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Research article

Endotyping Cellular and Humoral Cross-reactivity against Soybean (Glycine max) and Red Beans (Phaseolus vulgaris) in Non-IgE-mediated Food Protein-Induced Gastrointestinal Allergies

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Abstract

Background: Patients with sensitivity to legume lectins may present several phenotypes regarding clinical severity and features, suggesting the existence of several endotypes underlying clinical symptoms. However, the immunoreactivity produced by these carbohydrate-ligand proteins has not yet been thoroughly investigated.
Aim: To evaluate the potential of the Tube Titration of Precipitins (TTP) and the Leukocyte Adherence Inhibition Test (LAIT) to differentiate cellular and humoral immunoreactivity against lectin-rich related legumes (Glycine max and Phaseolus vulgaris), challenging their extracts against blood samples of patients with non–IgE-mediated food protein-induced gastrointestinal allergic diseases.
Study Design: We examined retrospectively the medical charts of two cohorts of patients clinically diagnosed with non–IgE-mediated food protein-induced gastrointestinal allergic diseases related to consumption of soybean and/or red bean, who were investigated with the help of TTP or LAIT.
Methodology: The registered results of the TTP and LAIT against 1 mg/mL soybean extract and 1 mg/mL red bean extract were distributed in ranges through a cascade distribution chart to outline the variability of the results within the first cohort. The statistical differences within these cohorts were calculated. The Pearson correlation test was calculated between paired results.
Results: The paired t-test indicated a non-significant difference between the LAIT results of soybean and red bean extracts (p-value = 0.74). Pearson's correlation indicated a significant moderate positive relationship between LAIT results of soybean and red bean extracts, $r(98) = 0.314$, $p = 0.001$. The paired t-test indicated a non-significant difference between the TTP results of soybean and red bean extracts (p-value = 0.655). The Pearson correlation indicated a significantly weak positive relationship between TTP results of soybean and red bean extracts; $r(98) = 0.228$, $p = 0.022$.
Conclusion: Our preliminary results support that the TTP and LAIT performed with soybean and red bean extracts may differentiate diverse humoral and cellular immunoreactivity degrees, as well as demonstrating a significant correlation between the humoral and cellular immunoreactivity between the two extracts in patients suffering from Non-IgE-mediated FPI-GIA.

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Abbreviations

FA: Food Allergies
FPI-GIA: Food Protein-Induced Gastrointestinal Allergies
LAI: Leukocyte Adherence Inhibition
LAIT: Leukocyte Adherence Inhibition Test
LMIT: Leukocyte Migration Inhibition Test
TTP: Tube Titration of Precipitins

1. Introduction

IgE-mediated and non-IgE-mediated Food allergies (FA) are among the most common non-communicable diseases [1, 2]. While IgE-mediated food allergies are easily diagnosed, the management of non-IgE-mediated food allergies still poses significant dilemmas to physicians treating gastrointestinal allergic diseases because of the wide variety of hypersensitivity mechanisms (endotypes) and clinical presentations (phenotypes), which may be involved [3–5].

Food Protein-Induced Gastrointestinal Allergies (FPI-GIA) may present themselves in a diversity of phenotypes, such as food protein-induced proctocolitis, food protein-induced enterocolitis, food protein-induced enteropathy, food protein-induced gastroesophageal reflux disease, eosinophilic esophagitis, eosinophilic gastroenteritis, celiac disease, multiple food protein intolerance of infancy, and food protein-induced gastrointestinal motility disorders [6, 7]. Sometimes, gastrointestinal food allergies may be produced by IgE-dependent mechanisms; however, most cases are produced by hypersensitive endotypes not mediated by IgE, and sometimes, mixed endotypes may take part in the production of the clinical conditions [8]. The FPI-GIA may manifest as an individual phenotype, or sometimes as a combination of associated overlapping phenotypes, through mild, moderate, or severe symptoms [9].

Gastrointestinal disorders may be produced by ingesting inedible vegetables such as raw legumes, a nutritional barrier transposed when humanity learned to use fire to cook foods and initiated the domestication of bean crops [10, 11]

Modern cultivated soybean (Glycine max) crops were believed to have been domesticated from wild soybeans in East Asia about six to nine thousand years ago [12]. Similarly, mitochondrial restriction fragment length polymorphism studies suggest that the domestication of the wild bean (Phaseolus vulgaris) first occurred in the Peru-Bolivia Andean mountains [13].

Lectins were one of the first food elements recognized as responsible for producing gastrointestinal disorders [14].

