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Proteomic analysis of ovomucoid hypersensitivity in mice by two-dimensional difference gel electrophoresis (2D-DIGE)

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Abstract

There is a need to develop reliable methods to assess the safety of genetically modified and other novel foods. The aim of this study was to identify protein biomarkers of food allergy in mice exposed to ovomucoid (OVM), a major food allergen found in chicken egg white. BALB/c mice were repeatedly sensitized by gavage with OVM and cholera toxin (CT) and control mice were exposed to a mixture of amino acids with CT. At the endpoint, all mice were challenged intraperitoneally with OVM and alum. Type-1 hypersensitivity was confirmed in OVM-sensitized mice by observation of clinical signs of anaphylaxis and elevated levels of plasma histamine, OVM-specific IgE and OVM-specific IgG by ELISA. Differential protein expression was assessed in albumin-depleted plasma as well as in mesenteric lymph node, liver, spleen, and ileum by two-dimensional difference gel electrophoresis (2D-DIGE). Differentially expressed proteins were identified by liquid chromatography with tandem mass spectrometry. Plasma proteins overexpressed in OVM-sensitized mice included haptoglobin (41-fold), serum amyloid A (19-fold) and peroxiredoxin-2 (1.9-fold). Further validation of these plasma proteins in other animal models of food allergy with different food allergens is required to assess their potential as candidate biomarkers for use in evaluating the allergenicity of novel foods.
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1. Introduction

Food allergies affect an estimated 3.7% of adults (Sampson, 2005) and 4.0% of infants (Venter et al., 2006). Adults are most often affected from eating fish or shellfish (2.3%) while infants frequently develop allergies to eggs 1.6% and milk 1.1% (Eggesbo et al., 2001a,b; Sicherer et al., 2004). Although most food allergies in humans cause mild reactions, on rare occasions they can be fatal and are now the leading cause of anaphylaxis in many countries (Sampson, 2003). Current knowledge of allergens, tolerance, effector mechanisms and predisposing conditions does not allow for accurate prediction of allergenicity in humans without food trials or prior accidental exposure. Consequently, there has been recent heightened interest to develop a validated animal model to objectively assess the allergenicity of foods for safety testing and immunotherapy development. While various models of human food allergy have been investigated none have yet been validated (Knippels and Penninks, 2005).

Abbreviations: 2D-DIGE, two-dimensional difference gel electrophoresis; CT, cholera toxin; Hp, haptoglobin; OVM, ovomucoid; PRXII, peroxiredoxin-2; SAA2, serum amyloid A2.

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Serum allergen-specific IgE titers are used as an indicator of sensitization and confirmation of anaphylaxis (Dearman et al., 2003) and have been suggested as a clinical aid to predicting allergy or tolerance in children (Shek et al., 2004; Eigenmann, 2005). Antigen-specific IgE titers have excellent positive predictive value for clinical allergic reactivity in individuals with markedly increased titers but poor negative predictive value in those with low titers, necessitating food trials for these individuals (Sampson, 2005). Moreover, analysis of IgE titers and passive cutaneous skin assays require repetitive prior exposure for development of sensitization. Thus, there is a need for a rapid and accurate screening assay with a high negative predictive value of allergenicity for the valuation of allergenicity of novel foods or the effects of novel food processing methods on common foods. These biomarkers should be derived from reliable, validated, and relevant animal models and should be quantifiable and proportional to the degree of allergenicity of the food and should be detectable during both sensitization and post-challenge anaphylaxis. Newly identified biomarkers may eventually be used in an allergy screening panels with known mediators of allergy including histamine, antigen-specific IgE, and IL-4. Panels of biomarkers would then require validation with different food allergens in various animal models before applying them to the safety testing of novel foods.

Ovomucoid (OVM) is a heat stable glycoprotein comprising about 11% of chicken egg white, and is the major cause of allergic reactions in children with egg allergies (Bernhisel-Broadbent et al., 1994). OVM allergenicity has previously been characterized in a cholera-toxin-based BALB/c model (Kroghsbo et al., 2003; Adel-Patient et al., 2005) and we have previously demonstrated that OVM challenge increases plasma histamine, OVM-specific IgE and OVM-specific IgG levels and the secretion of type-2 cytokines such as IL-4 by cultured splenic lymphocytes (Rupa and Mine, 2006). BALB/c mice have often been used as animal models allergy, because they are high IgE-responders and are predisposed to developing Th2 responses more readily than other mouse strains (Jyonouchi et al., 2001).

