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Effect of heat denaturation of egg white proteins ovalbumin and ovomucoid on CD4⁺ T cell cytokine production and human mast cell histamine production

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ABSTRACT

The conformational epitopes of egg glycoproteins ovalbumin (OVA) and ovomucoid (OVM) recognized by IgE antibodies in an allergic response can be denatured at high temperatures. This structural change results in a decreased allergenicity and has earlier been used to induce clinical tolerance for specific oral immunotherapy in allergic patients via consumption of cooked egg products. We investigated the difference in allergic response when consuming heat-treated and native egg proteins by examining the cytokine production of CD4⁺ T cells and mast cell histamine release. Cultures of mouse CD4⁺ T cells and dendritic cells were stimulated with native and heat denatured OVA and OVM (HD-OVA and HD-OVM) and various cytokine and histamine concentrations were measured using ELISA. An increased concentration of IL-12, IL-17 and IL-10 and a decreased concentration of IL-4 were detected in both HD-OVA and HD-OVM ($p < 0.05$). A decreased concentration of histamine was detected in HD-OVM ($p < 0.05$) with human KU812 basophil like cells. Heat denatured egg proteins OVA and OVM demonstrated a skewed type 1 CD4⁺ T cell cytokine response and inhibited intracellular Ca²⁺ concentration. This study provides insights into understanding the mechanisms behind induced tolerance of allergic individuals when consuming cooked egg.

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1. Introduction

Food allergy is a growing health concern that affects 3% to 4% of adults and up to 6% of children (Sicherer & Sampson, 2010). Over the past few decades both the prevalence and severity of food allergies have increased (Sampson, 2004). Immunoglobulin E (IgE)-mediated immune response to proteins in food

can be life-threatening, leading to responses ranging from vomiting to closure of the airway (Colgan & Hankel, 2010; Macdougall, Cant, & Colver, 2002).

Eight major foods are responsible for 90% of food allergies. Egg is one of the most common allergens, especially in young children who tend to outgrow it later in life (Caubet & Wang, 2011; Savage, Matsui, Skripak, & Wood, 2007). There has been evidence suggesting that 70–80% of children who are

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Abbreviations: OVA, ovalbumin; OVM, ovomucoid; SOIT, specific oral immunotherapy techniques; DC, dendritic; HD-OVA, denatured OVA; HD-OVM, denatured OVM; IL, Interleukin

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affected by egg allergies can consume baked egg products without having an allergic reaction (Des Roches, Nguyen, Paradis, Primeau, & Singer, 2006; Leonard et al., 2012; Lieberman, Huang, Sampson, & Nowak-Węgrzyn, 2012). Inducing clinical tolerance is an area of interest to researchers as the regular consumption of baked egg has the possibility to induce desensitization or a clinical tolerance (Golias et al., 2012; Watanabe et al., 2013).

The egg white contains many glycoproteins that have been identified as allergens, with ovalbumin (OVA) and ovomucoid (OVM) playing a prominent role in the allergic response pathway (Honma et al., 1991). The ability of a protein to withstand heat is a major factor in the classification of its allergenicity (Mills, Sancho, Rigby, Jenkins, & Mackie, 2009). Ovomucoid has a fairly strong resistance to heat, as well as digestive enzymes, and therefore has a strong allergenicity (Bartnikas, Sheehan, & Phipatanakul, 2013; Matsuda, Watanabe, & Nakamura, 1983). On the other hand, ovalbumin does not have a high heat tolerance (Joo & Kato, 2006). This would leave it to be suggested that an individual with IgE antibodies specific for OVA would possess the ability to tolerate egg that has been denatured by heat (Martos, Lopez-Exposito, & Nowak-Węgrzyn, 2011).

IgE antibodies can recognize either sequential or conformational epitopes of ovalbumin and ovomucoid (Bernhisel-Broadbent, Dintzis, Dintzis, & Sampson, 1994). Epitopes are irreversibly altered and denatured as a result of high temperatures (Urisu et al., 1997). Golias et al. (2012) have recently demonstrated changes in structure caused by heating thereby decreasing its allergenicity. Heating also enhanced the digestibility of OVA *in-vitro* (Lemon-Mule et al., 2008) rendering the heated proteins less capable of activating basophils.

In the allergic response pathway cluster of differentiation (CD) 4⁺ T cells play an important role (Mosmann & Coffman, 1987). These types of T cells are classified as T helper cells with four main subsets: Th1, Th2, Th3 and T reg (Kidd, 2003). Naive T helper cells are differentiated into a Th2 cell, which releases cytokines such as interleukin (IL)-4 that signals activated B cells to produce IgE antibodies (Kidd, 2003). In case of allergies, T cell response is imbalanced, showing preference towards Th2 (anti-inflammatory cytokine production) as opposed to Th1 (pro inflammatory cytokine production) (Maggi, 1998). Also structural changes in antigen conformation due to heating could potentially cause differentiated activation and cytokine release of the CD4⁺ T cells.

