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Contribution of the Leukocyte Adherence Inhibition Test to the Diagnosis of Non–IgE-mediated Immunoreactivity against *Staphylococcus aureus* in Patients with Intrinsic Atopic Dermatitis

Celso Eduardo Olivier [a*], Daiana Guedes Pinto [a], Ana Paula Monezzi Teixeira [a], Jhéssica Letícia Santos Santana [a], Raquel Acácia Pereira Gonçalves Santos [a] and Regiane Patussi Santos Lima [b]

[a] Instituto Alergoimuno de Americana, Brazil.
[b] Lavoisier's Laboratories, São Paulo, Brazil.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**Corresponding author: E-mail: celso@alergoimuno.med.br;*

ABSTRACT

Aims: The study aims to evaluate the potential of the Leukocyte Adherence Inhibition Test (LAIT) to discriminate non–IgE-mediated immunoreactivity against *Staphylococcus aureus* in patients with Intrinsic Atopic Dermatitis (IAD).
Study Design: We retrospectively examined the medical charts of 200 patients diagnosed with IAD who were investigated with an *ex vivo* challenge monitored by LAIT against an extract of *S. aureus.*
Place and Duration of Study: The study was conducted at Instituto Alergoimuno de Americana – São Paulo – Brazil – between January 2018 and October 2023.
Methodology: The percentage of Leukocyte Adherence Inhibition (LAI) promoted by the *ex vivo* challenges with *S. aureus* extract was distributed in ranges through a cascade distribution chart to outline the variability of the results.
Results: The LAI mean was 39.3%; SD 27.6%; ranging from 0% to 100%; mode = 0% (appeared 33 times). There was a wide range of distribution of LAI results, suggesting that some patients had immunoreactivity against the *S. aureus* allergens while others did not.
Conclusion: Our preliminary results support that the LAIT performed with *S. aureus* may differentiate diverse degrees of *ex vivo* immunoreactivity against their allergens in IAD patients.

Keywords: Allergy; Staphylococcus aureus; atopic dermatitis; diagnosis; hypersensitivity; leukocyte adherence inhibition test; non–IgE-mediated immunoreactivity.

1. INTRODUCTION

Atopic Dermatitis (AD) is one of the multiple clinical presentations produced by systemic immune hypersensitivity conditions, sharing common etiologic features with allergic rhinitis, allergic pharyngitis, allergic laryngitis, allergic bronchitis, allergic conjunctivitis, allergic esophagitis, allergic gastroenteritis, and so on [1]. Several known allergens may aggravate AD conditions by IgE-mediated or non–IgE-mediated immune mechanisms [2]. When patients with AD do not present IgE-mediated mechanisms, they are diagnosed with Intrinsic Atopic Dermatitis (IAD) [3]. Microorganisms are among the leading producers of exogenous antigens (allergens) that (locally and systemically) elicit hypersensitivity immune reactions, increasing dermal inflammation and flaring AD symptoms [4]. The skin microbiome of AD patients is remarkably different from that of healthy individuals [5]. The most prevalent human skin-colonizing bacteria belong to the Staphylococci group [6]. Staphylococci are members of the Staphylococcaceae family of bacteria that have a thick layer of peptidoglycan in their cell wall that retains the primary dye crystal violet (not washable by ethanol) at Gram's stain, as seen at optical microscopy with a purple coloration, and so, broadly classified as Gram-positive cocci [7,8]. Among Staphylococcaceae family, *Staphylococcus aureus* is the most dangerous pathogen to humans [9]. Its versatility to evade the human immune system allows this species to produce long-term asymptomatic colonization, developing light to moderate chronic and recurrent infections that, unusually, may turn into severe acute lethal diseases [10]. Therefore, it is classified as a pathobiont, i.e., a microorganism usually found as a commensal in human skin and mucosal surfaces, but that may become pathogenic [11]. Although *S. aureus* may circulate through the blood, the superficial epidermis is a particularly remarkable location to produce damage, mainly through their exfoliative toxins that, specifically cleaving human desmoglein, produce blisters by separation of the keratinocytes of the granular cell layer [12]. Only some strains of *S. aureus* can produce epidermolysins, which can be assessed in broth cultures through flow-cytometry-assisted multiplex immunoassay [13]. Some particularly invasive strains of *S. aureus* may produce proteolytic enzymes that can cleave antibodies, disarming the host's defenses [14]. The final success or failure of *S. aureus* to produce colonization or disease depends on the resultant balance between host defenses, the competing resident microorganisms, the expression of the microbial surface components that allow the adherence to host cells, and its self-downregulation of virulent genes [15]. Usually, the strains of *Staphylococci* that produce infections are the same that colonize the host during disease-free periods [16]. The prolonged evolutionary interplay between humans and Staphylococci developed in these microorganisms a wide variety of mechanisms to evade host defenses and resist antimicrobial peptides produced by human epithelia [17].

