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Anti-*Saccharomyces cerevisiae* Antibodies (ASCA) Researched by Tube Precipitins are Elevated in Patients with Dermatologic and Gastrointestinal Non-Ige-Mediated Hypersensitivity

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ABSTRACT

Background: **The presence of anti-*Saccharomyces cerevisiae* antibodies (ASCA) has been reported in the serum of patients with several immune-inflammatory diseases.**

Objective: **To evaluate the presence of ASCA in patients with non-IgE-mediated hypersensitivity.**

Methods: **A group of 222 patients with non-IgE-mediated hypersensitivity was divided into three groups according to dermatologic, gastrointestinal, and respiratory symptoms. Group D was composed of 163 patients with dermatologic symptoms diagnosed as intrinsic atopic dermatitis and/or non-IgE-mediated urticaria. Group G was composed of 23 patients diagnosed with non-IgE-mediated gastrointestinal food allergies. Group R was composed of 36 patients with respiratory symptoms diagnosed as non-IgE-mediated rhinitis and/or non-IgE-mediated asthma.**

Results: **The Wilcoxon-Mann-Whitney U test comparing the precipitin's titers of group G and group D showed a non-significant p-value of 0.83366. The Wilcoxon-Mann-Whitney U test comparing the precipitin's titers of group R and group G showed a significant p-value of 0.00034. The Wilcoxon-Mann-Whitney U test comparing the precipitin's titers of group R and group D showed a significant p-value < 0.0001.**

***Conclusion*: The patients with respiratory symptoms diagnosed as non-IgE-mediated rhinitis and/or asthma presented significantly less humoral immunoreactivity against *S. cerevisiae* than patients with non-IgE-mediated food allergy and patients with intrinsic atopic dermatitis and/or non-IgE-mediated urticaria. The elevation of ASCA titers may be an unspecific marker of intestinal hyperpermeability, and possibly may participate in Gell and Coomb's types II and/or type III hypersensitivity reactions responsible for the patient's dermatologic and gastrointestinal symptoms.**

Keywords: Allergy, antigen-antibody complex, atopic dermatitis, hypersensitivity, immune complex diseases, non-IgE-mediated hypersensitivity, precipitins, precipitins tests, *Saccharomyces cerevisiae*; urticaria.

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I. Introduction

Humans have evolved and co-adapted to fungi over millions of years. Several fungi are commensal, and several are used for nourishment. There is evidence, since the Neolithic era, that yeasts of the Saccharomyces *sensu stricto* complex, have been used to ferment cereals, honey, and fruits to produce bread, beer, sake, mead, cider, and wine [1], [2]. The fermentation may occur in the presence or absence of oxygen. The anaerobic fermentation produces ethanol. Aerobic fermentation reduces the ethanol to carbon dioxide, which is referred to as the Crabtree effect [3]. Ethanol has been pursued by mankind for its recreational euphoric effects, chemical properties, and medicinal purposes [4], [5]. Carbon dioxide has been sought after by cookers mainly to raise dough [6]. Until the appearance of optical microscopy and sterilization techniques, the fermentation "starts" were a mix of yeasts and bacteria (mainly of the *Lactobacillus* genus, which originated the sourdough by co-producing lactic acid) [7]. In the late 1800s, after the appearance of laboratory techniques to cultivate pure cultures, *Saccharomyces cerevisiae* begin to be produced on an industrial scale and chosen as the "baker's and brewer's yeast" [8]. The presence of fungi in the luminal sector of the mammalian intestine is considered physiologic. As well the bacterial microbiome, there is an equivalent mycobiome, which, as well as its counterpart, may exert a protective effect against inflammatory agents [9]. *S. cerevisiae* is a live commensal of

