Egg Desensitization and induction of tolerance in Children

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1 Proposed Research Questions

1. What is the rate of desensitization in children allergic to egg?

2. What are the predictors associated with success or failure of desensitization in children?

3. What are the molecular mechanisms involved in desensitization?

2 Research Objectives and Significance

We are proposing to initiate a study assessing desensitization for egg

This study would enable us to better determine the potential benefit of desensitization in individuals with egg allergy.

More specifically, we will address the following research objectives:

Objectives

A. To determine the rate of desensitization to egg..

B. To characterize predictors of successful desensitization.

C. To characterize molecular mechanisms involved in the process of desensitization.

3. Gaps and Unmet Needs in Existing Knowledge on food desensitization

3.i Why do we need trials on food desensitization.

Foods are reported to be primary inciting allergens for anaphylaxis and account for 33.2% to 56% of all anaphylaxis cases.[1-4] Further, food-induced anaphylaxis hospital admissions are reported to have increased, mainly in the first 2 decades of life.[5;6]

At present, the only treatment for food allergy is to avoid the allergy-causing food, while the only treatment for an allergic reaction is prompt administration of intramuscular epinephrine. [7;8] Primary anaphylaxis prevention is based on allergen desensitization through immunotherapy and until recently was not available for food allergies. Although there are a few recent research protocols exploring desensitization for foods and although these have so far been largely successful, [9;10] up to this point there are no known guidelines describing the optimal candidate for desensitization nor are there criteria for the safest and most effective dosing schedule. Further, many of these studies have a very small sample size and do not use a control group. Hence, the efficacy of these protocols is hard to interpret [11-14] and additional larger scale randomized control studies are required.

3.ii Why Egg.

Cow milk allergy (CMA) and egg allergies are the most common food allergies in children and recent reports suggest that rates of resolution are considerably lower than previous estimates. [15-17] Further, both CMA and egg allergy are associated with severe anaphylaxis[18;19] and due their ubiquitous use in our diet are almost impossible to strictly avoid.[20-22] In line with these, several studies suggest that quality of life of patients with milk/egg allergy and their families is severely impaired.[23-25]

With the support of AllerGen NCE and CIHR we have initiated in 2014 the first cross Canada study to assess milk desensitization. This study now includes almost 50 patients,

and our preliminarily results reveal an almost 80% success rate . We are now proposing to initiate an egg desensitization study based on the expertises, recruitment skills and data collected in our first 2 years of milk desensitization.

We hypothesize that there will be at least 50% improvement in the ability to tolerate egg. E.g. a child that was able to tolerate only 40 mg of merengue will be able to tolerate at least 60 mg subsequent to desensitization

The trial design is an RCT aiming to demonstrate superiority of the desensitization protocol to an avoidance strategy regarding the tolerated quantity of egg . The ratio of patients to control is 1:1 . Patients will be block randomized in groups of 2 according to age and sex.

Egg allergy is the most common IgE mediated food allergy in children. Approximately 30% of children suffering from moderate to severe atopic dermatitis present an associated food allergy and egg is reported to be the major food allergen triggering IgE mediated allergic reactions in those with atopic dermatitis.[26] The major allergens in egg are known as Gal d 1-5 and include Ovomucoid which is the dominant egg allergen,(Gal d 1) and is a protease inhibitor, Ovalbumin (Gal d 2) & Ovotransferrin (Gal d3) and lysozyme (Gal d4).[27] Egg allergens are altered by heat and gastric enzymes and 55%-73% of those with egg allergy will be able to ingest cooked egg.[28;29] However, ovomucoid, the main egg allergen, is not affected by heating.[30] Different egg whites (e.g, goose , duck and turkey) were all found to contain proteins cross-reacting with most of the allergens in hen's egg white. [31] Presence of egg allergy has also major implications regarding safe administration of vaccines. Several vaccines are manufactures on egg containing media including yellow fever and Q fever and may induce an allergic reaction in those that are egg allergic.[32-35]Hence, use of rapid and effective desensitization to egg may be extremely beneficial prior to travel to yellow fever endemic areas. In addition, although

it was reported that the median age for tolerance to egg is 6 years ± 6 months, [36] recent studies suggest a lower resolution rate of 68% by age 16 years.[17]

Given the importance of egg allergy, protocols aiming to induce desensitization were recently explored.[37;38] These protocols reveal encouraging results with significantly increased thresholds to food-induced allergic reactions after oral immunotherapy in 100% of those with egg allergy. [12] Recently rush[11;13] and modified rush protocols[12] were suggested for egg but again studies exploring these protocols are limited by small sample size and by lack of controls as well as absence of methodologies assessing the molecular processes underlying these successful desensitization. It is suspected that the nature of this increased threshold is transient and reflects desensitization rather than true tolerance given that avoidance of these foods was shown to increase sensitization as well as to lower the threshold of subsequent reactivity.[39;40] In conclusion, the efficacy of the immunotherapy, extent of desensitization versus tolerance, the quantity/ frequency of allergen consumption required to maintain this effect and molecular mechanisms involved in the desensitization process are currently unknown.

