Assessment of Immunoreactivity against Therapeutic Options Employing the Leukocyte Adherence Inhibition Test as a Tool for Precision Medicine

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ABSTRACT

Background: The Precision Medicine’s approach employs the endotype concept as a central feature to personalize medical treatment. Individual immunoreactivity, alongside characteristics such as genetics, environment, and diet, is one of the factors that differentiates the therapeutic-driven endotypes.

Objective: To evaluate the opportunity of the Leukocyte Adherence Inhibition test to differentiate the immunoreactivity between two similar therapeutic agents employed on Allergen Immunotherapy.

Methods: Side by side Leukocyte Adherence Inhibitions tests were performed with ovalbumin and carbamylated ovalbumin on a population of 33 self-reported egg-allergic individuals.

Results: The results showed two endotypes inside the immune response of the studied groups: The first endotype was defined by the 16 individuals that presented a significant decrease in ovalbumin’s immunoreactivity after carbamylation (mean of differences = 35%; p = 0.002). The second endotype was defined by 17 individuals that presented a significant increase in ovalbumin’s immunoreactivity after carbamylation (mean of differences = 32%; p = 0.001).

Conclusion: The Leukocyte Adherence Inhibition test was able to differentiate two distinct immunoreactivity patterns when comparing two similar therapeutic agents suggesting, as proof of concept, a potential role to be employed as a Precision Medicine tool.

Keywords: Allergoids, Leukocyte Adherence Inhibition test, Hypersensitivity, Precision Medicine.

Abbreviations:
LA: Leukocyte Adherence
LAR: Leukocyte Adherence Ratio
LAI: Leukocyte Adherence Inhibition
OVA: ovalbumin
cOVA: carbamylated ovalbumin
pOVA: phosphorylated OVA

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I. INTRODUCTION

Among the main causes of allergic symptoms that
deserve a Precision Medicine approach are food allergies [1]-
[5]. The Precision Medicine Initiative is a worldwide effort
created to provide personalized care to patients based on the
concept of the existence of different endotypes inside the
larger phenotype groups as defined by modern Medicine [6]-
[8]. The Precision Medicine approach also embraces the
“complex endotype” concept, consisting of several sub-
endotypes, instead of a single simplified endotype molecular
mechanism [9]. Precision Medicine is mainly focused on
therapeutic-driven endotypes, i.e. particular phenotype-
subgroups that will better respond to a given therapy, instead
of another [10]. The main concern is to increase efficacy and
decrease collateral effects in the assumption that the
peculiarities of each patient will produce diverse beneficial as
well different collateral responses to the same therapeutic, as
observed inside the whole population [11]. The development
of Precision Medicine is based on the development of
diagnostic assays to predict the specific response of each
patient to a potential therapy, before the prescription of the
treatment [12]. This concern, in the field of Allergology, has
already originated the conception of more immunogenic and
less allergenic alternatives to allergen-specific
immunotherapy: the elaboration of modified allergens, the
allergoids [13]. The use of allergoids for desensitization of
allergic diseases has been reported since 1938 when Sledge
et al. treated their hay fever patients sensitive to grass and
ragweed with alum-precipitated pollen extracts, asserting that
this modification provided better results and a significant
reduction of “constitutional reactions” when compared with the
natural extracts [14]. However, despite the alum-
precipitated extracts could act better for some patients, some
individuals presented allergic symptoms to the alum itself,
characterizing a novel endotype among the patients belonging
to this allergic phenotype [15]. Since then, dozens of
techniques and adjuvants were described to decrease
allergenicity and increase the immunogenicity of allergoids
to provide a safer, more comfortable, personalized, and
effective desensitization to allergic patients. One of the
techniques employed to produce allergoids is protein
carbamylation [16]. Protein carbamylation is a phenomenon
that occurs naturally inside urea producers’ organisms. The
electrophilic species in equilibrium with urea: cyanate and
isocyanate can react with lysine residues of proteins in an
irreversible posttranslational modification, producing a
carbamoyl group [17]. This spontaneous carbamylation
occurring into physiologic systems (mainly in individuals
with the uremic syndrome) may be assayed by colorimetric
methods and is also considered a signal of molecular aging,
related to degenerative disorders such as atherosclerosis and
rheumatic inflammation [18], [19]. Minor changes in
tridimensional conformation, such as the produced by
carbamylation, can alter immunoreactivity, which depends on
the antigen’s conformational epitope [20]. This modification
done in vitro can impair the binding of a reaginic antibody to
the natural antigen, converting the desensitization’s allergen
into an allergoid [21]. Theoretically, this may not necessarily
alter its tolerogenic capacity, which depends on the linear
epitopes produced by Dendritic Cell’s intracellular digestion
that are presented to the naïve T Cells into the MHC class II
context [22].