the human intestine that usually exerts a benefic effect by positively stimulating the mucosal immune cells [10]. Fecal samples of psoriatic patients demonstrated depletion of *S. cerevisiae* abundance when compared with control individuals [11]. Like any living organism, *S. cerevisiae* possesses a metabolic activity that may eventually disturb the host, such as the production of benzoic acid, from the catabolism of biphenyl, a fungistatic applied on citrus fruits [12]. Benzoic acid is a compound associated with hypersensitivity reactions in allergic individuals [13]. Fungi rarely produce diseases in healthy humans. The main conditions associated with diseases produced by fungi are immunodeficiencies and allergies. The primary immune responses elicited by fungi are innate. Fungal cell walls expose specific polysaccharides (glycans, glycoproteins, and glycolipids) not found in mammals, collectively named Pathogen-Associated Molecular Patterns (PAMPs). PAMPs are recognized by cognate molecules, collectively named Pattern Recognition Receptors (PRRs) responsible for the activation of innate immune responses [14]. The first discovered and better-studied PRR evolved in the innate immune activity against fungi is the transmembrane signaling receptor Dectin-1. Dectin-1 mediates fungal binding, uptake, and killing, associated with the production of several cytokines and chemokines. First discovered in dendritic cells, Dectin-1 was further found to be expressed not only by innate immune cells, such as macrophages, monocytes, neutrophils, mast cells, epithelial cells, and keratinocytes but also in adaptive immune cells such as B cells and T cells [15]. The PAMP recognized by Dectin-1 (and several other PRRs) is the fungi membrane-associated β-glucan [16]. Mannan Binding Lectin (MBL) is a liver-produced serum circulating PRR that acts as an opsonin, labeling the fungi cellular wall and activating the lectin pathway of the Complement system. It is thought that the MBL deficiency is associated with the production of anti-*Saccharomyces cerevisiae* antibodies (ASCA) associated with several inflammatory diseases, such as Inflammatory Bowel Diseases (IBDs-comprised of Crohn's Disease and Ulcerative Colitis), intestinal Behcet's syndrome, and Intestinal Tuberculosis [17]-[22]. Further, several autoimmune diseases have also been associated with the presence of ASCA, as well as the accuracy of the immunoassays employed to research them [23]-[25]. IgE-mediated sensitization against *S. cerevisiae* was first described in 1995 in patients with Atopic Dermatitis (AD) by German allergologists from Leipzig University [26]. Later, in 2001, this same group also described IgG-mediated sensitization against *S. cerevisiae* in their group of AD patients [27]. Now a day, the research of sensitization to fungal allergens is an elaborated practice that permeates all fields of allergology [28]. Innate immunity usually restricts the commensal fungi to the external environment. When the innate immune system fails to block the entrance of fungi into the internal environment, as happens in some disease states, the adaptive response is also activated, and consequently, anti-fungi antibodies appear in the serum of allergic and/or immunocompromised patients [29]. Allergic sensitization is a condition that facilitates the invasion of pathogenic fungi, as noted by the use of serologic tests identifying IgG and IgE-specific antibodies against molds because it impairs an effective immune response due to the excessive inflammatory status [30]. There are several methods to research antibodies to diagnose immune responses against fungi. Some methods, by their complexity, are only available in reference laboratories. The more simplified method described in the literature, easily performed in the dependencies of a local clinic, is the tube precipitins research, which can act as a triage test, before the more sophisticated studies [31]-[33]. As a proof-of-concept, we performed at our primary medical facility the research of tube precipitins against a locally prepared *S. cerevisiae* extract, in serum collected from patients diagnosed with diverse allergic conditions.