3. iii. Do we achieve only desensitization or do we induce also tolerance.

Desensitization refers to a change in the amount of food antigen needed to cause allergic symptoms; this state is dependent on regular antigen exposure. In contrast, *tolerance* refers to long-term immunologic changes associated with the ability to ingest a food without symptoms and without ongoing therapy. Desensitization is a worthwhile therapeutic goal as it allows individuals freedom from the risk of accidental ingestion in everyday settings; achieving long-term clinical tolerance would allow safe food ingestion

without ongoing therapy by inducing lasting immunologic changes.[41] Trials so far suggest that there might be loss of tolerance within 48 h following elimination of the food from the diet. [42] However, these trials are flawed by their small sample size (3 patients). [42] Given that more recent trials suggest that as long as participants ingested the maintenance dose of at least twice a week no allergic reactions are likely to occur[13] and given the abundance of egg products in our diet , it is crucial, to explore such an approach in a large scale clinical trial.

3. iv Gaps regarding the roles of mast cells in desensitization.

Given that food desensitization is a relatively rapid process, mast cell are implicated to have a key role in this process. Compared to activated cells, desensitized Mast cells were shown to have had impaired degranulation, calcium flux, secretion of arachidonic acid products.[43] However, the effect of food desensitization on mast cells was not explored so far in clinical trials.

3v. Gaps regarding the role of T regulatory cells in desensitization.

Although several studies in humans report that food allergens induce specific effector T cells, there are significant controversies between studies regarding the T cell phenotype dominating that response. Some studies report a Th2 dominated response in children with food allergies whereas Th1- responses underlie oral tolerance [44]. Other studies suggest that food antigens induce a Th2 response regardless of the presence of food allergy[45]. Further, it is proposed that Treg cells may be crucial to control food related allergic reactions. A potential suppressive role of Treg in food allergies was exemplified in both animal models [46-48] and in human studies. Increased numbers of Treg cells in

peripheral blood in children who outgrew milk allergy was reported [49;50] and decreased Treg suppression was associated with egg allergy in neonates [51]. Cord blood from offspring of atopic mothers showed fewer innate induced Treg cells and impaired suppression[52]. In line with these, an association with severe food allergy was described in patients with profound dysfunction of T reg cells that characterizes IPEX syndrome (immunodysregulation polyendocrinopathy enteropathy X-linked syndrome)[53].

In contrast 1 study looking at the number of Tregs in 10 children undergoing milk desensitization reports no enhancement of CD4+CD25+Foxp3+ T(reg) levels in peripheral blood and suggests that there is unlikely a role for long-lasting systemic immunologic changes in desensitization. However, this study is limited by a small sample size and relatively short period of follow up (6 months). [54] Additionally, data now suggests that Foxp3-ve Tregs may also play a role in tolerance.

3.vi Gaps regarding the role of demethylation in desensitization.

The rising incidence of food allergies is occurring more rapidly than changes to the genome sequence would allow[55] and epigenetic regulation (heritable changes in gene expression that occur in the absence of alterations in DNA sequences) may in part mediate the complex gene-by-environment interactions that can lead to asthma.[56]

Experimental studies provide substantial in vitro data indicating that DNA methylation of genes critical to T-helper cell differentiation may induce polarization toward or away from an allergic phenotype. Methylation of DNA and resulting changes in chromatin structure have been shown to initiate the process by which the Th cells lose their plasticity and differentiate productively toward the Th1 versus the proallergic Th2 pattern

of cytokine gene expression.[57-59] So far no studies have explored the role of DNA methylation in short term processes such as desensitization for food allergens.

3.vii. **Gaps in role of allergen specific salivary IgA.**

We hypothesize that development of an enhanced antigen specific mucosal IgA response is associated with reduced responses to oral antigen challenge in food allergy in children. Antigens delivered to the gastrointestinal mucosa are subject to a variety of barriers before they come into contact with effector cells such as mast cells, loaded with antigen specific IgE, that may trigger an adverse allergic reaction. Since the majority of allergic effector cells within the intestinal tract are below the epithelial barrier, and IgA is in much higher concentration than IgE, we may enhance blockade against allergic reactions in previously sensitized individuals through increases in mucosal IgA, and especially through an increased IgA:IgE ratio. While this has been examined experimentally in mice, the relationship between mucosal IgA and desensitization in the clinical setting is not defined.