Legumes such as soybeans (Glycine max) and red beans (Phaseolus vulgaris) contain prominent levels of lectins in the raw state. They significantly lose their lectin content by soaking and cooking [15]. Lectins from red beans and soybeans can be inactivated by heating presoaked beans at $100^{\circ}C$ for 15 min or at $80^{\circ}C$ for 2 hours or by pressure cooking (15 psi) for 45 minutes.

Lectins are a generic class of proteins (or glycoproteins) present in high concentrations in leguminous seeds, which share the capacity of reversibly binding with specific carbohydrates or carbohydrate residues [16]. Lectins on the cell surface act as recognition molecules binding to carbohydrate structures on other cells, mediating cell-to-cell interactions, playing essential roles in innate immunity for infection defense, and cell cycle regulation [17].

Lectins found in Glycine max and Phaseolus vulgaris beans have an affinity for specific sugar groups (particularly components of other molecules and cell membranes), prone to cause agglutination and precipitation of cells, glycoconjugates, and polysaccharides [18].

Soybean lectin (also known as soybean agglutinin) is a tetrameric-specific N-acetyl-galactosamine/galactose binding lectin considered the main responsible for the deleterious effects of raw soybean ingestion [19].Phaseolus vulgaris lectins found in red kidney beans are present in two main types: A) Erythroagglutinin (because it agglutinates red blood cells), also known as Phytohemagglutinin E (PHA-E), and B) Leucoagglutinin (because it stimulates lymphocyte proliferation), also known as Phytohemagglutinin L (PHA-L) [20]. Phytohemagglutinins are long-term known to have a stimulatory effect on humoral and cell-mediated immunoreactivity [21, 22].

Besides lectins, leguminous seeds produce several conspicuous proteins that can produce antibody-mediated immune responses.

The Allergen Nomenclature Sub-Committee of the World Health Organization and International Union of Immunological Societies (WHO/IUIS) has recognized so far eight allergens weighing from 7 KDa to 76 KDa, identified from the soybean (Glycine max) [23].

The Allergen Nomenclature Sub-Committee of the World Health Organization and International Union of Immunological Societies (WHO/IUIS) has recognized so far one allergen (with one variant) from the common bean (Phaseolus vulgaris) [24].

Besides the well-characterized IgE-specific allergens (and the lectins), many other proteins can produce immune reactions. When studying the potential epitope motifs in soybean proteins, German scientists validated eighty-three soybean peptides by microarray analysis using sera from people allergic to soybeans. Among these, 55 were bound by serum IgE, 43 by serum IgG, and 30 by both, with several individual-specific epitope patterns [25].

Humoral immunoreactivity against food allergens and aeroallergens has been classically evaluated by research on precipitins [26–30]. The semi-quantitative titration of precipitins is a pioneering lab exam that laid the fundamental basis of immunology [31]. Precipitating antibodies suggest the presence of a humoral immune response against the tested antigens [32]. Before the discovery of IgE, the research on precipitins was the leading way to realize in vitro diagnosis of immunoreactivity against allergenic agents [33]

We routinely employ the Tube Research of Precipitins (TTP) in our facilities as a triage to evaluate humoral non–IgE-mediated immunoreactivity against suspected allergens before performing more exhaustive in vivo provocation tests [34–38].

The Leukocyte Adherence Inhibition Test (LAIT) and its similar assay, the Leukocyte Migration Inhibition Test (LMIT), have also classically been used to differentiate non–IgE-mediated immunoreactivity against food allergens [39–43].

Non–IgE-mediated cellular immunoreactivity against food allergens had also been reported by our group with the help of the LAIT [44–46]. We also routinely employ the LAIT in our facilities as a triage to evaluate non–IgE-mediated immunoreactivity against suspected allergens before performing more exhaustive in vivo provocation tests [47–50].

To evaluate the potential of the LAIT and TTP to endotyping Non–IgE-mediated cellular and humoral immunoreactivity against soybean and red bean extract, we retrospectively compiled the electronic medical charts of patients diagnosed with non–IgE-mediated FIP-GIA who were investigated for immunoreactivity simultaneously by one of these assays.

The present study is a proof-of-concept that hypothesizes that LAIT and the TTP may demonstrate a correlation between cellular and/or humoral immunoreactivity against soybean and red bean proteins in patients suffering from non–IgE-mediated FIP-GIA.

2. Materials and Methods

2.1. Subjects

After receiving Institutional Review Board approval from the Instituto Alergoimuno de Americana (Brazil; 06/2025), we reviewed the electronic chart of 10.700 outpatients who attended our facility from January 2018 to August 2025.