These defense mechanisms allow these microorganisms long-term colonization and, sometimes, persistent or recurrent skin infections [18]. The antimicrobial peptides produced by the human epithelia and immune cells usually have cationic properties, i.e., present a surface-active cation that interacts with the usually negatively charged phospholipids of the bacteria's cytoplasmic membrane [19]. These cationic antimicrobial peptides, such as the defensins, the cathelicidins, the thrombocidins, and the eosinophilic cationic protein, are innate immune peptides produced by epithelia, platelets, and immune cells that act directly on bacteria, disrupting their membranes, as well assisting the function of immune cells [20]. Human eosinophilic cationic protein has a particular pore-formation mechanism producing channels at the target membrane that are relatively resistant to the effects of changes in the electrical field, resembling the channels produced by Complement C9 polymerization [21]. Eosinophilic cationic protein is one of the most frequently reported biomarkers for AD severity [22]. Human immunity against *Staphylococci* is based primarily on the innate immune system and secondarily on the adaptive immune system [23-25]. The antibody-independent cytotoxicity activity of normal circulating human leukocytes is a long-time described phenomenon that most properly can explain the immune mechanisms involved in the pathogenesis of AD [26]. Another antimicrobial mechanism probably associated is autophagy [27]. Autophagy is a well-described immune mechanism that mediates tolerance to *S. aureus* to limit the damage inflicted by its alpha-toxin to endothelial cells [28]. Also called efferocytosis, autophagy is an innate defense mechanism of engulfing infected apoptotic cells by primary human macrophages developed to limit intra-cellular bacterial infection [29]. C1q has a particular function in autophagy since it directly binds to the modified lipoproteins of apoptotic cell membranes, acting as an opsonin to enhance their macrophage uptake [30].

The C1q opsonization also signals the change of the lipid-loaded macrophages towards an anti-inflammatory-resolving phenotype [31]. Most patients with AD are colonized and/or infected with *S. aureus* [32]. The dysbiosis and the temporal shifts in the skin microbiome, with an increased proportion of *S. aureus* and decreased skin bacterial diversity, are particularly related to AD's inflammatory flares [33]. This vicious cycle of antigen-specific activation, tolerization, and reactivation of immune pathways is a particularly well-described dose-dependent phenomenon induced by *S. aureus* superantigens [34]. Clonal anergy may be induced by high doses of specific antigens, blocking the production of T-cell cytokines, which is studied under the perspective of T-cell exhaustion [35,36]. Activating the silencing adaptive pathways may turn the innate immune system too sensitive to antigenic stimuli, leading to a significant immune checkpoint error and changing the protective activity into a deleterious inflammatory disease [37].

The Leukocyte Adherence Inhibition Test (LAIT) is an *ex vivo* challenge laboratory procedure made with viable leukocytes, demonstrating non-specific immunoreactivity against specific microbial allergens [38-43]. Our facility employs the LAIT as a triage test to discriminate the presence of non–IgE-mediated immune activity against suspected allergens before *in vivo* provocation tests or empirical antibiotic treatment.

To evaluate the potential of the LAIT to discriminate non–IgE-mediated immunoreactivity against *S. aureus*, we retrospectively examined the medical charts of patients investigated with an *ex vivo* challenge monitored by LAIT against an *S. aureus* extract. These patients, diagnosed with Intrinsic Atopic Dermatitis (IAD), had clinical suspicion of skin dysbiosis, non-reactive skin tests, and undetectable specific IgE against *S. aureus*.