4. Methodological Approach/Study Design

Thirty four boys and girls with between 6 to 18 years of age, diagnosed with egg allergy will be approached by our study group. Participants will be recruited from the allergy clinic at the Montreal Children's Hospital.

Inclusion criteria: Children 6 years and older who satisfy all the following criteria will be included:

a. A history suggestive of immediate allergy to egg. A convincing clinical history of an IgE mediated reaction to a specific food will be defined as a minimum of 2 mild signs/symptoms or 1 moderate or 1 severe sign/symptom that was likely IgE mediated

and occurred within 120 minutes after ingestion or contact (or inhalation in the case of fish and shellfish). Reactions will be considered mild if they involve pruritus, urticaria, flushing, or rhinoconjunctivitis; moderate if they involve angioedema, throat tightness, gastrointestinal complaints, or breathing difficulties (other than wheeze); and severe if they involve wheeze, cyanosis, or circulatory collapse.[64-67]

b. The presence of at least one of the following confirmatory tests:

- (a) Positive SPT to egg or its proteins (weal diameter 3 mm larger than that of the normal saline control). The allergens used will be commercial extracts of egg (Omega Labs, Toronto, Ontario).
- (b) Detection of serum specific IgE (>0.35 kU/L) to egg or any of its proteins (ovalbumin, ovomucoid, lysozyme and conalbumin), measured by fluorescence enzyme immunoassay (Phadia, CAP System, Uppsala, Sweden).
- (c) Positive oral challenge test to egg. Oral challenges will be performed with Meringue Powder according to the recommendations of the position paper of the European Academy of Allergology and Clinical Immunology [68]and in an open manner (appendix C). When subjective symptoms will appear (oral pruritus, abdominal discomfort), the challenge tests will be blinded.

Informed consent form signed by the parents or legal guardian (appendix B).

Exclusion criteria.

1. Patients who are unstable from a respiratory point of view .

2. Patients who present with intercurrent disease at the time of starting desensitization.

4. Non-IgE-mediated or non-immunological adverse reactions to egg.

5. Malignant or immunopathological diseases and/or severe primary or secondary immune deficiencies.

6. Patients receiving immunosuppressor therapy.

7. Patients receiving β-blockers (including topical formulations).

8. Associated diseases contraindicating the use of epinephrine: cardiovascular disease or severe hypertension.

Subject Recruitment and Retention

Consent will be obtained by the study coordinator
Potential participants are referred to the study team by their treating allergist. They are given information about the study and a follow-up conversation - to review the study aims and procedures - with the study coordinator is scheduled. Typically, families of the potential participants will be given a week to review the study information. Following this, if potential participants are still interested in participating, they will meet with one of the investigators to review the potential benefits and risks of participation

It has been our experience that with this highly motivated group of participnats, it has not been necessary to implement procedures to monitor compliance.

Through a process of stratified randomization (according to sex), participants will be randomly assigned to one of 2 study groups. The first group will receive active desensitization , and the second group will not receive any treatment but will be followed up (appendix B protocols) .

The desensitization protocol will be performed at the Center of Innovative medicine (CIM) as is currently done for our milk and peanut desensitization study, under the direct supervision of the medical and nursing staff, and with full cardiopulmonary resuscitation measures available for the treatment of possible allergic reactions that could occur during

the procedure. Oral and intravenous doses of diphenhydramine and intramuscular epinephrine were at the bedside at all times.

Desensitization to egg .[12]

Desensitization will begin with combined challenge/rush desensitization. After placement of an intravenous catheter, 20 ml blood samples will be collected, skin prick test (SPT) to egg will be performed and 5 ml of saliva will be collected. These samples will be used to establish participants' baselines for the various biological markers being examined during the study. The modified challenge will begin with a first single dose of 0.1 mg of placebo, alternating with powdered egg white in the form of Meringue powder. After the initial dose, subjects will receive approximately doubling doses every 30 minutes until the highest tolerated single dose will be determined (see Appendix B for Challenge/Rush doses). For doses of 25 mg and greater, powdered meringue will be dispensed from individual preweighed containers.. After the initial dose, subjects will receive approximately doubling doses every 30 minutes until the highest tolerated single dose was determined. All doses will be mixed with an acceptable vehicle food chosen by the subject and his or her parent. Subjects treated for reactions will be observed for 2 hours, and if there is no evidence of an allergic reaction, they will be discharged home. Self-administered epinephrine will be provided to all patients' caregivers, along with instructions and indications for administration and education about the nature of possible reactions to OIT. To ensure there were no ill effects of the daily dosing, each subject will return to the CIM on the second day for an observed administration of the starting daily dose.