Mast cells also play a crucial role in the allergic response pathway. IgE antibodies produced in response to exposure to an allergen will bind to the high affinity FcεR1 constitutively expressed on the surface of mast cells, causing them to become sensitized (Arias et al., 2009; Kondo, Nishi, & Sugahara, 2015). Upon subsequent exposure to the allergen, the peptide will bind directly to IgE antibodies on mast cell surfaces, leading to a cascade of intracellular events causing mast cell degranulation. It is possible that a change in antigen conformation could alter its ability to activate mast cells to degranulate (Arias et al., 2009).

The purpose of this research is to investigate the difference in response at the molecular level comparing heat-treated versus native egg proteins by looking at the cytokine production of CD4⁺ T cells and the histamine production of mast cells. This work should give us further insights into mecha-

nisms at the molecular level of the allergic response to egg and assist in the success of specific oral immunotherapy techniques (SOIT) using heated egg (Martos et al., 2011). This study was conducted to test the hypothesis that denaturation of egg white proteins ovalbumin and ovomucoid may result in a decrease in allergenicity by inducing a type-1 skewed cytokine response by T cells and a drop in histamine release by mast cells.

2. Materials and methods

2.1. Animals

Female Balb/c mice were purchased from Charles River Laboratories (Montreal, QC, Canada) at 6–8 weeks of age and spleens were collected. Mice were euthanized prior to spleen collection. Spleens were placed in spleen dissociation media and kept on ice after removal. All procedures were performed in accordance with the guidelines established by the Canadian Council of Animal Care (CCAC) and approved by the Animal Care Committee at the University of Guelph.

2.2. Splenocyte isolation

Spleen from individual mice (n = 3) was aseptically removed into ice-cold RPMI-1640 medium (Gibco Invitrogen, New York, NY, USA) containing NaHCO₃ (1.5 g/L), glucose (4.5 g/L), L-glutamine (2 mM), sodium pyruvate (1 mM), penicillin (50 U/ml) and streptomycin (50 mg/ml). Collected spleens were placed in a Petri dish and 4 ml of spleen dissociation media was added. Spleens were then minced and filtered twice using a nylon mesh filter. The cell suspensions were passed through a 100 μm nylon membrane cell strainer and transferred to 15 ml conical centrifuge tubes and centrifuged for 10 min at 500 g at 4 °C. 80 μl of 0.5 M EDTA (Sigma, Oakville, ON, Canada) was added and splenocytes were incubated for 5 min on a horizontal rocker. The splenocytes were washed twice with 10 ml RPMI by centrifugation at 1200 g for 8 min at 4 °C. Supernatant was then discarded and splenocytes were resuspended in 10 ml of medium [RPMI 1640 supplemented with 8% fetal bovine serum (FBS)] (Hyclone, Fisher, Mississauga, ON, Canada) and cell viability was assessed by trypan blue exclusion and were used for purification of dendritic cells and CD4⁺ T cells.

2.3. CD4⁺ T cell and CD11c⁺ (dendritic-DC) cell isolation

Both the T cells and DCs were isolated using EasySep Positive Selection Kit for Mouse CD4⁺ and CD11c⁺ respectively (STEMCELL Technologies, Vancouver, BC, Canada). The manufacturer's protocols for the Purple EasySep Magnet were followed. A PE labelling reagent containing an antibody for the target cell's receptor was first added, followed by a selection cocktail containing bound antibodies for PE and Dextran. Magnetic Dextran nanoparticles were added which bound the dextran antibody from the cocktail. Final concentration of 4.53×10^6 cells/mL of CD11c⁺ cells and a final concentration of 1.29×10^7 cells/mL of CD4⁺ T cells were obtained and grown in individual flasks at

37 °C, with 5% CO₂ for 2 ½ weeks, with the media being changed every 2–3 days. After 2 ½ weeks, 50 µL of 1 mg/mL filtered cholera toxin was added to activate the immature DCs into fully maturing. After 3 weeks of cell culture, the cells reached approximately 100% confluency.

2.4. Preparation of heat denatured OVA and OVM

Pure OVA and OVM at 1 mg/mL concentration were filtered and denatured in a water bath at 100 °C for 5 minutes based on the protocol used by [Watanabe et al. \(2013\)](#).

2.5. Antigen stimulation

Once the cell cultures reached 100% confluency, the media was removed and cells were trypsinized with 0.25% Trypsin EDTA and left for 2–3 min in a 37 °C incubator. RPMI media (5 mL) was added and cells were scraped off the flask walls. The two flasks for each cell type were then pooled and centrifuged. The cells were further washed with the media and a final concentration of 2.5×10^5 cells/mL of CD11c⁺ cells and T cells were added to the plate and stimulated with 100 µg of OVA, HD-OVA, OVM and HD-OVM in triplicates. The plate was incubated for 72 hours at 37 °C in 5% CO₂. After 72 hours, the supernatants were collected and samples were stored at –80 °C until further analysis.