As a proof of concept, we compared the immunoreactivity
of self-reported egg-allergic individuals against a natural
allergen: the ovalbumin (Gal d 2) and its corresponding
carbamylated allergoid. Ovalbumin is the main egg white
hen’s protein with an approximate molecular weight of 43
kDa and consists of 385 amino acids single chain, 19 of which
are lysine residues [23]. Meta-analyses comparing diverse
diagnostic criteria (self-reported, skin tests, specific-IgE, and
double-blind placebo-controlled provocation tests (DBPCPT)
estimated that hypersensitivity to the egg is among the three
most common causes of food allergic reactions [24]. The
prevalence of food allergic reactions is relatively greater
when the self-reported method is employed, compared with
the objective diagnostic methods, but interestingly, the
prevalence’s ranking among the eliciting allergens is
consistently maintained when comparing the objective and
the self-reported criteria. This is understandable when we
consider that allergy may be mediated by immune
mechanisms not dependent on IgE and that food
hypersensitivity reactions may also be dependent on
thresholds, drugs, and physiologic stimuli, not always
employed by in vivo provocation’s tests [25]-[28].

Ovalbumin is not the most allergenic protein of hen’s egg (the
most allergenic is ovomucoid), but it is the most abundant and
accessible protein in albumen [29]. Additionally, albumins
are an interesting model to study immunoreactivity because
they are produced by the human liver (human serum
albumin), are present in significant amounts in human blood,
and can be found in several edible animals and plants with a
high degree of conservative evolution, resulting in both the
possibilities of natural immunotolerance or cross-reactive
hypersensitivity [30], [31]. Additionally, ovalbumin is also a
very well-studied experimental inducer of tolerogenic
Dendritic Cells [32]-[34]. One of the main functions of the
albumins are the internal transport of ligands due to their
conformational flexibility, another factor to alter their
allergenicity [35]. There are three subclasses in the purified
ovalbumin, according to their phosphate group content (two,
one, or none) [36]. Four interchangeable secondary structures
are found in an ovalbumin sample: alpha-helix, beta-sheet,
random coil, and beta-turns. The heating stimulates
denaturation, decreasing the alpha-helices and increasing the
aggregational beta-sheets (beta-aggregation) [37]. These
characteristics predict a broad specter of possibilities for
immune reactions and sensitization mechanisms. To compare
the general immunoreactivity against this complex allergen
and its correspondent carbamylated allergoid, we used the
Leukocyte Adherence Inhibition Test (LAI test) employing
heparinized plasma of human patients with self-reported egg
allergy. Leukocytes are naturally programmed to adhere to
glass in physiologic conditions, which can be easily observed
with an optical microscope and a glass surface
hemocytometer chamber [38]. When functionally activated,
they lose this capability, which allowed Halliday to design an
antigen-specific ex vivo challenge named Leukocyte
Adherence Inhibition test (LAI test) [39]. Challenged by
specific antigens, leukocytes release paracrine soluble factors
that interfere with glass adherence of nearby leukocytes, a
phenomenon that can be quantified with a concomitant assay
done with unchallenged plasma. Several immune
mechanisms can produce this phenomenon, which seems to
be just the final indicator of the antigen-specific leukocyte
activation or, in other words, the immunoreactivity [40]-[43].