All challenges and escalation doses will take place in the Center of Innovative medicine (CIM) as is currently done for our milk and peanut desensitization study . The CIM services include equipment and medications required to monitor and treat potential reactions . The CIM nurse that will assist in the desensitization protocol is a nurse that has been trained to work in an intensive care unit. The patients will not be admitted, but will remain under observation for 7 h each day, with the possibility for increasing this period if required.

SPT with the allergens listed above will be performed on all patients before starting desensitization, and the tests will be repeated at 3, 6 and 12 months. Blood tests will be also performed to measure sIgE and sIgG, T cell phenotypes (including regulatory Tcells), salivary IgA and DNA methylation before desensitization, at 3 weeks and at 3, 6 and 12 months.

Reactions during the desensitization protocol will be classified according to the categories proposed by Perry et al.[(69)]: mild reactions are defined when symptoms are limited to the oral mucosa or the skin; severe reactions include cardiovascular or respiratory symptoms or involvement of any four systems; all other reactions will classified as moderate, although we will consider isolated abdominal discomfort as mild when it lasted for 30 min or less (appendix C protocols).

Build-up phase

Subjects will begin daily home dosing based on the highest dose tolerated during the modified rushof powdered egg white in 1 to 2 tablespoons of baby food or other acceptable vehicle food. Home doses will be provided in individual pre-weighed containers, and subjects will be instructed to ingest 1 dose daily for 2 weeks. As long as

subjects tolerate current doses, the doses will be increased every 2 weeks (see dosing schedule Appendix B) until reaching 150 mg and then increased by 50 mg at each visit until reaching maintenance (300 mg). Dose escalations will be made at visits in the research unit to ensure that subjects tolerate increases, and subjects will be observed for at least 2 hours after increases for signs of clinical reactions. Subjects will be given diaries to track their dose-related symptoms during the study

The protocol will be individualized according to each patient's tolerance to egg:

- Mild allergic reactions will be treated when necessary and the regimen will be continued when the patient becomes asymptomatic. The previously tolerated dose will be repeated before resuming the process.
- Moderate reactions will be treated and desensitization would be restarted on the following day at the previously tolerated dose.
- Severe reactions will be treated with the necessary measures and in the appropriate department, followed by an assessment of whether to interrupt desensitization or reduce the dose on the following day to 1/10 of the dose that caused the reaction.

The patients' parents will be instructed verbally and in writing about the recommendations to be followed after desensitization and how to treat possible allergic reactions. They will also given a direct telephone line for consultation. Patients will be instructed not to perform physical exercise [70;71] for 2 h after eating egg and not to take non-steroidal anti-inflammatory drugs for 3 h before or after ingestion. No special recommendations will be given for viral infections.[11] Participants will be given

symptom diaries and instructed to complete it daily to keep track of their allergic symptoms during the desensitization process.

Participants who are not able to achieve desensitization (desensitization defined as being able to tolerate the 300 mg maintenance dose) by 18 months following the initiation of the desensitization process will be considered treatment failures and discontinued from the study.

Maintenance/ Follow-up Phase

Once subjects reached the daily dose of 300 mg, they will be instructed to take this daily for the length of the study (12 months). The subjects will be also instructed to otherwise continue an egg-elimination diet for the duration of the study.Subjects and families will be asked to complete daily home diaries to document that daily doses were taken, as well as to report accidental ingestions, problems with dose administration, or related symptoms. Follow-up visits in addition to and in conjunction with the biweekly visits for dose escalation will be planned at 3, 6, 9, 12 months from the second egg challenge. Each visit will involve a medical history and physical examination. egg specific skin prick testing (SPT), a blood draw similar to that at the challenge to measure serum egg-specific IgE and IgG concentrations, T cell penotyping and DNA methylation. A 5 ml saliva sample will be collected to assess salivary IgA

Food challenges

One month after subjects reach the 300 mg dose, they will undergo a second food challenge to determine his or her allergenic reactivity to egg protein. Subjects will continue OIT dosing through the day before the challenge. The challenge will consist of 10 doses of powdered egg white given every 10 to 20 minutes in increasing amounts up to a total of 8 g of powdered egg white (6 g of egg protein).

After their year in the control arm, , oral desensitization will be offered to the control patients

Variables measured. The following variables will be measured in each patient:

A. The primary study variable will be the presence of desensitization defined by the ability to tolerate egg 1 year after the start of the trial. This is a qualitative variable with three categories: total desensitization (300mg of egg); partial desensitization (30-300mg of egg); and failed desensitization (<30 mg of egg). Only patients presenting total desensitization were regarded as being successfully desensitized.

B. Secondary variables will be:

1. Number and severity of adverse reactions occurring after oral exposure to egg /milk during the desensitization and follow-up phases.