2.6. Cytokine ELISA

ELISA Ready-Set-Go! Kits (Affymetrix, eBioscience, San Diego, CA, USA) were used to test for IL-2, IL-12, IL-10, IFN-γ and IL-17 concentration in supernatant samples according to the manufacturer's guidelines. In brief, a 96 well plate was coated with 100 µL/well of capture antibody in 1X coating buffer and was sealed and incubated overnight at 4 °C. The wells are then aspirated and washed with wash buffer and wells are blocked with 1X assay buffer and incubated at room temperature for 1 h. Standards were diluted performing 2-fold serial dilutions and 100 µL/well of each dilution were added to the wells. Also 100 µL/well of the supernatant culture samples were added to the wells. The plate was sealed and incubated for 2 h then aspirated and washed. 100 µL/well of detection antibody diluted in 1X of assay buffer was added and incubated for 1 h followed by aspiration and washing. Avidin-HRP diluted in 1X ELISA diluent was then added at 100 µL/well and incubated for 30 min. The plate was then aspirated and washed and 100 µL/well of TMB solution was added and then incubated for about 15 minutes. Once a colour change was seen in the samples 50 µL/well of H₂SO₄ stop solution was added and optical density was measured at 450 nm (iMark; BioRad, Mississauga, ON, Canada). Data were expressed as sample means for test replicates.

2.7. Mast cell

KU812 basophil like cells (ATCC, Cedarlane, Burlington, ON, Canada) were thawed and 10 mL of RPMI-1640 medium was added prior to spinning down at 2000 rpm. Supernatant was removed and 5 mL RPMI-1640 medium were then added to get a final cell concentration of 1.0×10^5 cells/mL. Cells were allowed to grow in a flask and incubated at 37 °C, with 5% CO₂ for 2 ½

weeks, with the media being changed every 2–3 days. The culture reached 100% confluency at 10 days.

2.8. Histamine assay

The confluent cells were spun down and dissolved in 12 mL of RPMI. The cells were added to a 12 well plate at a concentration of 1.0×10^6 cells/mL and left overnight. The cells were pooled and spun down, supernatant was removed and 12 mL HBSS was added. The cells were then plated in the 12 well plate again (1.0×10^6 cells/mL) and 5 mM of calcium ionophore (Sigma) and 1.5 µg/mL of test antigen proteins (native and heat denatured OVA and OVM) were added to the wells. The plate was incubated for 20 min at 37 °C and then for 15 min at 4 °C. The wells were pooled and the supernatant isolated and stored at –80 °C until further use. Histamine ELISA Kit (LDN, Nordhorn, Germany) was used to test for histamine concentration in supernatant samples as per manufacturer's guidelines. To prepare the samples, 25 µL of standard, controls and supernatant samples were plated followed by the addition of 25 µL acylation buffer and 25 µL of acylation reagent. The plate was incubated for 45 minutes, 200 µL distilled water was added to each well and the plate was incubated for 15 min. Then 25 µL of standards, controls and samples were taken and plated in microtiter strips. 100 µL of histamine antiserum was added to each well and incubated for 3 h while covered. The wells were then washed prior to adding 100 µL enzyme conjugate followed by 30 min incubation. The wells were washed again, 100 µL of substrate was added and then incubated for 20–30 min until a colour change was observed. 100 µL of stop solution was added and the plate was read at 450 nm optical density.

2.9. Measurement of intracellular calcium ion concentration

KU812 cells (1×10^6 /mL) were suspended in Tyrode's buffer (134 mM NaCl, 1 mM CaCl₂, 12 mM NaHCO₃, 2.9 mM KCl, 0.34 mM Na₂HPO₄, 1 mM MgCl₂ and 0.055 mM glucose, supplemented with 10 mM HEPES) containing 2.5 mM probenecid and 2 µmol/L fluo-3/AM (Dojindo, Kumamoto, Japan) and incubated at room temperature for 40 min. The cells were again washed and resuspended in fresh Tyrode's buffer and were pre-treated with various test antigens (treated with 100 µg/mL of native and denatured OVA and OVM) and incubated for 30 min. The control sample was treated with equal volume of Tyrode's buffer alone. After washing with Tyrode's buffer, the cells were stimulated with anti-IgE (10 µg/mL) and concentration was immediately detected at a 485-nm excitation wavelength and a 535-nm emission wavelength at 37 °C with Perkin Elmer Victor2 TM 1420 Multiple Counter (PerkinElmer). Results are represented as fluorescence intensity units.