2. MATERIALS AND METHODS

2.1 Subjects

After receiving Institutional Review Board approval from the Instituto Alergoimuno de Americana (Brazil; 08/2023), we proceeded with the electronic chart review of 7,800 allergic patients who attended our outpatient facility from January 2018 to October 2023. A cohort of 200 patients had been submitted to an *ex vivo* allergen challenge test with *S. aureus* extract monitored with LAIT. The cohort counted 82 males; mean age 52.3 years; SD 19,1 years; range 18 to 93 years. We offered this procedure to patients with IAD who had an inconclusive investigation performed with allergic skin tests and undetectable specific IgE against *S. aureus* performed with ImmunoCAP® [44].

2.2 Antigen Extract

The *S. aureus* extract was acquired from CEMA – Centro de Manipulações em Alergia (NOVAK) – Álvaro de Carvalho – SP – Brazil and used to perform the LAIT and allergic skin tests [44].

2.3 *Ex vivo* Investigation: Leukocyte Adherence Inhibition Test

The LAIT was performed as we described previously (Olivier *et al.*) [45-53]. Shortly, each donor's fresh plasma was divided into two parts and used in paralleled *ex vivo* challenging tests with *S. aureus* extract and the unchallenged plasma assay. We collected the 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 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 1mg/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, we counted the leukocytes, removed the coverslip, and washed the chamber by immersion in a beaker with PBS at 37 °C. Then, we added a drop of PBS to the hemocytometer's chamber and allocated a clean coverslip 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 estimated 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 calculate the Leukocyte Adherence Inhibition (LAI) further, 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.

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. The LAI mean was 39.3%; SD 27.6%; ranging from 0% to 100%; mode = 0% (appeared 33 times).

There was a wide range of distribution of LAI results, as outlined by the cascade distribution chart in Fig. 1. Thirty-three patients ignored the presence of the allergen on the plasma and presented no inhibition of leukocyte adherence after contact with the *S. aureus* extract (16.5 % of the tests). Some patients showed low or moderate immunoreactivity during the *ex vivo* challenge test against the *S. aureus* extract. In contrast, others displayed strong immunoreactivity that possibly would reflect the S. aureus allergens' participation in the dermal inflammatory condition.

4. DISCUSSION

S. aureus is a master commensal able to turn on the production of several virulence factors to survive when stressed by the host's immune defenses. When activated, these virulence factors transform *S. aureus* into an ultimate pathogen able to produce from chronic mild conditions to debilitant and severe diseases or even acute lethal infections [54].

The immune activity testified by the LAIT may represent the stress pressed upon the pathobiont *S. aureus* by the host immune system. This immune activity may activate *S. aureus* virulence and contribute to the inflammation responsible for AD symptoms, turning the condition into a vicious circle that progressively aggravates the symptoms. AD is a condition where colonization of commensal bacteria such as *S. aureus* is both a cause and a consequence of allergic inflammation [55,56]. More than 90% of adults with AD have either *S. aureus* detected in their nares or skin [57]. *S. aureus* is a remarkable concern for physicians managing AD since reducing its colonization through systemic and topical antibiotic treatment decreases the inflammatory flares [58,59]. Phytotherapy exploits herbal options such as Tea Tree essential oil (*Melaleuca alternifolia*) to control the development of staphylococcal and streptococcal infections [60-62].

The propaedeutic investigation of hypersensitivity against *S. aureus* is routinely performed through allergic skin tests and dosage of specific IgE, which are limited tools that evaluate the humoral component of the adaptive immune arm [63,64]. The laboratory research of specific IgE is mainly directed against the superantigens secreted by *S. aureus* [65]. However, the innate immune arm performs the primary immune reconnaissance of *S. aureus* [23]. The employment of the LAIT explores the involvement of innate immune mechanisms in search of a demonstration of the hyperactivity of these pathways. The concept of "innate hypersensitivity reaction" is not new, but it was first elaborated by Rajan, who re-elaborated the Gell and Coombs classification, defining the "type V" hypersensitivity reaction as a deleterious innate reaction against pathogenic antigens [66]. Nowadays, the interaction between the innate and adaptive arms to produce hypersensitivity reactions may be appreciated by recent mouse models demonstrating that the inflammatory flares of AD skin are related to the activity of $CD4^+$ Tissue-Resident Memory cells recruiting the infiltration of neutrophils [67]. More recently, the Gell and Coombs classification was extended into nine different types and subtypes comprising three types of antibody-mediated hypersensitivity reactions (I-III); three subtypes of cell-mediated hypersensitivity reactions (subtypes IV, a to c); two types of tissue-driven mechanisms (V-VI) hypersensitivity reactions; and the ninth type of mechanism as a direct response to chemicals (VII). It is recognized that in the clinical set, several combinations of mixed types may produce the final symptoms [68]. The significant variability in immunoreactivity found in our study corroborates this viewpoint.