## II. METHODS

### A. Subjects

After receiving Institutional Review Board approval, from the Instituto Alergoimuno de Americana (Brazil), 33 consecutive outpatients complaining of self-reported challenge-proofed clinical symptoms compatible with hen’s egg allergy, were invited, with informed consent formularies, to voluntarily provide blood samples to perform ex vivo challenge tests, according to the principles of Helsinki and the International Committee of Medical Journal Editors requirements of privacy. All patients had non-detectable specific IgE to ovalbumin and inconclusive skin tests (unreactive histamine controls) [44]. The study was purely descriptive and did not interfere with the patient’s treatment or the assistant physician’s diagnosis. All relevant and mandatory laboratory health and safety measures have complied within the complete course of the experiments.

### B. Carbamylation

Ovalbumin was purchased as a powder from Sigma-Aldrich Brasil Ltda EPP (Cotia – SP – Brazil). The carbamylation was performed after dilution of ovalbumin in a borax buffer (4 mg/mL) followed by the addition of potassium cyanate (0.5 M) and kept overnight at 40 °C [45], [46]. The final solution was submitted to NaCl (0.15M) and EDTA (10g/L) buffer dialysis adjusted to pH 5.0 for 24 hours to remove residual cyanate ions. Two blank ovalbumin solutions were prepared with (and without) potassium chloride (0.5 M) instead of potassium cyanate. The three final solutions were adjusted to pH 7.5 with HCl and to 1 mg/mL as quantified by Bradford methodology [47]. The phosphated ovalbumin (pOVA) was used only to control the electrophoretic running. The LAI tests were done parallelly with the purified ovalbumin (OVA) and the carbamylated ovalbumin (cOVA).

### C. Sodium Dodecyl Sulfate (SDS)-Polyacrylamide Electrophoresis Gel

We compared, side by side, the purified, the phosphated, and the carbamylated ovalbumin utilizing a Coomassie® Blue stained 6% resolving sodium-dodecyl sulfate acrylamide (6%) / bis-acrylamide (40%) electrophoresis gel (SDS-PAGE) performed with a Mini Protean Tetra Cell apparatus (Bio-Rad, CA, USA) [48]. Aliquots of 5 μL of each extract were applied to each lane. A 10-180 KDa molecular mass Thermo Scientific prestained protein ladder (PageRuler™, Thermo Fisher, MA, USA) was used to identify approximate molecular weights. After electrophoresis, the gel was stained with Thermo Fisher Coomassie® Blue to identify the protein molecular weight distribution.

### D. Leukocyte Adherence Inhibition Test

Plasma samples were collected in heparinized collection tubes. The ex vivo challenge tests were performed as described previously [49]. Each patient’s fresh plasma was divided into three parts and used in two simultaneous and paralleled ex vivo challenging tests with purified ovalbumin (OVA) and the carbamylated ovalbumin (cOVA), both controlled by the third unchallenged plasma assay. The plasma with high leukocyte content (buffy coat) was collected from the heparinized tube after one hour of sedimentation at 37 °C and aliquots of 100 μL were distributed into Eppendorf tubes kept under agitation for 30 minutes (200 rpm at 37 °C) with (or without, as used as control) antigen extract (10μL of a solution with 1 mg/mL and pH 7.5). After incubation, the plasma was allocated into a standard Neubauer hemocytometer counting chamber with a plain, non-metallic glass surface and left to stand for 2 hours at 37 °C in the humidified atmosphere of the covered water bath to allow leukocytes to adhere to the glass. Next, leukocytes were counted, the coverslip was removed, and the chamber was washed by immersion in a beaker with PBS at 37 °C. A drop of PBS was added to the hemocytometer chamber and a clean coverslip was placed over it. The remaining cells were counted in the same squares as previously examined. The percentage of Leukocyte Adherence (LA) of each assay was estimated as: (the number of leukocytes observed on the hemocytometry chamber after washing divided by the number of leukocytes observed on the hemocytometry chamber before washing) and multiplied by 100 (%). The Leukocyte Adherence Ratio (LAR) was calculated based on the ratio between the LA from the antigen-specific challenged groups and the LA from the unchallenged control group: LAR = LA of the challenged sample divided by LA of unchallenged control sample; multiplied by 100 (%). To further calculate the Leukocyte Adherence Inhibition (LAI) the LAR was subtracted from 100 (%).