2.10. Statistical analysis

Data were expressed as calculation means ± SEM. Endpoint differences were compared with a level of $p < 0.05$ to determine significance. All calculations were carried out with GraphPad Prism software 4.0 (GraphPad Software Inc., San Diego, CA, USA).

Results were analysed using one-way ANOVA test with Tukey's post hoc multiple comparison test.

3. Results

3.1. IL-2 and IFN- γ cytokine production

There was no IL-2 or IFN- γ detected in a readable range in any of the supernatant samples stimulated with the native and heat denatured OVA and OVM (HD-OVA and HD-OVM). There was no variance in concentration in comparison to the unstimulated cells (data not shown).

3.2. IL-12 cytokine production

An increased concentration of IL-12 was detected in both the HD-OVA and HD-OVM stimulated samples (Fig. 1A). Splenocytes produced IL-12 in response to native OVA (30.25 ± 48.25 pg/mL), HD-OVA (705.88 ± 8.125 pg/mL) and HD-OVM (433.38 ± 3.712 pg/mL). No IL-12 was produced by native OVM stimulation of splenocytes. HD-OVA IL-12 concentration was statistically significantly higher in comparison to native OVA ($p < 0.05$). HD-OVM IL-12 concentration was also statistically significantly higher in comparison to native OVM ($p < 0.05$). Splenocytes that were not stimulated with any of the egg white proteins did not produce any detectable levels of IL-12 (Fig. 1A).

3.3. IL-17 cytokine production

An increased concentration of IL-17 was detected in both the HD-OVA and HD-OVM stimulated samples (Fig. 1B). Splenocytes produced IL-17 in response to HD-OVA (779.86 ± 104.286 pg/mL) and HD-OVM (586.64 ± 67.5 pg/mL). No IL-17 was produced by native OVA or OVM stimulation of splenocytes. HD-OVA and HD-OVM IL-17 concentrations were statistically significantly higher in comparison to native OVA and OVM respectively ($p < 0.05$) (Fig. 1B).

3.4. IL-4 cytokine production

A decreased concentration of IL-4 was detected in both native OVA and OVM stimulated samples (Fig. 1C). Splenocytes produced IL-4 in response to native OVA (67.76 ± 3.33 pg/mL), HD-OVA (16.09 ± 1.36 pg/mL), OVM (101.55 ± 1.67 pg/mL) and HD-OVM (25.03 ± 2.12 pg/mL). Native OVA concentration was statistically significantly lower than HD-OVA concentration ($p < 0.05$). Native OVM concentration was also statistically significantly lower than HD-OVM concentration ($p < 0.05$) (Fig. 1C).

3.5. IL-10 cytokine production

A decreased concentration of IL-10 was detected in both native OVA and OVM stimulated samples (Fig. 1D). Splenocytes produced IL-10 in response to native OVA (716.00 ± 20 pg/mL), HD-OVA (1708.50 ± 2.50 pg/mL), OVM (628.50 ± 12.50 pg/mL) and