Performing the *ex vivo* challenge test with the leukocyte buffy coat allows the exploitation of an extensive range of immune possibilities of interactions among innate and adaptive immune cells and humoral factors with the allergens, practically covering all types of hypersensitivity reactions [69-74]. Remarkably, the LAIT alone is not able to conclude a diagnosis. The clinical diagnosis is accomplished by the responses to the in vivo challenges, the skin colonization, the exclusion of the allergens by use of antibiotic therapy, and the close observance of the symptoms after its discontinuation.

The results from the *ex vivo* challenge test monitored by LAIT against *S. aureus* extract ranged through all the possible extension spectrum (from 0% in 16.5% of patients to 81-100% in 8.5% of patients), demonstrating a significant variability of immunoreactivity in a group of patients with IAD on this retrospective preliminary survey (see Fig. 1). The results suggest that most patients already had a previous immunological experience with their antigens, while others did not (or tolerated it). We employed LAIT as a complementary triage test to select worthwhile antigens to proceed with more laborious *in vivo* provocations when the specific IgE is undetectable. More studies with prospective larger double-blind cohorts need to evaluate the potential contribution of LAIT in managing patients with *S. aureus* non–IgE-mediated hypersensitivity and IAD.

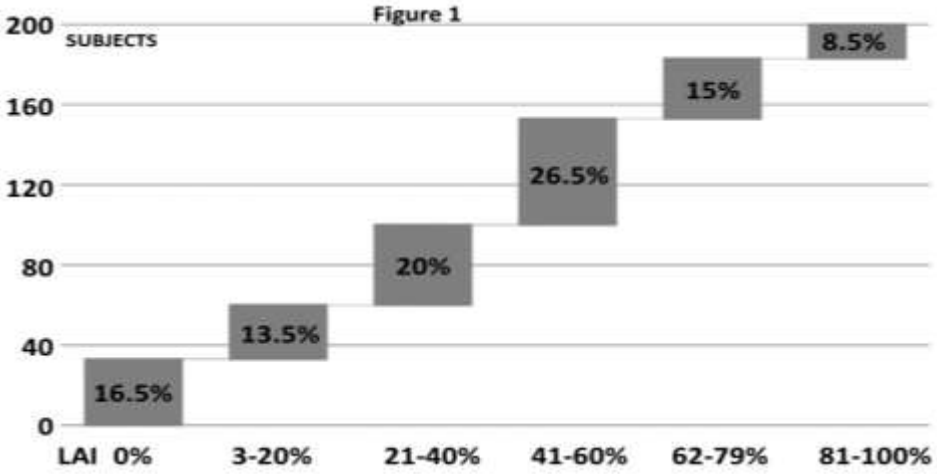

Fig. 1. Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition (LAI) results (x-axis %) of *ex vivo S. aureus* extract challenges monitored by the Leukocyte Adherence Inhibition Test (LAIT), according to the respective percentage of outcomes over 200 tests (y-axis)

5. CONCLUSION

Our preliminary results (Fig. 1) support that the LAIT may differentiate diverse *ex vivo* degrees of leukocyte adherence inhibition against the *S. aureus* extract, suggesting a previous immune experience with this agent, either toward a complete tolerance or a mild, moderate, or strong immunoreactivity. The LAIT positivity does not necessarily prove that the complaints presented by the patient were due to this specific tested antigen. The clinical diagnosis is accomplished by the responses to the *in vivo* challenges, the skin colonization, the exclusion of the allergens by use of antibiotic therapy, and the close observance of the symptoms after its discontinuation. More studies with prospective larger double-blind cohorts need to evaluate the potential contribution of LAIT in identifying patients with *S. aureus* non–IgE-mediated hypersensitivity.

CONSENT

As a retrospective survey of results recorded *incognito*, consent was given collectively by the institution's ethics committee following the principles of the Declaration of Helsinki [75].