2. Minimum dose of egg white that triggered symptoms during the desensitization protocols or challenge tests.

3. Days until desensitization was achieved, excluding weekends.

4. Indices of desensitization :

SPT weal size and levels of sIgE and sIgG before desensitization, during follow-up and at the end of the study.

5. Mast cell activation.

Mast cell activation will be assessed through a proxy measure: the basophil activation test. This test is based on the percentage of CD63-expressing basophils detected by flowcytometry . This test is done by incubating basophils from highly sensitized atopic donors or preferably after priming them with Il3 (regardless of basophil source) [72;73] with sera from patients . We will assess the expression of CD63 on basophils after addition of egg/milk antigens respectively as well as an irrelevant antigen (gliadin and tetnus toxoid) The expression of CD63 on these cells is compared to values acquired after incubation with healthy controls and the same antigens. Values above the mean of CD63+ cells + 2 SD induced will be considered as positive. [74]

6. Indices of T cell tolerance :

Levels of Treg and cytokine release at base line and uring desensitization. In order to characterise molecular pathways associated with desensitization , we will draw approximately 20 ml cc of blood from each participant and check their T cell phenotype including their Treg cells and the response to egg and milk proteins. As control the

response to an irrelevant antigen (gliadin) and to tetanus toxoid will be MEDICed. We will invite participants for annual follow ups in the first 5 years after study entry. We will also offer the desensitization to the control group at the end of the study.

7. Egg specific salivary IgA

At the initial visit and at all follow-up visits, participants will be asked to produce 5 ml of saliva. Our work in previous desensitization studies has demonstrated that even children as young as 6 years can produce this volume of saliva. Egg-specific salivary IgA will be determined by ELISA

5. Statistical Considerations

Given that previous studies in adults have attributed at least 50% improvement in the ability to tolerate egg ,[75] with an α of 0.05 and a power (1- β) of 0.80, a sample of 17 cases and 17 controls will be recruited. Statistical analyses will be performed using paired t-tests. A P value of <0.05 will be considered significant.

Descriptive statistics of the variables including means, standard deviations and frequencies were computed for all study variables. The concentrations of egg -sIgE and sIgG , the weal size , Mast cell, salivary IgA, T cell and DNA methylation status at the different study time-points will be compared with their baseline values using the one-sample paired t-test and to the control using a 2 sample t-test .

Multiple regression models will be used to assess factors associated with response to desensitization (i.e. baseline demographic characteristics; gender; age; baseline basophile

activation; T cell status, baseline SPT and specific IgE, salivary IgA, and DNA methylation status,).

6. Ethical Considerations

In the consent form given at study enrolment, participants will be advised that they will be randomly assigned to either desensitization treatment or observation alone for one year, (appendix B). Participants will also be advised that their data will be shared only among the study members. Participants who will be at the control group will be told that they will be offered the desensitization protocol one year after study entry.

7. Participant Confidentiality and Record Retention

All participants will be assigned a unique study code that will be used on all study documents. The link between the participants' code and their name will be stored on a password protected computer in the MUHC's Centre for Innovative Medicine, which is a restricted access unit. All study documents will be kept in a locked filing cabinet in the Centre for Innovative Medicine. Only members of the study team, and authorized oversight bodies will be allowed access to the study documents.

Study documents will be kept at the Centre for Innovative Medicine for two years after the end of the study, then transferred to Iron Mountain for long-term storage off-site. All study documents will be stored for 25 years

8. Research Deliverables and Milestones

Timeframe	Deliverables	Milestones

011/17 – 03/ 18	Develop sampling frame; ethics approval;	Ready to recruit participants
03/18 –07/20	Collect data from participants	12 weeks of data collection in treated participants completed
07/20– 12/24	Knowledge translation activities: dissemination workshops & manuscript preparation	Preparation of abstracts and manuscript

9. Requirement for networking across Disciplines and Sites

Our researchers have a legacy of working across disciplines, across sites, and across sectors. For this study, we have assembled a multi-institutional research team with cross-disciplinary expertise Dr Ben-Shoshan (Montreal Children's Hospital, McGill University) and Dr Mazer (Montreal Children's Hospital) and Dr Stuart Carr (f Department of Pediatrics, University of Alberta, Edmonton, Canada)bring expertise in clinical immunology to the research team. Dr. Ann Clarke (Montreal General Hospital) will bring expertise in the field of clinical allergy and together with the co-investigator, Dr. Joseph, a biostatistician, will assisted with the study design and will supervise the statistical analyses. Dr Ciriaco Piccirillo (Departments of Microbiology & Immunology and Medicine, McGill University, Montreal, Quebec) will contribute their expertise in the areas of T cell phenotyping and DNA methylation status. Epidemiologic and

biostatistician skills are essential to undertaking this program of research and are represented in the persons of Moshe Ben-Shoshan (McGill) and Mr .Duncan Lejtenyi (both Msc in Epidemiology). Mr Duncan Lejtenyi will also be responsible for the laboratory evaluation of CD63 levels. Finally, as incorporated in our protocol, we will share the results of this research with representatives of key constituencies involved in the data collection –patients with food allergies and their families, physicians and allied professionals (e.g. nurses) through scientific meetings, our website and publications in medical journals.

