

PHARMACOLOGY AND TOXICOLOGY

Effects of Plant Water-Soluble Polysaccharides on the Production of Immunoglobulins E and G1 by Lymphocytes of Mice Sensitized with Ovalbumin

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Were studied the effects of water-soluble plant polysaccharides isolated from pharmacopoeic raw material on anaphylactic shock and production of IgE and IgG1 by lymphocytes from mice immunized with ovalbumin. Course treatment with polysaccharides from coltsfoot, sweet flag, clover, Artemisia, marigold, and elecampane reduced animal mortality after induction of anaphylactic shock. In addition, injection of these substances reduced serum concentrations of IgE and IgG1. These substances can be regarded as promising agents for the treatment of IgE-dependent diseases (atopic dermatitis, asthma, atopic rhinitis, urticaria, food allergies, *etc.*).

Key Words: *atopy; immunoglobulins E and G1; anaphylactic shock*

Non-infectious diseases of the immune system, the most prevalent of which are various forms of immediate-type hypersensitivity reactions (atopic dermatitis, asthma, atopic rhinitis, urticaria, food allergies, *etc.*), are an important medicosocial problem. The diseases of this group are caused by excessive Th2 response characterized by intensive secretion of IL-4, IL-5, and IL-13 and allergen-specific IgE and IgG4 (IgE and IgG1 in mice).

The most prevalent therapy for IgE-dependent pathologies is allergen-specific treatment (for example, with histamine receptor blockers, glucocorti-

coids, mast cell membrane stabilizers) aimed at prevention or reduction of the negative effects of IgE; specific immunotherapy (SIT), switching over antibody production from IgE and IgG4 to IgG1 due to repeated desensitizing immunizations with the allergen. Despite high efficiency of SIT, it has important flaws: the method is expensive, a positive effect can be attained after several courses (3-5 years), and the antigen is to be precisely identified, which is not always possible [5]. Recently one more drug based on monoclonal antibodies to IgE appeared: omalizumab, or E25. High efficiency of this preparation was demonstrated [3,4,6,12]. However, its use is limited because of its high price and need of reinjections every 2-3 weeks in order to maintain the clinical effect. No drugs aimed at modification of the key component of the pathogenesis (sup-

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pression of excessive Th2 cytokine secretion), allowed for medical use, was created by the present time [1], while the need in such drugs is obvious.

The aim of this study was to detect substances inhibiting the Th2 immune response and thus suppressing the production of IgE and IgG1. We studied water-soluble plant polysaccharides (PS), because these substances exhibit a wide spectrum of immunomodulating effects. For example, they modify the production of some cytokines, expression of some adhesion molecules [9,11]. However, the data on PS effects on the immune response are contradictory: they can activate Th1 and suppress Th2 in parallel with this [10] and can stimulate Th2 [2].

MATERIALS AND METHODS

Experiments were carried out on male and female BALB/cY mice (8-12 weeks) from Breeding Center of Institute of Pharmacology. The animals were kept in an incomplete barrier system at 12:12 day: night regimen on granulated fodder and drinking water acidified with hydrochloric acid (pH 4-5).

The Th2 type immune response was induced by the common method (by ovalbumin) [7,8]. The mice were subcutaneously injected with 100 µg ovalbumin (Sigma) with 5 mg aluminum hydroxide (Sigma) in 0.1 ml isotonic NaCl. Repeated immunization was carried out after 14 days. Controls were injected with the same volume of saline according to the same protocol. Anaphylactic shock was induced by intravenous injection of ovalbumin (10 µg in 0.2 ml saline) into the retroorbital sinus.

Water-soluble PS were isolated from pharmacopoeic raw material at Department of Chemistry of Siberian State Medical University by the standard method. Water-soluble PS were isolated from the following plants: coltsfoot, birch, sweet flag, clover, Artemisia, marigold, and elecampane. The studied substances were dissolved in saline and injected intraperitoneally in a dose of 10 mg/kg (the dose was selected in preliminary experiments). In case of double immunization, PS were injected for 5 days after each injection of ovalbumin and 5 days before the first immunization. In case of single immunization, the studied substances were injected 10 times starting 5 days before ovalbumin injection. Muramyl dipeptide (MDP; N-acetylmuramyl-L-alanine-D-isoglutamine; Calbiochem) served as the positive control. It was injected according to the same protocol in a dose of 2 mg/kg (optimal dose selected in preliminary experiments).