ETHICAL APPROVALS

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

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mesjasz A, Zawadzka M, Chałubiński M, Trzeciak M. Is Atopic Dermatitis Only a Skin Disease? Int J Mol Sci. 2023;24(1):837.
2. Olivier CE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS. Intrinsic Atopic Dermatitis: Titration of Precipitins in the Screening of Food Allergens for Prescription of Elimination Diets and Desensitization Strategies. Eur J Clin Med. 2021;2(6):1-9.
3. Roguedas-Contios AM, Misery L. What is Intrinsic Atopic Dermatitis? Clin Rev Allergy Immunol. 41(3):233.
4. Ashman RB, Papadimitriou JM, Ott AK, Warmington JR. Antigens and immune responses in Candida albicans infection. Immunol Cell Biol. 1990;68(1):1-13.
5. Shi B, Bangayan NJ, Curd E, Taylor PA, Gallo RL, Leung DYM, et al. The skin microbiome is different in pediatric versus adult atopic dermatitis. J Allergy Clin Immunol. 2016;138(4):1233-1236.
6. Otto M. Staphylococcus colonization of the skin and antimicrobial peptides. Exp Rev Dermatol. 2010;5(2):183-195.
7. Family Staphylococcaceae. List of Prokaryotic names with Standing in Nomenclature (LPSN). Retrieved October 22, 2023;
   Available:https://lpsn.dsmz.de/family/staphylococcaceae.
8. Coico R. Gram Staining. Current Protocols in Microbiology, 00: A.3C.1-A.3C.2. Retrieved on October 22, 2023.
   Available:https://doi.org/10.1002/9780471729259.mca03cs00.
9. Lowy FD. Staphylococcus aureus Infections. N Eng J Med. 1998;339(8):520-532.
10. Frank DN, Feazel LM, Bessesen MT, Price CS, Janoff EN, Pace NR. The Human Nasal Microbiota and Staphylococcus aureus Carriage. PLoS ONE. 2010;5(5):e10598.
11. Li Z, Peres A, Damian A, Madrenas J. Immunomodulation and Disease Tolerance to Staphylococcus aureus. Pathogens. 2015;4(4):793-815.
12. Hanakawa Y, Schechter NM, Lin C, Garza L, Li H, Yamaguchi T, et al. Molecular mechanisms of blister formation in bullous impetigo and staphylococcal scalded skin syndrome. J. Clin. Invest. 2002;110(1):53-60.
13. Joubert O. Keller D. Pinck A, Monteil H, Prevost G. Sensitive and Specific Detection of Staphylococcal Epidermolysins A and B in Broth Cultures by Flow Cytometry-Assisted Multiplex Immunoassay. J Clin Microbiol. 2005:43(3):1076-1080.