II. Methods

A. Subjects

After receiving Institutional Review Board approval, from the Instituto Alergoimuno de Americana (Brazil), a group of 222 outpatients (75 male; 18-90 years old; mean age = 45.58 years, SD =16.47 years), were invited to voluntarily provide blood samples to perform *in vitro S. cerevisiae* precipitation titrations, according to the principles of the World Medical Association Declaration of Helsinki and the International Committee of Medical Journals Editors requirements of privacy [34]. The individuals were divided into three broad groups, according to their major symptoms, as follows. Group D was composed of 163 patients with dermatologic symptoms diagnosed as intrinsic atopic dermatitis and/or non-IgE-mediated urticaria (53 male; 18-90 years old; mean age = 46.5 years, SD =16.0 years). Group G was composed of 23 patients diagnosed with non-IgE-mediated gastrointestinal food allergies (5 male; 21-77 years old; mean age = 43.9 years, SD =15.0 years). All patients in group G had been previously submitted to gastrointestinal endoscopies and colonoscopies that had found no abnormality. Group R was composed of 36 patients with respiratory symptoms diagnosed as non-IgE-mediated rhinitis and/or non-IgE-mediated asthma (17 male; 18-87 years old; mean age = 42.1 years, SD =18.4 years). The diagnosis of these conditions was done according to our previous publications [35]-[40]. All individuals had normal-range total IgE, non-detectable *S. cerevisiae* specific-IgE, and non-reactive skin tests against the *S. cerevisiae* extract. None of the patients had a history or evidence of any rheumatologic auto-immune condition. The study was descriptive, and retrospective, 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 been complied with within the complete course of the experiments.

B. Preparation of the S. cerevisiae Extract

In a beaker were added 50 mg of *S. cerevisiae* (purchased in powder form from a local food merchant) and 25 mL of extracting solution (Propylparaben 0.5 g; Methylparaben 1g; Sorbitol 30 g; NaCl 5 g; $NaHCO_3$ 2.5 g; 1,000 mL H_2O). The sample was homogenized and then left for 48 hours at 4 °C for protein extraction. After 48 hours, the supernatant was diluted in 25 mL of antigen dilution solution (NaCl 10 g; KH_2PO_4 0.72 g; Na_3PO_4 2.86 g; methylparaben 1 g; propylparaben 0.5 g; glycerin 400 mL; H_2O 600 mL). This

solution was used for the research of precipitins and the immediate skin tests, done as previously reported [41].

C. Tube Precipitins in transparent solution

The semi-quantitative tube titration of precipitins against the *S. cerevisiae* extract in a transparent solution was performed as previously reported [35]. Shortly, the patient's blood was collected in a clot-activator collecting tube. After serum separation, the tube was centrifugated at 2,000 rpm for 10 minutes. The allergen extracts were allocated in sets of nine glass tubes at progressive duplicated serum dilutions. The progressive dilutions were combined with the 15 µL of the antigen (1 mg/mL) 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; and 1:128. The first tube was a blank control done just with the serum to observe occasional spontaneous precipitation (cryoglobulins). After 24 hours, the tubes were examined by one of us and the titers (the highest dilution factor that yields a positive reading) were recorded [42].

D. Graphic Presentation of Data and Statistics

The smallest and largest precipitin's titer, the median, the mode, the mean titer, and the standard deviation (SD) were calculated for each group. Three 3D graphs were constructed to allow an overview and comparison of the distribution of the percentages of each precipitin's titers inside each group. The data of the independent groups were compared by the non-parametric Wilcoxon-Mann-Whitney U test [43], [44]. The comparisons were considered significant when the p-value was < 0.001.

III. Results

The estimated mean precipitin's titer of Group D was 1:81 (range: negative to 1:128; median = 1:128; mode = 1:128 with 93 appearances; SD = 1:55).

The estimated mean precipitin's titer of Group G was 1:85 (range: negative to 1:128; median = 1:128; mode = 1:128 with 12 appearances; SD = 1:48).

The estimated mean precipitin's titer of Group R was 1:35 (range: negative to 1:128; median = 1:8; mode = negative with 8 appearances; SD = 1:48).

The percentage of each precipitin's titer in each group is presented in Fig. 1 (group D); Fig. 2 (group G); and Fig. 3 (group R).

The two-tailed Wilcoxon-Mann-Whitney U test comparing the precipitin's titers of group G and group D showed a distribution approximately normal. The value of U was 1823. The z-score was -0.211. The non-significant p-value was 0.83366.

