Plasma Derived IgA from Healthy Donors Binds to Peanut Extract and Inhibits Peanut-Induced Rat Basophil Activation

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Abstract

Rationale: IgA and IgE are both mucosal immunoglobulins. We hypothesize that food specific IgA is capable of interfering with IgE mediated allergy effector cell activation. Oral administration of food specific secretory IgA prepared from plasma IgA may ameliorate food allergy symptoms.

Methods: IgA from more than 3000 healthy plasma donors was purified from a by-product of intravenous immunoglobulin manufacture. The ability of this pooled IgA to bind to crude peanut antigen was determined using an ELISA. Specificity of binding was confirmed using an ELISA inhibition assay. Monomeric and dimeric IgA were separated by size exclusion chromatography. Binding of IgA monomers and IgA dimers to peanut were each determined independently using an ELISA. Leuciferase transfected rat basophil leukemia cells were sensitized with peanut specific IgE using serum from a peanut allergic subject. Inhibition of peanut-induced rat basophil leukemia activation by plasma derived IgA was determined using luciferase-produced light emission.

Results: Pooled plasma IgA from healthy plasma donors binds to crude peanut extract. The binding is inhibited in a concentration dependent manner by soluble peanut antigen. Both IgA monomers and IgA dimers bind to peanut extract. Peanut extract pre-incubated with plasma derived IgA partially inhibits the ability of peanut to activate IgE sensitized rat basophil leukemia cells in culture.

Conclusion: Both IgA monomers and IgA dimers derived from pooled healthy donor plasma binds to crude peanut extract. Plasma derived anti-peanut IgA partially inhibits peanut-induced rat basophil leukemia cell activation demonstrating that it is physiologically active.

Rationale

8% of American children (5.9 million patients) have food allergy (1, 2)

>1% of Americans (~3 million) are allergic to peanuts (3, 4).

Peanut allergy is the leading cause of anaphylaxis-related U.S. emergency room visits (5, 6)

IgA can neutralize food antigens and prevent the initiation of food antigen-induced mast cell and basophil activation.

- **Antigen-specific secretory IgA blocking of IgE mediated histamine release has been demonstrated *in vitro* (7).**
- **IgA blocks IgE mediated reactions *in vivo*. Antigen specific IgA blocks passive cutaneous anaphylaxis in mice (8).**
- **IgA prevents ragweed induced airway hyperresponsiveness (9)**
- **IgA ameliorates ovalbumin-induced systemic anaphylaxis in mice (10)**

Objectives

Demonstrate that human plasma derived IgA

- **Has antigenic specificity for peanut antigen**
- **Neutralizes IgE mediated rat basophil leukemia cell activation**

Methods: IgA Isolation

IgA from Cohn fraction III precipitate (12) byproduct of IVIg production.
From the pooled plasma of up to 5000 donors.
Suspended in PBS; "Filter aides" (diamataceous earth) removed
Viral inactivation by solvent—detergent (1% tri (N butyl) phosphate, 1% Triton X-100) treatment.
IgA was isolated by jacalin affinity chromatography (12). Separation of IgA monomer and dimer by size exclusion chromatography. a 85.5 cm long Sephacryl 300 column on an FPLC (BioCAD Workstation)