14. Brezski RJ, Jordan RE. Cleavage of IgGs by proteases associated with invasive diseases: an evasion tactic against host immunity? MAbs. 2010;2(3):212-20.
15. Liu GY. Molecular pathogenesis of Staphylococcus aureus infection. Ped Res. 2009;65(5):71R-77R.
16. Von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of Staphylococcus aureus bacteremia. N Eng J Med. 2001;344(1):11-16.
17. Peschel A. How do bacteria resist human antimicrobial peptides? Trends Microbiol. 2002;10(4):179-186.
18. Lei J, Sun L, Huang S, Zhu C, Li P, He J, et al. The antimicrobial peptides and their potential clinical applications. Am J Transl Res. 2019;11(7):3919-3931.
19. Mahlapuu M, HÃ¥kansson J, Ringstad L, BjÃ¶rn C. Antimicrobial Peptides: An Emerging Category of Therapeutic Agents. Front Cell Infect Microbiol. 2016;6(article 194).
20. Hancock RE, Chapple DS. Peptide antibiotics. Antimicrob Ag Chemother. 1999;43(6):1317-1323.
21. Young JDE, Peterson CGB, Venge P, Cohn ZA. Mechanism of membrane damage mediated by human eosinophil cationic protein. Nature. 1986;321(6070):613-616.
22. Thijs J, Krastev T, Weidinger S, Buckens CF, de Bruin-Weller M, Bruijnzeel-Koomen C, et al. Biomarkers for atopic dermatitis: a systematic review and meta-analysis. Curr Opin Allergy Clin Immunol. 2017:15(5):453-460.
23. Fournier B, Philpott DJ. Recognition of Staphylococcus aureus by the innate immune system. Clin Microbiol Rev. 2005;18(3):521-540.
24. Karauzum H, Datta SK. Adaptive Immunity Against Staphylococcus aureus. Curr Top Microbiol Immunol. 2017;409:419-439.
25. Brandt SL, Putnam NE, Cassat JE, Serezani CH. Innate Immunity to Staphylococcus aureus: Evolving Paradigms in Soft Tissue and Invasive Infections. J Immunol. 2018;200(12):3871-3880.
26. Richter M, Banerjee D, Sklar S. The antibody-independent cytotoxic activity of normal circulating human leucocytes. II. Failure to demonstrate effector cell-target cell interaction and target cell specificity of the circulating cytotoxic-enhancing factor. Immunology. 1981;44(1):109-118.
27. Gomes LC, Dikic I. Autophagy in Antimicrobial Immunity. Molecular Cell. 2014;54(2):224-233.
28. Maurer K, Reyes-Robles T, Alonzo F, Durbin J, Torres VJ, Cadwell, K. Autophagy Mediates Tolerance to Staphylococcus aureus Alpha-Toxin. Cell Host Microbe. 2015;17(4):429-440.
29. Pan Z, Dumas E, Lawrence C, Pate L, Longobardi S, Wang X, et al. Bacillus anthracis Edema Toxin Inhibits Efferocytosis in Human Macrophages and Alters Efferocytic Receptor Signaling. Int J Mol Sci. 2019;20(5):1167.
30. Fraser DA, Curiel L, Budin R, Pulanco MC. Complement protein C1q modulates lipoprotein metabolism in macrophage foam cells. J Immunol. 2017:198(Sup 1):208.10-208.10.
31. Ho MM, Manughian-Peter A, Spivia WR, Taylor A, Fraser DA. Macrophage molecular signaling and inflammatory responses during ingestion of atherogenic lipoproteins are modulated by complement protein C1q. Atherosclerosis. 2016;253:38-46.
32. Ali HA, El-Mahdy RH, Gaballah MA. Community-acquired methicillin-resistant Staphylococcus aureus colonization in atopic dermatitis patients in Mansoura, Egypt. Biomed Dermatol. 2019;3(1):2.
33. Kong HH, Oh J, Deming C, Conlan S, Grice EA, Beatson MA, et al. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. Gen Res. 2012;22(5):850-859.
34. Röcken M, Urban J, Shevach EM. Antigen-specific activation, tolerization, and reactivation of the interleukin 4 pathway in vivo. J Exp Med. 1994;179(6):1885-1893.
35. Mueller DL, Chiodetti L, Bacon PA, Schwartz RH. Clonal anergy blocks the response to IL-4, as well as the production of IL-2, in dual-producing T helper cell clones. J Immunol. 1991;147(12):4118-4125.
36. Wherry EJ. T cell exhaustion. Nat Immunol. 2011;12(6):492-499.
37. Hünefeld C, Mezger M, Röcken M, Röcken M. The Three Dimensions of Functional T-Cell Tolerance: From Research to

Practice. J Invest Dermatol. 2012;132(3): 508-511.