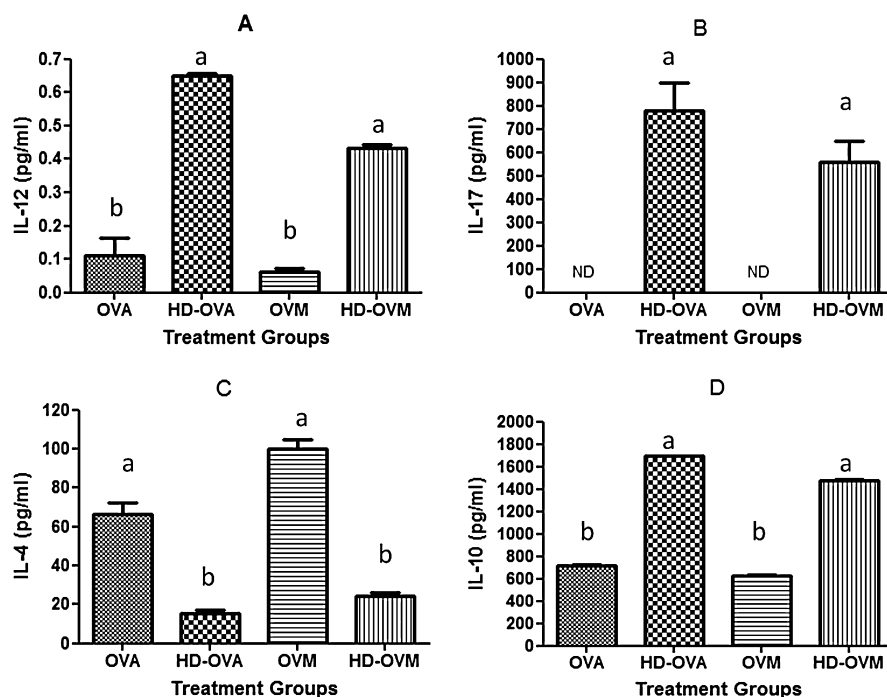


Fig. 1 – The influence of heat denaturation of egg white proteins OVA and OVM on CD4⁺ T cell cytokine production. Cultured CD4⁺ T cells and myeloid dendritic cells were stimulated with native OVA, heat denatured OVA (HD-OVA, 100 °C, 5 min), native OVM, and heat denatured OVM (HD-Ovm, 100 °C, 5 min). A – IL-12 concentration; B – IL-17 concentration; C – IL-4 concentration; D – IL-10 concentration. Cytokine concentration of cell culture supernatants were measured by ELISA. Data represent the average of duplicate samples for each treatment $\pm$ SEM. ND denotes values that were not determined. Different letters represent statistical significance at $p < 0.05$.

HD-OVM (1478.50 ± 17.50 pg/mL). Native OVA concentration was statistically significantly lower than HD-OVA concentration ($p < 0.05$). Native OVM concentration was also statistically significantly lower than HD-OVM concentration ($p < 0.05$) (Fig. 1D).

3.6. Histamine production

A decreased concentration of histamine was detected in HD-OVM stimulated samples (Fig. 2). Human basophil like cells produced histamine in response to OVA (0.20 ± 0.05 ng/mL), HD-OVA (0.39 ± 0.013 ng/mL), OVM (7.37 ± 0 ng/mL) and HD-OVM (0.929 ± 0.199 ng/mL). HD-OVM histamine concentration was statistically significantly lower than native OVM concentration ($p < 0.05$). HD-OVA concentration was not statistically significantly lower than native OVA concentration ($p < 0.05$) (Fig. 2).

3.7. Denaturation inhibits intracellular-calcium ion production

As shown in Fig. 3 relative to positive control cells (with no antigen) and to the native OVA and OVM treatment groups, intracellular Ca^{2+} concentration was significantly lower with both HD-OVA and HD-OVM ($p < 0.05$) (Fig. 3).

4. Discussion

In this research, CD4^+ T cell cytokine production and human mast cell histamine production were used as indicators to test the effects of both native and heat denatured egg white proteins ovalbumin and ovomucoid on allergenicity. The varying concentration of different type 1 and type 2 cytokines as well

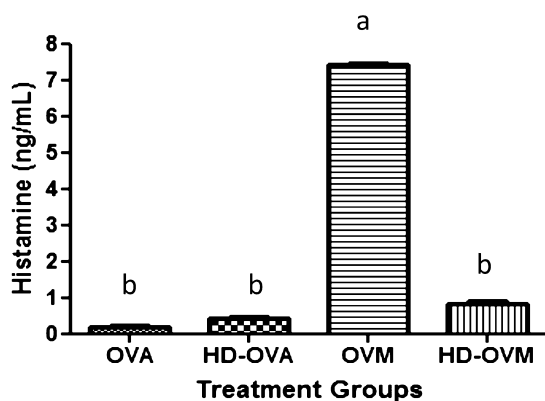


Fig. 2 – The influence of heat denaturation of egg white proteins OVA and OVM on human mast cell histamine production. Cultured human basophil like cells were stimulated with native OVA, heat denatured OVA (HD-OVA, 100 °C, 5 min), native OVM, and heat denatured OVM (HD-Ovm, 100 °C, 5 min). Histamine concentration of cell culture supernatants were measured by ELISA. Data represent the average of duplicate samples for each treatment $\pm$ SEM. Different letters represent statistical significance at $p < 0.05$.

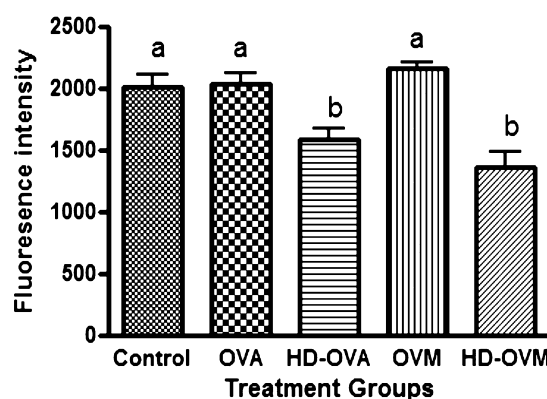


Fig. 3 – Measurement of calcium ion concentration. Effect of denatured antigens on KU812 cell on in vitro calcium ion concentration was measured using fluorescence activation by Ca^{2+} indicator Fluo-4/AM after treatment with native and HD OVA and OVM.

as histamine indicates the effect heat treatment of egg has on the allergic response at a molecular level.

