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SHORT COMMUNICATION

Low-dose intragastric administration of *Phaseolus vulgaris* agglutinin (PHA) does not induce immunoglobulin E (IgE) production in Sprague-Dawley rats

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Native *Phaseolus vulgaris* agglutinin (PHA) poses a potential health threat, when ingested with improperly cooked red kidney beans. Since PHA triggers human basophilic granulocytes in culture to rapidly release considerable amounts of interleukin-(IL-)4 and IL-13, key cytokines for inducing immunoglobulin E (IgE) production, the question was addressed whether this lectin can evoke *in vivo* IgE production. IgE-low-responder (Sprague-Dawley) rats received PHA (6 mg/rat/day) intragastrically by gavage over a period of 10 days. Up to day 35, there was no IgE induction regardless of whether the animals were boostered subcutaneously with PHA or not, indicating that PHA cannot be regarded as a general IgE inducer in rats.

Keywords: allergy, lectin, immunoglobulin E, interleukin-4, phytohemagglutinin

Abbreviations: Con A, concanavalin A; IgE, immunoglobulin E; IL-, interleukin-; PHA, *Phaseolus vulgaris* agglutinin.

Introduction

The mechanisms by which legumes induce IgE-mediated allergy are currently not known. We have recently observed that various legume-derived lectins such as PHA and concanavalin A (Con A) can trigger human basophilic granulocytes to release interleukin- (IL-)4 and IL-13, the key cytokines for inducing IgE production [1]. Furthermore, in various mouse strains Con A has been found to be allergenic [2] or to enhance reaginic (*i.e.* IgE) antibody formation [3]. Herein, we report the effects of intragastric administration of a non-toxic PHA dose to rats to clarify whether this lectin might induce IgE production *in vivo* and, thus, possibly be involved in the initiation of IgE-mediated allergy.

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Materials and methods

Preparation of PHA

The complete mixture of PHA isolectins was purified by affinity chromatography with ovomucoid as ligand glycoprotein immobilized on Sepharose 4B and elution using 150 mM sodium tetraborate, pH 8.0. Quality control of purity to exclude presence of further proteins except for the lectin fraction was performed by one- and two-dimensional gel electrophoresis and functional activity was determined by hemagglutination assays. IL-4-inducing activity of PHA was checked using the procedure described in [1].

Animals and sensitization

Three independent experiments were performed with male Sprague-Dawley rats (n = 26) weighing 120 g (Charles River, Sulzfeld, Germany). In all three experiments groups of animals (n = 4) received PHA (6 mg/day) intragastrically (i.g.) by gavage in 1 ml physiologic saline over a period of 10 days. For

one group food was withheld from the animals three hours prior to the experiment until one hour thereafter to exclude the possibility of undesired binding of PHA to food components and neutralization of its biologic effect. The PHA-fed animals of experiment 1 were given an additional booster dose of 0.6 mg PHA subcutaneously (s.c.) in 0.5 ml saline at days 14, 21 and 28. Experiment 2 contained two PHA-fed groups: one of them with and one without additional booster. In experiment 3 the group of PHA-fed animals was not boostered. Control animals (experiment 1: n = 2; experiments 2 and 3, respectively: n = 4) received saline solution only by gavage at days 1–10 and in addition (experiments 1 and 2) subcutaneously at days 14, 21 and 28. Heparinized venous blood was taken from the tail vein of all animals at days 0, 14, 21, 28 and 35 to obtain 50–100 µl plasma for IgE determination.