38. Kuratsuji T. Studies on leukocyte adherence inhibition test. Part II. Clinical applications of LAI test to detect delayed type hypersensitivity in infants and children. Keio J Med. 1981;30(2):65-9.
39. Olivier CE, Pinto DG. Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS, et al. Evaluating Non-IgE-mediated Allergens' Immunoreactivity in Patients with "Intrinsic" Persistent Rhinitis with Help of the Leukocyte Adherence Inhibition Test. Eur J Med Health Sci. 2023;5(1):17-22.
40. Olivier CE, Pinto DG. Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS, et al. Evaluating Non-IgE-Mediated Allergens' Immunoreactivity in Patients Formerly Classified as "Intrinsic" Asthmatics with Help of the Leukocyte Adherence Inhibition Test. Eur J Clin Med. 2023;4(2):1-7.
41. Olivier CE, Pinto DG. Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS, et al. Contribution of the Leukocyte Adherence Inhibition Test to the Diagnosis of Innate Non–IgE-mediated Immunoreactivity against Alternaria alternata. Asian J Immunol. 2023;6(1):243-251.
42. Olivier CE, Pinto DG. Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS. Contribution of the Leukocyte Adherence Inhibition Test to the Diagnosis of Innate Non–IgE-mediated Immunoreactivity against Saccharomyces cerevisiae. Asian J Immunol. 2023;6(1):234-241.
43. Powell AE, Sloss AM, Smith RN. Leukocyte-Adherence Inhibition: A Specific Assay of Cell-Mediated Immunity Dependent on Lymphokine-Mediated Collaboration between T Lymphocytes. J Immunol. 1978;120(6):1957-1966.
44. Olivier CE, Argentão DGP, Santos RAPG, Silva MD, Lima RPS, Zollner RL. Skin scrape test: an inexpensive and painless skin test for recognition of immediate hypersensitivity in children and adults. Open Allergy J. 2013;6:9-17.
45. Olivier CE, Lima RPS, Pinto DG, Santos RAPG, Silva GKM, Lorena SLS, 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. 2012;67(10):1171-1179.
46. Olivier CE, Santos RAPG, Lima RPS, Argentão DGP, Silva GKM, Silva MD. A Novel Utility for an Old Method: The Leukocyte Adherence Inhibition Test Is an Easy Way to Detect the Immunoreactive Interference of the Collection Tube Anticoagulant on Cellular Immunoassays. J Cell Adhesion; 2014. article ID 860427

Available:http://dx.doi.org/10.1155/2014/860427), 1-6.
47. Olivier CE, Pinto DG, Lima RPS, Silva MD, Santos RAPG, Teixeira APM, et al. Assessment of Immunoreactivity against Therapeutic Options Employing the Leukocyte Adherence Inhibition Test as a Tool for Precision Medicine. Eur J Clin Med. 2021;2(3):40-45.
48. Olivier CE, Pinto DG, Santos RAPG, Lima RPS. Dextran's interference over the Leukocyte Adherence Inhibition Test. Academia Letter. 2021 article 3792.
49. Olivier CE, Pinto DG. Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS. Immunoreactivity against Dermatophagoides pteronyssinus Assessed by the Leukocyte Adherence Inhibition Test in Patients with Intrinsic Atopic Dermatitis and Correlated "Intrinsic" Non–IgE-mediated Allergic Conditions. Eur J Clin Med. 2021;2(6):45-50.
50. Olivier CE, Pinto DG. Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS. Contribution of the Leukocyte Adherence Inhibition Test to the Evaluation of Cellular Immunoreactivity against Latex Extracts for Non—IgE-Mediated Latex-Fruit-Pollen Syndrome in Allergic Candidates to Exclusion Diets and Allergic Desensitization. Eur J Clin Med. 2022;3(1):11-17.
51. Olivier CE, Pinto DG. Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS. Contribution of the Leukocyte Adherence Inhibition Test for the evaluation of immunoreactivity against gluten extracts in non—IgE-mediated / non-autoimmune Gluten-Related Disorders. Eur J Clin Med. 2022;3(2):1-7.
52. Olivier CE, Pinto DG. Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS. Leukocyte Adherence Inhibition Test to the Assessment of Immunoreactivity Against Cow's Milk Proteins in Non—IgE-Mediated

Gastrointestinal Food Allergy. Eur J Clin Med. 2022;3(2):38-43.