A cohort of 100 consecutive outside patients (TTP cohort) had been submitted to TTP simultaneously with soybean and red bean extracts for presenting non–IgE-mediated FPI-GIA. This cohort counted 27 males; mean age 37.4 years; the standard deviation was 21.8 years; range 1 to 88 years; median 37.5 years; modes = 36 (appeared five times); geometric mean = 26.8 years.

A cohort of 100 consecutive outside patients (LAIT cohort) had been submitted to LAIT simultaneously with soybean and red bean extracts for presenting non–IgE-mediated FPI-GIA. This cohort counted 34 males; mean age 38.8 years; the standard deviation was 21 years; range 1 to 84 years; median 37 years; modes = 36 (appeared five times); geometric mean = 30 years.

This study did not include patients under biological and/or systemic anti-inflammatory therapy. These procedures were offered to patients with FPI-GIA with clinical suspicion of soybean and/or red bean hypersensitivity who demonstrated a non-reactive or inconclusive skin test against the extracts of these allergens [51].

2.2. Extracts

Red bean extract

Red beans (200g) acquired from the local market were presoaked overnight. Half of the grains were cooked for 10 minutes in a pressure cooker, and half were not. After they were assembled, they were crushed, homogenized, and left for 48 hours in a Coca-based extractor solution (propylparaben 0.5g, methylparaben 1g, sorbitol 30g, NaCl 5g, $NaHCO_3$ 2.5g, 1,000mL H_2O) at $4°C$ for protein extraction before centrifugation and separation of the water-soluble fraction from solid particles and oily fraction [52]. The protein quantification of the allergen extracts was done according to Bradford's protein-dye binding methodology [53]. The solution was diluted in an antigen dilution solution (NaCl 10g; KH_2PO_4 0.72g; Na_3PO_4 2.86g; methylparaben 1g; propylparaben 0.5g; glycerin 400mL; H_2O 600mL) to an estimated protein concentration of 1 mg/mL and stored at $4°C$ in amber opaque glass vials. The extracted solution was used to perform the allergic skin tests, TTP, and LAIT. All relevant and mandatory laboratory health and safety measures have been complied with during the experiments.

Soybean extract

Soybeans (200g) acquired from the local market were prepared analogously for red bean extract.

2.3. LAIT: Ex vivo Investigation: Leukocyte Adherence Inhibition Test

LAIT: Procedure for allergen ex vivo challenging

We performed the LAIT as previously described [54–58]. Shortly, each donor's fresh plasma was divided into two parts and used in parallel ex vivo challenging tests with the allergen extract and the unchallenged plasma (added with antigen dilution solution as a control). We collected plasma with high leukocyte content (buffy coat) from the heparinized tube after one hour of sedimentation at $37°C$. Then, we distributed aliquots of 100 μL into Eppendorf tubes with (or without) the challenging extract and kept them under agitation for 30 minutes (200 rpm at $37°C$).

LAIT: Procedure for adherence assay

After incubation, the challenged 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, we counted the leukocytes, removed the coverslip, and washed the chamber by immersion in a beaker with phosphate buffer saline (PBS) at $37°C$. Then, we added a drop of PBS to the hemocytometer's chamber and placed a clean coverslip over it. The remaining cells were counted in the same squares as previously examined.

LAIT: Procedure for calculation

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 estimated based on the ratio between the LA from the antigen-specific challenged plasma and the LA from the unchallenged control plasma: LAR = LA of the challenged sample divided by LA of unchallenged control plasma multiplied by 100 (%). To further calculate the Leukocyte Adherence Inhibition (LAI), we subtracted the LAR from 100 (%). We employed the LAI results for the cascade distribution chart and the statistics calculations, both performed with the help of the Microsoft Excel® statistical package.

2.4. TTP: In vitro Investigation: Tube Titration of Precipitins

As previously reported, the semi-quantitative TTP was performed in a transparent vitreous tube array [59]. Shortly, the patient's blood was collected in a clot-activator collecting tube. After separation, the serum was centrifuged at 2,000 rpm for 10 minutes. Each allergen extract was allocated in sets of eleven glass tubes at progressively duplicated serum dilutions. The progressive dilutions were combined with separated aliquots of 15 μL of the allergen extract with 250 μL of the patient's serum, progressively diluted into physiological saline solution (NaCl 0,9%) in the dilution ratios of 1:1; 1:2; 1:4; 1:8; 1:16; 1:32; 1:64; 1:128; 1:256; and 1:512. One tube was a blank control done with water and serum to observe occasional spontaneous precipitation (Sia Test). After 24 hours, the tubes were examined, and the titers (the highest dilution factor that yields a positive reading) were recorded [60].