The advent of proteomics technology has greatly facilitated profiling of plasma and tissues for disease-related biomarkers. Two-dimensional difference gel electrophoresis (2D-DIGE) is an efficient and accurate technology that efficiently separates proteins in complex mixtures and quantifies differential expression in treated and control samples (Alban et al., 2003). 2D-DIGE is very useful as a broad-based screening tool to identify differentially expressed proteins of interest, however, the technique has limited capability in resolving very small, very large, or highly basic, acidic, or hydrophobic proteins. 2D-DIGE is best applied in pH ranges approximating physiological pH and narrowing the range increases separation and resolution of protein spots.

To the best of our knowledge, proteomic profiling to detect biomarkers of food allergy has not been previously reported in mice or other animal models. The aim of this study was to identify protein biomarkers of OVM allergy in a mouse model for eventual use in the assessment of allergic potential of human foods during food safety testing.

2. Materials and methods

2.1. Materials

Immobilized pH gradient strips 13 cm pH 4–7 and pH 3–11, Cy2, Cy3, Cy5, 2D-Clean Up Kit, pharmalytes 3–10, Nuclease mix, and lower molecular weight markers were purchased from GE Healthcare (Montreal, QC). Urea, thiourea, ASB 14, DMF, trifluoroacetic acid, goat anti-mouse-IgG, and pNPP were acquired from Sigma (St. Louis, MO); iodacetamide, CHAPS and DTT from UBS (Cleveland, OH); Qproteome Murine Albumin Depletion Kit from Qiagen (Mississauga, ON); Biorad protein assay, BSA protein standard, 40% acrylamide, and Sypro Ruby stain; Bio-Scale S5 column from Bio-Rad Laboratories (Hercules, CA); 2% alhydrogel from Superfos Biosector (Denmark); histamine ELISA assay kit from Neogen Corporation (Lexington, KY); acetonitrile and water from Fisher Scientific (Ottawa, ON); formic acid from VWR (Mississauga, ON); rat anti-mouse-IgE and purified mouse IgE from BD Pharmingen (San Diego, CA); and purified mouse IgG from AbD Serotec (Raleigh, NC).

2.2. Animal treatments and sample collection

Thirty, 6–8 week old, female, BALB/c mice were purchased from Charles River (Montreal, QC, Canada) randomly divided into two groups (15 control and 15 OVM-sensitized) and conventionally housed 3 per cage at the Central Animal Facility at the University of Guelph. Mice were fed, exclusively, ad libitum, acidified bottled water along with 2014 Teklad Global 14% Protein Rodent Maintenance Diet which has a fixed formula and is free of all animal protein including egg and fish meal. Animal studies were conducted in accordance with the guidelines of the Canadian Council on Animal Care.

Whole OVM antigen consisting of all three domains was isolated from chicken egg white according to an earlier described procedure (Fredericq and Deutsch, 1949) and further purified using HPLC ion-exchange chromatography. Fifty milligram of crude OVM was dissolved in 5 ml of 20 mM sodium acetate buffer, pH 4.0, and applied to a Bio-Scale S5 column equilibrated with the same buffer. The column was eluted with a linear gradient of 0–1.0 M NaCl in 20 mM sodium acetate buffer, pH 4.0, at a flow rate of 1.0 ml/min using a Bio-Rad Biologic HPLC system.

Mice were gavaged twice weekly for 5 weeks with OVM (1 mg/mouse/gavage) whereas control mice were gavaged with a mixture of amino acids (1 mg) in a ratio similar to OVM. Both gavages contained cholera toxin (10 μg) as a mucosal adjuvant in sterile water for a total volume of 100 μL/mouse/gavage. At week 6, all mice were challenged with an intraperitoneal (IP) injection of OVM (1 mg) in sterile water (50 μl) and aluminum hydroxide adjuvant (50 μl, 2% alhydrogel) to induce an immediate type-1 hypersensitivity reaction. Clinical responses were scored for anaphylaxis (see below) and at 40 min post-challenge, mice were euthanized with CO_2, whole blood was collected by cardiac puncture into citrated tubes and samples of liver, spleen, ileum, and mesenteric lymph node were flash frozen in liquid nitrogen and stored at −70 °C. Plasma was obtained by centrifugation at 2000*g* for 15 min, pooled by cage (3 mice/cage/group) and stored at −70 °C. Additional liver, ileum and spleen samples were fixed in 10% v/v buffered formalin, paraffin-embedded, and 5 μm sections were stained with hematoxylin and eosin and evaluated in a blinded fashion by two independent pathologists.

3. Immunologic analysis of OVM allergy

3.1. Anaphylaxis scoring

Clinical responses in all mice were assessed from 0 to 40 min post IP challenge by three independent

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