10. Organization of Research Team

The nominated principal applicant, Dr. Moshe Ben-Shoshan, and co-principal applicants, Dr. Bruce Mazer, will oversee all aspects of the proposed research. The collaborators, Dr Stark, Dr Piccirillo will aid in the recruitment of patients and the assessment T cells and DNA methylation status respectively.

Mr Duncan Lejtenyi, who had worked with our team in numerous studies will serve as research coordinator.

11. External Research Partnerships and funding

As indicated above, external research partnerships are considered essential to the activities of our research team. We will apply to receive substantial financial support from the CIHR and from AllerGen (appendix D budget). We will work closely with client organizations such as the Anaphylaxis Canada , AAIA (allergy/Asthma Information Association) , and the AQAA (Association québécoise des allergies

alimentaires). These support a cyber-society of people with food allergies and will provide support for our research activities from their limited budgets, again indicating the relevance of our research efforts to their needs.

Name of Partner	Nature of participation	Contribution (CA$)		Letter yes /no
		Cash	In-kind	
Anaphyalxis Canada			1000	Y
AAIA			1000	Y
AQAA			1000	Y
Total value of partnerships				

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Appendix A : Disease burden and gaps.

Table 2. **Primary and secondary prevention measures for food allergies**[a]

Trigger	Primary prevention	Route of desensitization	Reference number	Secondary prevention	Reference number
Foods	Milk	PO, SL	(14;76-78)	Avoidance of allergenic food; education of allergic individuals and their care-givers on importance of avoidance, improved labeling of prepackaged foods for allergens, wearing of Medic-Alert bracelet stating specific food allergy	154,155
	Egg	PO	(11-13)		
	Peanut	PO	(39)		
	Tree nut	SL	(79)		
	Peach	SL	(80)		

[a] only the most common foods , drugs and insect desensitization approaches are mentioned.

PO, **Per os**; SL, **Sublingual**; SC,

Table 2.Environmental factors associated with food allergy.

Factor	study	Type of study	Effect	Reference number
Season	Vassallo MF	Case control	Children younger than 5 years born in fall or winter had a 53% higher odds of food allergy compared with controls.	[81]
Drugs	Palli-Scholl	Case control	The relative risk to develop food-specific IgE after anti-acid therapy was 10.5 (95% CI,1.44,76.48).	[82]
Microbial exposure	Gourbeyre	Review of case control and cohort studies	No clear conclusion regarding probiotic beneficial effects on the prevention or treatment of allergy .	[83]
Food consumption (quantity and timing)	Poole JA	Cohort	After adjusting for breastfeeding duration, introduction of rice cereal, family history of allergy, and history of food allergy before 6 months of age, age at initial exposure to cereal grains continued to be strongly associated with wheat allergy (≥7 months: adjusted OR: 3.8; 95% CI, 1.18,12.28)	[84]

	Du Toit	Case control	After adjustment for atopy, other food allergies, age, and sex, the RR for peanut allergy in the UK vs Israel is 5.8 (95% CI, 2.8,11.8), and largest and most significant difference in weaning between the UK and Israel was observed in the age of introduction of peanut (P < .0001). By 9 months of age, 69% of Israelis were eating peanut compared with only 10% of UK infants.	[85]
	Katz	Cohort	The OR was 19.3 (95% CI, 6.0,62.1) for development of IgE mediated CMA among infants with exposure to cow milk protein at the age of 15 days or more (P < .001) vs those introduced to cow milk protein before 15 days.	[86]
	Joseph	Cohort	Early feeding reduced the risk of peanut sensitization among children with a parental history [adjusted OR, 0.2 (95% CI, 0.1,0.7); P = .007]. The relationship also became significant for egg when a cutoff for IgE of ≥0.70 IU/mL was used [adjustedOR, 0.5 (95% CI, 0.3,0.9)].	[87]
	Koplin	Case control	Introduction of cooked egg at age 4 to 6 months, vs later exposure reduced the risk of egg allergy [OR, 0.2 (95% CI, 0.06-0.71)].	[88]
	Des Roches	Case control	The reported consumption of peanuts during pregnancy and breastfeeding was higher in the case group (those who developed peanut allergy and associated with an increased risk of peanut allergy in offspring [OR, 4.22 (95%CI, 1.57,11.30) and OR, 2.28 (95% CI, 1.31,3.97) for pregnancy and breastfeeding, respectively].	[89]