2.5. Statistical analyses

The results of each group were presented individually in cascade distribution graphs, and correlation graphs were compared with the paired analyses. The Pearson correlation test was applied between them. The coefficient (r) is considered weak when r = 0.2 to 0.5, moderate when r = 0.5 to 0.7, and strong when r = 0.7 to 1. A value close to 0 indicates no correlation.

3. Results

As a retrospective survey, there was no research protocol; therefore, we report the incidental immune investigation as registered in the digital medical charts.

TTP for the soybean extract showed a distribution concentrated on the higher dilutions Figure 1. There was no negative result. The titers varied from 1:32 to 1:512. The mean was estimated at 1:290; the median was 1:256; the standard deviation was estimated at 1:177; the mode was 1:512 (appeared 36 times).

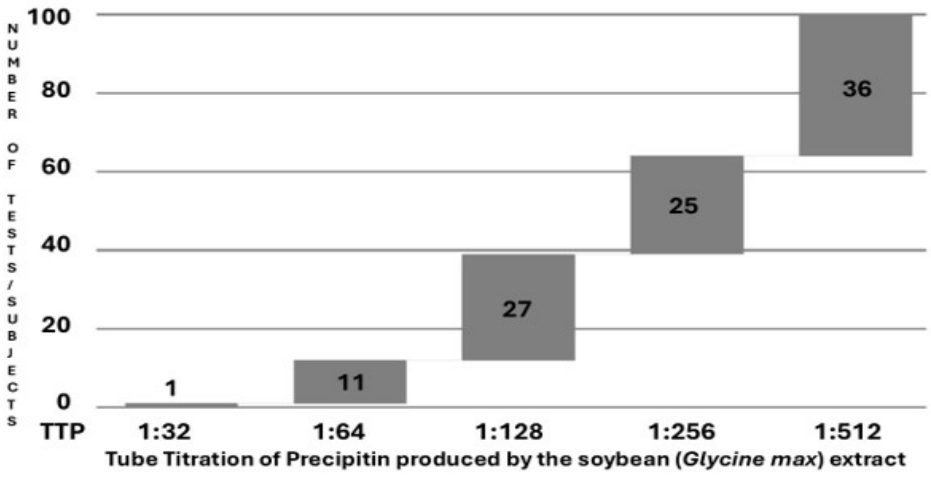

Figure 1: Cascade distribution chart of the tube titration of precipitins (x-axis %) resulting from the soybean (Glycine max) extract against the serum of the TTP cohort of 100 tests/subjects (y-axis)

TTP for the red bean extract showed a distribution concentrated on the higher dilutions Figure 2. There was no negative result. The titers varied from 1:32 to 1:512. The mean was estimated at 1:280; the median was 1:256; the standard deviation was estimated at 1:176; the mode was 1:512 (appeared 33 times).

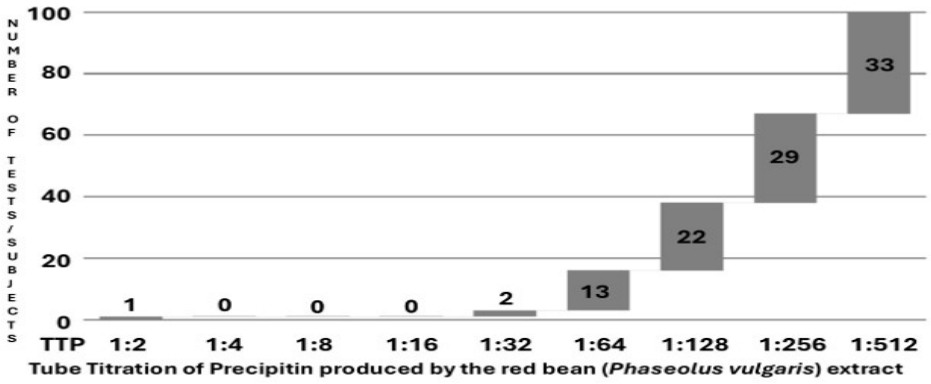

Figure 2: Cascade distribution chart of the tube titration of precipitins (x-axis %) resulting from the red bean (Phaseolus vulgaris) extract against the serum of the TTP cohort of 100 tests/subjects (y-axis)

The paired t-test indicated a non-significant difference between the TTP results of soybean and red bean extracts (p-value = 0.655). The Pearson correlation indicated a significantly weak positive relationship between TTP results of soybean and red bean extracts; r(98) = 0.228, p = 0.022. See Figure 3.