53. Olivier CE, Pinto DG. Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS. Contribution of the Leukocyte Adherence Inhibition Test to the Diagnosis of Immunoreactivity against Cobalt. Asian J Immunol. 2023;6(1):174-184.
54. Schlievert PM, Strandberg KL, Lin YC, Peterson ML, Leung DY. Secreted virulence factor comparison between methicillin-resistant and methicillin-sensitive Staphylococcus aureus, and its relevance to atopic dermatitis. J Allergy Clin Immunol. 2010;125(1):39-49.
55. Lin YT, Wang CT, Chiang BL. Role of bacterial pathogens in atopic dermatitis. Clin Rev Allergy Immunol. 2007;33(3):167-77.
56. Kim J, Kim BE, Ahn K, Leung DYM. Interactions Between Atopic Dermatitis and Staphylococcus aureus Infection: Clinical Implications. Allergy Asthma Immunol Res. 2019;11(5):593-603.
57. Brown AF, Leech JM, Rogers TR, McLoughlin RM. Staphylococcus aureus Colonization: Modulation of Host Immune Response and Impact on Human Vaccine Design. Front Immunol. 2014;4(article 507):1-20.
58. Geoghegan JA, Irvine AD, Foster TJ. Staphylococcus aureus and Atopic Dermatitis: A Complex and Evolving Relationship. Trends Microbiol. 2018;26(6):484-497.
59. Huang JT, Abrams M, Tlougan B, Rademaker A, Paller AS. Treatment of Staphylococcus aureus Colonization in Atopic Dermatitis Decreases Disease Severity. Pediatrics. 2009;123(5):e808-e814.
60. Halcón L, Milkus K. Staphylococcus aureus and wounds: A review of tea tree oil as a promising antimicrobial. Am J Infect Control. 2004;32(7):402-408.
61. Carson CF, Hammer KA, Riley TV. Melaleuca alternifolia (Tea Tree) Oil: a Review of Antimicrobial and Other Medicinal Properties. Clin Microbiol Rev. 2006;19(1):50-62.
62. Carson CF, Mee BJ, Riley TV. Mechanism of Action of Melaleuca alternifolia (Tea Tree) Oil on Staphylococcus aureus Determined by Time-Kill, Lysis, Leakage, and Salt Tolerance Assays and Electron Microscopy. Antimicrob Ag Chemother. 2002;46(6):1914-1920.
63. Bachert C, Humbert M, Hanania NA, Zhang N, Holgate S, Buhl R, et al. Staphylococcus aureus and its IgE-inducing enterotoxins in asthma: current knowledge. Eur Respir J. 2020;55(4):1901592.
64. Hauser C, Wuethrich B, Matter L, Wilhelm JA, Schopfer K. Immune Response to Staphylococcus aureus in Atopic Dermatitis. Dermatologica. 2009;170(3):114-120.
65. Leung DYM. Pathogenesis of atopic dermatitis. J Allergy Clin Immunol. 1999;104(3):S99-S108.
66. Rajan TV. The Gell-Coombs classification of hypersensitivity reactions: a re-interpretation. Trends Immunol. 2003;24(7):376-9.
67. Zheng C, Cao T, Ye C, Zou Y. Neutrophil recruitment by CD4 tissue-resident memory T cells induces chronic recurrent inflammation in atopic dermatitis. Clin Immunol. 2023;109805.
68. Jutel M, Agache I, Zemelka-Wiacek M, Akdis M, Chivato T, Del Giacco S, Gajdanowicz P, et al. Nomenclature of allergic diseases and hypersensitivity reactions: Adapted to modern needs: An EAACI position paper. Allergy; 2023.

Available:https://onlinelibrary.wiley.com/doi/epdf/10.1111/all.15889.
69. Shirakawa T, Kusaka Y, Fujimura N, Goto S, Morimoto K. The existence of specific antibodies to cobalt in hard metal asthma. Clin Exp Allergy. 1988;18(5):451-460.
70. Olivier CE, Lima RPS, Pinto DG, Santos RAPG. The Plasma Preincubation with Papain Before the Assay Suggests that a Gell and Coombs Type II Reaction is Been Demonstrated by the Leukocyte Adherence Inhibition Test. Biomedic J Sci Tech Res. 2021;36(3):28647-28655.
71. Thomson DMP. Assessment of immune status by the leukocyte adherence inhibition test. Academic Press: New York. 1982; 380 p.
72. Tong AW, Burger DR, Finke P, Barney C, Vandenbark AA, Vetto RM. Assessment of the mechanism of the leukocyte adherence inhibition test. Cancer Res. 1979;39(2): 597-603.

73. Fink A, Heller L, Eliraz A, Weisman Z, Miskin A, Schlezinger M, et al. Allergen-specific leukocyte adherence inhibition (LAI) assay: sensitivity, specificity and mechanism. Immunol Lett. 1987;16(1):65-70.

74. Halliday WJ, Maluish A, Miller S. Blocking and unblocking of cell-mediated anti-tumor immunity in mice, as detected by the leucocyte adherence inhibition test. Cell Immunol. 1974;10(3):467-475.

75. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-4.

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