IL-12 was seen to have a much higher concentration in splenocytes stimulated with heat denatured ovalbumin (OVA) and ovomucoid (OVM) in comparison to stimulation by their native counterparts. No IL-12 production was detected in splenocytes that were not stimulated by any antigen and native OVM. This is indicative of a shifted type 1 cytokine response, as IL-12 is a Th1 cytokine (Lucey, 1999). IL-12 produced by antigen presenting cells, such as dendritic cells (Athie-Morales, Smits, Cantrell, & Hilken, 2004), plays a key role in developing a Th1 pro inflammatory response by differentiating naïve Th cells into Th1 cells, thus skewing the T helper cell response (Athie-Morales et al., 2004).

IL-17 is a controversial cytokine, whose role in the allergic response pathway remains to be elucidated (Oboki, Ohno, Saito, & Nakae, 2008). IL-17 is produced by Th17 cells, which are a distinct T cell population from Th1 and Th2. Significantly reduced Th17 population in children with food allergies, as well as impaired IL-17 production in allergic individuals was reported recently (Bin et al., 2013). IL-17 is a potential biomarker for food allergen tolerance, which is supported by our findings of increased IL-17 concentration in HD-OVA and HD-OVM treated T cells.

IL-10 is an anti-inflammatory cytokine produced by T reg cells, which control peripheral immune responses (Hawrylowicz, 2005) and plays a role in protection against allergic disease as individuals with a mutation in FOXP3 (T reg transcription factor) suffer from food allergies (Hawrylowicz, 2005). Immunotherapy for treating allergies has also shown to increase levels of IL-10, which works by inhibiting Th2 cell activity (Akdis, Blesken, Akdis, Wuthrich, & Blaser, 1998). Kearley, Barker, Robinson, and Lloyd (2005) found a decrease in Th2 cytokine production in mice sensitized to ovalbumin when administering purified T reg cells prior to antigen exposure; however, they found that IL-10 production was not restricted to T reg cells. Our findings of increased IL-10 concentration in HD-OVA and HD-OVM treated CD4^+ T cells indicate that heat denaturation is causing a protective decreased Th2 response to allergens.

IL-4 is produced by Th2 cells and plays a role in antigen-specific B cell production of IgE antibodies in the allergic response (Dourado et al., 2010; Mathias et al., 2011). Previous studies have found that heat denaturation of OVA has led to significantly decreased levels of IL-4 in mice (Sicherer & Sampson, 2010). Our results concur with these findings, as IL-4 concentration was decreased in both HD-OVA and HD-OVM groups.

Histamine is a key preformed mediator found in mast cell granules that is released during antigen specific IgE binding and plays a major role in induction of the adverse physiological symptoms of an allergic reaction (Cianferoni & Muraro, 2012). We had earlier demonstrated that Quillaja saponin had inhibitory effects on IgE-mediated response in rat basophil cells (Zhang, Yang, Rupa, Jiang, & Mine, 2012). Calcium influx is essential for FcεRI-mediated mast cell activation and allergic reaction (Baba et al., 2008). Decreased calcium ion concentration was observed with both HD-OVA and HD-OVM. Lower concentration of histamine was observed with HD-OVM, but not with HD-OVA in comparison to native OVM. Previous studies have found that HD-OVA leads to a lessened mast cell response; however, this was by measuring MMCP-1 as a biomarker of mast cell activation (Sicherer & Sampson, 2010). Therefore, it is possible that histamine is not a good indicator of mast cell degranulation in regard to OVA or experimental error could have caused these results. This finding could also be due to the fact that native OVA did not stimulate a significant histamine release by mast cells.

These findings provide evidence towards the role of heat-treated eggs in producing tolerance in allergic individuals (Golias et al., 2012). The molecular basis behind this induced tolerance is not well understood. Increased production of IL-12, IL-17 and IL-10 and a decreased production of IL-4 by splenocytes, and low concentration histamine by mast cells in HD-OVM validates this understanding. An increased Th1 response diminishes the Th2 allergic response as Th1 cells produce IFN-γ, which inhibits Th2 cell activation (Macdougall et al., 2002). Further understanding of CD4⁺ T cell cytokine production and mast cell histamine production in relation to the molecular allergic response when stimulated by heat denatured versus native egg proteins will provide more information on how specific oral immunotherapy using cooked and baked egg helps to produce clinical tolerance and desensitization. This research and the insight that it provides is quite important for immunotherapy to help fight allergies. The findings from this study provide some insight into the molecular mechanisms behind SOIT and can help in the creation of other therapeutic measures.

5. Conclusion

Heat treatment of eggs causes denaturation of their key proteins ovalbumin and ovomucoid, leading to a decrease in allergenicity. Cooked and baked eggs have also been used in specific oral immunotherapy treatment for anaphylactic individuals and have induced clinical tolerance. In an attempt to understand the molecular mechanisms behind these findings, this study observed CD4⁺ T cell cytokine production and

human mast cell histamine production. Cytokine production indicating a shift from a type 2 cytokine response to a type 1 response was observed in heat denatured ovalbumin and ovomucoid stimulated cells. Reduced allergic response was also observed by decreased histamine concentration in heat denatured ovalbumin stimulated cells. This knowledge can help with the development of future immunotherapy methods. Future research conducted in this area could look at differential cytokine response in humans and focus on other areas of the allergic response pathway.