The two-tailed Wilcoxon-Mann-Whitney U test comparing the precipitin's titers of group R and group G showed a distribution approximately normal. The value of U was 183. The z-score was -3.58237. The significant p-value was 0.00034.

The two-tailed Wilcoxon-Mann-Whitney U test comparing the precipitin's titers of group R and group D showed a distribution approximately normal. The value of U was 1529. The z-score was 4.4911. The significant p-value was < 0.0001.

IV. Discussion

When professor Hulusi Behçet, from the Faculty of Istanbul, first described their patients with the triple complex syndrome, in 1937, his first theory was that it probably was an infectious disease [45]-[47]. He described in his papers, how difficult was for dermatologists and ophthalmologists to understand a connection between oral and genital ulcerations with diverse ocular inflammatory conditions described as iritis, iridocyclitis, neuritis, retinitis, neuroretinitis, and/or conjunctivitis. The observation that some patients also presented arthritis and erythema nodosum, further complicated the clinical interpretation of the patient's condition. This "soup" of symptoms was further grouped under the name of Behçet's syndrome, and, since then, medical scientists have searched for a common explanation for the combination of these conditions. Despite being named after Behçet, this unexpected symptoms association had already been described by Hippocrates (5th century BC) [48]. Later, it was also noted that a subset of these patients also presented severe gastrointestinal symptoms, which was called "intestinal Behçet'syndrome" [49].

The association of severe intestinal inflammation to a systemic condition such as the Behçet syndrome approached this last condition the "Inflammatory Bowel Diseases" (IBDs), which usually present mainly local digestive symptoms, despite several extra-intestinal manifestations that have been described [50]. Since then, a common immune marker was intensively researched between these conditions. The main serologic link found between these conditions was the ASCA [51]. The presence of ASCA was reported to have a prevalence of about 44% in patients with intestinal Behcet's syndrome, compared with 3% in patients with Behçet's syndrome without intestinal involvement [52].

The participation of the ASCA in the etiology of the inflammation of immune diseases is not yet fully comprehended. The surgical resection of the compromised intestine of patients with Crohn's Disease led to a reduction of ASCA titers and suggested that the production of ASCA was a response to the increased permeability due to a disrupted mucosal barrier [53]. A simple way to figure out the association of antibodies against *S. cerevisiae* in inflammatory and allergic conditions is the ability of the mannoprotein to act as an emulsifier [54]. Emulsifiers, also called surfactants, have been associated with the disruption of the intestinal mucous barrier, with may produce intestinal hyperpermeability [55], [56]. The disruption of the intestinal mucous layer impacts the gut microbiota and enhances the penetration of bacterial toxins, which is associated with colitis, metabolic syndrome, and food allergy [57], [58]. Intestinal hyperpermeability may be a cause and a consequence of immune-inflammatory conditions, such as those observed in food-allergic syndromes [59]. Patients diagnosed with IBD had a significant increase in disease activity when receiving a supplement of *S. cerevisiae* over a low-yeast diet [60]. One must also remember that the ethanol, present in *S. cerevisiae* fermented beverages, also increases intestinal hyperpermeability [61]. Our results showed no significant difference between the group with dermatologic symptoms and the group with gastrointestinal symptoms. However, there was a significant difference between the group with respiratory symptoms and the group with

dermatologic symptoms, as well as a significant difference between the group with respiratory symptoms and gastrointestinal symptoms. If we consider the respiratory group R as a "control group" to groups D and G, since their participants did not present either gastrointestinal or dermatologic complaints, we can appreciate a significant difference between the distribution of the precipitin's titers, between these groups. This suggests that patients with exclusively respiratory symptoms diagnosed as non-IgE-mediated rhinitis and/or non-IgE-mediated asthma present significantly less humoral immunoreactivity against *S. cerevisiae* than patients with non-IgE-mediated food allergy and patients diagnosed with as intrinsic atopic dermatitis and/or non-IgE-mediated urticaria. In this discussion, two types of interpretations cannot be neglected; to wit, the perspective that the presence of ASCA may be an unspecific marker of intestinal hyperpermeability in patients with inflammatory intestinal conditions; and the perspective that ASCA may be contributing to patient's symptoms through the participation in Gell and Coomb's types II and/or type III hypersensitivity reactions by mean of cell-bound antibodies and antigen-antibody complexes, respectively, when the *S. cerevisiae* allergens are been ingested in sufficient amounts [35], [62].