### E. Statistical Analyses

Statistical analyses were performed using GraphPad Prism software (version 5.0; GraphPad Software, Inc., San Diego, CA, USA). The data were reported as arithmetic means with 95% confidence intervals (CI) and standard deviations (SD). Differences in the means of matched samples were assessed by paired t-tests [50]. For all analyses, a p-value of less than 0.05 was considered significant. Two whiskers-and-box plot graphs were generated by the software.

## III. RESULTS

The Coomassie® Blue-stained gel similarly showed two groups of proteins at the purified ovalbumin lane, the carbamylated ovalbumin lane, and the phosphated ovalbumin lane. The lighter group observed at approximately 43 kDa corresponds to the monomeric alpha-helix. The heavier group with approximately 86 kDa corresponds to the aggregated beta-sheet. The carbamylation interfered with the binding of the dye to the proteins but did not affect significantly their molecular weight (see Fig. 1).

When comparing the paired LAI responses inside the whole tested population, there was no significant difference by the t-test between OVA and cOVA immunoreactivity. However, we observed two distinct groups according to the results. The first group was defined by the 16 individuals that presented a significant decrease in ovalbumin’s immunoreactivity (as assumed by the LAI) after carbamylation (mean of differences = 35%; p = 0.002) as displayed in Fig. 2. The second group was defined by 17
individuals that presented a significant increase in ovalbumin’s immunoreactivity (as assumed by the LAI) after carbamylation (mean of differences = 32%; p = 0.001) as displayed in Fig. 3. The main indicator that the carbamylation interfered randomly in the immunoreactivity to OVA was the fact that the previously paired challenge tests were considered “not paired” by the t-tests after carbamylation.

IV. DISCUSSION

The design and the production of less allergenic food is a great concern not only for nutritional purposes but also for therapeutic interventions on food allergic people, mainly in this era characterized by the production of chemically, enzymatically, and/or genetically modified foods [51]. Usually, the concept of immunoreactivity employed by most food researchers with a nutritional background is exploited in its deleterious sense, referring to a reaginic immune reaction, usually mediated by IgE or IgG antibodies, associated with diseases, hypersensitivity or, at least, adverse reactions that can produce undesirable symptoms associated with the ingestion or even mere contact with specific food proteins [52]. But an immunologist must remember that the first and desirable immune reaction to food is immunotolerance. Tolerance to food is not the passive “absence” of immune reactions or unresponsiveness, but instead, is the resulting of an active and orchestrated interplay that establishes a chain of recognition of specific nutritional proteins that the immune system identifies as beneficial to the body physiology. The major participants of this complex task are the tolerogenic Dendritic Cells, the regulatory T and B lymphocytes (Treg and Breg), the TGF-β, the IL-10, and the IgA [53]-[57]. The main determinant of the kind of immune response to be developed is the environment in which the allergens are collected by the Dendritic Cells before being presented to the naïve T Cells inside the lymph organs. According to the antigens’ collecting environment, the Dendritic Cell expresses tolerogenic or costimulatory cytokines during the antigen presentation that drive the naïve T Cell to differentiate to a regulatory (tolerogenic) or a helper (inflammatory) phenotype [58]. When employing the LAI test to evaluate immunoreactivity we are not predicting the subsequent direction towards immunotolerance or hypersensitivity, but just the ability of the immune cells to recognize and respond to the specific antigen to which it is exposed. The inhibition of the leukocytes’ glass adherence is just the final indicator of a non-specific release of cytokines. Therefore, this immunoassay is not designed to evaluate the utility of the LAI test to diagnosis allergy or to predict the patients’ clinical response to the natural allergen or the carbamylated allergoid but to testify the first steps that drive the endotype differentiation. To predict the clinical response, it is necessary to employ information provided by others in vivo, in vitro, and ex vivo assays, such as the allergy cutaneous tests, the research of specific antibodies, the lymphocytes proliferation tests, or the basophils challenge tests, for instance. This experiment had the sole objective to explore the phenotype/endotype concept inside a Precision Medicine perspective. When evaluating the entire population, the statistical analysis found no significant difference in the immune response comparing the natural ovalbumin and the carbamylated allergoid. However, when the two different endotypes were defined, the statistical analyses revealed a significant difference characterizing the two trends of response. In conclusion, the objective of this work is to present a different perspective to evaluate the data, and instead of simply disqualify the mixed results, we propose to study more deeply the differences among the immune responses to extract additional information with the potential to improve and personalize the treatment of the therapeutic-
driven endotypes.