In order to measure the levels of IgG1 and IgE, the animals were sacrificed by cervical dislocation 7 days after the second ovalbumin injection and 10

days after single immunization. The blood was collected from the heart, serum was divided into aliquots, and stored at -20°C. Serum immunoglobulins were measured by ELISA with respective test systems (Immunology Consultants Lab.) according to the instructions.

The results were statistically processed using Student's *t* test.

RESULTS

In order to evaluate the capacity to modify the Th2-dependent immune response, we studied changes in the mortality from anaphylactic shock in animals receiving a course of PS injections. Two series of experiments were carried out, 10 animals per series. The PS from birch increased animal mortality by 33%, while other PS and MDP decreased it: MDP by 10%, coltsfoot PS by 33%, sweet flag PS by 25%, clover PS by 37.5%, Artemisia PS by 12.5%, and elecampane PS by 20%. All PS except birch PS were taken for further studies.

Course treatment with water-soluble plant PS reduced serum IgE level in mice receiving single

TABLE 1. Serum IgE Concentrations in Ovalbumin-Immunized Mice after a Course of PS and MDP Injections ($\bar{X} \pm m$)

Substance	Number of immunizations	Group	Concentration of IgE, µg/ml
MDP	1	Control	4.90±0.64
		Experiment	4.36±1.11
	2	Control	7.36±0.81
		Experiment	2.17±0.47*
Sweet flag PS	1	Control	4.90±0.64
		Experiment	2.92±0.45*
	2	Control	7.36±0.81
		Experiment	2.05±0.49*
Coltsfoot PS	1	Control	4.90±0.64
		Experiment	3.11±0.21*
	2	Control	7.36±0.8
		Experiment	2.80±0.55*
Artemisia PS	1	Control	4.90±0.64
		Experiment	2.06±0.36*
Marigold PS	1	Control	4.90±0.64
		Experiment	2.41±0.63*
Elecampane PS	1	Control	4.90±0.64
		Experiment	2.60±0.43*
Clover PS	1	Control	4.90±0.64
		Experiment	2.10±0.23*

Note. Here and in Table 2: **p*<0.05 compared to the control.

TABLE 2. Serum IgG1 Concentrations in Mice after Two Immunizations with Ovalbumin and a Course of PS or MDP Injections ($X \pm m$)

Substance	Group	Concentration of IgG1, $\mu\text{g/ml}$
MDP	Control	4.22 \pm 0.28
	Experiment	2.38 \pm 0.32*
Sweet flag PS	Control	4.22 \pm 0.28
	Experiment	2.00 \pm 0.50*
Coltsfoot PS	Control	4.22 \pm 0.28
	Experiment	3.14 \pm 0.15*
Artemisia PS	Control	3.99 \pm 0.34
	Experiment	2.52 \pm 0.42*
Marigold PS	Control	3.99 \pm 0.34
	Experiment	2.15 \pm 0.56*
Elecampane PS	Control	3.99 \pm 0.34
	Experiment	2.04 \pm 0.35*
Clover PS	Control	3.99 \pm 0.34
	Experiment	1.85 \pm 0.34*

or double immunization with ovalbumin (Table 1). This parameter did not change after single sensitization with MDP, while double immunization with MDP reduced the parameter by 3.4 times. Plant PS reduced the concentration of IgE: sweet flag PS 1.7 and 3.4 times, coltsfoot PS 1.6 and 2.6 times (after a single and double immunization, respectively). Artemisia PS reduced IgE concentration 2.4 times, marigold PS 2-fold, elecampane PS 1.9 times, and clover PS reduced it 2.3 times after single immunization.

Course treatment with all test substances reduced the concentrations of IgG1 in animals receiving two immunizations (Table 2). MDP reduced this parameter by 1.8 times, sweet flag PS by 2.1 times, coltsfoot PS by 1.3 times, Artemisia PS by 1.6 ti-

mes, marigold PS by 1.9 times, elecampane PS 2-fold, and clover PS by 2.2 times reduced the concentration of IgG1.

These results indicate that water soluble PS from sweet flag, coltsfoot, Artemisia, marigold, elecampane, and clover inhibit the production of IgE and IgG1 by B lymphocytes and hence, reduce the severity of anaphylactic shock. Hence, these substances are prospective agents for the therapy of IgE-dependent pathologies.

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