IgE ELISA

Microtiter plates (Maxisorp F96, Nunc, Roskilde, Denmark) were coated overnight with mouse anti-rat IgE mAb (clone B41-1, Pharmingen, San Diego, CA; diluted 1:200 in TBS (0.1 M Tris/HCl, 0.1 M NaCl, 2.5 mM $MgCl_2$, pH 7.5)) at 4°C. All following steps were performed at room temperature. Serial dilutions of a rat IgE standard (rat myeloma IgE kappa, PRPO7, Serotec, Oxford, UK; from 100 to 0.14 ng/ml) or of rat serum samples, respectively, were added for 90 min. To block any PHA activity contaminating the serum samples, the plates were incubated thereafter with skimmed milk (5% milk powder in TBS/0.05% Tween 20 (TBS-T)) for 60 min Biotinylated anti-rat IgE mAb (clone B41-3, Pharmingen, San Diego, CA; diluted 1:400 in TBS-T containing 0.5% BSA) was added for 60 min. Then, streptavidin-alkaline phosphatase (diluted 1:1000 in TBS-T/0.5% BSA) was added for 60 min, and finally, substrate-chromogen solution (1 mg/ml p-nitrophenylphosphate (Sigma 104), Sigma-Aldrich, Taufkirchen, Germany; in 0.1 M TBS/HCl, 0.1 M NaCl, 5 mM $MgCl_2$, pH 9.5). Absorption was read at 405 nm. Between all incubation steps the plates were washed 7× with TBS-T. The assay had a sensitivity of 0.4 ng/ml rat IgE.

Results and discussion

To exclude the possibility that systemically absorbed PHA present in rat sera might artificially affect IgE determination,