**REFERENCES**

[1] F. Vega, C. Panizo, M. T. Dordal, M. L. González, E. Velázquez, A. Valero, et al. “Relationship between respiratory and food allergy and evaluation of preventive measures,” *Allergologia et Immunopathologia*, vol. 44, no. 3, pp. 263-275, 2016.

[2] A. K. Sood, and A. M. Scurluck, “Food allergy oral immunotherapy,” *Journal of Food Allergy*, vol. 2, no. 1, pp. 75-80, 2020.

[3] O. M. Schluss, “A Case of Allergy to Common Foods,” *Am J Dis Child*, vol. III, no. 6, pp. 341-3416, June 1, 1912, 1912.

[4] C. W. Canonica, C. R. Bachert, P. Helbesch, D. Ryan, E. Valvortia, M. Wickmann, O. De Beaumont, and J. Bouquet, “Allergen Immunotherapy (AIT): a prototype of Precision Medicine,” *World Allergy Organ J*, vol. 8, no. 1, pp. 015-0799, 2015.

[5] F. S. Collins, and H. Varmus, “A New Initiative on Precision Medicine,” *New England Journal of Medicine*, vol. 372, no. 9, pp. 795-795, 2015.

[6] P. L. Sankar, and L. S. Parker, “The Precision Medicine Initiative’s All of Us Research Program: an agenda for research on its ethical, legal, and social issues,” *Genetics in Medicine*, vol. 19, no. 7, pp. 743-750, 2017.

[7] C. Song, Y. Kong, L. Huang, H. Luo, and X. Zhu, “Big data-driven precision medicine: Starting the custom-made era of iatromodology,” *Biomedicine & Pharmacotherapy*, vol. 129, pp. 110445, 2020.

[8] L. Chen, J. E. Manautou, T. P. Rasmussen, and X.-b. Zhong, “Development of precision medicine approaches based on individual variability of BCRP/ABCG2,” *Acta Pharmacologica Sinica*, vol. 9, no. 4, pp. 659-674, 2019.

[9] I. Agache, and C. A. Akdis, “Endotypes of allergic diseases and asthma: An important step in building blocks for the future of precision medicine,” *Allergology International*, vol. 65, no. 3, pp. 243-252, 2020.

[10] A. Muraro, R. F. Lemanske, P. W. Helings, C. A. Akdis, T. Bieber, T. B. Casale, et al. “Precision medicine in patients with allergic diseases: Airway diseases and atopic dermatitis; PRACTALL document of the European Academy of Allergy and Clinical Immunology and the American Academy of Allergy, Asthma & Immunology,” *Journal of Allergy and Clinical Immunology*, vol. 137, no. 5, pp. 1347-1358, 2016.