	Sicherer	Case control	Multivariate analysis including clinical, laboratory, and demographic variables showed frequent peanut consumption during pregnancy {OR, 2.9(95% CI, 1.7,4.9)] to be associated with peanut IgE ≥5 kUA/L.	[90]
Food processing	Chung	Laboratory analysis	After curing and roasting, mature peanuts exhibited approximately 20% higher levels of advanced glycation end adducts and higher IgE binding vs immature peanuts.	[91]
	Yadzir	Laboratory analysis	Extracts from raw shrimp bound higher IgE than extracts from boiled shrimp, but the purified boiled tropomysoin (the main shrimp allergen) demonstrates higher IgE binding vs raw shrimp.	[92]
	Samson	Laboratory analysis	Thermal processing can lead to the formation of new antigenic structures.	[93]
Vitamin D	Milner	Cohort	Early vitamin D use (within the first 6 months of life) was associated with a higher risk for food allergies in the exclusively formula-fed population [OR,1.63(95% CI,1.21,2.20)]. Vitamin use at 3 years of age was associated with increased risk for food allergies but not asthma in both breastfed [OR,1.62(95% CI,1.19,2.21)]and exclusively formula-fed infants [OR, 1.39(95% CI,1.03,1.88)].	[63]
	Cramago	Ecologic study	Strong north-south gradient for the prescription of EpiPens in the United	[60]

			States, with the highest rates found in New England. [adjusted β for New England vs the rest of the US, 4.07 (95%CI, 2.77,5.36)]	
	Mulins et al	Ecologic study	Using multivariate analysis , EpiPen prescription rates were higher in southern latitudes (less sunlight) compared with northern regions [β, −19.22(95% CI, −26.71 , −11.73)].	(61)
	Mulins et al	Ecologic study	Southern latitudes were associated with higher hypoallergenic formulae prescription rates [beta, -147.98(95% CI,-281.83 , -14.14)].	(62)

OR, odds ratio;RR, Relative Risk; CI,confidence interval ;CMA, Cow's Milk Allergy

Appendix B. protocols.

Initial Egg Challenge/Desensitization doses [12]*:

Dose (n:0)	Mg egg protein	Dosage form
1	**0.1**	Excipient microcrystalline cellulose
2	**0.2**	_”_
3	**0.4**	_”_
4	**0.8**	_”_
5	**1.5**	_”_
6	**3**	_”_
7	**6**	_”_
8	**12**	Dose of the powder
9	**25**	_”_
10	**50**	_”_
11	**75**	_”_
12	**100**	_”_
13	**125**	_”_
14	**150**	_”_
15	**200**	_”_
16	**250**	_”_

17	**300**	**_”_**

Increasing doses are given approximately every 30 minutes. If the subject does not tolerate a given dose and symptoms are mild, then that dose or the previously tolerated one is repeated, and the protocol proceeds as outlined. If the subject experiences significant symptoms, then the protocol is stopped, and the highest tolerated dose is used as the starting daily dose.

Post – Escalation Egg challenge (Egg Protein):

Number of dose	**Egg protein mg at each challenge dose**	**Cumulative dose**
1	**1mg**	**1**
2	**3mg**	**4**
3	**10mg**	**14**
4	**30mg**	**44**
5	**100mg**	**144**
6	**300mg**	**443**
7	**600mg**	**1043**
8	**1000mg**	**2043**
9	**2000mg**	**4043**
10	**2000mg**	**6043**

10 doses of powdered Meringue given every 10 to 20 minutes in increasing amounts up to a total of 10 g of powdered egg white (6.04 g of egg protein which is equivalent to 1 egg).

Scale for Grading Reaction Severity and management

Score	Symptom	Action
Mild	Pruritus, Urticaria, Flushing, Rhinoconjunctivitis	Observe May give Antihistamine (e.g. Benadryl or Reactin as prescribed **Call Research Team** Research team will evaluate if dose adjustment is needed and if next dose will be given at home or in hospital.
Moderate	Angioedema, Throat tightness, Gastrointestinal complaints (cramping, . pain,vomiting,diarrhea) Respiratory symptoms (Cough, Mucous production)	Give epinephrine IM as per protocol Give Antihistamine (e.g. Benadryl or Reactin as prescribed Seek urgent care (hospital emergency room) Call Research team To give next adjusted dose in hospital research unit (CIM)
Severe	Wheeze, Respiratory Distress Hypoxia, Cyanosis, Hypotension Circulatory collapse (Shock)	Give epinephrine IM as per protocol Give Antihistamine (e.g. Benadryl or Reactin as prescribed Call 911 Seek urgent care (transfer to hospital emergency room) Call Research team; if the symptoms are not improving within 10 minutes of the first dose, instructions will be given from the team regarding use of a second dose of epinephrine.