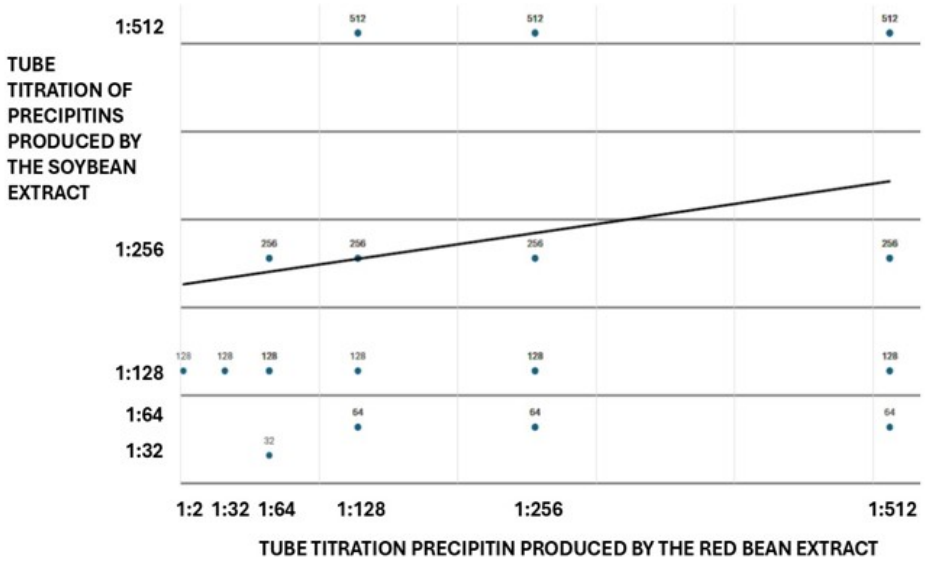

Figure 3: Dispersion chart of the Tube Titration of Precipitins (TTP) results against red bean (Phaseolus vulgaris) extract (x-axis %), plotted against the TTP results against soybean (Glycine max) extract (y-axis%)

The LAIT for the soybean extract showed a wide distribution range of results. There were eleven negative results. The LAI ranged from 0% to 99%. The mean was 60%; the median was 71%; the standard deviation was 32.6%; the mode was 0% (appeared eleven times). The cascade distribution demonstrates a wide range of LAI results Figure 4. Some patients showed low or moderate immunoreactivity during the ex vivo challenge test. In contrast, others displayed strong immunoreactivity, which could reflect the participation of soybean allergens in a Non–IgE-mediated hypersensitivity condition in these patients.

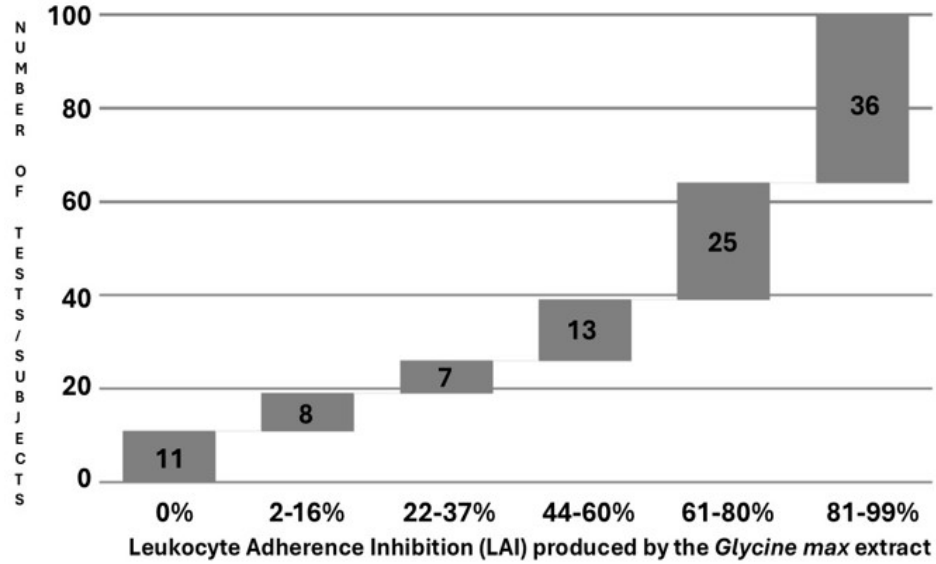

Figure 4: Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition (LAI) results (x-axis %) of the ex vivo challenge test against soybean (Glycine max) monitored by the Leukocyte Adherence Inhibition Test (LAIT), according to the respective number of outcomes over the LAIT cohort with 100 tests/subjects (y-axis)