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REFERENCES

- Akdis, C. A. T., Blesken, M., Akdis, B., Wuthrich, B., & Blaser, K. (1998). Role of interleukin 10 in specific immunotherapy. *The Journal of Clinical Investigation*, 102, 98–106.
- Arias, K., Baig, M., Bhsc, M. C., Chu, D., Walker, T., Goncharova, S., Coyle, A., Vadas, P., Wasserman, S., & Jordana, M. (2009). Concurrent blockade of platelet-activating factor and histamine prevents life-threatening peanut-induced anaphylactic reactions. *Journal of Allergy and Clinical Immunology*, 124, 307–314.
- Athie-Morales, V., Smits, H. H., Cantrell, D. A., & Hilken, C. M. U. (2004). Sustained IL-12 signaling is required for Th1 development. *The Journal of Immunology*, 172, 61–69.
- Baba, Y., Nishida, K., Fujii, Y., Hirano, T., Hikida, M., & Kurosaki, T. (2008). Essential function of the calcium sensor STIM1 in mast cell activation and anaphylaxis responses. *Nature Immunology*, 9, 81–88.
- Bartnikas, L. M., Sheehan, W. J., & Phipatanakul, W. (2013). Ovomucoid is not superior to egg white testing in predicting tolerance to baked egg. *Journal of Allergy and Clinical Immunology*, 1, 354–360.
- Bernhisel-Broadbent, J., Dintzis, H. M., Dintzis, R. Z., & Sampson, H. A. (1994). Allergenicity and antigenicity of chicken egg ovomucoid (Gal-D-III) compared with ovalbumin (Gal-D-I) in children with egg allergy and in mice. *Journal of Allergy and Clinical Immunology*, 93, 1047–1059.
- Bin, D. K., Hennezel, E., Ben-Shoshan, M., McCusker, C., Clarke, A., Fiset, P., Mazer, B., & Piccirillo, C. A. (2013). Altered T helper 17 responses in children with food allergy. *International Archives of Allergy and Immunology*, 162, 318–322.
- Caubet, J. C., & Wang, J. (2011). Current understanding of egg allergy. *Pediatric Clinics of North America*, 58, 427–443.
- Cianferoni, A., & Muraro, A. (2012). Food-induced anaphylaxis. *Immunology and Allergy Clinics of North America*, 32, 165–195.
- Colgan, J. D., & Hankel, I. L. (2010). Signaling pathways critical for allergic airway inflammation. *Current Opinion in Allergy and Clinical Immunology*, 10, 42–47.
- Des Roches, A., Nguyen, M., Paradis, L., Primeau, M. N., & Singer, S. (2006). Tolerance to cooked egg in an egg allergic population. *Allergy*, 61, 900–901.