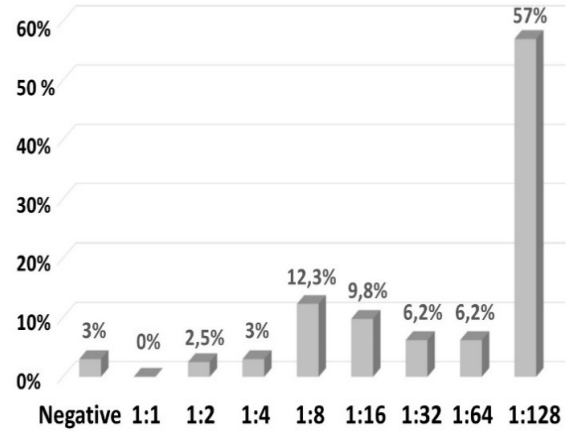

Fig. 1. Column comparison chart distributing percentages of precipitin's titers results of 163 patients with dermatologic symptoms diagnosed as intrinsic atopic dermatitis and/or non-IgE-mediated urticaria (Group D).

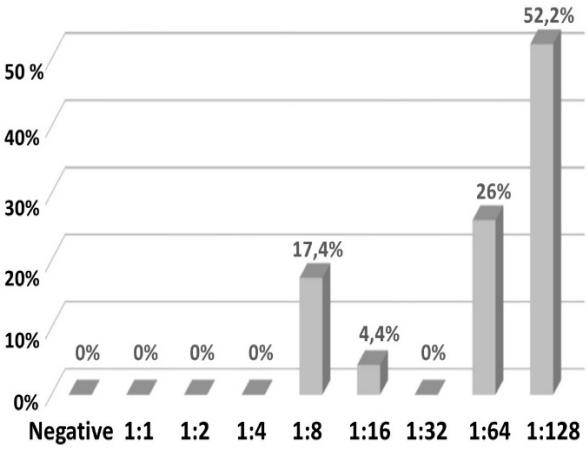

Fig. 2. Column comparison chart distributing percentages of precipitin's titers results composed of 23 patients diagnosed with non-IgE-mediated food allergy (Group G).

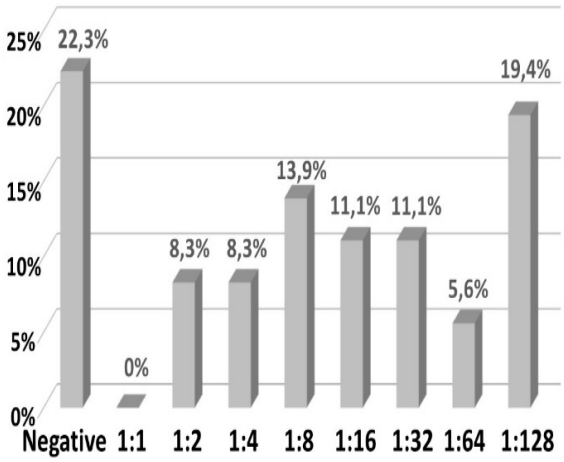

Fig. 3. Column comparison chart distributing percentages of precipitin's titers results of a group of 36 patients with respiratory symptoms diagnosed as non-IgE-mediated rhinitis and/or non-IgE-mediated asthma (Group R).

ABBREVIATIONS

ASCA: Anti-*Saccharomyces cerevisiae* antibodies
IBD: Inflammatory Bowel Diseases
PAMP: Pathogen-Associated Molecular Pattern
PRR: Pattern Recognition Receptor

CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

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