The LAIT for the red bean extract showed a wide distribution range of results. There were eight negative results. The LAI ranged from 0% to 99%. The mean was 53.5%; the median was 51.5%; the standard deviation was 29.8%; the mode was 0% (appeared eight times). The cascade distribution demonstrates a wide range of LAI results Figure 5. Some patients showed low or moderate immunoreactivity during the ex vivo challenge test. In contrast, others displayed strong immunoreactivity, which could reflect the participation of red bean allergens in a Non–IgE-mediated hypersensitivity condition in these patients.

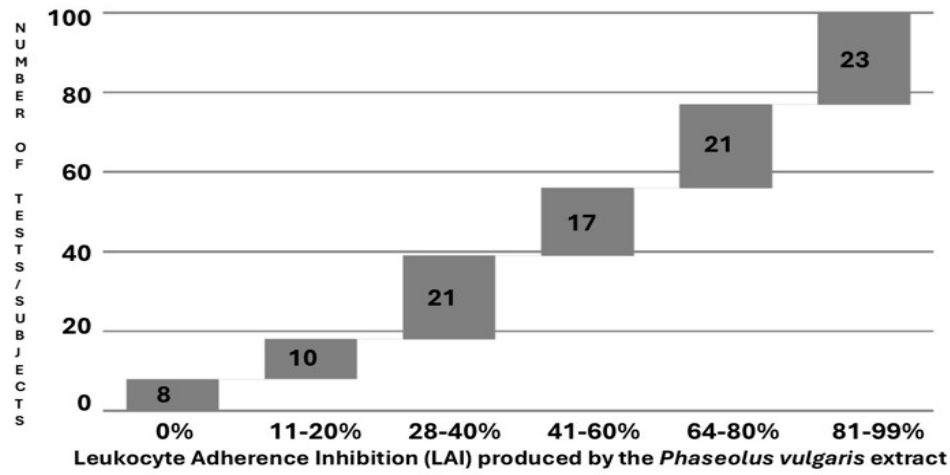

Figure 5: Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition (LAI) results (x-axis %) of the ex vivo challenge test against red bean extract (Phaseolus vulgaris) monitored by the Leukocyte Adherence Inhibition Test (LAIT), according to the respective number of outcomes over the LAIT cohort with 100 tests/subjects (y-axis)

The paired t-test indicated a non-significant difference between the LAIT results of soybean and red bean extracts (p-value = 0.74). Pearson's correlation indicated a significant moderate positive relationship between LAIT results of soybean and red bean extracts, $r(98) = 0.314$, $p = 0.001$. See Figure 6.

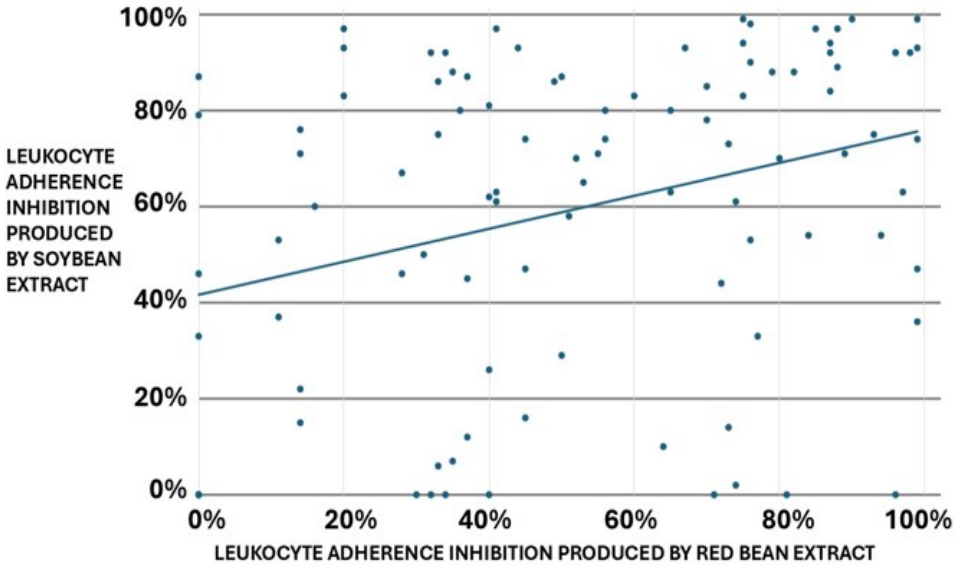

Figure 6: Dispersion chart of the Leukocyte Adherence Inhibition (LAI) results of the ex vivo challenge test against soybean (Glycine max) extract (y-axis %), plotted against the LAI results of the ex vivo challenge test against red bean (Phaseolus vulgaris) extract (x-axis %)