[11] I. Miyagawa, and Y. Tanaka, “The approach to precision medicine for the treatment of psoriatic arthritis,” *Immunological Medicine*, vol. 43, no. 3, pp. 98-102, 2020.

[12] B. Shen, and J. Hwang, “The clinical utility of precision medicine: properly assessing the value of emerging diagnostic tests,” *Clin Pharmacol Ther*, vol. 88, no. 6, pp. 754-6, Dec, 2010.

[13] C. E. Olivier, “The use of allergoids and adjuvants in Allergen Immunotherapy,” *Arch Asthma Immunol*, vol. 1, pp. 40-60, 2017.

[14] R. F. Sledge, “Treatment of hay fever with alum-precipitated pollen extract,” *Journal of Allergy*, vol. 9, no. 4, pp. 424, 20170728, 1938.

[15] P. Y. Castelan, M. Castelan, D. Vervloet, L. Garbe, and B. Mallet, “Sensitization to aluminium by aluminium driven endotypes,” *Contact Dermatitis*, vol. 19, no. 1, pp. 50-88, 1988.

[16] C. Lombardi, S. Gargioni, A. Melchiorre, A. S. Ngarize, H. Herman, A. Adams, and N. Howell, “Comparison of Carbamylation in ESRD: surrogate markers or partners in crime?,” *Biochimica et biophysica acta*, vol. 1830, no. 12, pp. 5357-5381.

[17] F. J. Moreno, and A. Clemente, “2S Albumin Storage Proteins: What makes them Food Allergens?,” *The open biochemistry journal*, vol. 2, pp. 16-28, 2008.

[18] R. Thomé, L. G. R. Fernandes, M. F. Mineiro, P. U. Simionini, P. P. Joaozinho, and W. M. d. S. C. Tamashiro, “Oral tolerance and OVA-induced tolerogenic dendritic cells reduce the severity of collagen/ovalbumin-induced arthritis in mice,” *Cellular Immunology*, vol. 280, no. 1, pp. 113-123, 2012.

[19] P. U. Simionini, L. G. R. Fernandes, D. L. Gabriel, and W. M. S. C. Tamashiro, “Induction of Systemic Tolerance in Normal but not in Transgenic Mice Through Continuous Feeding of OvaLbumin,” *Scandinavian Journal of Immunology*, vol. 60, no. 3, pp. 257-266, 2004.

[20] L. N. Paiato, F. G. D. Silva, J. Bier, M. R. Brochetto-Braga, A. T. Yamada, W. M. S. C. Tamashiro, and P. U. Simionini, “Oral Tolerance Induced by OVA Intake Ameliorates TNBS-Induced Colitis in Mice,” *PLoS ONE*, vol. 12, no. 1, pp. e0170205, 2017.

[21] C.-H. Tang, and L. Shen, “Role of Conformational Flexibility in the Emulsifying Properties of Bovine Serum Albumin,” *Journal of Agricultural and Food Chemistry*, vol. 61, no. 12, pp. 3097-3110, 2013.

[22] Y. Mine, “Recent advances in the understanding of egg white protein functionality,” *Trends in Food Science & Technology*, vol. 6, no. 7, pp. 225-232, 1995.

[23] S. Ngarize, H. Herman, A. Adams, and N. Howell, “Comparison of Changes in the Secondary Structure of Unheated, Heated, and High-Pressure-Treated 1-Lactoglobulin and OvaLbumin Proteins Using Fourier Transform Raman Spectroscopy and Self-Deconvolution,” *Journal of Agricultural and Food Chemistry*, vol. 52, no. 21, pp. 6470-6477, 2004.

[24] D. M. P. Thomson, “Assessment of immune status by the leucocyte adherence inhibition test,” *New York: Academic Press*, 1982.

[25] W. J. Halliday, “Historical Background and Aspects of the Mechanism of Leukocyte Adherence Inhibition,” *Cancer Res*, vol. 39, no. 2, pp. 558-563, February 1, 1979, 1979.