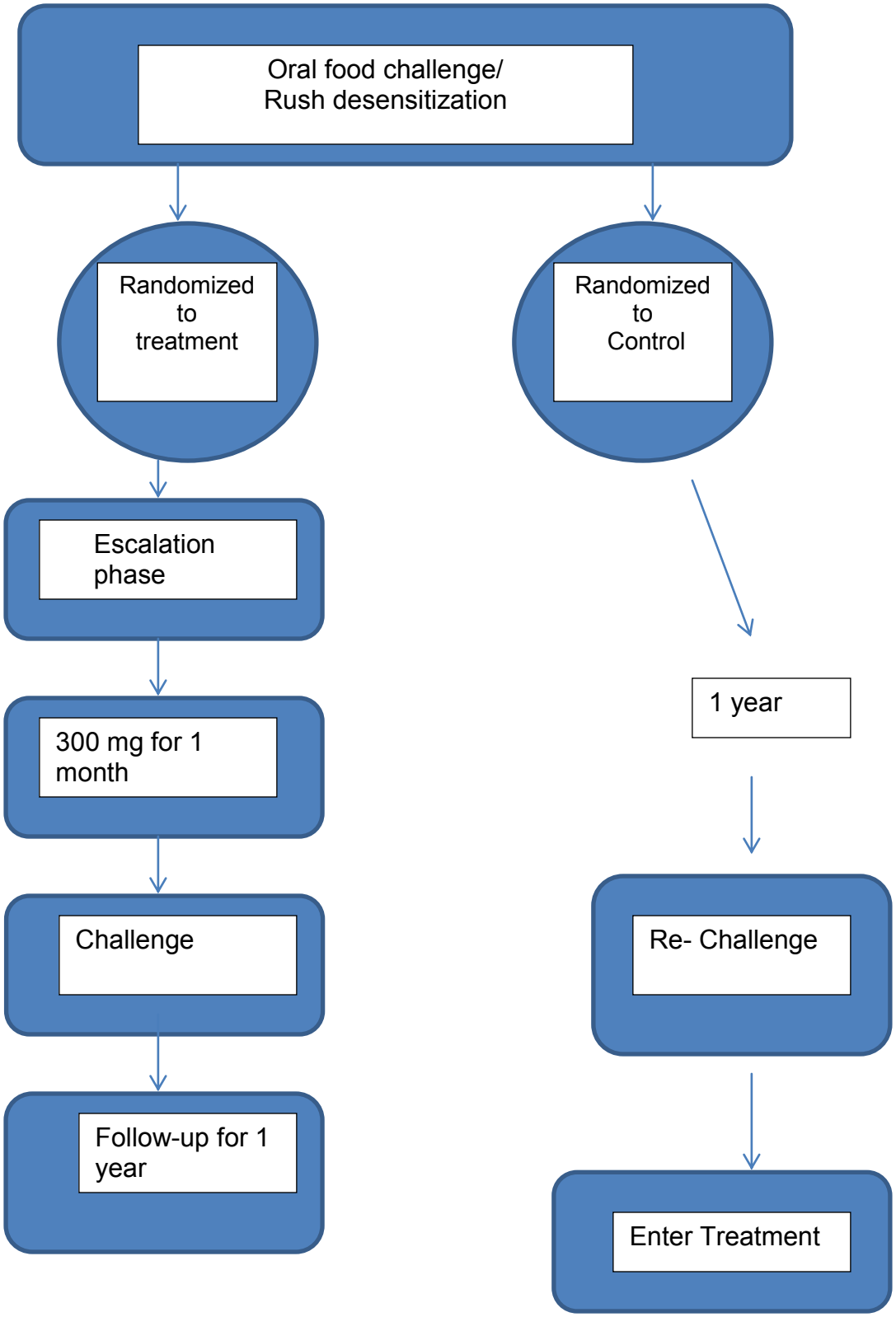
Appendix C. Budget

Total budget estimate: 78,000CAD per year including:

	Estimate (CAD)	comment

Yearly salary for an undergraduate student for expenses related to training in Lab evaluation mechanisms for auto-immune urticaria detection	35,000	
Study coordinator	40,000	Yearly salary with benefits
Paediatric test center (70CAD for each blood sample)	3000	
Staining with monoclonal Abs	3000	
Use of FACS	3000	
CBA	3500	
Culture materials and stimulating material	3500	
Travel expenses for presentation of results in scientific conferences	5000	
	78,000	yearly

Appendix D Study Schema


Oral food challenge/
Rush desensitization
Randomized to treatment
Randomized to Control
Escalation phase
300 mg for 1 month
Challenge
Follow-up for 1 year
1 year
Re- Challenge
Enter Treatment