4. Discussion

Gastrointestinal symptoms can be caused by various diseases of the most varied natures. They should preferably be initially addressed by gastroenterologists skilled in differential diagnosis by performing imaging tests to exclude severe and potentially fatal diseases such as malignant tumors, ulcers, and infections [61]. Typically, when initial imagery and laboratory tests do not demonstrate evidence of these kinds of diseases, the patients are referred to an allergist. Sometimes, before the allergologist's appointment, they have consulted a nutritionist, who invariably suggests empirical exclusion diets, trying to identify, through home provocation tests, a food culprit for their symptoms [62].

Provocation tests, whether in vivo Oral Challenge Tests or the ex vivo LAIT, do not necessarily diagnose immune hypersensitivity. They would rather demonstrate tolerance or a hyperergic response. Hyperergic responses are not necessarily due to any described hypersensitivity mechanism (allergic) or immune reactions. Intolerance reactions such as lactose intolerance, or glutamate intolerance may not be immune, despite their hyperergic characteristics [63, 64]. Hyperergic reactions elicited by cross-reactivity among gluten proteins and mucosal enzymes are diversified and sometimes classified as autoimmunity and immune hypersensitivity mechanisms [65, 66].

Cross-reactivity may be an undesirable property for antibodies, since specificity and affinity are compromised in cross-reactive immunoglobulins [67]. Immunological cross-reactivity is responsible for antigenic mimicry, producing transient allergic reactions to debilitating autoimmune diseases [68]. Celiac disease is a classic example where cross-reactivity may produce both allergic reactions and autoimmune conditions [69]. However, when dealing with highly mutagenic viruses, cross-reactivity may be advantageous to the host when fighting viruses with similar antigenic expressions [70].

Some patients already have a clear idea of the foods associated with their symptoms, some patients have suspicions, and others have no idea of the culprits, mainly because sometimes a given food produces symptoms and sometimes does not. Sometimes, the symptoms appear after a threshold is passed [71]. Sometimes, symptoms appear when associated with cofactors [72–76].

Lectins are unspecific ligands that may interact with glycoproteins, moieties of membrane receptors on immune cells (C-type lectin receptors), producing unexpected activation, non-IgE-mediated immune responses, inflammation, and, sometimes, annoying symptoms [77].

Lectins can be particularly blocked by their ligand. If a meal with a high content of lectins is accompanied by food high in carbohydrates that bind to the dietary lectin, the chance for this lectin to produce immune activation and inflammation is reduced [78]. In our region, people have a high consumption of red beans; however, the red bean is usually accompanied by rice, a food with high carbohydrate content, which could be a dietary explanation for the high tolerance to red beans among Brazilians. The combination classically called “rice and beans” (or “arroz-feijão” in Brazilian Portuguese) is a popular food base in our country, considered by Brazilian nutritionists as a ”perfect pairing” due to its complementarity in essential amino acids [79].

The lack of evidence of IgE-specific antibodies against these lectins has prompted a legitimate pursuit to determine the utility of the specific IgG to diagnose the non–IgE-mediated hypersensitivity conditions [80]. The research on specific IgG against food antigens is yet controversial since the IgG may also act as an allergen blocker, depending on its subclass and the participation of the other immune players [81]. IgG antibodies can participate in type II (antibody-dependent cell-mediated) and type III (immune complex disease) Gell and Coombs hypersensitivity reactions, which may be demonstrated by the LAIT and the TTP assays, respectively [56]. So, the gold standard for diagnosing food allergy is the oral food challenge test [82]. Demonstrating immunoreactivity against environmental and food allergens is crucial for prescribing exclusion diets and the development of allergen-specific and allergoid desensitization, especially in polysensitized patients [83, 84].

The LAIT is an ex vivo challenge test that facilitates the interaction of all immune-circulating participants with the allergens, performed with a viable leukocyte buffy coat that can theoretically explore most known immune pathways, as it allows the interaction of all immune-circulating participants with the allergens [56]. It is not pathway-specific since activation of several immune pathways inhibits leukocyte adherence [85–88].