[26] T. Appelboom, J. P. Famaey, R. Gortz, and J. Wybran, “Effect of levamisole on leukocyte adherence inhibition,” *Agents Action*, vol. 11, no. 6-7, pp. 604-5, Dec, 1981.

[27] A. Fink, H. Bibi, A. Elizar, E. Tabachnik, and Z. Bentwich, “Leukotrienes (LT4, LT4D) confer glass non-adherence on leucocytes of asthmatic individuals. Dependency on cyclooxygenase products and calcium ion,” *Immunol Lett*, vol. 10, no. 6, pp. 319-323, 1995.

[28] A. Fink, R. Shahin, A. Elizar, H. Bibi, H. Berkenstadt, S. Levin, and Z. Bentwich, “Interferon modulates the leukotriene C4-induced non-adherence properties of leucocytes: acquisition of an asthmatic phenotype,” *Immunol Lett*, vol. 10, no. 3-4, pp. 159-63, 1985.

[29] K. Iwabuchi, and T. Yamasita, “Platelet-derived neutrophil adherence-inhibiting factor in humans,” *Blood*, vol. 76, no. 11, pp. 2308-23, Dec 1, 1990.
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A. D. E. Lachalan, “Boxor as a Standard Buffer Solution,” Nature, vol. 154, no. 3914, pp. 577–577, 1944.

M. M. Bradford, “A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding,” Anal Biochem, vol. 72, pp. 248–54, 1976.

K. Weber, J. R. Pringle, and M. Osbom, “Measurement of molecular weights by electrophoresis on SDS-acrylamide gel,” Methods Enzymol, vol. 26 PC, pp. 3–27, 1972.

C. E. Olivier, R. P. S. Lima, D. G. Pinto, R. A. P. G. Santos, G. K. M. Silva, S. L. S. Lorena, et al., “In search of a tolerance-induction strategy for cow’s milk allergies: significant reduction of beta-lactoglobulin allergenicity via transglutaminase/cysteine polymerization,” Clinics, vol. 67, no. 10, pp. 1171–1179, 2012.

W. S. Gossel (Student), “The probable error of a mean,” Biometrika, vol. 6, no. 1, pp. 1–25, 1908.

J. Leszczynska, A. Lacka, and M. Bryszewska, “The use of transglutaminase in the reduction of immunoreactivity of wheat flour,” Food and Agricultural Immunol, vol. 17, no. 2, pp. 105–113, 2006.

A. F. Allakhverb, “Foods Causing Highest IgG Immune Response in Saudi Arabia,” Annual Research & Review in Biology, pp. 115–127, 2020.

A. M. Mowat, “Anatomical basis of tolerance and immunity to intestinal antigens,” Nature Reviews Immunol, vol. 3, no. 4, pp. 331–341, 2003.

M. C. Berin, and A. S. Hugh, “Mucosal Immunology of Food Allergy,” Current Biology, vol. 23, no. 9, pp. R389–R400, 2013.

J. Pier, E. G. Liu, S. Eisenbarth, and K. M. Järvinen, “The Role of Intestinal Antigens,” Annual Research & Review in Biology, vol. 2021, no. 3, pp. 00090–00090, 2021.

B. Bohle, T. Kinaciyan, M. Grestmyar, A. Radakivics, B. Jahn-Schmid, and C. Eber, “Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergen-specific T-cell tolerance, and immune deviation,” J Allergy Clin Immunol, vol. 120, no. 3, pp. 707–13, Sep. 2007.

O. U. Soyer, M. Akdis, J. Ring, H. Behrendt, R. Cramer, R. Lauener, and C. A. Akdis, “Mechanisms of peripheral tolerance to allergens,” Allergy, vol. 68, no. 2, pp. 161–170, 2012.

R. Hinterleitner, and B. Jabbi, “A dendritic cell subset designed for oral tolerance,” Nat Immunol, vol. 17, no. 5, pp. 474–6, 2016.