Methods

- **Peanut extract (Ludmilla Bardina, Mount Sinai, NY) from roasted ground peanuts defatted with >20 vol (wt/vol) acetone, dried, extracted with PBS.**
- **High binding ELISA plates (Microfluor 2) coated with 1 μg/mL blocked with KemEnTec block buffer; HRP-conjugated goat anti-human IgA (Zymed); fluorogenic Amplex Red (Invitrogen) used for detection. Plates were read on a fluorescent plate reader (7620 FluoroCount, Packard Instruments).**
- **IgA inhibition of peanut-induced cell activation was measured using a rat basophil leukemia cell line. The RS-ATL8 cell line (Dr. Ryosuke Nakamura (National Institute of Health Sciences, Tokyo, Japan) (Allergy 2010:1266-1273). These cells are a luciferase transfected RBL-SX38 rat basophil leukemia cell line (Beth Israel Deaconess Medical Center, Boston, MA). Cultured in poly-D-lysine coated white clear bottom 96 well tissue culture plates (Corning) and then sensitized with Class IV positive peanut allergic serum (Warde Medical Laboratory, Ann Arbor, MI). IgG was removed by passage through Protein A slurry (protocol from Dr. Stephen Dreskin, U of CO Denver School of Medicine) (Int. Arch. Allergy Immunol. 104 (1994) 204– 206). The serum used at 1:80 dilution for cell sensitization. Peanut extract was used at final concentration of 700 ng/mL. IgA 10 mg/mL was incubated at a 4000:1 IgA:peanut ratio for neutralization peanut antigen. Luminescence was generated using luciferase substrate (ONE-Glo™ Luciferase Assay System, Promega). Luminescence was read in an MLX Microplate Luminometer (Dynex Technologies, Chantilly, VA).**

Results

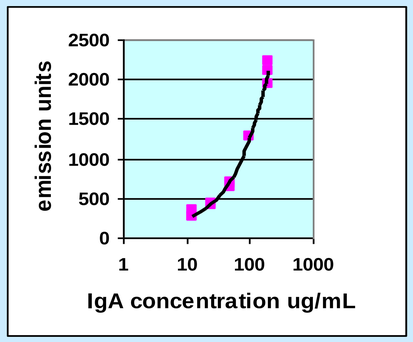

Fig 1 IgA binds to peanut in IgA ELISA

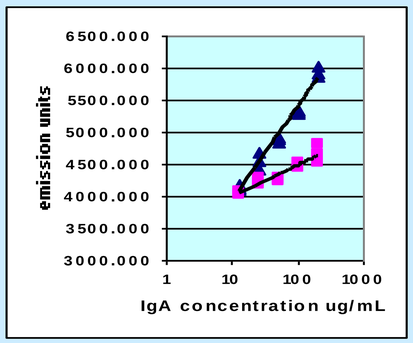

Fig 2 IgA monomer and IgA dimer anti-peanut ELISA

Results (continued)

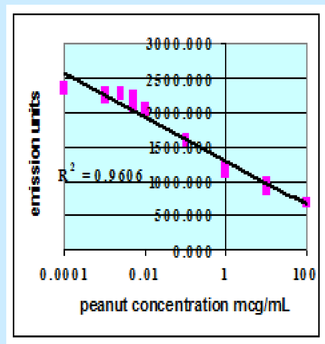

Fig 3. Competitive inhibition of IgA binding to plate bound peanut by soluble peanut

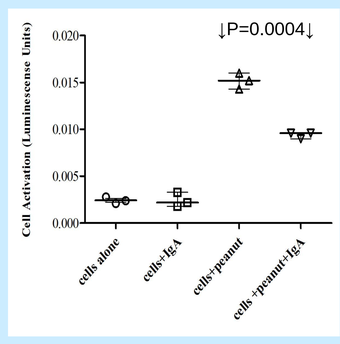

Figure 4. Polyclonal plasma IgA partially inhibits peanut-induced activation of RS-ATL8 rat mast cell leukemia

- **Pooled healthy donor plasma monomeric and dimeric IgA binds to peanut.**
- **Binding is inhibited in a concentration dependent manner by soluble peanut antigen thereby confirming specificity.**
- **Peanut pre-incubated with IgA partially inhibits the peanut activation of IgE sensitized rat basophil leukemia cells.**

Discussion

- **Secretory IgA can be synthesized from dimeric plasma IgA and recombinant human secretory component (11, 12)**
- **Secretory IgA1 is resistant to digestion (11, 13)**
- **We hypothesize that this semisynthetic secretory IgA can be used as an orally administered passive immunization.**

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