The present study is a proof-of-concept that hypothesizes that LAIT and the TTP may differentiate diverse degrees of cellular and humoral immunoreactivity against soybean and red bean allergens among patients suffering from non–IgE-mediated FIP-GIA. As the tests were performed simultaneously with the same venous sample with the two allergens, it was possible to calculate a correlation test to distinguish some order of cross-reactivity between them.

The retrospective compilation of our data showed a large distribution of results when we ascertained the results of TTP and LAIT to explore humoral and cellular immunoreactivity against the two tested allergens. These immunoassays did not precisely identify the mechanisms responsible for the clinical condition. Instead, they provide evidence about cellular and humoral immunoreactivity distributed into an extensive spectral range that may suggest immune tolerance or hypersensitivity.

This preliminary retrospective survey demonstrated extensive results from the TTP, and the ex vivo challenge test monitored by LAIT against soybean and fed bean allergens in two cohorts of non–IgE-mediated food allergic patients. TTP and LAIT are complementary triage tests used at our facilities to select worthwhile antigens to proceed with more laborious in vivo provocation tests when the specific IgE is undetectable. None of our patients presented an exclusive reaction to these allergens. Every patient was simultaneously tested for several chemical and biological allergens, demonstrating positive results for some of them. Our results suggest that soybean allergic patients may experience worsening symptoms due to additional cross-immunoreactivity against red bean allergens and vice versa.

5. Limitations

This study is a retrospective analysis of data collected over seven years. There was no protocol research, and the subject’s data was limited to the essentials available on our electronic sheets. Therefore, we could not establish a cross-comparison between positive and negative controls to validate the results. The number of subjects is appropriate for a preliminary study; however, future studies must be more comprehensive. The lack of a research protocol implies the possibility of a bias produced by the point of view of the physician who indicated the exam (CEO) based on a clinical suspicion led purely by the anamnesis and physical examination. The study lost many of these patients to follow-up, so assuring the relationship between the immunoassay results and the patient’s clinical outcome is not possible yet. Unfortunately, it was impossible to compare the two procedures with paired tests because they were taken from distinct groups of patients.

6. Conclusion

Our preliminary results support that the TTP and LAIT performed with soybean and red bean extracts may differentiate diverse humoral and cellular immunoreactivity degrees, as well as demonstrating a significant correlation between the humoral and cellular immunoreactivity between the two extracts in patients suffering from Non-IgE-mediated FPI-GIA. LAIT and TTP are inexpensive, can be performed with minimum laboratory equipment, and can be incorporated into strategies to address health disparities in respiratory and food allergy [89]. As a preliminary report, the propaedeutic meaning of the presented results and the possibility of interferents must be yet established [90]. More studies focused on the quality-by-design approach with larger prospective double-blind cohorts need to evaluate the potential contribution of LAIT and TTP for endotyping cellular and humoral immunoreactivity in patients with hyperergic responses against soybean and red bean allergens [91].

7. Future Directions and Recommendations For Clinical Practice

The primary intended use of in vitro or ex vivo allergen challenge tests is to spare the patients from being submitted to unnecessary, exhaustive, and dangerous in vivo challenge tests. Exploring the humoral and the cellular arms of immune systems, the TTP and LAIT alone or combined may represent, in the near future, a tool for allergists to construct an etiologic diagnosis from their patients, as well as determine the endotypes (mechanisms) of hypersensitivity, in order to choose more convenient and personalized therapies for them. Adding data provided by TTP and LAIT may also contribute to streamlining biomedical research and improving tools such as Large Language

Models, usually used by clinicians as a decision support system to enhance diagnostic accuracy [92].

Article Information

Consent: As a retrospective survey of results recorded in cognito, consent was given collectively by the institution's ethics committee following the principles of the Declaration of Helsinki [93].

Ethical Approvals: The authors have collected and preserved written ethical approval per international standards.

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Author's Contributions: The authors conducted this work in collaboration. The author CEO is responsible for the conceptualization, data curation, formal analysis, literature review, and writing the original draft. Authors DGP, APMT, CSM, JLSS, and RPSL performed laboratory procedures. Author RAPGS performed cutaneous tests.

Competing Interests: The authors have declared that no competing interests exist.

Disclaimer (Artificial Intelligence): Authors hereby declare that NO generative AI technologies, such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators, have been used during the writing or editing of this manuscript.

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