- Dourado, L. P. A., Saldanha, J. C., Gargiulo, D. L., Noviello, M., Brant, C. C., Reis, M. L. C., Fernandes de Souza, R. M., Faria, A. M. C., de Souza, D. da G., & Cara, D. C. (2010). Role of IL-4 in aversion induced by food allergy in mice. *Cellular Immunology*, 262, 62–68.
- Golias, J., Schwarzer, M., Wallner, M., Kverka, M., Kozakova, H., Klimesova, D. S. K., Sotkovsky, P., Ferreira, L.P.-J.F., & Tuckova, L. (2012). Heat-induced structural changes affect OVA-antigen processing and reduce allergic response in mouse model of food allergy. *PLoS ONE*, 7, e37156.
- Hawrylowicz, C. M. (2005). Regulatory T cells and IL-10 in allergic inflammation. *The Journal of Experimental Medicine*, 202, 1459–1463.
- Honma, K., Aoyagi, M., Saito, K., Nishimuta, T., Sugimoto, K., Tsunoo, H., Niimi, H., & Kohno, Y. (1991). Antigenic determinants on ovalbumin and ovomucoid: Comparison of the specificity of IgG and IgE antibodies. *Arerugi*, 40, 1167–1175.
- Joo, K., & Kato, Y. (2006). Assessment of allergenic activity of a heat-coagulated ovalbumin after in vivo digestion. *Bioscience, Biotechnology, and Biochemistry*, 70, 591–597.
- Kearley, J., Barker, J. E., Robinson, D. S., & Lloyd, C. M. (2005). Resolution of allergen-induced airway inflammation and hyperreactivity by transfer of allergen-specific CD4⁺ CD25⁺ regulatory T cells in vivo: Role of IL-10. *The Journal of Experimental Medicine*, 202, 1539–1547.
- Kidd, P. (2003). Th1/Th2 balance: The hypothesis, its limitations, and implications for health and disease. *Alternative Medicine Review: A Journal of Clinical Therapeutic*, 8, 223–246.
- Kondo, M., Nishi, K., & Sugahara, T. (2015). Ishizuchi dark tea suppresses IgE-mediated degranulation of RBL-2H3 cells and nasal rubbing behavior of pollinosis in mice. *Journal of Functional Foods*, 14, 659–669.
- Lemon-Mule, H., Sampson, H. A., Sicherer, S. H., Shreffler, W. G., Noone, S., & Nowak-Węgrzyn, A. (2008). Immunologic changes in children with egg allergy ingesting extensively heated egg. *Journal of Allergy and Clinical Immunology*, 122, 977–983.
- Leonard, S. A., Sampson, H. A., Sicherer, S. H., Noone, S., Moshier, E. L., Godbold, J., & Nowak-Węgrzyn, A. (2012). Dietary baked egg accelerates resolution of egg allergy in children. *Journal of Allergy and Clinical Immunology*, 130, 473–480.
- Lieberman, J. A., Huang, F. R., Sampson, H. A., & Nowak-Węgrzyn, A. (2012). Outcomes of 100 consecutive open, baked-egg oral food challenges in the allergy office. *Journal of Allergy and Clinical Immunology*, 129, 1682–1684.
- Lucey, D. R. (1999). Evolution of the type-1 (Th1)–type-2 (Th2) cytokine paradigm. *Infectious Disease Clinics of North America*, 13, 1–9.
- Macdougall, C. F., Cant, A. J., & Colver, A. F. (2002). How dangerous is food allergy in childhood? The incidence of severe and fatal allergic reactions across the UK and Ireland. *Archives of Disease in Childhood*, 86, 236–239.
- Maggi, E. (1998). The TH1/TH2 paradigm in allergy. *Immunotechnology: An International Journal of Immunological Engineering*, 3, 233–244.
- Martos, G., Lopez-Exposito, I., & Nowak-Węgrzyn, A. (2011). Mechanisms underlying differential food-allergic response to heated egg. *The Journal of Allergy and Clinical Immunology*, 127, 990–997.
- Mathias, C. B., Hobson, S. A., Garcia-Lloret, M., Lawson, G., Poddighe, D., Freyschmidt, E. J., Xing, W., Gurish, M. F., Chatila, T. A., & Oettgen, H.C. (2011). IgE-mediated systemic anaphylaxis and impaired tolerance to food antigens in mice with enhanced IL-4 receptor signaling. *Journal of Allergy and Clinical Immunology*, 127, 795–805.
- Matsuda, T., Watanabe, K., & Nakamura, R. (1983). Immunochemical and physical properties of peptic-digested ovomucoid. *Journal of Agricultural and Food Chemistry*, 31, 942–946.
- Mills, E. N., Sancho, A. I., Rigby, N. M., Jenkins, J. A., & Mackie, A. R. (2009). Impact of food processing on the structural and allergenic properties of food allergens. *Molecular Nutrition and Food Research*, 53, 963–969.
- Mosmann, T. R., & Coffman, R. L. (1987). Two types of mouse helper T-cell clones. *Immunology Today*, 8, 223–227.
- Oboki, K., Ohno, T., Saito, H., & Nakae, S. (2008). Th17 and allergy. *Allergology International*, 57, 121–134.
- Sampson, A. (2004). Update on food allergy. *The Journal of Allergy and Clinical Immunology*, 113, 805–819.
- Savage, J. H., Matsui, E. C., Skripak, J. M., & Wood, R. A. (2007). The natural history of egg allergy. *Journal of Allergy and Clinical Immunology*, 120, 1413–1417.
- Sicherer, S. H., & Sampson, H. A. (2010). Food allergy. *The Journal of Allergy and Clinical Immunology*, 125, S116–S125.
- Urisu, A., Ando, H., Morita, Y., Wada, E., Yasaki, T., Yamada, K., Komada, K., Torii, S., Goto, M., & Wakamatsu, T. (1997). Allergenic activity of heated and ovomucoid-depleted egg white. *Journal of Allergy and Clinical Immunology*, 100, 171–176.
- Watanabe, H., Toda, M., Sekido, H., Wellner, A., Fujii, T., Henle, T., Hachimura, S., & Nakajima-Adachi, H. (2013). Heat treatment of egg white controls allergic symptoms and induces oral tolerance to ovalbumin in a murine model of food allergy. *Molecular Nutrition and Food Research*, 58, 394–404.
- Zhang, T., Yang, C., Rupa, P., Jiang, B., & Mine, Y. (2012). Inhibitory effects of Quillaja saponin on IgE-mediated degranulation of rat basophilic leukemia RBL-2H3 Cells. *Journal of Functional Foods*, 4, 864–871.