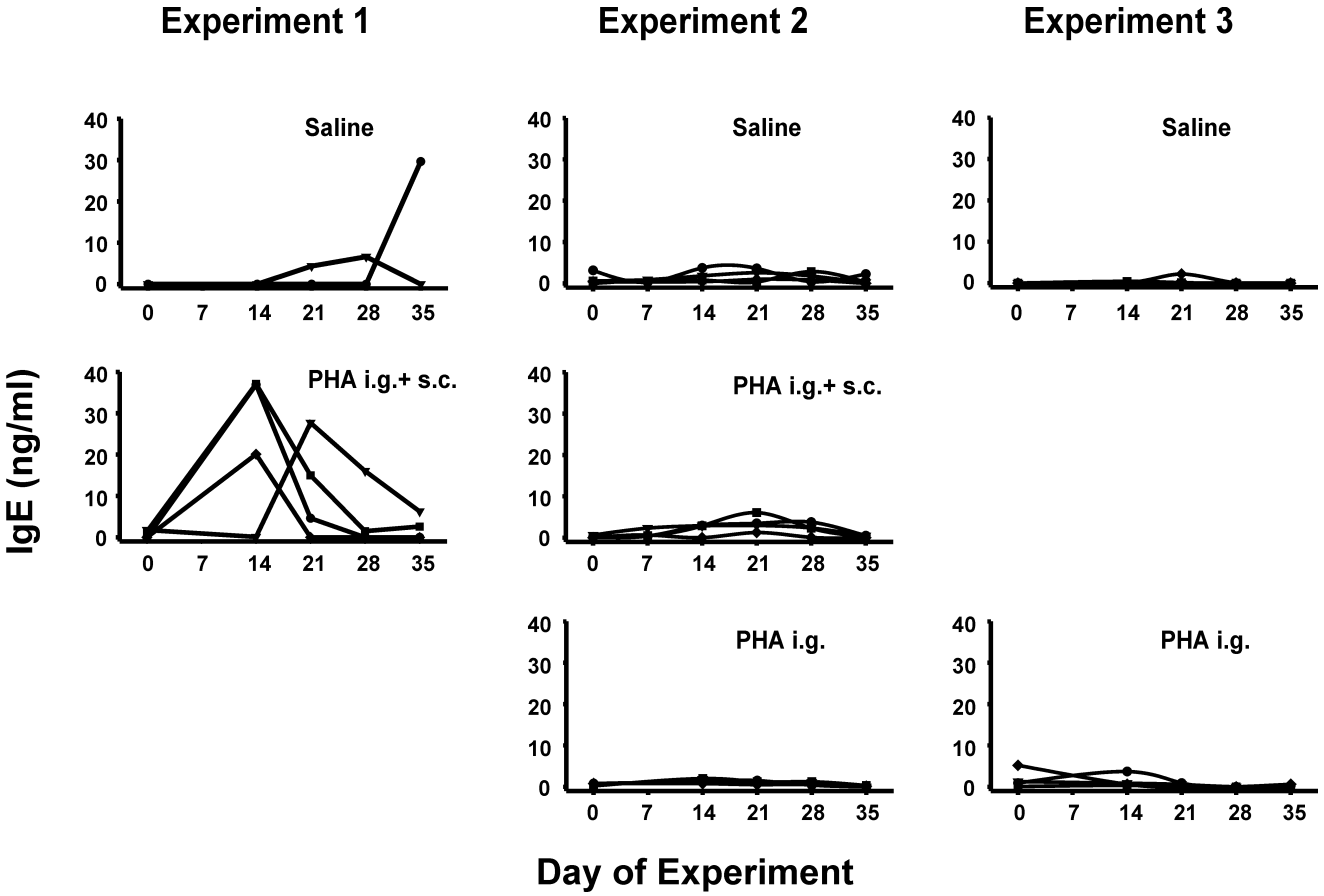

Figure 1. Serum IgE levels in PHA-treated rats. Three groups of Sprague-Dawley rats received PHA (or saline as control) intragastrically (i.g.; Experiment 1–3) and subcutaneously (s.c.; Experiment 1 + 2). Serum IgE concentrations were determined at days 0, 14, 21, 28 and 35. Only minimal (up to 40 ng/ml) increases for the IgE level were observed.

an incubation step with skimmed milk as a blocking agent was added to the protocol of the IgE ELISA. Under these conditions, IgE determination in a control rat serum spiked with a minute amount of rat IgE (2 ng/ml) was neither artificially increased nor decreased by various amounts (0.14–100 µg/ml) of PHA (not shown).

Using this modified ELISA, we found that intragastric PHA administration to Sprague-Dawley rats did not cause any significant IgE induction (Figure 1). IgE concentrations were very low and did not exceed 40 (experiment 1) or 10 ng/ml (experiments 2 and 3), respectively, with no clear difference between PHA-treated and control animals. Evidently, PHA – although triggering significant amounts of the IgE-inducing cytokines IL-4 and IL-13 from human basophils in culture – does not induce elevated IgE levels in Sprague-Dawley rats under these conditions. A conceivable explanation for this lack of effect in animals could be the impact of PHA on the cytokine network with inherent possibilities for antagonism. In fact, PHA triggers also the release both of interferon-γ [4, and own unpublished observation] and of IL-12 [4] from peripheral blood mononuclear cells, *i.e.* of cytokines that antagonize the effect of IL-4 and IL-13. In addition, rat strain selection can account for the lack of response. Sprague-Dawley rats are known to be low-IgE responders [5]. A positive result in these rats would have indicated, that the effect will not be restricted to a small population of IgE-high-responder strains which barely had significant meaning.

The selected dose of PHA administered in this study (6 mg/rat/day) was lower than previously employed in a study assessing the biological effects of PHA in the pancreas (*e.g.* 42 mg/rat/day) [6], yet still is in the range of significantly stimulating small bowel growth (3.4 and 6.7 mg/rat/day) as assessed by measuring the small bowel wet weight [7]. At the selected dose we did not observe histological changes by standard light microscopy. Considering, that regular cooking mostly destroys PHA, the relatively low and non-toxic dose of PHA was chosen as a model for the situation in humans.

Furthermore, we employed a mixture of PHA isolectins composed of different ratios of an erythroagglutinating (E) and/or a leucoagglutinating (L) subunit resulting in five possible hololectin types: E4, E3L1, E2L2, ElL3 and L4 [8]. Although purified E4 (also called PHA-E) induces higher amounts of IL-4 from basophils than L4 (PHA-L) *in vitro* [4], the complete mixture of isolectins after affinity chromatography was used for this study to determine its activity level reflecting the occurrence of isolectins in the food source.

In summary, PHA, a lectin which triggers production of the IgE-inducing cytokines IL-4 and IL-13 but also of the IgE-antagonistic cytokines IL-12 and interferon-γ in vitro, does not elicit IgE production in Sprague-Dawley rats after intragastric administration at a non-toxic dose.

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