube for 14 d: 7 d before and 7 d after the OVA gavage ($n = 7$ for CP-treated group, $n = 7$ for AP-treated group). Thus the intake of pectin was 1 mg/d or 40–50 mg/kg. Untreated mice ($n = 7$) received PBS.

To assess OT, delayed type hypersensitivity (DTH) and serum Ab responses were measured in control, tolerant untreated, tolerant CP-treated, and tolerant AP-treated mice. The mice were injected with 25 μg of heat-aggregated OVA in 25 μL of PBS in the right footpad 14 d after subcutaneous immunization; PBS was injected in the left footpad. The volume of the injected paws was measured by using a water-displacement plethysmograph (Ugo Basil, Comerio, Italy) 24 h after injection and was recorded as the difference between the left and right footpads [12].

To determine Ab responses, mice were bled with cardiopuncture after the DTH measurement and blood was centrifuged for 5 min at 400 $\times$ *g*. Serum was collected from these samples and stored frozen at −20°C until assayed.

Antibody titers to OVA were assessed using an enzyme-linked immunosorbent assay (ELISA) as described in detail elsewhere [13]. Briefly, flat-bottom polystyrene plates (Costar, NY, USA) were coated overnight at 4°C with 5 μg of OVA diluted in 0.1 mL of 0.05 mol/L $NaHCO_3$ buffer (pH 9.6) per well, washed with saline containing 0.05% (v/v) Tween-20, blocked with 1% BSA in PBS, washed again, and then the mouse serum samples with serial dilutions starting at 1:10 were added to the wells. After incubation for 2 h at 25°C, HRP-labeled goat anti-mouse IgG Abs (1/2000) were added to wells and incubated for 2 h at 25°C. The color reaction was developed by the addition of H_2O_2 and *ortho*-phenylene-diamine. The reaction was interrupted after 15 min by the addition of 0.1 mL of 2.5 mol/L HCl and absorbance (OD) was read at 492 nm (Power Wave 200, BioTek Instruments, Winooski, VT, USA). Endpoint Ab titers were expressed as the last dilution producing an OD 0.1 U above negative control values at 492 nm.

Sandwich ELISA was used for measurement of total IgE. Monoclonal affinity-purified anti-mouse IgE antibodies (23G3; eBiosciences, San Diego, CA, USA) were used as the capture Abs for IgE, with biotin-conjugated anti-mouse IgE Abs as the detection Abs. Indirect ELISA was used for measurement of serum OVA-specific IgE. To generate a standard curve, the upper two rows of each plate were coated with the purified goat anti-mouse IgE (23G3; eBioscience). HRP-labeled streptavidin (BD Pharmingen, San Jose, CA, USA) was used for detection. Biotin-conjugated monoclonal Abs specific for γ1 (LO-MG1-2) or γ2a (LO-MG2a-9; Acris Antibodies GmbH, Hiddenhausen, Germany) were used for analysis of IgG1 and IgG2a Ab subclasses [14].

Detection of serum-immunoreactive OVA

In experiment 2, mice treated with 1 mg/d of CP or AP for 7 d received 20 mg of OVA and were sacrificed 0.5, 1, 3, 6, or 24 h thereafter. Serum was collected and immunoreactive OVA was measured by indirect competitive ELISA. Microplates with 96 wells (Costar, USA) were coated for 18 h at 4°C with 100 μL/well of a 25-μg/mL solution of OVA in carbonate buffer, pH 9.6. The plates were washed four times with PBS containing 0.05% (v/v)